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ACE2 Research Literatures

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Abstract: Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus is mainly spread during close contact and via respiratory droplets that are produced when a person talks, coughs, or sneezes. Respiratory droplets may be produced during breathing, however, current research indicates that the virus is not considered airborne. People may also contract COVID-19 by touching a contaminated surface (Fomite) and then inadvertently transfer the pathogen to a mucous membrane (such as the eyes, nose, or mouth). It is most contagious when people are symptomatic, although spread may be possible before symptoms appear. The virus can live on surfaces up to 72 hours. Time from exposure to onset of symptoms is generally between two and fourteen days, with an average of five days. The standard method of diagnosis is by reverse transcription polymerase chain reaction (rRT-PCR) from a nasopharyngeal swab. The infection can also be diagnosed from a combination of symptoms, risk factors and a chest CT scan showing features of pneumonia. This article introduces recent research reports as references in the related studies.
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Key words: ACE2; life; research; literature

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus is mainly spread during close contact and via respiratory droplets that are produced when a person talks, coughs, or sneezes. Respiratory droplets may be produced during breathing, however, current research indicates that the virus is not considered airborne. People may also contract COVID-19 by touching a contaminated surface (Fomite) and then inadvertently transfer the pathogen to a mucous membrane (such as the eyes, nose, or mouth). It is most contagious when people are symptomatic, although spread may be possible before symptoms appear. The virus can live on surfaces up to 72 hours. Time from exposure to onset of symptoms is generally between two and fourteen days, with an average of five days. The standard method of diagnosis is by reverse transcription polymerase chain reaction (rRT-PCR) from a nasopharyngeal swab. The infection can also be diagnosed from a combination of symptoms, risk factors and a chest CT scan showing features of pneumonia. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Abdel-Fattah, M. M., et al. (2018). "Modulation of brain ACE and ACE2 may be a promising protective strategy against cerebral ischemia/reperfusion injury: an experimental trial in rats." Naunyn Schmiedebergs Arch Pharmacol **391**(9): 1003-1020.

The brain renin-angiotensin system (RAS) is considered a crucial regulator for physiological homeostasis and disease progression. We evaluated the protective effects of the angiotensin receptor blocker (ARB) telmisartan and the angiotensin-converting enzyme 2 (ACE2) activator xanthenone on experimental cerebral ischemia/reperfusion (I/R) injury. Rats were divided into a sham control, a cerebral I/R control, a standard treatment (nimodipine, 10 mg/kg/day, 15 days, p.o.), three telmisartan treatments (1, 3, and 10 mg/kg/day, 15 days, p.o.), and three xanthenone treatments (0.5, 1, and 2 mg/kg/day, 15 days, s.c.) groups. One hour after the last dose, all rats except the sham control group were exposed to 30-min cerebral ischemia followed by 24-h reperfusion. Brain ACE and ACE2 activities and the apoptotic marker caspase-3 levels were assessed. Glutathione (GSH), malondialdehyde (MDA), and nitric oxide end products (NOx) as oxidative markers and tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), and IL-10 as immunological markers were assessed. Histopathological examination and immunohistochemical evaluation of glial fibrillary

acidic protein (GFAP) were performed in cerebral cortex and hippocampus sections. Telmisartan and xanthenone in the higher doses restored MDA, NOx, TNF-alpha, IL-6, caspase-3, ACE, and GFAP back to normal levels and significantly increased GSH, IL-10, and ACE2 compared to I/R control values. Histopathologically, both agents showed mild degenerative changes and necrosis of neurons in cerebral cortex and hippocampus compared with I/R control group. Modulation of brain RAS, either through suppression of the classic ACE pathway or stimulation of its antagonist pathway ACE2, may be a promising strategy against cerebral I/R damage.

Abdelkader, N. F., et al. (2020). "Telmisartan/17beta-estradiol mitigated cognitive deficit in an ovariectomized rat model of Alzheimer's disease: Modulation of ACE1/ACE2 and AT1/AT2 ratio." Life Sci **245**: 117388.

AIMS: The higher incidence rate of Alzheimer's disease (AD) among women has led to explorations on the association between estrogen deficiency and AD. Also, usage of antihypertensive drugs has been suggested to reduce the incidence of AD in elderly hypertensive patients. Thus, this study aimed to investigate the effects of telmisartan and/or 17beta-estradiol on a cognitively impaired ovariectomized rat model of AD. MAIN METHODS: 75 female Wistar rats were randomly allocated into five groups. One group was sham operated and the other four groups were subjected to ovariectomy, received D-galactose and either untreated or treated with telmisartan and/or 17beta-estradiol for 6 weeks. KEY FINDINGS: Ovariectomized rats showed cognitive impairment in Morris water maze and novel object recognition tests, increasing inflammatory biomarkers (tumor necrosis factor-alpha, and interleukin-1beta), increasing AD biomarkers (amyloid beta1-42, and acetylcholine esterase), and over activation of classical arm of renin angiotensin system (RAS) (ACE1/Ang2/AT1) in hippocampi. Also, hippocampi histopathological examination revealed amyloid beta deposition. Whereas, administration of telmisartan and/or 17beta-estradiol improved animals' behavior, alleviated histopathological alterations and reduced the level of inflammatory and AD biomarkers, modulated RAS activity favoring the novel neuroprotective arm (ACE2/Ang (1-7)/MasR). SIGNIFICANCE: Our findings suggest that combined administration of both drugs has synergetic neuroprotective effects; supporting their potential application in AD treatment.

Abe, M., et al. (2015). "Urinary ACE2 is associated with urinary L-FABP and albuminuria in patients with chronic kidney disease." Scand J Clin Lab Invest **75**(5): 421-427.

AIM: Angiotensin-converting enzyme 2 (ACE2) is expressed in the kidney and may be a renoprotective enzyme since it converts angiotensin (Ang) II to Ang-(1-7). In addition, ACE2 has been detected in urine from patients with chronic kidney disease (CKD). The aim of this study was to determine the urinary ACE2 levels in patients with various stages of CKD and to identify the factors associated with the presence of ACE2. METHODS: We assessed 152 patients with CKD stage G1-G4. The patients were classified according to the presence or absence of diabetes mellitus (DM) (DM group, n = 72; non-DM group, n = 80) and according to the estimated glomerular filtration rate (CKD stage G1/2 group, n = 40; CKD stage G3 group, n = 74; and CKD stage G4 group, n = 38). Parameters were urinary ACE2, urinary albumin/creatinine ratio (UACR), urinary liver-type fatty acid binding protein (L-FABP), estimated glomerular filtration rate, and other factors determined to be associated with elevated urinary ACE2. RESULTS: Urinary ACE2 was significantly higher in patients with diabetes ($p = 0.01$) and in patients with CKD stage G4 compared with stages G1-G3 ($p < 0.0001$). Multivariable regression analysis revealed that urinary L-FABP and UACR were significantly associated with urinary ACE2 levels, indicating that urinary ACE2 is increased in patients with diabetes and advanced stage CKD. CONCLUSION: ACE2 might continuously protect from both glomerular and tubulointerstitial injury during CKD progression. Taken together, urinary ACE2 might be a marker of kidney renin-angiotensin system activation in such patients.

Aguilar, C., et al. (2011). "[Atorvastatin induced increase in homologous angiotensin I converting enzyme (ACE2) mRNA is associated to decreased fibrosis and decreased left ventricular hypertrophy in a rat model of diabetic cardiomyopathy]." Rev Peru Med Exp Salud Publica **28**(2): 264-272.

OBJECTIVES: This study has investigated the effect of atorvastatin on the progression of cardiac remodelling and ACE- 2 expression in diabetic myocardium in rats. MATERIALS AND METHODS: Diabetes was induced in Holtzman rats with an intraperitoneal injection of streptozotocin. The animals were divided into 3 groups: (1) normal control rats, (2) diabetic rats and (3) diabetic rats treated orally with atorvastatin (50 mg/kg/day). After eight weeks of treatment, the hearts were removed for morphometric studies, collagen content assay and genetic expressions of ACE and ACE2 mRNA. RESULTS: Myocardial hypertrophy index and collagen deposition were increased in diabetic rats, but not in the treated-diabetic rats, without producing changes in cholesterol levels. Myocardial ACE mRNA levels were increased while ACE2 mRNA levels were decreased in diabetic

rats. Atorvastatin administration attenuated overexpression of ACE mRNA and overexpression of ACE-2 mRNA in diabetic rats. CONCLUSIONS: Our results indicate that atorvastatin, independently of its cholesterol-lowering capacity, lowers the ACE/ACE2 ratio to normal values and attenuates the development of adverse remodeling in the diabetic heart.

Alenina, N. and M. Bader (2019). "ACE2 in Brain Physiology and Pathophysiology: Evidence from Transgenic Animal Models." Neurochem Res **44**(6): 1323-1329.

Angiotensin-converting enzyme 2 (ACE2) is a protein consisting of two domains, the N-terminus is a carboxypeptidase homologous to ACE and the C-terminus is homologous to collectrin and responsible for the trafficking of the neutral amino acid transporter B (0)AT1 to the plasma membrane of gut epithelial cells. The carboxypeptidase domain not only metabolizes angiotensin II to angiotensin-(1-7), but also other peptide substrates, such as apelin, kinins and morphins. In addition, the collectrin domain regulates the levels of some amino acids in the blood, in particular of tryptophan. Therefore it is of no surprise that animals with genetic alterations in the expression of ACE2 develop a diverse pattern of phenotypes ranging from hypertension, metabolic and behavioural dysfunctions, to impairments in serotonin synthesis and neurogenesis. This review summarizes the phenotypes of such animals with a particular focus on the central nervous system.

Alghamri, M. S., et al. (2013). "Enhanced angiotensin II-induced cardiac and aortic remodeling in ACE2 knockout mice." J Cardiovasc Pharmacol Ther **18**(2): 138-151.

Angiotensin-converting enzyme 2 (ACE2) is present in the heart and thought to exert protective functions. We conducted studies in ACE2 deficient mice to determine whether enzyme loss would exacerbate the cardiac and vascular pathological responses to chronic subcutaneous (sc) angiotensin II (Ang II) infusion. Eight-week-old male ACE2 knockout (KO) and wild type (WT) mice were infused with Ang II (1000 ng/kg per min, 4 weeks) using mini-osmotic pumps. Blood pressure (radiotelemetry), cardiac function (echocardiography, echo), cardiac/aortic structure (histology, collagen, and oxidative stress), and vascular inflammation were examined. Before Ang II infusion, ACE2 KO mice showed unaltered cardiac function and blood pressure. After 4 weeks of Ang II infusion, the mean arterial pressure (MAP) increased from 96 +/- 2 to 136 +/- 17 mm Hg (approximately 40%) in WT and from 104 +/- 5 to 141 +/- 13 mm Hg (approximately 35%) in ACE2 KO. While there were no differences in MAP between groups, the ACE2 KO responded differently to the hypertensive stimulus. Echo analysis revealed severe myocardial dysfunction in Ang II-infused ACE2 KO (Ang ACE2 KO). Ejection fraction was lower (39% versus 50%) as was fractional shortening (27% versus 38%) in ACE2 KO versus WT, respectively. Cardiac dysfunction was associated with hypertrophic cardiomyopathy shown by increased left-ventricular wall thickness, average cardiomyocyte cross-sectional area, and heart weight/body weight ratio. Collagen staining in the myocardium and aorta revealed increased collagen in Ang ACE2 KO, suggestive of remodeling. Results also showed enhanced oxidative stress in the myocardium and aorta of Ang ACE2 KO. There was a 3-fold elevation in macrophage inflammatory protein 1alpha (MIP 1alpha) in the aorta of ACE2 KO. Studies in the ACE2 KO model reveal the importance of ACE2 in the maladaptive cardiac and aortic responses to Ang II stimulation, seen as enhanced remodeling using physiological, structural, and biochemical markers. Results document a cardio- and vascular-protective role of ACE2 under pathological conditions.

Ali, Q., et al. (2013). "Chronic AT2 receptor activation increases renal ACE2 activity, attenuates AT1 receptor function and blood pressure in obese Zucker rats." Kidney Int **84**(5): 931-939.

Abnormal regulation of the renin angiotensin system such as enhanced renal AT1R function and reduced ACE2 activity contributes to obesity-related hypertension. Here, we tested whether long-term AT2R activation affects renal function in obesity using lean and obese Zucker rats treated with the AT2R agonist CGP42112A for 2 weeks. This caused blood pressure to decrease by 13 mm Hg, which was associated with increased urinary sodium excretion in the obese rats. Cortical ACE2 expression and activity, the Mas receptor (MasR), and its ligand angiotensin-(1-7) were all increased in CGP-treated obese compared with control rats. Candesartan-induced natriuresis, a measure of AT (1)R function, was reduced but cortical AT (1)R expression and angiotensin II levels were similar in CGP-treated obese compared with control rats. Renin and AT2R expression in obese rats was not affected by CGP treatment. In HK-2 cells in vitro, CGP treatment caused increased ACE2 activity and MasR levels but decreased AT (1)R levels and renin activity. Thus, long-term AT2R activation shifts the opposing arms of renin angiotensin system and contributes to natriuresis and blood pressure reduction in obese animals. Our study highlights the importance of AT2R as a target for treating obesity-related hypertension.

Ali, R. M., et al. (2018). "Role of Wnt4/beta-catenin, Ang II/TGFbeta, ACE2, NF-kappaB, and IL-18 in attenuating renal ischemia/reperfusion-induced injury in rats treated with Vit D and pioglitazone." Eur J Pharmacol **831**: 68-76.

Renal ischemia-reperfusion injury (I/RI) remains a critical clinical situation. Several evidence revealed the potential reno-protective effects of Vitamin D and/or pioglitazone, on renal I/RI. This study addresses the possible involvement of the Wnt4/beta-catenin signaling, p-S536NF-kappaBp65, PPARgamma, Ang II/TGF-beta, and ACE2 as potential effectors to vitamin D and pioglitazone-mediated renoprotective effects. Two sets of Sprague-Dawley rats (n=30 rat each), were randomized into sham, I/R, Vit D "alfacalcidol" (5ng/kg/day), pioglitazone (5mg/kg/day), and Vit D +pioglitazone groups. In all groups renal biochemical parameters, as well as inflammatory and structural profiles were assessed, besides the expression/contents of Wnt4/beta-catenin and pS536-NF-kappaBp65. All treatments started 7 days before I/RI and animals were killed 24h after I/RI in the first set, while those in the 2nd set continued their treatments for 14 days. After 24h, all pre-treatments impeded theI/R effect on neutrophils recruitment, p-S536NF-kappaBp65, IL-18, NGAL, caspase-3, AngII, ACE-2, PPARgamma and TGF-beta, besides the expression of Wnt4 and ACE-2 with notable reflection on histological changes. Two weeks after I/RI, except a marked up regulation in Wnt4 expression and a striking elevation in the beta-catenin content, the magnitude of the injurious events was relatively less pronounced, an effect that was mostly augmented by the different treatments. The current study pledges a promising and novel reno-protective role of the administration of Vit D and pioglitazone entailing a potential involvement of ICAM-1, MPO, NF-kappaB, Ang II, ACE2, TGFbeta, and a modulation of Wnt4/beta-catenin pathway.

Anguiano, L., et al. (2017). "Circulating ACE2 in Cardiovascular and Kidney Diseases." Curr Med Chem **24**(30): 3231-3241.

Angiotensin converting enzyme (ACE) 2 is a homologue of ACE that catalyzes the conversion of Angiotensin (Ang) II into Ang1-7, which induces vasodilation, anti-fibrotic, anti-proliferative and anti-inflammatory effects. Given that ACE2 counterbalances the effects of Ang II, it has been proposed as a biomarker in kidney disease patients. Circulating ACE2 has been studied in human and experimental studies under physiological and pathological conditions and different techniques have been assessed to determine its enzymatic activity. In patients with cardiovascular (CV) disease circulating ACE2 has been shown to be increased. In addition, hypertensive and diabetic patients have also shown higher circulating ACE2 activities. A study in type 1 diabetes patients found a negative association between circulating ACE2 and estimated glomerular filtration rate in male and female patients. Recently, it has been demonstrated that circulating ACE2 is increased in male patients with chronic kidney disease (CKD) and that it is independently associated with other classical CV risk factors, such as advanced age and diabetes. Furthermore, circulating ACE2 has been shown to be associated with silent atherosclerosis and CV outcomes in CKD patients. In diabetic nephropathy, experimental studies have demonstrated an increase in circulating ACE2 activity both at early and late stages of the disease, as well as a direct association with increased urinary albumin excretion, suggesting that it may be increased as a renoprotective mechanism in these patients. In this paper we will review the measurement of circulating ACE2 and its role in kidney disease, as well as its potential role as a renal and CV biomarker.

Aragao, D. S., et al. (2011). "Purification and characterization of angiotensin converting enzyme 2 (ACE2) from murine model of mesangial cell in culture." Int J Biol Macromol **49**(1): 79-84.

Angiotensin converting enzyme 2 (ACE2) is a component of the renin-angiotensin system (RAS) which converts Ang II, a potent vasoconstrictor peptide into Ang 1-7, a vasodilator peptide which may act as a negative feedback hormone to the actions of Ang II. The discovery of this enzyme added a new level of complexity to this system. The mesangial cells (MC) have multiple functions in glomerular physiology and pathophysiology and are able to express all components of the RAS. Despite of being localized in these cells, ACE2 has not yet been purified or characterized. In this study ACE2 from mice immortalized MC (IMC) was purified by ion-exchange chromatography. The purified enzyme was identified as a single band around 60-70 kDa on SDS-polyacrylamide gel and by Western blotting using a specific antibody. The optima pH and chloride concentrations were 7.5 and 200 mM, respectively. The N-terminal sequence was homologous with many species ACE2 N-terminal sequences as described in the literature. ACE2 purified from IMC was able to hydrolyze Ang II into Ang 1-7 and the K (m) value for Ang II was determined to be 2.87 +/- 0.76 muM. In conclusion, we purified and localized, for the first time, ACE2 in MC, which was able to generate Ang 1-7 from Ang II. Ang 1-7 production associated to Ang II degradation by ACE2 may exert a protective effect in the renal hemodynamic.

Arumugam, S., et al. (2012). "Candesartan cilexetil protects from cardiac myosin induced cardiotoxicity via reduction of endoplasmic reticulum stress and apoptosis in rats: involvement of ACE2-Ang (1-7)-mas axis." Toxicology **291**(1-3): 139-145.

Candesartan cilexetil, an angiotensin (Ang) II receptor 1 blocker was reported to suppress the myocardial damage in various cardiovascular complications but the mode by which it is effective in preventing the progression of dilated cardiomyopathy (DCM) is unknown. Emerging evidences suggest that, at least, part of the benefits observed with the use of AT1 receptor blockers could be attributed to the increased Ang (1-7) levels observed during administration of these agents. Identification of the novel components of the RAS, ACE2 and Ang (1-7) receptor mas, provided essential elements for considering the existence of a vasodilator arm of the RAS, represented by the ACE2-Ang (1-7)-mas axis. In this study, rat model of DCM was prepared by injection with porcine cardiac myosin. Twenty-eight days after immunization, candesartan cilexetil was administered intraperitoneally at 1 or 10mg/kg/day to rats for four weeks. Myocardial expression of Ang receptors and markers of calcium homeostasis, endoplasmic reticulum (ER) stress and apoptosis were measured by Western blotting and histopathological staining techniques. Candesartan improved the functional markers in a dose-dependent manner and also upregulated Ang (1-7), ACE2 and mas1 in the myocardium of DCM rats. Various ER stress and apoptosis markers were attenuated and the number apoptotic cells were significantly lower in the candesartan treated rats compared with those of the vehicle group. These findings suggest that candesartan treatment prevented the progression of DCM by activation of the counter regulatory arm of the RAS and possibly through modulation of ER stress and subsequently, cardiac apoptosis.

Badae, N. M., et al. (2019). "Is the cardioprotective effect of the ACE2 activator diminazene aceturate more potent than the ACE inhibitor enalapril on acute myocardial infarction in rats?" Can J Physiol Pharmacol **97**(7): 638-646.

Myocardial infarction is a major cause of cardiac dysfunction. All components of the cardiac renin-angiotensin system (RAS) are upregulated in myocardial infarction. Angiotensin-converting enzyme (ACE) and ACE2 are key enzymes involved in synthesis of components of RAS and provide a counter-regulatory mechanism within RAS. We compared the cardioprotective effect of the ACE2 activator diminazene aceturate (DIZE) versus the ACE inhibitor enalapril on post acute myocardial infarction (AMI) ventricular dysfunction in rats. Adult male rats received subcutaneous injections of either saline (control) or isoproterenol (85 mg/kg) to induce AMI. Rats with AMI confirmed biochemically and by ECG, were either left untreated (AMI) or administered DIZE (AMI + DIZE) or enalapril (AMI + enalapril) daily for 4 weeks. DIZE caused a significant activation of cardiac ACE2 compared with enalapril. DIZE caused a significantly greater enhancement of cardiac hemodynamics. DIZE also caused greater reductions in heart-type fatty acid binding protein (H-FABP), beta-myosin heavy chain (beta-MYH), and in heart mass to total body mass ratio. These results indicated that activation of cardiac ACE2 by DIZE enhanced the protective axis of RAS and improved myocardial function following AMI, whereas enalapril was not sufficient to restore all cardiac parameters back to normal.

Bader, M. (2013). "ACE2, angiotensin-(1-7), and Mas: the other side of the coin." Pflugers Arch **465**(1): 79-85.

The renin-angiotensin system (RAS) has recently been extended by the addition of a novel axis consisting of the angiotensin-converting enzyme 2 (ACE2), the heptapeptide angiotensin (1-7) (Ang-(1-7)), and the G protein-coupled receptor Mas. ACE2 converts the vasoconstrictive and pro-oxidative peptide angiotensin II (Ang II) into Ang-(1-7) which exerts vasodilatory and antioxidative effects via its receptor Mas. Thereby, ACE2 regulates the local actions of the RAS in cardiovascular tissues and the ACE2/Ang-(1-7)/Mas axis exerts protective actions in hypertension, diabetes, and other cardiovascular disorders. Consequently, this novel RAS axis represents a promising therapeutic target for cardiovascular and metabolic diseases.

Bai, F., et al. (2016). "Angiotensin II AT1 receptor alters ACE2 activity, eNOS expression and CD44-hyaluronan interaction in rats with hypertension and myocardial fibrosis." Life Sci **153**: 141-152.

AIM: This study tested the hypothesis that angiotensin II (Ang II) AT1 receptor is involved in development of hypertension and cardiac fibrosis via modifying ACE2 activity, eNOS expression and CD44-hyaluronan interaction. MAIN METHODS: Male Sprague-Dawley rats were subjected to Ang II infusion (500ng/kg/min) using osmotic minipumps up to 4weeks and the AT1 receptor blocker, telmisartan was administered by gastric gavage (10mg/kg/day) during Ang II infusion. KEY FINDINGS: Our results indicated that Ang II enhances AT1 receptor, downregulates AT2 receptor, ACE2 activity and eNOS expression, and increases CD44 expression and hyaluronidase activity, an enzyme for hyaluronan degradation. Further analyses revealed that Ang II

increases blood pressure and augments vascular/interstitial fibrosis. Comparison of the Ang II group, treatment with telmisartan significantly increased ACE2 activity and eNOS expression in the intracardiac vessels and intermyocardium. These changes occurred in coincidence with decreased blood pressure. Furthermore, the locally-expressed AT1 receptor was downregulated, as evidenced by an increased ratio of the AT2 over AT1 receptor (1.4+/-0.4% vs. 0.4+/-0.1% in Ang II group, P<0.05). Along with these modulations, telmisartan inhibited membrane CD44 expression and hyaluronidase activity, decreased populations of macrophages and myofibroblasts, and reduced expression of TGFbeta1 and Smads. Collagen I synthesis and tissue fibrosis were attenuated as demonstrated by the less extensive collagen-rich area. SIGNIFICANCE: These results suggest that the AT1 receptor is involved in development of hypertension and cardiac fibrosis. Selective activating ACE2/eNOS and inhibiting CD44/HA interaction might be considered as the therapeutic targets for attenuating Ang II induced deleterious cardiovascular effects.

Bai, S., et al. (2013). "Effects of felodipine combined with puerarin on ACE2-Ang (1-7)-Mas axis in renovascular hypertensive rat." Regul Pept **184**: 54-61.

This study aimed to investigate the effect of combination of felodipine+puerarin on ACE2-Ang (1-7)-Mas axis, and to explore the protective effect of the combination against kidney in renovascular hypertensive rats. Goldblatt rats were randomly divided into 5 groups as follows: 4 groups which were treated with felodipine (Felo), puerarin (Pue), Felo+Pue, and Felo+captopril (Cap), respectively, and a control group of animals that were administrated with distilled water. Contents of Ang II and Ang (1-7) in renal tissues were determined by ELISA kit. The mRNA expression of ACE2/Mas and ACE/AT1 in kidneys was analyzed by RT-PCR. After 8weeks of treatment, compared with Goldblatt group, Felo+Pue reduced SBP, DBP and HR ($p<0.01$ or $p<0.05$), ameliorated renal interstitial fibrosis, decreased the level of Ang II and increased that of Ang (1-7), upregulated mRNA expression of ACE2 and Mas, decreased that of ACE and AT1, and downregulated protein expression of TGF-beta1 in kidneys ($p<0.01$). Compared with Felo group, Felo+Pue decreased DBP and HR more markedly, attenuated fibrosis, decreased Ang II levels and increased those of Ang (1-7), upregulated mRNA expression of ACE2 in bilateral kidneys and that of Mas in ischemic kidney, downregulated that of ACE in bilateral kidneys and that of AT1 in ischemic kidney, and decreased expression of TGF-beta1 protein significantly. In a word, a combination of Felo+Pue has a more efficient therapeutic effect on DBP and HR, and contributes to a better protection against renal interstitial fibrosis.

Basu, R., et al. (2017). "Roles of Angiotensin Peptides and Recombinant Human ACE2 in Heart Failure." J Am Coll Cardiol **69**(7): 805-819.

BACKGROUND: The renin-angiotensin system (RAS) is activated in heart failure (HF) and inhibition of RAS is a mainstay therapy for HF. Angiotensin-converting enzyme 2 (ACE2) and its product, angiotensin 1-7 (Ang-[1-7]), are important negative regulators of the RAS. OBJECTIVES: A comprehensive examination of angiotensin peptide levels and therapeutic effects of recombinant human ACE2 (rhACE2) on peptide metabolism was evaluated in human plasma and explanted heart tissue from patients with HF. METHODS: Using prospective cohorts with chronic (n = 59) and acute (n = 42) HF, plasma angiotensin analysis was performed using a unique liquid chromatography-mass spectrometry/mass spectroscopy method quantifying circulating and equilibrium levels. Angiotensin II (Ang II) metabolism was examined in human explanted hearts with dilated cardiomyopathy (n = 25). RESULTS: The dynamic range of the RAS was large, with equilibrium angiotensin levels being 8- to 10-fold higher compared with circulating angiotensin levels. In chronic HF patients receiving ACE inhibition, plasma Ang II was suppressed and plasma Ang-(1-7) was elevated, whereas acute HF and patients receiving angiotensin receptor blocker had higher plasma Ang II with lower Ang-(1-7) levels. Suppressed Ang-(1-7)/Ang II ratio was associated with worsening HF symptoms and longer hospitalization. Recombinant human ACE2 effectively metabolized Ang-(1-10) and Ang II into Ang-(1-9) and Ang-(1-7), respectively. Myocardial Ang II levels in explanted human hearts with dilated cardiomyopathy were elevated despite ACE inhibition with elevated chymase activity, and Ang II was effectively converted to Ang-(1-7) by rhACE2. CONCLUSIONS: Plasma angiotensin peptides represent a dynamic network that is altered in HF and in response to rhACE2. An increased plasma Ang-(1-7) level is linked to ACE inhibitor use, whereas acute HF reduced Ang-(1-7) levels and suppressed the Ang-(1-7)/Ang II ratio. Increased chymase activity elevated Ang II levels in failing human hearts. Use of rhACE2 effectively normalized elevated Ang II while increasing Ang-(1-7) and Ang-(1-9) levels.

Benjafield, A. V., et al. (2004). "No association of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms with essential hypertension." Am J Hypertens **17**(7): 624-628.

Recent intriguing findings from genetic linkage, knockout, and physiologic studies in mice and rats led us to conduct the first investigation of the novel angiotensin-converting enzyme 2 gene (ACE2) in human hypertension (HT). We genotyped four single nucleotide polymorphisms (SNP) (A-->G at nucleotide 1075 in intron 1, G-->A at nucleotide 8790 in intron 3, C-->G at nucleotide 28330 in intron 11, and G-->C at nucleotide 36787 in intron 16) in HT (n = 152) and normotensive (NT, n = 193) groups having inherently high biological power (>80%) due to our inclusion only of subjects whose parents had the same BP status as themselves. The SNPs were in linkage disequilibrium (D' = 54% to 100%, P =.05 to 0.0001). Because ACE2 is on the X chromosome, data for each sex were analyzed separately. Minor allele frequencies in HT versus NT were as follows: for the intron 1 variant 0.21 versus 0.17 in female subjects (P =.31) and 0.25 versus 0.29 in male subjects (P =.60); intron 3 variant 0.22 versus 0.18 in female subjects (P =.35) and 0.15 versus 0.20 in male subjects (P =.47); intron 11 variant 0.39 versus 0.46 in male subjects (P = 0.17) and 0.31 versus 0.30 in male subjects (P =.96); intron 16 variant 0.20 versus 0.19 in female subjects (P =.72) and 0.17 versus 0.17 in male subjects (P =.95). Haplotype analysis was also negative. These data provide little support for ACE2 in genetic predisposition to HT.

Bennion, D. M., et al. (2015). "Neuroprotective mechanisms of the ACE2-angiotensin-(1-7)-Mas axis in stroke." Curr Hypertens Rep **17**(2): 3.

The discovery of beneficial neuroprotective effects of the angiotensin converting enzyme 2-angiotensin-(1-7)-Mas axis [ACE2-Ang-(1-7)-Mas] in ischemic and hemorrhagic stroke has spurred interest in a more complete characterization of its mechanisms of action. Here, we summarize findings that describe the protective role of the ACE2-Ang-(1-7)-Mas axis in stroke, along with a focused discussion on the potential mechanisms of neuroprotective effects of Ang-(1-7) in stroke. The latter incorporates evidence describing the actions of Ang-(1-7) to counter the deleterious effects of angiotensin II (AngII) via its type 1 receptor, including anti-inflammatory, anti-oxidant, vasodilatory, and angiogenic effects, and the role of altered kinase-phosphatase signaling. Interactions of Mas with other receptors, including bradykinin receptors and AngII type 2 receptors are also considered. A more complete understanding of the mechanisms of action of Ang-(1-7) to elicit neuroprotection will serve as an essential step toward research into potential targeted therapeutics in the clinical setting.

Bernardi, S., et al. (2015). "ACE2 deficiency shifts energy metabolism towards glucose utilization." Metabolism **64**(3): 406-415.

BACKGROUND: This study aimed at investigating the effects of genetic angiotensin-converting enzyme (ACE) 2 deficiency on glucose homeostasis in the pancreas and skeletal muscle and their reversibility following ACE inhibition. PROCEDURES: ACE2-knockout and C57bl6J mice were placed on a standard diet (SD) or a high-fat diet (HFD) for 12 weeks. An additional group of ACE2-knockout mice was fed a SD and treated with the ACE inhibitor, perindopril (2 mg kg (-1)day (-1)). Glucose and insulin tolerance tests, indirect calorimetry measurements and EchoMRI were performed. Non-esterfied 'free' fatty acid oxidation rate in skeletal muscle was calculated by measuring the palmitate oxidation rate. beta-cell mass was determined by immunostaining. Insulin, collectrin, glucose transporter protein, and peroxisome proliferator-activated receptor-gamma expression were analysed by RT-PCR. Markers of mithocondrial biogenesis/content were also evaluated. MAIN FINDINGS: ACE2-knockout mice showed a beta-cell defect associated with low insulin and collectrin levels and reduced compensatory hypertrophy in response to a HFD, which were not reversed by perindopril. On the other hand, ACE2 deficiency shifted energy metabolism towards glucose utilization, as it increased the respiratory exchange ratio, reduced palmitate oxidation and PCG-1alpha expression in the skeletal muscle, where it up-regulated glucose transport proteins. Treatment of ACE2-knockout mice with perindopril reversed the skeletal muscle changes, suggesting that these were dependent on Angiotensin II (Ang II). PRINCIPAL CONCLUSIONS: ACE2-knockout mice display a beta-cell defect, which does not seem to be dependent on Ang II but may reflect the collectrin-like action of ACE2. This defect seemed to be compensated by the fact that ACE2-knockout mice shifted their energy consumption towards glucose utilisation via Ang II.

Bernardi, S., et al. (2012). "High-salt diet increases glomerular ACE/ACE2 ratio leading to oxidative stress and kidney damage." Nephrol Dial Transplant **27**(5): 1793-1800.

BACKGROUND: Angiotensin II (AngII) contributes to salt-driven kidney damage. In this study, we aimed at investigating whether and how the renal damage associated with a high-salt diet could result from changes in the ratio between angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2). METHODS: Forty-eight rats randomly allocated to three different dietary contents of salt were studied for 4 weeks after undergoing a left

uninephrectomy. We focussed on kidney functional, structural and molecular changes. At the same time, we studied kidney molecular changes in 20 weeks old Ace2-knockout mice (Ace2KO), with and without ACE inhibition. RESULTS: A high salt content diet significantly increased the glomerular ACE/ACE2 ratio. This was associated with increased oxidative stress. To assess whether these events were related, we measured renal oxidative stress in Ace2KO, and found that the absence of ACE2 promoted oxidative stress, which could be prevented by ACE inhibition. CONCLUSION: One of the mechanisms by which a high-salt diet leads to renal damage seems to be the modulation of the ACE/ACE2 ratio which in turn is critical for the cause of oxidative stress, through AngII.

Bernardi, S., et al. (2012). "Characterization and significance of ACE2 and Mas receptor in human colon adenocarcinoma." J Renin Angiotensin Aldosterone Syst **13**(1): 202-209.

INTRODUCTION: A new arm of the renin-angiotensin system (RAS) has been recently characterized; this includes angiotensin converting enzyme (ACE)2 and angiotensin (Ang)1-7, a heptapeptide acting through the Mas receptor (MasR). Recent studies show that Ang1-7 has an antiproliferative action on lung adenocarcinoma cells. The aim of this study was to characterize RAS expression in human colon adenocarcinoma and to investigate whether Ang1-7 exerts an antiproliferative effect on human colon adenocarcinoma cells. MATERIALS AND METHODS: Gene, protein expression and enzymatic activity of the main components of the RAS were determined on non-neoplastic colon mucosa as well as on the tumor mass and the mucosa taken 5 cm distant from it, both collected from patients with colon adenocarcinoma. Two different human colon cancer cell lines were treated with AngII and Ang1-7. RESULTS: The novel finding of this study was that MasR was significantly upregulated in colon adenocarcinoma compared with non-neoplastic colon mucosa, which showed little or no expression of it. ACE gene expression and enzymatic activity were also increased in the tumors. However, AngII and Ang1-7 did not have any pro-/antiproliferative effects in the cell lines studied. CONCLUSIONS: The data suggest that upregulation of the MasR could be used as a diagnostic marker of colon adenocarcinoma.

Bessa, A. S. M., et al. (2019). "Stimulation of the ACE2/Ang-(1-7)/Mas axis in hypertensive pregnant rats attenuates cardiovascular dysfunction in adult male offspring." Hypertens Res **42**(12): 1883-1893.

The aim of this study was to investigate whether treatment with diminazene aceturate (DIZE), a putative ACE2 activator, or with angiotensin-(1-7) during pregnancy could attenuate the development of cardiovascular dysfunction in the adult offspring of spontaneously hypertensive rats (SHRs). For this, pregnant SHRs received DIZE or Ang-(1-7) throughout gestation. The systolic blood pressure (SBP) was measured in the male offspring from the 6th to16th weeks of age by tail-cuff plethysmography. Thereafter, the left ventricular contractile function and coronary reactivity were evaluated by the Langendorff technique. Samples of the left ventricles (LVs) and kidneys were collected for histology and western blot assay in another batch of adult rat offspring. Maternal treatment with DIZE or Ang-(1-7) during pregnancy attenuated the increase in SBP in adult offspring. In addition, both DIZE and Ang-(1-7) treatments reduced the cardiomyocyte diameter and fibrosis deposition in the LV, and treatment with Ang-(1-7) also reduced the fibrosis deposition in the kidneys. Maternal treatment with DIZE, as well as Ang-(1-7), improved the coronary vasodilation induced by bradykinin in isolated hearts from adult offspring. However, no difference was observed in the contractile function of the LVs of these animals. The expression levels of AT1 and Mas receptors, ACE, ACE2, SOD, and catalase in the LV were not modified by maternal treatment with Ang-(1-7), but this treatment elicited a reduction in AT2 expression. These data show that treatment with DIZE or Ang-(1-7) during gestation promoted beneficial effects of attenuating hypertension and cardiac remodeling in adult offspring.

Bindom, S. M., et al. (2010). "Angiotensin I-converting enzyme type 2 (ACE2) gene therapy improves glycemic control in diabetic mice." Diabetes **59**(10): 2540-2548.

OBJECTIVE: Several clinical studies have shown the benefits of renin-angiotensin system (RAS) blockade in the development of diabetes, and a local RAS has been identified in pancreatic islets. Angiotensin I-converting enzyme (ACE)2, a new component of the RAS, has been identified in the pancreas, but its role in beta-cell function remains unknown. Using 8- and 16-week-old obese db/db mice, we examined the ability of ACE2 to alter pancreatic beta-cell function and thereby modulate hyperglycemia. RESEARCH DESIGN AND METHODS: Both db/db and nondiabetic lean control (db/m) mice were infected with an adenovirus expressing human ACE2 (Ad-hACE2-eGFP) or the control virus (Ad-eGFP) via injection into the pancreas. Glycemia and beta-cell function were assessed 1 week later at the peak of viral expression. RESULTS: In 8-week-old db/db mice, Ad-hACE2-eGFP significantly improved fasting glycemia, enhanced intraperitoneal glucose tolerance, increased islet insulin content and

beta-cell proliferation, and reduced beta-cell apoptosis compared with Ad-eGFP. ACE2 overexpression had no effect on insulin sensitivity in comparison with Ad-eGFP treatment in diabetic mice. Angiotensin-(1-7) receptor blockade by D-Ala (7)-Ang-(1-7) prevented the ACE2-mediated improvements in intraperitoneal glucose tolerance, glycemia, and islet function and also impaired insulin sensitivity in both Ad-hACE2-eGFP- and Ad-eGFP-treated db/db mice. D-Ala (7)-Ang-(1-7) had no effect on db/m mice. In 16-week-old diabetic mice, Ad-hACE2-eGFP treatment improved fasting blood glucose but had no effect on any of the other parameters. CONCLUSIONS: These findings identify ACE2 as a novel target for the prevention of beta-cell dysfunction and apoptosis occurring in type 2 diabetes.

Bindom, S. M. and E. Lazartigues (2009). "The sweeter side of ACE2: physiological evidence for a role in diabetes." Mol Cell Endocrinol **302**(2): 193-202.

Diabetes mellitus is a growing problem in all parts of the world. Both clinical trials and animal models of type I and type II diabetes have shown that hyperactivity of angiotensin-II (Ang-II) signaling pathways contribute to the development of diabetes and diabetic complications. Of clinical relevance, blockade of the renin-angiotensin system prevents new-onset diabetes and reduces the risk of diabetic complications. Angiotensin-converting enzyme (ACE) 2 is a recently discovered mono-carboxypeptidase and the first homolog of ACE. It is thought to inhibit Ang-II signaling cascades mostly by cleaving Ang-II to generate Ang-(1-7), which effects oppose Ang-II and are mediated by the Mas receptor. The enzyme is present in the kidney, liver, adipose tissue and pancreas. Its expression is elevated in the endocrine pancreas in diabetes and in the early phase during diabetic nephropathy. ACE2 is hypothesized to act in a compensatory manner in both diabetes and diabetic nephropathy. Recently, we have shown the presence of the Mas receptor in the mouse pancreas and observed a reduction in Mas receptor immuno-reactivity as well as higher fasting blood glucose levels in ACE2 knockout mice, indicating that these mice may be a new model to study the role of ACE2 in diabetes. In this review we will examine the role of the renin-angiotensin system in the physiopathology and treatment of diabetes and highlight the potential benefits of the ACE2/Ang-(1-7)/Mas receptor axis, focusing on recent data about ACE2.

Bodiga, S., et al. (2011). "Enhanced susceptibility to biomechanical stress in ACE2 null mice is prevented by loss of the p47(phox) NADPH oxidase subunit." Cardiovasc Res **91**(1): 151-161.

AIMS: Angiotensin-converting enzyme 2 (ACE2) is an important negative regulator of the renin-angiotensin system. Loss of ACE2 enhances the susceptibility to heart disease but the mechanism remains elusive. We hypothesized that ACE2 deficiency activates the NADPH oxidase system in pressure overload-induced heart failure. METHODS AND RESULTS: Using the aortic constriction model, we subjected wild-type (Ace2(+/y)), ACE2 knockout (ACE2KO, Ace2(-/y)), p47(phox) knockout (p47(phox)KO, p47(phox-) (/-)), and ACE2/p47(phox) double KO mice to pressure overload. We examined changes in peptide levels, NADPH oxidase activity, gene expression, matrix metalloproteinases (MMP) activity, pathological signalling, and heart function. Loss of ACE2 resulted in enhanced susceptibility to biomechanical stress leading to eccentric remodelling, increased pathological hypertrophy, and worsening of systolic performance. Myocardial angiotensin II (Ang II) levels were increased, whereas Ang 1-7 levels were lowered. Activation of Ang II-stimulated signalling pathways in the ACE2-deficient myocardium was associated with increased expression and phosphorylation of p47(phox), NADPH oxidase activity, and superoxide generation, leading to enhanced MMP-mediated degradation of the extracellular matrix. Additional loss of p47(phox) in the ACE2KO mice normalized the increased NADPH oxidase activity, superoxide production, and systolic dysfunction following pressure overload. Ang 1-7 supplementation suppressed the increased NADPH oxidase and rescued the early dilated cardiomyopathy in pressure-overloaded ACE2KO mice. CONCLUSION: In the absence of ACE2, biomechanical stress triggers activation of the myocardial NAPDH oxidase system with a critical role of the p47(phox) subunit. Increased production of superoxide, activation of MMP, and pathological signalling leads to severe adverse myocardial remodelling and dysfunction in ACE2KO mice.

Brosnihan, K. B., et al. (2008). "Tissue-specific regulation of ACE/ACE2 and AT1/AT2 receptor gene expression by oestrogen in apolipoprotein E/oestrogen receptor-alpha knock-out mice." Exp Physiol **93**(5): 658-664.

Angiotensin-converting enzyme (ACE) and ACE2 and the AT1 and AT2 receptors are pivotal points of regulation in the renin-angiotensin system. ACE and ACE2 are key enzymes in the formation and degradation of angiotensin II (Ang II) and angiotensin-(1-7) (Ang-(1-7)). Ang II acts at either the AT1 or the AT2 receptor to mediate opposing actions of vasoconstriction or vasodilatation respectively. While it is known that oestrogen acts to downregulate ACE and the AT (1) receptor, its regulation of ACE2 and

the AT2 receptor and the involvement of a specific oestrogen receptor subtype are unknown. To investigate the role of oestrogen receptor-alpha (ERalpha) in the regulation by oestrogen of ACE/ACE2 and AT1/AT2 mRNAs in lung and kidney, ovariectomized female mice lacking apolipoprotein E (ee) with the ERalpha (AAee) or without the ERalpha (alphaalphaee) were treated with 17beta-oestradiol (6 microg day (-1)) or placebo for 3 months. ACE, ACE2, AT1 receptor and AT2 receptor mRNAs were measured using reverse transcriptase, real-time polymerase chain reaction. In the kidney, 17beta-oestradiol showed 1.7-fold downregulation of ACE mRNA in AAee mice, with 2.1-fold upregulation of ACE mRNA in alphaalphaee mice. 17beta-Oestradiol showed 1.5- and 1.8-fold downregulation of ACE2 and AT1 receptor mRNA in AAee mice; this regulation was lost in alphaalphaee mice. 17beta-Oestradiol showed marked (81-fold) upregulation of the AT (2) receptor mRNA in AAee mice. In the lung, 17beta-oestradiol treatment had no effect on AT1 receptor mRNA in AAee mice, but resulted in a 1.5-fold decreased regulation of AT1 mRNA in alphaalphaee mice. There was no significant interaction of oestrogen with ERalpha in the lung for ACE, ACE2 and AT2 receptor genes. These studies reveal tissue-specific regulation by 17beta-oestradiol of ACE/ACE2 and AT1/AT2 receptor genes, with the ERalpha receptor being primarily responsible for the regulation of kidney ACE2, AT1 receptor and AT2 receptor genes.

Brosnihan, K. B., et al. (2003). "Enhanced renal immunocytochemical expression of ANG-(1-7) and ACE2 during pregnancy." Hypertension **42**(4): 749-753.

Previously we demonstrated that kidney concentration and urinary excretion of angiotensin-(1-7) are increased during normal pregnancy in rats. Since this finding may reflect local kidney production of angiotensin-(1-7), we determined the immunocytochemical distribution of angiotensin-(1-7) and its newly described processing enzyme, ACE2, in kidneys of virgin and 19-day-pregnant Sprague-Dawley rats. Sprague-Dawley rats were killed at the 19th day of pregnancy, and tissues were prepared for immunocytochemical by using a polyclonal antibody to angiotensin- (1-7) or a monoclonal antibody to ACE2. Angiotensin-(1-7) immunostaining was predominantly localized to the renal tubules traversing both the inner cortex and outer medulla. ACE2 immunostaining was localized throughout the cortex and outer medulla and was visualized in the renal tubules of both virgin and pregnant rats. The quantification of angiotensin-(1-7) and ACE2 immunocytochemical staining showed that in pregnant animals, the intensity of the staining increased by 56% and 117%, respectively ($P<0.05$). This first demonstration of the immunocytochemical distribution of angiotensin-(1-7) and ACE2 in kidneys of pregnant rats shows that pregnancy increases angiotensin-(1-7) immunocytochemical expression in association with increased ACE2 intensity of staining. The findings suggest that ACE2 may contribute to the local production and overexpression of angiotensin-(1-7) in the kidney during pregnancy.

Bruce, E. B., et al. (2018). "ACE2 activator diminazene aceturate reduces adiposity but preserves lean mass in young and old rats." Exp Gerontol **111**: 133-140.

The obesity epidemic is multi-generational and is particularly debilitating in the aging population, necessitating the use of pharmaceutical interventions. Recent evidence suggests that increasing the activity of the angiotensin converting enzyme-2 [ACE2]/angiotensin-(1-7) [Ang-(1-7)]/Mas receptor (MasR) axis in obese animal models leads to significant reductions in body weight. It was hypothesized that activation of ACE2 via diminazene aceturate (DIZE) will significantly reduce body weight of rats fed a high fat diet. Young and old (4 and 23months, respectively) male Fisher 344xBrown Norway rats were fed 60% high fat diet for one week, and subsequently given either 15mg/kg/day DIZE s.c. or vehicle for three weeks. DIZE treatment resulted in a significant reduction of food intake and body weight in both young and old animals. However, that decrease was so dramatic in the older animals that they all nearly stopped eating. Interestingly, the TD-NMR assessments revealed that the weight-loss was primarily a result of decreased body fat percentage, with a relative preservation of lean mass. Tissue weights confirm the significant loss of white adipose tissue (WAT), with no change in muscle weights. Gene expression and serum ACE2 activity analyses implied that increased activation of the ACE2/Ang-(1-7)/MasR axis plays a role in reducing fat mass. Collectively, our results suggest that DIZE may be a useful tool in the study of obesity; however, caution is recommended when using this compound in older animals due to severe anorectic effects, although there is a mechanism by which muscle is preserved.

Bukowska, A., et al. (2017). "Protective regulation of the ACE2/ACE gene expression by estrogen in human atrial tissue from elderly men." Exp Biol Med (Maywood) **242**(14): 1412-1423.

Data from animal experiments and clinical investigations suggest that components of the renin-angiotensin system are markedly affected by sex hormones. However, whether estrogen affects human atrial myocardium has not been investigated yet. In

this study, we determined the effects of estrogen on key components of atrial renin-angiotensin system: angiotensin-converting enzyme, responsible for generation of angiotensin II and angiotensin-converting enzyme 2, counteracting majority of AngII effects, and different renin-angiotensin system receptors, AT1R, AT2R, and MAS. First, the expression levels of estrogen receptors mRNA were determined in right atrial appendages obtained from patients undergoing heart surgery. The amounts of estrogen receptor alpha and estrogen receptor beta mRNA were similar between women (n = 14) and men (n = 10). Atrial tissue slices (350 microm) were prepared from male donors which were exposed to estrogen (1-100 nM; n = 21) or stimulated at 4 Hz for 24 h in the presence or absence of 100 nM estrogen (n = 16), respectively. The administration of estrogen did not change mRNA levels of estrogen receptors, but activated MAP kinases, Erk1/2. Furthermore, estrogen increased the amounts of angiotensin-converting enzyme 2-mRNA (1.89 +/- 0.23; $P < 0.05$) but reduced that of angiotensin-converting enzyme-mRNA (0.78 +/- 0.07, $P < 0.05$). In addition, the transcript levels of AT2R and MAS were upregulated by estrogen. Pacing of tissue slices significantly increased the angiotensin-converting enzyme/angiotensin-converting enzyme 2 ratio at both the mRNA and protein level. During pacing, administration of estrogen substantially lowered the angiotensin-converting enzyme/angiotensin-converting enzyme 2 ratio at the transcript (0.92 +/- 0.21 vs. 2.12 +/- 0.27 at 4 Hz) and protein level (0.94 +/- 0.20 vs. 2.14 +/- 0.3 at 4 Hz). Moreover, estrogen elicited anti-inflammatory and anti-oxidative effects on renin-angiotensin system-associated downstream effectors such as pro-oxidative LOX-1 and pro-inflammatory ICAM-1. An antagonist of estrogen receptor alpha reversed these anti-inflammatory and anti-oxidative effects of estrogen significantly. Overall, our results demonstrated that estrogen modifies the local renin-angiotensin system homeostasis and achieves protective effects in atrial myocardium from elderly men. Impact statement The present study demonstrates that estrogen affects the human atrial myocardium and mediates protective actions through estrogen receptors-(ER) dependent signaling. Estrogen substantially modulates the local RAS via downregulation of ACE and simultaneous upregulation of ACE2, AT2R and MAS expression levels. This is indicative of a shift of the classical RAS/ACE axis to the alternative, protective RAS/ACE2 axis. In support of this view, estrogen attenuated the expression of RAS-associated downstream effectors, LOX-1, and ICAM-1. A specific antagonist of ERalpha reversed the anti-inflammatory and anti-oxidative effects of estrogen in paced and non-paced atrial tissue slices. In summary, our data demonstrate the existence of protective effects of estrogen in atrial tissue from elderly men which are at least in part, mediated by the regulation of local RAS homeostasis.

Burrell, L. M., et al. (2013). "The ACE2 gene: its potential as a functional candidate for cardiovascular disease." Clin Sci (Lond) **124**(2): 65-76.

The RAS (renin-angiotensin system) plays an important role in the pathophysiology of CVD (cardiovascular disease), and RAS blockade is an important therapeutic strategy in the management of CVD. A new counterbalancing arm of the RAS is now known to exist in which ACE (angiotensin-converting enzyme) 2 degrades Ang (angiotensin) II, the main effector of the classic RAS, and generates Ang-(1-7). Altered ACE2 expression is associated with cardiac and vascular disease in experimental models of CVD, and ACE2 is increased in failing human hearts and atherosclerotic vessels. In man, circulating ACE2 activity increases with coronary heart disease, as well as heart failure, and a large proportion of the variation in plasma ACE2 levels has been attributed to hereditary factors. The ACE2 gene maps to chromosome Xp22 and this paper reviews the evidence associating ACE2 gene variation with CVD and considers clues to potential functional ACE2 variants that may alter gene expression or transcriptional activity. Studies to date have investigated ACE2 gene associations in hypertension, left ventricular hypertrophy and coronary artery disease, but the results have been inconsistent. The discrepancies may reflect the sample size of the studies, the gender or ethnicity of subjects, the cardiovascular phenotype or the ACE2 SNP investigated. The frequent observation of apparent sex-dependence might be of special importance, if confirmed. As yet, there are no studies to concurrently assess ACE2 gene polymorphisms and circulating ACE2 activity. Large-scale carefully conducted clinical studies are urgently needed to clarify more precisely the potential role of ACE2 in the CVD continuum.

Burrell, L. M., et al. (2004). "ACE2, a new regulator of the renin-angiotensin system." Trends Endocrinol Metab **15**(4): 166-169.

Angiotensin-converting enzyme (ACE) is a zinc metalloproteinase and a key regulator of the renin-angiotensin system (RAS). ACE2 is a newly described enzyme identified in rodents and humans with a more restricted distribution than ACE, and is found mainly in heart and kidney. ACE2 cleaves a single residue from angiotensin I (Ang I) to generate Ang 1-9, and degrades Ang II, the main effector of the RAS, to the vasodilator Ang 1-7. The importance of ACE2 in

normal physiology and pathophysiological states is largely unknown. ACE2 might act in a counter-regulatory manner to ACE, modulating the balance between vasoconstrictors and vasodilators within the heart and kidney, and playing a significant role in regulating cardiovascular and renal function.

Burrell, L. M., et al. (2005). "Myocardial infarction increases ACE2 expression in rat and humans." Eur Heart J **26**(4): 369-375; discussion 322-364.

AIMS: Angiotensin converting enzyme (ACE) 2 catalyses the cleavage of angiotensin (Ang) I to Ang 1-9 and of Ang II to Ang 1-7. ACE2 deficiency impairs cardiac contractility and upregulates hypoxia-induced genes, suggesting a link with myocardial ischaemia. We studied the expression of ACE2 after myocardial infarction (MI) in the rat as well as in human failing hearts. METHODS AND RESULTS: Rats were killed at days 1, 3, and 28 after MI, or treated for 4 weeks with the ACE inhibitor ramipril (1 mg/kg). Cardiac gene and protein expression of ACE and ACE2 were assessed by quantitative real-time reverse transcriptase-polymerase chain reaction and immunohistochemistry/activity assays/in vitro autoradiography, respectively. Both ACE (P = 0.022) and ACE2 (P = 0.015) mRNA increased in the border/infarct area compared with the viable area at day 3 after MI. By day 28, increases in ACE (P = 0.005) and ACE2 (P = 0.006) mRNA were also seen in the viable myocardium of MI rats compared with myocardium of control rats. ACE2 protein localized to macrophages, vascular endothelium, smooth muscle, and myocytes. Ramipril attenuated cardiac hypertrophy and inhibited cardiac ACE. In contrast, ramipril had no effect on cardiac ACE2 mRNA, which remained elevated in all areas of the MI rat heart. Immunoreactivity of both ACE and ACE2 increased in failing human hearts. CONCLUSION: The increase in ACE2 after MI suggests that it plays an important role in the negative modulation of the renin angiotensin system in the generation and degradation of angiotensin peptides after cardiac injury.

Butler, G. and D. J. Thiele (1991). "ACE2, an activator of yeast metallothionein expression which is homologous to SWI5." Mol Cell Biol **11**(1): 476-485.

Transcription of the Saccharomyces cerevisiae metallothionein gene CUP1 is induced in response to high environmental levels of copper. Induction requires the ACE1 gene product, which binds to specific sites in the promoter region of the CUP1 gene. In this study, we found that deleting the entire coding sequence of the ACE1 gene resulted in a decrease in basal-level transcription of CUP1 to low but detectable levels and conferred a copper-sensitive phenotype to the cells. We have isolated a gene, designated ACE2, which when present on a high-copy-number plasmid suppresses the copper-sensitive phenotype of an ace1-deletion strain. The presence of multiple copies of the ACE2 gene enhanced expression of an unlinked CUP1-lacZ fusion integrated in the yeast genome and resulted in an increase in the steady-state levels of CUP1 mRNA in an ace1-deletion background. A large deletion of the coding region of the genomic copy of ACE2 resulted in a decrease in steady-state levels of CUP1 mRNA, indicating that ACE2 plays a role in regulating basal-level expression of CUP1. The ACE2 open reading frame encodes a polypeptide of 770 amino acids, with putative zinc finger structures near the carboxyl terminus. This protein is 37% identical to the SWI5 gene product, an activator of HO gene transcription in S. cerevisiae, suggesting that ACE2 and SWI5 may have functional similarities.

Byrnes, J. J., et al. (2009). "Effects of the ACE2 inhibitor GL1001 on acute dextran sodium sulfate-induced colitis in mice." Inflamm Res **58**(11): 819-827.

OBJECTIVE AND DESIGN: Angiotensin-converting enzyme 2 (ACE2) is expressed in gastrointestinal tissue. Previous studies of GL1001, a potent and selective ACE2 inhibitor, have revealed anti-inflammatory activity in the mouse digestive tract. We hypothesized that GL1001 might also produce beneficial effects in a mouse DSS model of inflammatory bowel disease. MATERIALS: Female mice were used for study. TREATMENT: Animals were treated for 5 days with 5% DSS in the drinking water to induce colitis. For the following 9 days, animals were treated twice daily with GL1001 (30, 100, 300 mg/kg, s.c.), sulfasalazine (150 mg/kg, p.o.), or vehicle. METHODS: Throughout the experiment, body weight, rectal prolapse, stool consistency, and fecal occult blood were monitored. At termination, colon length, histopathology, and myeloperoxidase activity were assessed. RESULTS: High-dose GL1001 ameliorated DSS-induced disease activity, including rectal prolapse and intestinal bleeding. The most robust effect of GL1001 was observed 48-96 h post DSS treatment and was comparable in magnitude to that of sulfasalazine. Colon pathology and myeloperoxidase activity were also markedly attenuated by high-dose GL1001 treatment, with the most profound effects observed in the distal segment. CONCLUSIONS: The findings support the previously observed anti-inflammatory effects of ACE2 inhibition in gastrointestinal tissue and suggest that GL1001 may have therapeutic utility for inflammatory bowel disease.

Calderon-Norena, D. M., et al. (2015). "A single nucleotide polymorphism uncovers a novel function

for the transcription factor Ace2 during Candida albicans hyphal development." PLoS Genet **11**(4): e1005152.

Candida albicans is a major invasive fungal pathogen in humans. An important virulence factor is its ability to switch between the yeast and hyphal forms, and these filamentous forms are important in tissue penetration and invasion. A common feature for filamentous growth is the ability to inhibit cell separation after cytokinesis, although it is poorly understood how this process is regulated developmentally. In C. albicans, the formation of filaments during hyphal growth requires changes in septin ring dynamics. In this work, we studied the functional relationship between septins and the transcription factor Ace2, which controls the expression of enzymes that catalyze septum degradation. We found that alternative translation initiation produces two Ace2 isoforms. While full-length Ace2, Ace2L, influences septin dynamics in a transcription-independent manner in hyphal cells but not in yeast cells, the use of methionine-55 as the initiation codon gives rise to Ace2S, which functions as the nuclear transcription factor required for the expression of cell separation genes. Genetic evidence indicates that Ace2L influences the incorporation of the Sep7 septin to hyphal septin rings in order to avoid inappropriate activation of cell separation during filamentous growth. Interestingly, a natural single nucleotide polymorphism (SNP) present in the C. albicans WO-1 background and other C. albicans commensal and clinical isolates generates a stop codon in the ninth codon of Ace2L that mimics the phenotype of cells lacking Ace2L. Finally, we report that Ace2L and Ace2S interact with the NDR kinase Cbk1 and that impairing activity of this kinase results in a defect in septin dynamics similar to that of hyphal cells lacking Ace2L. Together, our findings identify Ace2L and the NDR kinase Cbk1 as new elements of the signaling system that modify septin ring dynamics in hyphae to allow cell-chain formation, a feature that appears to have evolved in specific C. albicans lineages.

Callera, G. E., et al. (2016). "Differential renal effects of candesartan at high and ultra-high doses in diabetic mice-potential role of the ACE2/AT2R/Mas axis." Biosci Rep **36**(5).

High doses of Ang II receptor (AT1R) blockers (ARBs) are renoprotective in diabetes. Underlying mechanisms remain unclear. We evaluated whether high/ultra-high doses of candesartan (ARB) up-regulate angiotensin-converting enzyme 2 (ACE2)/Ang II type 2 receptor (AT2R)/Mas receptor [protective axis of the of the renin-angiotensin system (RAS)] in diabetic mice. Systolic blood pressure (SBP), albuminuria and expression/activity of RAS components were assessed in diabetic db/db and control db/+ mice treated with increasing candesartan doses (intermediate, 1 mg/kg/d; high, 5 mg/kg/d; ultra-high, 25 and 75 mg/kg/d; 4 weeks). Lower doses candesartan did not influence SBP, but ultra-high doses reduced SBP in both groups. Plasma glucose and albuminuria were increased in db/db compared with db/+ mice. In diabetic mice treated with intermediate dose candesartan, renal tubular damage and albuminuria were ameliorated and expression of ACE2, AT2R and Mas and activity of ACE2 were increased, effects associated with reduced ERK1/2 phosphorylation, decreased fibrosis and renal protection. Ultra-high doses did not influence the ACE2/AT2R/Mas axis and promoted renal injury with increased renal ERK1/2 activation and exaggerated fibronectin expression in db/db mice. Our study demonstrates dose-related effects of candesartan in diabetic nephropathy: intermediate-high dose candesartan is renoprotective, whereas ultra-high dose candesartan induces renal damage. Molecular processes associated with these effects involve differential modulation of the ACE2/AT2R/Mas axis: intermediate-high dose candesartan up-regulating RAS protective components and attenuating pro-fibrotic processes, and ultra-high doses having opposite effects. These findings suggest novel mechanisms through the protective RAS axis, whereby candesartan may ameliorate diabetic nephropathy. Our findings also highlight potential injurious renal effects of ultra-high dose candesartan in diabetes.

Calo, L. A., et al. (2010). "ACE2 and angiotensin 1-7 are increased in a human model of cardiovascular hyporeactivity: pathophysiological implications." J Nephrol **23**(4): 472-477.

BACKGROUND: ACE and ACE2 produce angiotensin II (Ang II), a vasopressor that induces cardiovascular remodeling, and Ang 1-7, a vasodilator with an antiremodeling effect. While Ang 1-7 has antiarrhythmic properties, at higher concentration it may induce ventricular tachycardia and sudden death. ACE2, therefore, may play an essential role in blood pressure homeostasis, in the long-term complications of hypertension (cardiovascular remodeling), and in the induction of cardiac electric abnormalities. This study evaluated the levels of ACE2 and Ang 1-7 in Bartter's/Gitelman's patients (BS/GS) who have elevated Ang II and endogenous blockade of Ang II type 1 receptor signaling compared with healthy subjects (C) and essential hypertensives (EH). BS/ GS patients were also considered because of their predisposition to cardiac arrhythmias, which has yet to be completely clarified. METHODS: Mononuclear cell ACE2 and Ang 1-7 were evaluated using western

blot. RESULTS: One-way ANOVA showed that ACE2 and Ang 1-7 levels were significantly different between the three groups (p=0.0074 and p=0.0001, respectively). Post-hoc analysis (Tukey's HSD) showed that both ACE2 (1.59+/-0.63) and Ang1-7 (2.26+/-1.18) were significantly elevated in BS/GS compared with either C (0.98+/-0.45; p=0.008; 1.12+/-0.48, p=0.002, respectively) or EH (1.06+/-0.24; p=0.043; 0.72+/-0.28; p=0.0001, respectively). ACE2 and Ang 1-7 directly correlated only in BS/GS (r=0.91, p<0.0003). CONCLUSIONS: The elevated ACE2 and Ang 1-7 in BS/ GS patients mirror those in hypertensives and are in line with the clinical, hemodynamic and pathophysiological characteristics of BS/GS, likely contributing to them. In consideration of the clinical picture of these syndromes, the opposite of hypertension, the results of this study further strengthen the importance of the ACE2/Ang 1-7 system in the regulation of vascular tone and cardiovascular biology.

Camargo, S. M., et al. (2009). "Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations." Gastroenterology **136**(3): 872-882.

BACKGROUND & AIMS: Hartnup amino acid transporter B (0)AT1 (SLC6A19) is the major luminal sodium-dependent neutral amino acid transporter of small intestine and kidney proximal tubule. The expression of B (0)AT1 in kidney was recently shown to depend on its association with collectrin (Tmem27), a protein homologous to the membrane-anchoring domain of angiotensin-converting enzyme (ACE) 2. METHODS: Because collectrin is almost absent from small intestine, we tested the hypothesis that it is ACE2 that interacts with B (0)AT1 in enterocytes. Furthermore, because B (0)AT1 expression depends on an associated protein, we tested the hypothesis that Hartnup-causing B (0)AT1 mutations differentially impact on B (0)AT1 interaction with intestinal and kidney accessory proteins. RESULTS: Immunofluorescence, coimmunoprecipitation, and functional experiments using wild-type and ace2-null mice showed that expression of B (0)AT1 in small intestine critically depends on ACE2. Coexpressing new and previously identified Hartnup disorder-causing missense mutations of B (0)AT1 with either collectrin or ACE2 in Xenopus laevis oocytes showed that the high-frequency D173N and the newly identified P265L mutant B (0)AT1 transporters can still be activated by ACE2 but not collectrin coexpression. In contrast, the human A69T and R240Q B (0)AT1 mutants cannot be activated by either of the associated proteins, although they function as wild-type B (0)AT1 when expressed alone. CONCLUSIONS: We thus show that ACE2 is necessary for the expression of the Hartnup transporter in intestine and suggest that the differential functional association of mutant B (0)AT1 transporters with ACE2 and collectrin in intestine and kidney, respectively, participates in the phenotypic heterogeneity of human Hartnup disorder.

Campbell, D. J., et al. (2004). "Evidence against a major role for angiotensin converting enzyme-related carboxypeptidase (ACE2) in angiotensin peptide metabolism in the human coronary circulation." J Hypertens **22**(10): 1971-1976.

OBJECTIVE: To investigate the role of angiotensin-converting enzyme-related carboxypeptidase (ACE2) in angiotensin peptide metabolism in the human coronary circulation. METHODS: Angiotensin I and angiotensin II, and their respective carboxypeptidase metabolites, angiotensin-(1-9) and angiotensin-(1-7), were measured in arterial and coronary sinus blood of heart failure subjects receiving angiotensin-converting enzyme (ACE) inhibitor therapy and in normal subjects not receiving ACE inhibitor therapy. In addition, angiotensin I, angiotensin II and angiotensin-(1-7) were measured in arterial and coronary sinus blood of subjects with coronary artery disease before, and at 2, 5 and 10 min after, intravenous administration of ACE inhibitor. RESULTS: In comparison with normal subjects, heart failure subjects receiving ACE inhibitor therapy had a greater than 40-fold increase in angiotensin I levels, but angiotensin-(1-9) levels were low (1-2 fmol/ml), and similar to those of normal subjects. Moreover, angiotensin-(1-7) levels increased in parallel with angiotensin I levels and the angiotensin-(1-7)/angiotensin II ratio increased by 7.5-fold in coronary sinus blood. Intravenous administration of ACE inhibitor to subjects with coronary artery disease rapidly decreased angiotensin II levels by 54-58% and increased angiotensin I levels by 2.4- to 2.8-fold, but did not alter angiotensin-(1-7) levels or net angiotensin-(1-7) production across the myocardial vascular bed. CONCLUSIONS: The failure of angiotensin-(1-9) levels to increase in response to increased angiotensin I levels indicated little role for ACE2 in angiotensin I metabolism. Additionally, the levels of angiotensin-(1-7) were more linked to those of angiotensin I than angiotensin II, consistent with its formation by endopeptidase-mediated metabolism of angiotensin I, rather than by ACE2-mediated metabolism of angiotensin II.

Cao, X., et al. (2014). "The ACE2/Ang-(1-7)/Mas axis can inhibit hepatic insulin resistance." Mol Cell Endocrinol **393**(1-2): 30-38.

Blocking the renin-angiotensin system (RAS) can reduce the risk of diabetes. Meanwhile, the angiotensin

(Ang)-converting enzyme-2 (ACE2)/Ang-(1-7)/Mas axis has recently been proposed to function as a negative regulator of the RAS. In previous studies, we first demonstrated that ACE2 knockout (ACE2(-/) (y)) mice exhibit impaired glucose tolerance or diabetes. However the precise roles of ACE2 on glucose metabolism are unknown. Here we show that the ACE2/Ang-(1-7)/Mas axis can ameliorate insulin resistance in the liver. Activation of the ACE2/Ang-(1-7)/Mas axis increases glucose uptake and decreases glycogen synthesis in the liver accompanied by increased expression of glucose transporters, insulin receptor substrates and decreased expression of enzymes for glycogen synthesis. ACE2 knockout mice displayed elevated levels of oxidative stress and exposure to Ang-(1-7) reduced the stress in hepatic cells. As a consequence of anti-oxidative stress, activation of the ACE2/Ang-(1-7)/Mas axis led to improved hepatic insulin resistance through the Akt/PI3K/IRS-1/JNK insulin signaling pathway. This is the first time documented that the ACE2/Ang-(1-7)/Mas axis can ameliorate insulin resistance in the liver. As insulin resistance in the liver is considered to be the primary cause of the development of type 2 diabetes, this axis may serve as a new diabetes target.

Castro-Chaves, P., et al. (2010). "New pathways of the renin-angiotensin system: the role of ACE2 in cardiovascular pathophysiology and therapy." Expert Opin Ther Targets **14**(5): 485-496.

IMPORTANCE OF THE FIELD: The renin-angiotensin system (RAS) is nowadays an important target in cardiovascular diseases and we are currently on the verge of a new interpretation of its role in cardiovascular homeostasis, mainly due to the identification of the new axis ACE2/angiotensin 1 - 7/Mas receptor. AREAS COVERED IN THIS REVIEW: The main aspects related to ACE2 role in cardiovascular physiology and possible pathological and therapeutic implications are reviewed. WHAT THE READER WILL GAIN: A description of the new view of the RAS, along with the key findings that support it. In the cardiovascular system, the ACE2/angiotensin 1 - 7/Mas axis, mainly through the inhibition of fibrosis, inflammation, thrombosis and cell proliferation, modulates RAS activity with significant pathophysiological implications in clinical conditions such as hypertension, myocardial ischemia and heart failure. A more complete understanding of this axis has significant therapeutic relevance and a major effort is being carried out in order to pursue this objective. TAKE HOME MESSAGE: There is increasing evidence that ACE2/angiotensin 1 - 7/Mas receptor axis has a key role in RAS activity regulation with significant pathophysiological implications in several disease states. A therapeutic intervention at this level may open new doors and change the current approach to RAS targeting.

Cervenka, L., et al. (2015). "Combined suppression of the intrarenal and circulating vasoconstrictor renin-ACE-ANG II axis and augmentation of the vasodilator ACE2-ANG 1-7-Mas axis attenuates the systemic hypertension in Ren-2 transgenic rats exposed to chronic hypoxia." Physiol Res **64**(1): 11-24.

The aim of the present study was to test the hypothesis that chronic hypoxia would aggravate hypertension in Ren-2 transgenic rats (TGR), a well-defined monogenetic model of hypertension with increased activity of endogenous renin-angiotensin system (RAS). Systolic blood pressure (SBP) in conscious rats and mean arterial pressure (MAP) in anesthetized TGR and normotensive Hannover Sprague-Dawley (HanSD) rats were determined under normoxia that was either continuous or interrupted by two weeks hypoxia. Expression, activities and concentrations of individual components of RAS were studied in plasma and kidney of TGR and HanSD rats under normoxic conditions and after exposure to chronic hypoxia. In HanSD rats two weeks exposure to chronic hypoxia did not alter SBP and MAP. Surprisingly, in TGR it decreased markedly SBP and MAP; this was associated with substantial reduction in plasma and kidney renin activities and also of angiotensin II (ANG II) levels, without altering angiotensin-converting enzyme (ACE) activities. Simultaneously, in TGR the exposure to hypoxia increased kidney ACE type 2 (ACE2) activity and angiotensin 1-7 (ANG 1-7) concentrations as compared with TGR under continuous normoxia. Based on these results, we propose that suppression of the hypertensiogenic ACE-ANG II axis in the circulation and kidney tissue, combined with augmentation of the intrarenal vasodilator ACE2-ANG 1-7 axis, is the main mechanism responsible for the blood pressure-lowering effects of chronic hypoxia in TGR.

Chang, S. Y., et al. (2011). "Angiotensin II type II receptor deficiency accelerates the development of nephropathy in type I diabetes via oxidative stress and ACE2." Exp Diabetes Res **2011**: 521076.

Since the functional role (s) of angiotensin II (Ang II) type II receptor (AT (2)R) in type I diabetes is unknown, we hypothesized that AT (2)R is involved in decreasing the effects of type I diabetes on the kidneys. We induced diabetes with low-dose streptozotocin (STZ) in both AT (2)R knockout (AT (2)RKO) and wild-type (WT) male mice aged 12 weeks and followed them for 4 weeks. Three subgroups nondiabetic, diabetic, and insulin-treated diabetic (Rx

insulin implant) were studied. Systolic blood pressure (SBP), physiological parameters, glomerular filtration rate (GFR), renal morphology, gene expression, and apoptosis were assessed. After 4 weeks of diabetes, compared to WT controls, AT (2)RKO mice clearly developed features of early diabetic nephropathy (DN), such as renal hypertrophy, tubular apoptosis, and progressive extracellular matrix (ECM) protein accumulation as well as increased GFR. AT (2)RKO mice presented hypertension unaffected by diabetes. Renal oxidative stress (measured as heme oxygenase 1 (HO-1) gene expression and reactive oxygen species (ROS) generation) and intrarenal renin angiotensin system components, such as angiotensinogen (Agt), AT (1)R, and angiotensin-converting enzyme (ACE) gene expression, were augmented whereas angiotensin-converting enzyme2 (ACE2) gene expression was decreased in renal proximal tubules (RPTs) of AT (2)RKO mice. The renal changes noted above were significantly enhanced in diabetic AT (2)RKO mice but partially attenuated in insulin-treated diabetic WT and AT (2)RKO mice. In conclusion, AT (2)R deficiency accelerates the development of DN, which appears to be mediated, at least in part, via heightened oxidative stress and ACE/ACE2 ratio in RPTs.

Chappel, M. C. and C. M. Ferrario (2006). "ACE and ACE2: their role to balance the expression of angiotensin II and angiotensin-(1-7)." Kidney Int **70**(1): 8-10.

The discovery of angiotensin-converting enzyme 2 (ACE-2) has revealed a far more complex enzymatic cascade that may influence the renin-angiotensin system within the kidney, specifically the expression of the functional products angiotensin II (Ang II) and Ang-(1-7). The regulation of this critical system involved in blood pressure control must now encompass the integral relationship of ACE and ACE-2 activities.

Chen, I. Y., et al. (2010). "Upregulation of the chemokine (C-C motif) ligand 2 via a severe acute respiratory syndrome coronavirus spike-ACE2 signaling pathway." J Virol **84**(15): 7703-7712.

Severe acute respiratory syndrome coronavirus (SARS-CoV) was identified to be the causative agent of SARS with atypical pneumonia. Angiotensin-converting enzyme 2 (ACE2) is the major receptor for SARS-CoV. It is not clear whether ACE2 conveys signals from the cell surface to the nucleus and regulates expression of cellular genes upon SARS-CoV infection. To understand the pathogenesis of SARS-CoV, human type II pneumocyte (A549) cells were incubated with the viral spike protein or with SARS-CoV virus-like particles containing the viral spike protein to examine cytokine modulation in lung cells. Results from oligonucleotide-based microarray, real-time PCR, and enzyme-linked immunosorbent assays indicated an upregulation of the fibrosis-associated chemokine (C-C motif) ligand 2 (CCL2) by the viral spike protein and the virus-like particles. The upregulation of CCL2 by SARS-CoV spike protein was mainly mediated by extracellular signal-regulated kinase 1 and 2 (ERK1/2) and AP-1 but not the IkappaBalpha-NF-kappaB signaling pathway. In addition, Ras and Raf upstream of the ERK1/2 signaling pathway were involved in the upregulation of CCL2. Furthermore, ACE2 receptor was activated by casein kinase II-mediated phosphorylation in cells pretreated with the virus-like particles containing spike protein. These results indicate that SARS-CoV spike protein triggers ACE2 signaling and activates fibrosis-associated CCL2 expression through the Ras-ERK-AP-1 pathway.

Chen, J., et al. (2017). "Protective effect of diminazene attenuates myocardial infarction in rats via increased inflammation and ACE2 activity." Mol Med Rep **16**(4): 4791-4796.

The present study aimed to investigate whether diminazene attenuates myocardial infarction (MI) in rats. In addition, the present study investigated whether ACE2 signaling was involved in the effects of diminazene on protein function. A rat model of acute myocardial infarction (AMI) was established by occlusion of the left anterior descending coronary artery. The AMI model rats received intraperitoneal injections of diminazene (5 mg/kg/day) for 3 days. Treatment with diminazene significantly inhibited the expression of casein kinase and lactate dehydrogenase, and reduced infarct size in AMI rats. The findings indicated that diminazene significantly reduced the levels of inflammatory factors including tumor necrosis factoralpha and interleukin6, suppressed the protein expression of cytochrome c oxidase subunit 2 (COX2) and inducible nitric oxide synthase (iNOS), and activated angiotensinconverting enzyme 2 (ACE2), angiotensin II receptor type 1 (AT1R) and MAS1 protooncogene, G proteincoupled receptor (MasR) protein expression in AMI model rats. In conclusion, the present study demonstrated that diminazene attenuated AMI in rats via suppression of inflammation, reduction of COX2 and iNOS expression, and activation of the ACE2/AT1R/MasR signaling pathway.

Chen, J., et al. (2014). "Neuronal over-expression of ACE2 protects brain from ischemia-induced damage." Neuropharmacology **79**: 550-558.

Angiotensin (Ang) II exaggerates cerebral injury in ischemic damage. Angiotensin-converting enzyme

type 2 (ACE2) converts Ang II into Ang (1-7) and thus, may protect against the effects of Ang II. We hypothesized that neuronal ACE2 over-expression decreases ischemic stroke in mice with Ang II overproduction. Human renin and angiotensinogen double transgenic (RA) mice and RA mice with neuronal over-expression of ACE2 (SARA) were used for the study. The mean arterial pressure (MAP) was calculated from telemetry-recorded blood pressure (BP). SARA mice were infused peripherally with Norepinephrine to "clamp" the BP, or intracerebroventricularly-infused with a Mas receptor antagonist (A-779). Middle cerebral artery occlusion (MCAO) surgery was performed to induce permanent focal ischemic stroke. Cerebral blood flow (CBF) and neurological function were determined. Two days after surgery, brain samples were collected for various analyses. Results showed: 1) When compared to chronically hypertensive RA mice, SARA mice had lower basal MAP, less MCAO-induced infarct volume, and increased CBF, neurological function and cerebral microvascular density in the peri-infarct area; 2) These changes in SARA mice were not altered after MAP "clamping", but partially reversed by brain infusion of A-779; 3) Ang (1-7)/Ang II ratio, angiogenic factors, endothelial nitric oxide synthase (eNOS) expression and nitric oxide production were increased, whereas, NADPH oxidase subunits and reactive oxygen species were decreased in the brain of SARA mice. ACE2 protects brain from ischemic injury via the regulation of NADPH oxidase/eNOS pathways by changing Ang (1-7)/Ang II ratio, independently of MAP changes.

Chen, L. J., et al. (2015). "The ACE2/Apelin Signaling, MicroRNAs, and Hypertension." Int J Hypertens **2015**: 896861.

The renin-angiotensin aldosterone system (RAAS) plays a pivotal role in the development of hypertension. Angiotensin converting enzyme 2 (ACE2), which primarily metabolises angiotensin (Ang) II to generate the beneficial heptapeptide Ang-(1-7), serves as a negative regulator of the RAAS. Apelin is a second catalytic substrate for ACE2 and functions as an inotropic and cardiovascular protective peptide. The physiological effects of Apelin are exerted through binding to its receptor APJ, a seven-transmembrane G protein-coupled receptor that shares significant homology with the Ang II type 1 receptor (AT1R). The deregulation of microRNAs, a class of short and small noncoding RNAs, has been shown to involve cardiovascular remodeling and pathogenesis of hypertension via the activation of the Ang II/AT1R pathway. MicroRNAs are linked with modulation of the ACE2/Apelin signaling, which exhibits beneficial effects in the cardiovascular system and hypertension. The ACE2-coupled crosstalk among the RAAS, the Apelin system, and microRNAs provides an important mechanistic insight into hypertension. This paper focuses on what is known about the ACE2/Apelin signaling and its biological roles, paying particular attention to interactions and crosstalk among the ACE2/Apelin signaling, microRNAs, and hypertension, aiming to facilitate the exploitation of new therapeutic medicine to control hypertension.

Chen, Q., et al. (2019). "Sini decoction ameliorates sepsis-induced acute lung injury via regulating ACE2-Ang (1-7)-Mas axis and inhibiting the MAPK signaling pathway." Biomed Pharmacother **115**: 108971.

Sepsis, as life-threatening organ dysfunction caused by a dysregulated host response to infection, is characterized by the extensive release of cytokines and other mediators. Sini decoction (SND), a traditional Chinese prescription medicine, has been used clinically for the treatment of sepsis. But its explicit mechanism of action is still unclear. The present study aims to evaluate the potential protective effects of SND on sepsis-induced acute lung injury (ALI). After SND intervention, the lung tissues of each experimental group were collected. H & E sections were used to observe the pathological changes of lung tissue, and alveolar lavage fluid was collected to detect the infiltration of inflammatory cells. Level of inflammatory factors in lung tissue were analyzed by qRT-PCR. The change of Renin angiotensin system (RAS), as well as downstream MAPK/NF-kappaB signaling pathways were measured by Western blot. For in vitro experiments, human umbilical vein endothelial cells (HUVECs) were pretreated with lipopolysaccharide (LPS) and treated with SND. Subsequently, the expression levels of RAS and MAPK/NF-kappaB signaling pathways were measured by Western blot. In vivo, we found that SND significantly attenuated sepsis-induced pathological injury in the lung. SND also inhibited LPS-mediated inflammatory cell infiltration, the expression of pro-apoptotic proteins and the production of IL-6, IL-1beta, TNF-alpha and MCP-1. In vitro, experiments using a co-culture of HUVECs with SND showed that there was a decrease in pro-apoptotic protein and pro-inflammatory mediator. In this research, we also found that SND protective action could be attributed to the regulation of renin-angiotensin system (RAS). MAPKs and NF-kappaB pathways. To conclude, our study demonstrated that SND ameliorates sepsis-induced-ALI via regulating ACE2-Ang (1-7)-Mas axis and inhibiting the MAPK signaling pathway.

Chen, Q. F., et al. (2018). "Lipoxin A4 attenuates LPS-induced acute lung injury via activation of the

ACE2-Ang-(1-7)-Mas axis." Innate Immun **24**(5): 285-296.

Previous studies have reported that lipoxin A4 (LXA4) and the angiotensin I-converting enzyme 2 (ACE2), angiotensin-(1-7) [Ang-(1-7)], and its receptor Mas [ACE2-Ang-(1-7)-Mas] axis play important protective roles in acute lung injury (ALI). However, there is still no direct evidence of LXA4-mediated protection via the ACE2-Ang-(1-7)-Mas axis during ALI. This work was performed using an LPS-induced ALI mouse model and the data indicated the following. First, the animal model was established successfully and LXA4 ameliorated LPS-induced ALI. Second, LXA4 could increase the concentration and activity of ACE2 and the levels of Ang-(1-7) and Mas markedly. Third, LXA4 decreased the levels of TNF-alpha, IL-1beta, and reactive oxygen species while increasing IL-10 levels. Fourth, LXA4 inhibited the activation of the NF-kappaB signal pathway and repressed the degradation of inhibitor of NF-kappaB, the phosphorylation of NF-kappaB, and the translocation of NF-kappaB. Finally, and more importantly, BOC-2 (LXA4 receptor inhibitor), MLN-4760 (ACE2 inhibitor), and A779 (Mas receptor antagonist) were found to reverse all of the effects of LXA4. Our data provide evidence that LXA4 protects the lung from ALI through regulation of the ACE2-Ang-(1-7)-Mas axis.

Chen, Y. Y., et al. (2016). "Impact of ACE2 gene polymorphism on antihypertensive efficacy of ACE inhibitors." J Hum Hypertens **30**(12): 766-771.

Angiotensin-converting enzyme 2 (ACE2), a newly discovered member of renin-angiotensin-aldosterone system, counterbalances the actions of angiotensin-converting enzyme. The objective of our study was to assess the association between rs2106809 polymorphism in ACE2 gene and the blood pressure response to ACE inhibitors in untreated hypertensive patients. After a 2-week, double-blind placebo run-in period, either benazepril or imidapril was administered for 6 weeks to 497 patients with mild to moderate essential hypertension. The achieved changes in BP were analyzed for their association with genotypes at ACE2 gene loci. In female hypertensive patients, the genotype frequency of ACE2 rs2106809 was 36.7%, 45.2% and 18.1% for CC, CT and TT genotypes, respectively. After 6 weeks of treatment, the reductions in diastolic blood pressure were significantly greater in female patients carrying the CC or CT genotype compared with those carrying the TT genotype (9.62+/-6.83 or 10.2+/-7.2 versus 6.81+/-6.31 mm Hg, respectively; P=0.045, analysis of variance (ANOVA)). Moreover, the reductions in mean arterial pressure were significantly greater in female patients carrying the CC or CT genotype compared with those carrying the TT genotype (12.1+/-7.5 or 12.0+/-7.9 versus 8.38+/-6.83 mm Hg, respectively; P=0.035, ANOVA). In male hypertensive patients, the genotype frequency of ACE2 rs2106809 was 58.1% and 41.9% for C and T genotypes, respectively. However, no association could be observed in males. We conclude that ACE2 rs2106809 is an important predictive factor of the response to antihypertensive treatment with ACE inhibitors in Chinese female hypertensive patients.

Chen, Y. Y., et al. (2018). "Relationship between genetic variants of ACE2 gene and circulating levels of ACE2 and its metabolites." J Clin Pharm Ther **43**(2): 189-195.

WHAT IS KNOWN: Angiotensin-converting enzyme 2 (ACE2) plays an important role in the development of essential hypertension (EH). Genetic factors remarkably influence circulating ACE2 level. OBJECTIVE: Because heritability had remarkable effects on circulating ACE2, we designed this study to shed light on whether circulating levels of ACE2, angiotensin-(1-7) and angiotensin-(1-9) were linked to single nucleotide polymorphisms (SNPs) and haplotypes in ACE2 gene. METHODS: A total of 213 patients with newly diagnosed mild to moderate EH were enrolled in the present study. Four ACE2 tag SNPs (rs2074192, rs4646171, rs4646155 and rs2106809) were genotyped, and major haplotypes consisting of these 4 SNPs were reconstructed for all subjects. Circulating levels of ACE2, angiotensin-(1-7) and angiotensin-(1-9) were measured using enzyme-linked immunosorbent assay. RESULTS: In female subjects, linear regression analysis suggested that rare alleles of ACE2 rs2074192 and rs2106809 were associated with reduced circulating angiotensin-(1-7) levels (P=.007 and P=.006, respectively). ACE2 haplotype CAGC was associated with elevated circulating angiotensin-(1-7) levels (P=.03) whereas TAGT was associated with reduced circulating angiotensin-(1-7) levels in females (P<.001). Univariate linear regression analysis revealed that circulating ACE2 levels were positively associated with systolic blood pressure (P=.02), mean arterial pressure (P=.02) and serum creatinine (P<.001) in females whereas circulating ACE2 levels were positively associated with age (P<.001) and serum creatinine (P<.001) in males. WHAT IS NEW AND CONCLUSION: ACE2 SNPs and haplotypes are associated with circulating angiotensin-(1-7) levels. ACE2 genetic variants may be the determinants of circulating angiotensin-(1-7) levels in hypertensive females.

Cheng, Q., et al. (2016). "ACE2 overexpression inhibits acquired platinum resistance-induced tumor

angiogenesis in NSCLC." Oncol Rep **36**(3): 1403-1410.

Angiotensin II (AngII) is a multifunctional bioactive peptide in the renin-angiotensin system (RAS). Angiotensin-converting enzyme 2 (ACE2) is a newly identified component of RAS. We previously reported that ACE2 overexpression may inhibit cell growth and vascular endothelial growth factor (VEGF) production in vitro and in vivo. In the present study, we investigated the effect of ACE2 on tumor-associated angiogen-esis after the development of acquired platinum resistance in non-small cell lung cancer (NSCLC). Four NSCLC cell lines, A549, LLC, A549-DDP and LLC-DDP, were used in vitro, while A549 and A549-DDP cells were used in vivo. A549-DDP and LLC-DDP cells were newly established at our institution as acquired platinum-resistant sublines by culturing the former parent cells in cisplatin (CDDP)-containing conditioned medium for 6 months. These platinum-resistant cells showed significantly higher angiotensin II type 1 receptor (AT1R), ACE and VEGF production and lower ACE2 expression than their corresponding parent cells. We showed that ACE2 overexpression inhibited the production of VEGF in vitro and in vivo compared to their corresponding parent cells. We also found that ACE2 overexpression reduced the expression of AT1R and ACE. Additionally, we confirmed that ACE2 overexpres-sion inhibited cell growth and VEGF production while simultaneously suppressing ACE and AT1R expression in human lung cancer xenografts. Our findings indicate that ACE2 overexpression may potentially suppress angiogenesis in NSCLC after the development of acquired platinum resistance.

Cherney, D. Z., et al. (2014). "Urinary ACE2 in healthy adults and patients with uncomplicated type 1 diabetes." Can J Physiol Pharmacol **92**(8): 703-706.

Angiotensin-converting enzyme 2 (ACE2) is expressed in the kidney and may be renoprotective. We determined whether urinary ACE2 enzyme activity and protein levels (ELISA), as well as angiotensinogen and ACE, are elevated during clamped euglycemia (4-6 mmol.L (-1)) in patients with uncomplicated type 1 diabetes (T1D, n = 58) compared with normoglycemic controls (n = 21). We also measured the effect of clamped hyperglycemia (9-11 mmol.L (-1)) on each urinary factor in T1D patients. Urinary ACE2 activity and protein levels were higher during clamped euglycemia in T1D compared with the controls ($p < 0.0001$). In contrast, urinary angiotensinogen levels ($p = 0.27$) and ACE excretion ($p = 0.68$) did not differ. In response to clamped hyperglycemia in T1D, urinary ACE2 protein decreased ($p < 0.0001$), whereas urinary ACE2 activity as well as angiotensinogen and ACE levels remained unchanged. Urinary ACE2 activity and protein expression are increased in T1D patients prior to the onset of clinical complications. Further work is required to determine the functional role of urinary ACE2 in early T1D.

Chodavarapu, H., et al. (2016). "High-fat diet-induced glucose dysregulation is independent of changes in islet ACE2 in mice." Am J Physiol Regul Integr Comp Physiol **311**(6): R1223-R1233.

While restoration of ACE2 activity in the pancreas leads to improvement of glycemia in experimental models of Type 2 diabetes, global deficiency in ACE2 disrupts beta-cell function and impairs glucose tolerance in mice, demonstrating the physiological role of ACE2 in glucose homeostasis. Although the contribution of pancreatic ACE2 to glucose regulation has been demonstrated in genetic models of diabetes and in models with overexpression of the renin-angiotensin system (RAS), it is unclear whether islet ACE2 is involved in glycemic control in common models of human Type 2 diabetes. To determine whether diet-induced diabetes deregulates glucose homeostasis via reduction of ACE2 in the pancreatic islets, wild-type (WT) and ACE2 knockout (KO) male mice were fed a high-fat diet (HFD) for 16 wk. ACE2 KO mice were more susceptible than WT mice to HFD-mediated glycemic dysregulation. Islet ACE2 activity and expression of various genes, including ANG II type 1a receptor (mAT1aR) were then assessed. Surprisingly, we observed no change in islet ACE2 activity and expression despite local RAS overactivity, indicated by an upregulation of mAT1aR expression. Despite a predominant expression in islet alpha-cells, further investigation highlighted a minor role for ACE2 on glucagon expression. Further, pancreatic ACE2 gene therapy improved glycemia in HFD-fed WT mice, leading to enhanced glucose-stimulated insulin secretion, reduced pancreatic ANG II levels, fibrosis, and ADAM17 activity. Altogether, our study demonstrates that HFD feeding increases RAS activity and mediates glycemic dysregulation likely through loss of ACE2 present outside the islets but independently of changes in islet ACE2.

Chou, C. F., et al. (2006). "ACE2 orthologues in non-mammalian vertebrates (Danio, Gallus, Fugu, Tetraodon and Xenopus)." Gene **377**: 46-55.

Angiotensin-converting enzyme 2 (ACE2), a newly identified member in the renin-angiotensin system (RAS), acts as a negative regulator of ACE. It is mainly expressed in cardiac blood vessels and the tubular epithelia of kidneys and abnormal expression has been implicated in diabetes, hypertension and heart failure. The mechanism and physiological function of this zinc metallopeptidase in mammals are

not yet fully understood. Non-mammalian vertebrate models offer attractive and simple alternatives that could facilitate the exploration of ACE2 function. In this paper we report the in silico analysis of Ace2 genes from the Gallus (chicken), Xenopus (frog), Fugu and Tetraodon (pufferfish) genome assembly databases, and from the Danio (zebrafish) cDNA library. Exon ambiguities of Danio and Xenopus Ace2s were resolved by RT-PCR and 3'RACE. Analyses of the exon-intron structures, alignment, phylogeny and hydrophilicity plots, together with the conserved synteny among these vertebrates, support the orthologous relationship between mammalian and non-mammalian ACE2s. The putative promoters of Ace2 from human, Tetraodon and Xenopus tropicalis drove the expression of enhanced green fluorescent protein (EGFP) specifically in the heart tissue of transgenic Xenopus thus making it a suitable model for future functional genomic studies. Additionally, the search for conserved cis-elements resulted in the discovery of WGATAR motifs in all the putative Ace2 promoters from 7 different animals, suggesting a possible role of GATA family transcriptional factors in regulating the expression of Ace2.

Chou, C. H., et al. (2013). "Interaction between TGF-beta and ACE2-Ang-(1-7)-Mas pathway in high glucose-cultured NRK-52E cells." Mol Cell Endocrinol **366**(1): 21-30.

Transforming growth factor-beta (TGF-beta) is pivotal in diabetic nephropathy (DN). Angiotensin converting enzyme-2 (ACE2) converts angiotensin II (Ang II) to angiotensin 1-7 (Ang-(1-7)), which binds to Mas. Proximal tubular ACE2 is decreased in DN. ACE2 deficiency exacerbates whereas ACE2 overexpression attenuates DN. Thus, we investigated the mechanism of high glucose-decreased ACE2 in terms of the interaction between TGF-beta and ACE2-Ang-(1-7)-Mas in NRK-52E cells. We found that high glucose increased TGF-beta1. SB431542 attenuated high glucose-inhibited ACE2 and Mas and Ang-(1-7) conversion from Ang II while attenuating high glucose-induced fibronectin. TGF-beta1 also decreased ACE2 and Mas and Ang-(1-7) conversion from Ang II. A779 attenuated Ang-(1-7)-decreased TGF-beta1 and Ang-(1-7)-activated JAK2-STAT3. Moreover, A779, LY294002 and AG490 attenuated Ang-(1-7)-inhibited TGF-beta1. The combination of Ang-(1-7) and Mas attenuated TGF-beta1 (but not high glucose)-induced fibronectin. Thus, high glucose decreases ACE2 via TGF-betaR in NRK-52E cells. Additionally, there is a negative feedback function between TGF-beta and ACE2, and the combined inhibition of TGF-beta and activation of the ACE2-Ang-(1-7)-Mas may be useful for treating diabetic renal fibrosis.

Chu, P. L. and T. H. Le (2014). "Role of collectrin, an ACE2 homologue, in blood pressure homeostasis." Curr Hypertens Rep **16**(11): 490.

Collectrin (Tmem27) is a transmembrane glycoprotein that is highly expressed in the kidney and vascular endothelium. It is a homologue of the angiotensin-converting enzyme 2 (ACE2) but harbors no catalytic domain. In the extravascular tissues of the kidney, collectrin is localized to the proximal tubule and collecting duct. Collectrin-deficient mice are featured with hypertension and exaggerated salt sensitivity. These phenotypes are associated with impaired uptake of the nitric oxide precursor L-arginine and the expression of its amino acid transporters, CAT-1 and y (+)LAT1, in endothelial cells. In addition, collectrin-deficient mice display decreased dimerization of nitric oxide synthase and decreased nitric oxide synthesis, but enhanced superoxide generation, suggesting that deletion of collectrin leads to a state of nitric oxide synthase uncoupling. These findings suggest that collectrin plays a protective role against hypertension. The collectrin knockout mouse represents a unique model for hypertension research. Furthermore, collectrin may serve as a novel therapeutic target in the treatment of hypertension.

Clarke, N. E., et al. (2014). "Epigenetic regulation of angiotensin-converting enzyme 2 (ACE2) by SIRT1 under conditions of cell energy stress." Clin Sci (Lond) **126**(7): 507-516.

ACE2 (angiotensin-converting enzyme 2) counterbalances the actions of ACE (angiotensin-converting enzyme) by metabolizing its catalytic product, the vasoactive and fibrogenic peptide AngII (angiotensin II), into Ang-(1-7) [angiotensin-(1-7)]. Enhanced ACE2 expression may be protective in diabetes, cardiovascular disease and cancer. However, relatively little is known about the specific physiological factors regulating ACE2 expression. In the present paper, we show, by Western blotting and qPCR (quantitative real-time PCR), that ACE2 expression is increased under conditions of cell stress, including hypoxic conditions, IL (interleukin)-1beta treatment and treatment with the AMP mimic AICAR (5-amino-4-imidazolecarboxamide riboside). The NAD+-dependent deacetylase SIRT1 (silent information regulator T1) was found to be up-regulated after AICAR treatment but, conversely, was down-regulated after IL-1beta treatment. ChIP analysis demonstrated that SIRT1 bound to the ACE2 promoter and that binding was increased after AICAR treatment, but decreased after IL-1beta treatment. Inhibition of SIRT1 activity ablated the AICAR-induced increase in ACE2. In conclusion, we have

established that the expression of the ACE2 transcript is controlled by the activity of SIRT1 under conditions of energy stress.

Clarke, N. E., et al. (2012). "Angiotensin converting enzyme (ACE) and ACE2 bind integrins and ACE2 regulates integrin signalling." PLoS One **7**(4): e34747.

The angiotensin converting enzymes (ACEs) are the key catalytic components of the renin-angiotensin system, mediating precise regulation of blood pressure by counterbalancing the effects of each other. Inhibition of ACE has been shown to improve pathology in cardiovascular disease, whilst ACE2 is cardioprotective in the failing heart. However, the mechanisms by which ACE2 mediates its cardioprotective functions have yet to be fully elucidated. Here we demonstrate that both ACE and ACE2 bind integrin subunits, in an RGD-independent manner, and that they can act as cell adhesion substrates. We show that cellular expression of ACE2 enhanced cell adhesion. Furthermore, we present evidence that soluble ACE2 (sACE2) is capable of suppressing integrin signalling mediated by FAK. In addition, sACE2 increases the expression of Akt, thereby lowering the proportion of the signalling molecule phosphorylated Akt. These results suggest that ACE2 plays a role in cell-cell interactions, possibly acting to fine-tune integrin signalling. Hence the expression and cleavage of ACE2 at the plasma membrane may influence cell-extracellular matrix interactions and the signalling that mediates cell survival and proliferation. As such, ectodomain shedding of ACE2 may play a role in the process of pathological cardiac remodelling.

Clayton, D., et al. (2011). "beta-Amino acid substitution to investigate the recognition of angiotensin II (AngII) by angiotensin converting enzyme 2 (ACE2)." J Mol Recognit **24**(2): 235-244.

In spite of the important role of angiotensin converting enzyme 2 (ACE2) in the cardiovascular system, little is known about the substrate structural requirements of the AngII-ACE2 interaction. Here we investigate how changes in angiotensin II (AngII) structure affect binding and cleavage by ACE2. A series of C3 beta-amino acid AngII analogs were generated and their secondary structure, ACE2 inhibition, and proteolytic stability assessed by circular dichroism (CD), quenched fluorescence substrate (QFS) assay, and LC-MS analysis, respectively. The beta-amino acid-substituted AngII analogs showed differences in secondary structure, ACE2 binding and proteolytic stability. In particular, three different subsets of structure-activity profiles were observed corresponding to substitutions in the N-terminus, the central region and the C-terminal region of AngII. The results show that beta-substitution can dramatically alter the structure of AngII and changes in structure correlated with ACE2 inhibition and/or substrate cleavage. beta-amino acid substitution in the N-terminal region of AngII caused little change in structure or substrate cleavage, while substitution in the central region of AngII lead to increased beta-turn structure and enhanced substrate cleavage. beta-amino acid substitution in the C-terminal region significantly diminished both secondary structure and proteolytic processing by ACE2. The beta-AngII analogs with enhanced or decreased proteolytic stability have potential application for therapeutic intervention in cardiovascular disease.

Clayton, D., et al. (2015). "Structural determinants for binding to angiotensin converting enzyme 2 (ACE2) and angiotensin receptors 1 and 2." Front Pharmacol **6**: 5.

Angiotensin converting enzyme 2 (ACE2) is a zinc carboxypeptidase involved in the renin-angiotensin system (RAS) and inactivates the potent vasopressive peptide angiotensin II (Ang II) by removing the C-terminal phenylalanine residue to yield Ang1-7. This conversion inactivates the vasoconstrictive action of Ang II and yields a peptide that acts as a vasodilatory molecule at the Mas receptor and potentially other receptors. Given the growing complexity of RAS and level of cross-talk between ligands and their corresponding enzymes and receptors, the design of molecules with selectivity for the major RAS binding partners to control cardiovascular tone is an on-going challenge. In previous studies we used single beta-amino acid substitutions to modulate the structure of Ang II and its selectivity for ACE2, AT1R, and angiotensin type 2 (AT2R) receptor. We showed that modification at the C-terminus of Ang II generally resulted in more pronounced changes to secondary structure and ligand binding, and here, we further explore this region for the potential to modulate ligand specificity. In this study, (1) a library of 47 peptides derived from the C-terminal tetrapeptide sequence (-IHPF) of Ang II was synthesized and assessed for ACE2 binding, (2) the terminal group requirements for high affinity ACE2 binding were explored by and N- and C-terminal modification, (3) high affinity ACE2 binding chimeric AngII analogs were then synthesized and assessed, (4) the structure of the full-length Ang II analogs were assessed by circular dichroism, and (5) the Ang II analogs were assessed for AT1R/AT2R selectivity by cell-based assays. Studies on the C-terminus of Ang II demonstrated varied specificity at different residue positions for ACE2 binding and four Ang II chimeric peptides were identified as selective ligands for the

AT2 receptor. Overall, these results provide insight into the residue and structural requirements for ACE2 binding and angiotensin receptor selectivity.

Clotet-Freixas, S., et al. (2018). "Sex dimorphism in ANGII-mediated crosstalk between ACE2 and ACE in diabetic nephropathy." Lab Invest **98**(9): 1237-1249.

Angiotensin-converting enzyme (ACE) and ACE2 play a critical role in the renin-angiotensin system (RAS) by altering angiotensin II (ANGII) levels, thus governing its deleterious effects. Both enzymes are altered by sex and diabetes, and play an important role in the development of diabetic nephropathy (DN). Importantly, previous evidence in diabetic and ACE2-deficient (ACE2KO) males suggest a sex-dependent crosstalk between renal ACE and ACE2. In the present work, we aimed to study the sex-specific susceptibility to diabetes and direct infusion of ANGII in kidney disease progression, with a special focus on its link to ACE2 and ACE. In our mouse model, ANGII promoted hypertension, albuminuria, reduced glomerular filtration, and glomerular histological alterations. ANGII adverse effects were accentuated by diabetes and ACE2 deficiency, in a sex-dependent fashion: ACE2 deficiency accentuated ANGII-induced hypertension, albuminuria, and glomerular hypertrophy in diabetic females, whereas in diabetic males exacerbated ANGII-mediated glomerular hypertrophy, mesangial expansion, and podocyte loss. At the molecular level, ANGII downregulated renal ACE gene and enzymatic activity levels, as well as renin gene expression in ACE2KO mice. Interestingly, male sex and diabetes accentuated this effect. Here we show sex dimorphism in the severity of diabetes- and ANGII-related renal lesions, and demonstrate that ACE2- and ACE-related compensatory mechanisms are sex-specific. Supporting our previous findings, the modulation and ANGII-mediated crosstalk between ACE2 and ACE in DN progression was more evident in males. This work increases the understanding of the sex-specific role of ACE2 and ACE in DN, reinforcing the necessity of more personalized treatments targeting RAS.

Cocklin, R., et al. (2011). "New insight into the role of the Cdc34 ubiquitin-conjugating enzyme in cell cycle regulation via Ace2 and Sic1." Genetics **187**(3): 701-715.

The Cdc34 ubiquitin-conjugating enzyme plays a central role in progression of the cell cycle. Through analysis of the phenotype of a mutant missing a highly conserved sequence motif within the catalytic domain of Cdc34, we discovered previously unrecognized levels of regulation of the Ace2 transcription factor and the cyclin-dependent protein kinase inhibitor Sic1. In cells carrying the Cdc34(tm) mutation, which alters the conserved sequence, the cyclin-dependent protein kinase inhibitor Sic1, an SCF (Cdc4) substrate, has a shorter half-life, while the cyclin Cln1, an SCF (Grr1) substrate, has a longer half-life than in wild-type cells. Expression of the SIC1 gene cluster, which is regulated by Swi5 and Ace2 transcription factors, is induced in CDC34(tm) cells. Levels of Swi5, Ace2, and the SCF (Grr1) targets Cln1 and Cln2 are elevated in Cdc34(tm) cells, and loss of Grr1 causes an increase in Ace2 levels. Sic1 levels are similar in CDC34(tm) ace2Delta and wild-type cells, explaining a paradoxical increase in the steady-state level of Sic1 protein despite its reduced half-life. A screen for mutations that interact with CDC34(tm) uncovered novel regulators of Sic1, including genes encoding the polyubiquitin chain receptors Rad23 and Rpn10.

Coelho, M. S., et al. (2010). "High sucrose intake in rats is associated with increased ACE2 and angiotensin-(1-7) levels in the adipose tissue." Regul Pept **162**(1-3): 61-67.

Sucrose-fed rats, a model of metabolic syndrome, are characterized by insulin resistance, obesity, hypertension, and high plasma levels of triacylglycerols and angiotensin II (Ang II). However, whether tissue renin-angiotensin system (RAS) is altered in metabolic syndrome is unclear. To study this issue, food ad libitum and water (C) or 20% sucrose solution (SC) were given to adult male Wistar rats, for 30 days. Body weight (BW), blood pressure (BP), epididymal adipose tissue (EPI) mass, rate of in vivo fatty acid (FA) synthesis in EPI, circulating glucose, insulin, leptin, angiotensins I and II, triacylglycerols, and plasma renin (PRA) and angiotensin-converting enzyme (ACE) activities were evaluated. In kidneys and EPI, gene and protein expression of type 1 (AT (1)) and 2 (AT (2)) Ang II receptors, ACE, angiotensinogen (AGT) as well as protein expression of angiotensin-converting enzyme 2 (ACE2) were determined. In both tissues, Ang I, Ang II and Ang-(1-7) contents were also measured by HPLC. In SC rats higher BP, EPI mass, circulating triacylglycerols, insulin, leptin, PRA and, Ang II were found. In EPI, the rate of in vivo FA synthesis was associated with increased Ang-(1-7), protein expression of AT (1) and AT (2) receptors, ACE2, AGT, and gene expression of AGT although a reduction in ACE activity and in adipose Ang I and Ang II contents was observed. In kidneys, AT (1) and AT (2), ACE and AGT gene and protein expression as well as protein expression of ACE2 were unaltered while Ang II, Ang-(1-7) and ACE activity increased. These RAS component changes seem to be tissue specific and possibly are related to enhancement of FA synthesis, EPI mass and hypertension.

Cohall, D., et al. (2020). "Is hypertension in African-descent populations contributed to by an imbalance in the activities of the ACE2/Ang-(1-7)/Mas and the ACE/Ang II/AT1 axes?" J Renin Angiotensin Aldosterone Syst **21**(1): 1470320320908186.

INTRODUCTION: Low plasma renin activity hypertension is prevalent in Afro-Caribbean persons. Reduced angiotensin converting enzyme 2 activity from the counter angiotensin converting enzyme 2 /angiotensin-(1-7)/Mas receptor axis of the renin angiotensin aldosterone system has been reported in people with pre-hypertension, type 2 diabetes mellitus and chronic renal disease. This study investigates whether an imbalance in the regulatory mechanisms between the pressor arm of the renin angiotensin aldosterone system (angiotensin converting enzyme/angiotensin II/AT1 receptor) and the depressor axis (angiotensin converting enzyme 2/angiotensin-(1-7)/Mas receptor) predisposes persons of African descent to hypertension. METHODS: In total, 30 normotensives and 30 recently diagnosed hypertensives aged 18-55 of Afro-Caribbean origin who are naive to antihypertensive treatment will be recruited from public sector polyclinics in Barbados. Demographic and anthropometric data, clinical blood pressure readings, 24-hour urine collections and venous blood samples will be collected. Biological samples will be analysed for renin angiotensin aldosterone system peptide markers using radioimmunoassay. CONCLUSION: We describe the design, methods and rationale for the characterization of renin angiotensin aldosterone system mechanisms that may contribute to hypertension predisposition in persons of African descent. Our findings will characterize any imbalance in the counter axes of the renin angiotensin aldosterone system in hypertensive Afro-Caribbeans with a potential view of identifying novel approaches with the use of renin angiotensin aldosterone system and mineralocorticoid blockers to manage the condition.

Cole-Jeffrey, C. T., et al. (2015). "ACE2 and Microbiota: Emerging Targets for Cardiopulmonary Disease Therapy." J Cardiovasc Pharmacol **66**(6): 540-550.

The health of the cardiovascular and pulmonary systems is inextricably linked to the renin-angiotensin system (RAS). Physiologically speaking, a balance between the vasodeleterious (Angiotensin-converting enzyme [ACE]/Angiotensin II [Ang II]/Ang II type 1 receptor [AT1R]) and vasoprotective (Angiotensin-converting enzyme 2 [ACE2]/Angiotensin-(1-7) [Ang-(1-7)]/Mas receptor [MasR]) components of the RAS is critical for cardiopulmonary homeostasis. Upregulation of the ACE/Ang II/AT1R axis shifts the system toward vasoconstriction, proliferation, hypertrophy, inflammation, and fibrosis, all factors that contribute to the development and progression of cardiopulmonary diseases. Conversely, stimulation of the vasoprotective ACE2/Ang-(1-7)/MasR axis produces a counter-regulatory response that promotes cardiovascular health. Current research is investigating novel strategies to augment actions of the vasoprotective RAS components, particularly ACE2, in order to treat various pathologies. Although multiple approaches to increase the activity of ACE2 have displayed beneficial effects against experimental disease models, the mechanisms behind its protective actions remain incompletely understood. Recent work demonstrating a non-catalytic role for ACE2 in amino acid transport in the gut has led us to speculate that the therapeutic effects of ACE2 can be mediated, in part, by its actions on the gastrointestinal tract and/or gut microbiome. This is consistent with emerging data which suggest that dysbiosis of the gut and lung microbiomes is associated with cardiopulmonary disease. This review highlights new developments in the protective actions of ACE2 against cardiopulmonary disorders, discusses innovative approaches to targeting ACE2 for therapy, and explores an evolving role for gut and lung microbiota in cardiopulmonary health.

Currie, D., et al. (2010). "Investigation of ACE, ACE2 and AGTR1 genes for association with nephropathy in Type 1 diabetes mellitus." Diabet Med **27**(10): 1188-1194.

BACKGROUND: Polymorphisms in ACE and AGTR1 genes have been assessed in multiple studies for association with diabetic nephropathy; however, results are conflicting. The ACE2 gene has not been studied extensively for association with diabetic nephropathy. METHODS: We investigated variants in ACE, ACE2 and AGTR1 for association with nephropathy in a case-control group (1467 participants with Type1 diabetes, case subjects n=718; control subjects n=749) of white descent with grandparents born in the British Isles. All recruited individuals were carefully phenotyped and genotyping was performed using Sequenom, Taqman and gel electrophoresis methods. The chi (2) -test for contingency tables was used to compare genotype and allele frequencies in case and control groups. RESULTS: No departure from Hardy-Weinberg equilibrium was observed in cases or controls. Two variants within the ACE gene (rs4293, P (allelic) =0.02, P (genotypic) =0.008; rs4309, P (allelic) =0.02, P (genotypic) =0.01) were significantly associated with nephropathy at the 5% level. No variant remained statistically significant following adjustment for multiple comparisons. No single nucleotide polymorphisms in the ACE2 or

AGTR1 genes were significantly associated with nephropathy when analysed either by genotype or allele frequencies. CONCLUSIONS: Our independent case-control study provides no evidence that common variants in ACE, ACE2 and AGTR1 play a major role in genetic susceptibility to diabetic nephropathy in a white population with Type1 diabetes.

da Silva, J. S., et al. (2017). "Blunting of cardioprotective actions of estrogen in female rodent heart linked to altered expression of cardiac tissue chymase and ACE2." J Renin Angiotensin Aldosterone Syst **18**(3): 1470320317722270.

BACKGROUND: Diastolic dysfunction develops in response to hypertension and estrogen (E2) loss and is a forerunner to heart failure (HF) in women. The cardiac renin-angiotensin system (RAS) contributes to diastolic dysfunction, but its role with respect to E2 and blood pressure remain unclear. METHODS: We compared the effects of ovariectomy (OVX) or sham surgery on the cardiac RAS, left ventricular (LV) structure/function, and systemic/intracardiac pressures of spontaneously hypertensive rats (SHRs: n = 6 intact and 6 OVX) and age-matched Wistar-Kyoto (WKY: n = 5 intact and 4 OVX) controls. RESULTS: WKY rats were more sensitive to OVX than SHRs with respect to worsening of diastolic function, as reflected by increases in Doppler-derived filling pressures (E/e') and reductions in myocardial relaxation (e'). This pathobiologic response in WKY rats was directly linked to increases in cardiac gene expression and enzymatic activity of chymase and modest reductions in ACE2 activity. No overt changes in cardiac RAS genes or activities were observed in SHRs, but diastolic function was inversely related to ACE2 activity. CONCLUSION: Endogenous estrogens exert a more significant regulatory role upon biochemical components of the cardiac RAS of WKY versus SHRs, modulating the lusitropic and structural components of its normotensive phenotype.

da Silveira, K. D., et al. (2010). "ACE2-angiotensin-(1-7)-Mas axis in renal ischaemia/reperfusion injury in rats." Clin Sci (Lond) **119**(9): 385-394.

AngII (angiotensin II), ACE (angiotensin I-converting enzyme) and the AT1 receptor (AngII type 1 receptor) are associated with the inflammatory process and microvascular dysfunction of AKI (acute kidney injury) induced by renal I/R (ischaemia/reperfusion). However, Ang-(1-7) [angiotensin-(1-7)], ACE2 (angiotensin I-converting enzyme 2) and the Mas receptor also play a role in renal disease models. Therefore, in the present study, we have examined the renal profile of Ang-(1-7), ACE2 and the Mas receptor in renal I/R and compared them with that of AngII, ACE and the AT1 receptor. Male Wistar rats were submitted to left nephrectomy and ischaemia (45 min) followed by reperfusion (2 or 4 h) in the right kidney. At 4 h of reperfusion, renal AngII was increased ($P<0.01$) and renal Ang-(1-7) was decreased substantially ($P<0.05$), although plasma levels of both angiotensins were unchanged. In addition, renal I/R decreased the renal mRNA expression of renin ($P<0.05$), AT1 receptors ($P<0.001$) and ACE2 ($P<0.05$). At 2 and 4 h of reperfusion, renal ACE activity was reduced ($P<0.05$). On the other hand, renal expression of the Mas receptor was greatly increased at 4 h of reperfusion ($P<0.01$), which was confirmed by immunohistochemical and Western blot analysis. In conclusion, increased renal expression of the Mas receptor associated with changes in the RAS (renin-angiotensin system)-related peptidases support an important role for the ACE2-Ang-(1-7)-Mas axis in AKI.

Dales, N. A., et al. (2002). "Substrate-based design of the first class of angiotensin-converting enzyme-related carboxypeptidase (ACE2) inhibitors." J Am Chem Soc **124**(40): 11852-11853.

Angiotensin-converting enzyme-related carboxypeptidase (ACE2) is a recently identified zinc metalloprotease with carboxypeptidase activity that was identified using our genomics platform. We implemented a rational design approach to identify potent and selective ACE2 inhibitors. To this end, picomolar inhibitors of ACE2 were designed and synthesized.

Dalpiaz, P. L., et al. (2015). "Sex Hormones Promote Opposite Effects on ACE and ACE2 Activity, Hypertrophy and Cardiac Contractility in Spontaneously Hypertensive Rats." PLoS One **10**(5): e0127515.

BACKGROUND: There is growing interest in sex differences and RAS components. However, whether gender influences cardiac angiotensin I-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2) activity is still unknown. In the present work, we determined the relationship between ACE and ACE2 activity, left ventricular function and gender in spontaneously hypertensive rats (SHRs). METHODOLOGY/PRINCIPAL FINDINGS: Twelve-week-old female (F) and male (M) SHRs were divided into 2 experimental groups (n = 7 in each group): sham (S) and gonadectomized (G). Fifty days after gonadectomy, we measured positive and negative first derivatives (dP/dt maximum left ventricle (LV) and dP/dt minimum LV, respectively), hypertrophy (morphometric analysis) and ACE and ACE2 catalytic activity (fluorimetrically). Expression of calcium

handling proteins was measured by western blot. Male rats exhibited higher cardiac ACE and ACE2 activity as well as hypertrophy compared to female rats. Orchiectomy decreased the activity of these enzymes and hypertrophy, while ovariectomy increased hypertrophy and ACE2, but did not change ACE activity. For cardiac function, the male sham group had a lower +dP/dt than the female sham group. After gonadectomy, the +dP/dt increased in males and reduced in females. The male sham group had a lower -dP/dt than the female group. After gonadectomy, the -dP/dt increased in the male and decreased in the female groups when compared to the sham group. No difference was observed among the groups in SERCA2a protein expression. Gonadectomy increased protein expression of PLB (phospholamban) and the PLB to SERCA2a ratio in female rats, but did not change in male rats. CONCLUSION: Ovariectomy leads to increased cardiac hypertrophy, ACE2 activity, PLB expression and PLB to SERCA2a ratio, and worsening of hemodynamic variables, whereas in males the removal of testosterone has the opposite effects on RAS components.

Dang, Z., et al. (2020). "Tsantan Sumtang attenuated chronic hypoxia-induced right ventricular structure remodeling and fibrosis by equilibrating local ACE-AngII-AT1R/ACE2-Ang1-7-Mas axis in rat." J Ethnopharmacol **250**: 112470.

ETHNOPHARMACOLOGICAL RELEVANCE: Tsantan Sumtang, which consists of Choerospondias axillaris (Roxb.) Burtt et Hill, Myristica fragrans Houtt and Santalum album L, is a traditional and common prescription of Tibetan medicine. Tsantan Sumtang originates from Four Tantra with properties of nourishing heart and has been used as a folk medicine for cardiovascular diseases and heart failure in Qinghai, Tibet and Inner Mongolia. Our previous studies found that Tsantan Sumtang showed beneficial effects on right ventricular structure in hypoxia rats, while the underling mechanism remains unclear. AIM OF THE STUDY: To elucidate the underlying mechanisms of Tsantan Sumtang attenuated right ventricular (RV) remodeling and fibrosis of chronic hypoxia-induced pulmonary arterial hypertension (HPAH) rats. MATERIALS AND METHODS: Fifty male Sprague Dawley (SD) rats (170 +/- 20 g) were randomly divided into control group, hypoxia group, and hypoxia + Tsantan Sumtang groups (1.0g.kg (-1).day (-1), 1.25g.kg (-1).day (-1), 1.5g.kg (-1).day (-1)). Rats in the hypoxia group and hypoxia + Tsantan Sumtang groups were maintained in a hypobaric chamber by adjusting the inner pressure and oxygen content to simulate an altitude of 4500 m for 28 days. The mean pulmonary arterial pressure (mPAP), right ventricle hypertrophy index (RVHI), the ratio of RV weight to tibia length (TL) (RV/TL), heart rate (HR) and RV systolic pressure (RVSP) was determined. Histomorphological assay of RV structure was evaluated by hematoxylin and eosin (HE) staining. RV tissue fibrosis was assessed by collagen proportion area (CPA), collagen I, collagen III and hydroxyproline content. CPA was obtained by picro-sirius red staining (PSR). The expression of collagen I and collagen III were detected by immunohistochemistry and western blotting. The hydroxyproline content was detected by alkaline hydrolysis. In addition, the level of angiotensin II (AngII) and angiotensin 1-7 (Ang1-7) in RV tissue was tested by enzyme-linked immune sorbent assay (ELISA). Protein expression of angiotensin-converting enzyme (ACE), AngII, AngII type 1 receptor (AT1R), angiotensin-converting enzyme 2 (ACE2), Mas receptor (Mas) were determined by immunohistochemistry and western blotting. mRNA level of ACE, AT1R, ACE2, Mas were tested by qPCR. The chemical profile of Tsantan Sumtang was revealed by UHPLC-Q-Exactive hybrid quadrupole-orbitrap mass analysis. RESULTS: Our results showed that RVHI, RV/TL and RVSP were significantly increased in HPAH rat. Furthermore, levels of collagen I, collagen III and hydroxyproline were up-regulated in RV tissue under hypoxia. We found that RV hypertrophy and fibrosis were associated with increased expression of ACE, AngII, AT1R as well as decreased expression of ACE2, Ang1-7 and Mas. RV remodeling and fibrosis were attenuated after Tsantan Sumtang administration by up-regulating ACE2 and Mas level as well as down-regulating ACE, AngII and AT1R levels in RV tissue. 35 constituents in Tsantan Sumtang were identified. CONCLUSION: Tsantan Sumtang attenuated RV remodeling and fibrosis in rat exposed to chronic hypoxia. The pharmacological effect of Tsantan Sumtang was based on equilibrating ACE-AngII-AT1R and ACE2-Ang1-7-Mas axis of RV tissue in HPAH rat.

de Carvalho Fraga, C. A., et al. (2017). "Angiotensin-Converting Enzymes (ACE and ACE2) as Potential Targets for Malignant Epithelial Neoplasia: Review and Bioinformatics Analyses Focused in Oral Squamous Cell Carcinoma." Protein Pept Lett **24**(9): 784-792.

INTRODUCTION: The Renin-Angiotensin System (RAS) has emerged as being related to vascular disease. Recently the RAS has been associated with obesity, diabetes, and even cancer. OBJECTIVE: This review and Bioinformatics analyses focuses on the investigation of Angiotensinconverting enzymes (ACE and ACE2) as therapeutical targets for Malignant Epithelial Neoplasia, specifically for Oral Squamous Cell

Carcinoma (OSCC). CONCLUSION: The literature review and Bioinformatics analyses showed that ACE and ACE2 are interesting targets for OSCC treatment. Studies involving RAS and OSCC should be encouraged for experimental validation.

de Lang, A., et al. (2006). "Interferon-gamma and interleukin-4 downregulate expression of the SARS coronavirus receptor ACE2 in Vero E6 cells." Virology **353**(2): 474-481.

Interferons (IFNs) inhibit severe acute respiratory syndrome coronavirus (SARS-CoV) replication and might be valuable for SARS treatment. In this study, we demonstrate that treatment of Vero E6 cells with interleukin-4 (IL-4) decreased the susceptibility of these cells to SARS-CoV infection. In contrast to IFNs, IL-4 did not show antiviral activity when administered immediately after SARS-CoV infection, suggesting that IL-4 acts early during the SARS-CoV replication cycle. Indeed, binding of recombinant SARS-CoV spike protein to Vero E6 cells was diminished on cells treated with IL-4, but also on cells exposed to IFN-gamma. Consistent with these observations, IL-4 and IFN-gamma downregulated cell surface expression of angiotensin-converting enzyme 2 (ACE2), the SARS-CoV receptor. Besides diminished ACE2 cell surface expression, ACE2 mRNA levels were also decreased after treatment with these cytokines. These findings suggest that IL-4 and IFN-gamma inhibit SARS-CoV replication partly through downregulation of ACE2.

de Melo, L. A. and A. F. Almeida-Santos (2019). "Neuropsychiatric Properties of the ACE2/Ang-(1-7)/Mas Pathway: A Brief Review." Protein Pept Lett.

The current pharmacological strategies for the management of anxiety disorders and depression, serious conditions which are gaining greater prevalence worldwide, depend on only two therapeutic classes of mood-stabilizing drugs: Serotonin Reuptake Inhibitors (SSRIs) and Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs). Although first line agents with proven efficacy, their clinical success in the management of anxiety disorders and depression is still considered highly complex due to the multifaceted nature of such conditions. Several studies have shown a possible therapeutic target could be found in the form of the Angiotensin-Converting Enzyme [ACE] type 2 (ACE2), Angiotensin [Ang]-(1-7) and Mas receptor pathway of the Renin-Angiotensin System (RAS), which as will be discussed, has been described to exhibit promising therapeutic properties for the management of anxiety disorders and depression. In this article, the literature to describe recent findings related to the role of the RAS in anxiety and depression disorders was briefly revised. The literature used covers a time range from 1988 to 2019 and were acquired from the National Center for Biotechnology Information's (NCBI) PubMed search engine. The results demonstrated in this review are promising and encourage the development of new research for treatment of anxiety and depression disorders focusing on the RAS. In conclusion, the ACE2/Ang-(1-7)/Mas pathway may exhibit anxiolytic and anti-depressive effects through many possible biochemical mechanisms both centrally and peripherally, and result in highly promising mental health benefits which justifies further investigation into this system as a possible new therapeutic target in the management of neuropsychiatric disorders, including any as of yet undescribed risk-benefit analysis compared to currently-implemented pharmacological strategies.

de Moraes, P. L., et al. (2017). "Vasodilator Effect of Angiotensin-(1-7) on Vascular Coronary Bed of Rats: Role of Mas, ACE and ACE2." Protein Pept Lett **24**(9): 869-875.

BACKGROUND: Angiotensin (Ang)-(1-7) is a biologically active member of the reninangiotensin system that participates of the regulation of blood pressure. Although Ang-(1-7) is able to potentiate the vasodilator effect of bradykinin in coronary bed of rats, a direct vasodilator effect of Ang-(1-7) in this vascular bed has not been characterized. OBJECTIVES: The aim of this study was to evaluate the mechanisms involved in the vasodilator effect of Ang-(1-7) in the vasculature of isolated rat hearts perfused according to the Langendorff technique at constant flow. METHODS: Isolated hearts, after approximately 30 minutes of stabilization, were perfused with Krebs-Ringer solution (KRS) alone (control) or KRS containing Ang-(1-7). The participation of the Ang-(1-7) receptor Mas, AT1 receptor, angiotensin-converting enzyme (ACE) and ACE2 was evaluated perfusing hearts with a combination of Ang-(1-7) plus A779, Ang-(1-7) plus losartan, Ang-(1-7) plus captopril/enalapril and Ang-(1-7) plus DX-600, respectively. RESULTS: Ang-(1-7) induced a significant decrease in the perfusion pressure, indicating a direct vasodilatation action of this peptide in the coronary bed. This effect was abolished by A779, captopril, enalapril and DX-600 an ACE2-specific inhibitor. However, AT1 blockade did not blunt the Ang-(1-7) effect. No significant changes were observed in heart rate, as well as in contractile tension and +/-dT/dt. Moreover, immunohistochemical analysis showed the presence of Ang-(1-7) and Mas in coronary vessels. CONCLUSION: The Ang-(1-7) concentration used in this study was unable to induce changes in the cardiac function since no consistent alterations in contraction force and HR were viewed after Ang- (1-7) perfusion. In summary, this study showed that Ang-(1-7) induces vasodilation in the

coronary bed of rats and this effect involves coupling to Mas receptor and interaction with ACE and ACE2.

de Paula Gonzaga, A., et al. (2020). "ACE2/Angiotensin-(1-7)/Mas receptor axis in human cancer: Potential role for pediatric tumors." Curr Drug Targets.

BACKGROUND: Pediatric tumors remain the highest cause of death in developed countries. Research on novel therapeutic strategies with lesser side effects is of utmost importance. In this scenario, the role of Renin Angiotensin System (RAS) axes, the classical one formed by angiotensin converting enzyme (ACE), Angiotensin II and AT1 receptor and the alternative axis composed by ACE2, Angiotensin-(1-7) and Mas receptor, have been investigated in cancer. OBJECTIVE: This review aimed to summarize the pathophysiological role of RAS in cancer, evidence for anti-tumor effects of ACE2/Angiotensin-(1-7)/Mas receptor axis and future therapeutic perspectives for pediatric cancer. METHODS: Pubmed, Scopus and Scielo were searched in regard to RAS molecules in human cancer and in pediatric patients. The search terms were "RAS", "ACE", "Angiotensin-(1-7)", "ACE2", "Angiotensin II", "AT1 receptor", "Mas receptor", "Pediatric", "Cancer". RESULTS: Experimental studies have shown that Angiotensin-(1-7) inhibits the growth of tumors cells and reduces local inflammation and angiogenesis in several types of cancer. Clinical trials with Angiotensin-(1-7) or TXA127, a pharmaceutical grade formulation of the naturally occurring peptide, have reported promising findings, but not enough to recommend medical use in human cancer. In regard to pediatric cancer, only three articles that marginally investigated RAS components were found and none of them evaluated molecules of the alternative RAS axis. CONCLUSION: Despite the potential applicability of Angiotensin-(1-7) in pediatric tumors, the role of this molecule was never tested. Further clinical trials are necessary, also including pediatric patients, to confirm safety and efficiency and to define therapeutic targets.

Dean, R. G. and L. M. Burrell (2007). "ACE2 and diabetic complications." Curr Pharm Des **13**(26): 2730-2735.

Angiotensin converting enzyme (ACE) is a key enzyme in the renin angiotensin system (RAS) and converts angiotensin (Ang) I to the vasoconstrictor Ang II, which is thought to be responsible for most of the physiological and pathophysiological effects of the RAS. This classical view of the RAS was challenged with the discovery of the enzyme, ACE2 which both degrades Ang II and leads to formation of the vasodilatory and anti-proliferative peptide, Ang 1-7. Activation of the RAS is a major contributor to diabetic complications, and blockade of the vasoconstrictor and hypertrophic actions of Ang II, slows but does not prevent the progression of such complications. The identification of ACE2 in the heart and kidney adds further complexity to the RAS, provides the rationale to explore the role of this enzyme in pathophysiological states, including the microvascular and macrovascular complications of diabetes. It is believed that ACE2 acts in a counter-regulatory manner to ACE to modulate the balance between vasoconstrictors and vasodilators within the heart and kidney, and may thus play a significant role in the pathophysiology of cardiac and renal disease. Relatively little is known about ACE2 in diabetes, and this review will explore and discuss the data that is currently available. The discovery of ACE2 presents a novel opportunity to develop drugs that specifically influence ACE2 activity and/or expression, and it is possible that such compounds may have considerable clinical value in the prevention and treatment of the complications of diabetes.

Demogines, A., et al. (2012). "Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats." J Virol **86**(11): 6350-6353.

In 2002, severe acute respiratory syndrome (SARS)-coronavirus (CoV) appeared as a novel human virus with high similarity to bat coronaviruses. However, while SARS-CoV uses the human angiotensin-converting enzyme 2 (ACE2) receptor for cellular entry, no coronavirus isolated from bats appears to use ACE2. Here we show that signatures of recurrent positive selection in the bat ACE2 gene map almost perfectly to known SARS-CoV interaction surfaces. Our data indicate that ACE2 utilization preceded the emergence of SARS-CoV-like viruses from bats.

Der Sarkissian, S., et al. (2006). "ACE2: A novel therapeutic target for cardiovascular diseases." Prog Biophys Mol Biol **91**(1-2): 163-198.

Hypertension afflicts over 65 million Americans and poses an increased risk for cardiovascular morbidity such as stroke, myocardial infarction and end-stage renal disease resulting in significant mortality. Overactivity of the renin-angiotensin system (RAS) has been identified as an important determinant that is implicated in the etiology of these diseases and therefore represents a major target for therapy. In spite of the successes of drugs inhibiting various elements of the RAS, the incidence of hypertension and cardiovascular diseases remain steadily on the rise. This has lead many investigators to seek novel and innovative approaches, taking advantage of new pathways and technologies, for the control and

possibly the cure of hypertension and related pathologies. The main objective of this review is to forward the concept that gene therapy and the genetic targeting of the RAS is the future avenue for the successful control and treatment of hypertension and cardiovascular diseases. We will present argument that genetic targeting of angiotensin-converting enzyme 2 (ACE2), a newly discovered member of the RAS, is ideally poised for this purpose. This will be accomplished by discussion of the following: (i) summary of our current understanding of the RAS with a focus on the systemic versus tissue counterparts as they relate to hypertension and other cardiovascular pathologies; (ii) the newly discovered ACE2 enzyme with its physiological and pathophysiological implications; (iii) summary of the current antihypertensive pharmacotherapy and its limitations; (iv) the discovery and design of ACE inhibitors; (v) the emerging concepts for ACE2 drug design; (vi) the current status of genetic targeting of the RAS; (vii) the potential of ACE2 as a therapeutic target for hypertension and cardiovascular disease treatment; and (viii) future perspectives for the treatment of cardiovascular diseases.

Dhangadamajhi, G., et al. (2010). "Gene polymorphisms in angiotensin I converting enzyme (ACE I/D) and angiotensin II converting enzyme (ACE2 C-->T) protect against cerebral malaria in Indian adults." Infect Genet Evol **10**(2): 337-341.

To explore the hypothesis that angiotensin II may play a role in the susceptibility to cerebral malaria (CM), we performed a genetic association study of malaria patients in Orissa, India analyzing three SNPs (ACE2 C-->T, iNOS C-->T, eNOS Glu-->Asp) and two I/D polymorphisms (ACE I/D and IL-4 B1/B2). Our results showed that the 'D' allele of ACE I/D polymorphism, responsible for increased Ang II production had a significant association with mild malaria and the ACE2 C-->T substitution had gender specific effect of possibly reduced expression of ACE2 in presence of 'T' allele in women leading to increased level of Ang II and hence protection against CM. Combined genotype analysis of eNOS Glu-->Asp substitution responsible for increased NO production in Plasmodium falciparum infected individuals and ACE I/D polymorphism also showed stronger association of (Glu-Asp+Asp-Asp/ID+DD) genotypes with mild malaria ($P<0.0001$). Whether by its antiplasmodial activity and/or by some unknown mechanisms, Ang II protects from susceptibility to cerebral malaria remains to be investigated. These genetic findings may contribute to the understanding of malaria pathogenesis.

Dhawale, V. S., et al. (2016). "Activation of angiotensin-converting enzyme 2 (ACE2) attenuates allergic airway inflammation in rat asthma model." Toxicol Appl Pharmacol **306**: 17-26.

Angiotensin-I converting enzyme (ACE) is positively correlated to asthma, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS) and is highly expressed in lungs. ACE2, the counteracting enzyme of ACE, was proven to be protective in pulmonary, cardiovascular diseases. In the present study we checked the effect of ACE2 activation in animal model of asthma. Asthma was induced in male wistar rats by sensitization and challenge with ovalbumin and then treated with ACE2 activator, diminazene aceturate (DIZE) for 2weeks. 48h after last allergen challenge, animals were anesthetized, blood, BALF, femoral bone marrow lavage were collected for leucocyte count; trachea for measuring airway responsiveness to carbachol; lungs and heart were isolated for histological studies and western blotting. In our animal model, the characteristic features of asthma such as altered airway responsiveness to carbachol, eosinophilia and neutrophilia were observed. Western blotting revealed the increased pulmonary expression of ACE1, IL-1beta, IL-4, NF-kappaB, BCL2, p-AKT, p-p38 and decreased expression of ACE2 and IkappaB. DIZE treatment prevented these alterations. Intraalveolar interstitial thickening, inflammatory cell infiltration, interstitial fibrosis, oxidative stress and right ventricular hypertrophy in asthma control animals were also reversed by DIZE treatment. Activation of ACE2 by DIZE conferred protection against asthma as evident from biochemical, functional, histological and molecular parameters. To the best of our knowledge, we report for the first time that activation of ACE2 by DIZE prevents asthma progression by altering AKT, p38, NF-kappaB and other inflammatory markers.

Diez-Freire, C., et al. (2006). "ACE2 gene transfer attenuates hypertension-linked pathophysiological changes in the SHR." Physiol Genomics **27**(1): 12-19.

Recently discovered, angiotensin-converting enzyme-2 (ACE2) is an important therapeutic target in the control of cardiovascular diseases as a result of its proposed central role in the control of angiotensin peptides. Thus our objective in the present study was to determine whether ACE2 gene transfer could decrease high blood pressure (BP) and would improve cardiac dysfunctions induced by hypertension in the spontaneously hypertensive rat (SHR) model. Five-day-old SHR and normotensive WKY rats received a single intracardiac bolus injection of lentiviral vector containing either murine ACE2 (ACE2) or control enhanced green fluorescent protein (EGFP) genes.

Systolic BP, cardiac functions, and perivascular fibrosis were evaluated 4 mo after ACE2 gene transduction. ACE2 gene transfer resulted in a significant attenuation of high BP in the SHR (149 +/- 2 mmHg in lenti-ACE2 vs. 180 +/- 9 mmHg in lenti-EGFP, P < 0.01). In contrast, no significant effect of lenti-ACE2 on BP of WKY rats was observed. Lenti-ACE2-treated SHR showed an 18% reduction in left ventricular wall thickness (1.52 +/- 0.04 vs. 1.86 +/- 0.04 mm in lenti-EGFP, P < 0.01). In addition, there was a 12% increase in left ventricular end diastolic and a 21% increase in end systolic diameters in lenti-ACE2-treated SHR. Finally, lenti-ACE2 treatment resulted in a significant attenuation of perivascular fibrosis in the SHR. In contrast, ACE2 gene transfer did not influence any of these parameters in WKY rats. These observations demonstrate that ACE2 overexpression exerts protective effects on high BP and cardiac pathophysiology induced by hypertension in the SHR.

Dilauro, M., et al. (2010). "Effect of ACE2 and angiotensin-(1-7) in a mouse model of early chronic kidney disease." Am J Physiol Renal Physiol **298**(6): F1523-1532.

Angiotensin-converting enzyme 2 (ACE2) is expressed at high levels in the kidney and converts angiotensin II (ANG II) to ANG-(1-7). We studied the effects of ACE2 inhibition and ANG-(1-7) in the (5/6) nephrectomy ((5/6) Nx) mouse model of chronic kidney disease (CKD). Male FVB mice underwent sham surgery (Sham) or (5/6) Nx and were administered either vehicle, the ACE2 inhibitor MLN-4760 (MLN), the AT (1) receptor antagonist losartan, MLN plus losartan, or ANG-(1-7) for 4 wk. In (5/6) Nx mice with or without MLN, kidney cortical ACE2 protein expression was significantly decreased at 4 wk, compared with Sham. Inhibition of ACE2 caused a decrease in renal cortical ACE2 activity. Kidney cortical ACE expression and activity did not differ between groups of mice. In (5/6) Nx mice treated with MLN, kidney levels of ANG II were significantly increased, compared with Sham. (5/6) Nx induced a mild but insignificant increase in blood pressure (BP), a 50% reduction in FITC-inulin clearance, and a significant increase in urinary albumin excretion. ACE2 inhibition in (5/6) Nx mice did not affect BP or FITC-inulin clearance but significantly increased albuminuria compared with (5/6) Nx alone, an effect reversed by losartan. Treatment of (5/6) Nx mice with ANG-(1-7) increased kidney and plasma levels of ANG-(1-7) but did not alter BP, FITC-inulin clearance, or urinary albumin excretion, and it increased relative mesangial area. These data indicate that kidney ACE2 is downregulated in the early period after (5/6) Nx. Inhibition of ACE2 in (5/6) Nx mice increases albuminuria via an AT (1) receptor-dependent mechanism, independent of BP. In contrast, ANG-(1-7) does not affect albuminuria after (5/6) Nx. We propose that endogenous ACE2 is renoprotective in CKD.

Dimitrov, D. S. (2003). "The secret life of ACE2 as a receptor for the SARS virus." Cell **115**(6): 652-653.

The membrane-associated carboxypeptidase angiotensin-converting enzyme 2 (ACE2) is an essential regulator of heart function. Now, Li at al. identify and characterize an unexpected second function of ACE2 as a partner of the SARS-CoV spike glycoprotein in mediating virus entry and cell fusion.

Diniz, G. P., et al. (2016). "Cardiac ACE2/angiotensin 1-7/Mas receptor axis is activated in thyroid hormone-induced cardiac hypertrophy." Ther Adv Cardiovasc Dis **10**(4): 192-202.

OBJECTIVES: Thyroid hormone (TH) promotes marked effects on the cardiovascular system, including the development of cardiac hypertrophy. Some studies have demonstrated that the renin-angiotensin system (RAS) is a key mediator of the cardiac growth in response to elevated TH levels. Although some of the main RAS components are changed in cardiac tissue on hyperthyroid state, the potential modulation of the counter regulatory components of the RAS, such as angiotensin-converting enzyme type 2 (ACE2), angiotensin 1-7 (Ang 1-7) levels and Mas receptor induced by hyperthyroidism is unknown. The aim of this study was to investigate the effect of hyperthyroidism on cardiac Ang 1-7, ACE2 and Mas receptor levels. METHODS: Hyperthyroidism was induced in Wistar rats by daily intraperitoneal injections of T4 for 14 days. RESULTS: Although plasma Ang 1-7 levels were unchanged by hyperthyroidism, cardiac Ang 1-7 levels were increased in TH-induced cardiac hypertrophy. ACE2 enzymatic activity was significantly increased in hearts from hyperthyroid animals, which may be contributing to the higher Ang 1-7 levels observed in the T4 group. Furthermore, elevated cardiac levels of Ang 1-7 levels were accompanied by increased Mas receptor protein levels. CONCLUSION: The counter-regulatory components of the RAS are activated in hyperthyroidism and may be contributing to modulate the cardiac hypertrophy in response to TH.

Dohrmann, P. R., et al. (1992). "Parallel pathways of gene regulation: homologous regulators SWI5 and ACE2 differentially control transcription of HO and chitinase." Genes Dev **6**(1): 93-104.

Two independent pathways of transcriptional regulation that show functional homology have been identified in yeast. It has been demonstrated

previously that SWI5 encodes a zinc finger DNA-binding protein whose transcription and cellular localization both are cell cycle regulated. We show that ACE2, whose zinc finger region is nearly identical to that of SWI5, shows patterns of cell cycle-regulated transcription and nuclear localization similar to those seen previously for SWI5. Despite their similarities, SWI5 and ACE2 function in separate pathways of transcriptional regulation. SWI5 is a transcriptional activator of the HO endonuclease gene, whereas ACE2 is not. In contrast, ACE2 is a transcriptional activator of the CTS1 gene (which encodes chitinase), whereas SWI5 is not. An additional parallel between the SWI5/HO pathway and the ACE2/CTS1 pathway is that HO and CTS1 both are cell cycle regulated in the same way, and HO and CTS1 both require the SWI4 and SWI6 transcriptional activators. Overproduction of either SWI5 or ACE2 permits transcriptional activation of the target gene from the other pathway, suggesting that the DNA-binding proteins are capable of binding in vivo to promoters that they do not usually activate. Chimeric SWI5/ACE2 protein fusion experiments suggest that promoter specificity resides in a domain distinct from the zinc finger domain.

Dong, B., et al. (2008). "Overexpression of ACE2 enhances plaque stability in a rabbit model of atherosclerosis." Arterioscler Thromb Vasc Biol **28**(7): 1270-1276.

OBJECTIVE: The purpose of this study was to test the hypothesis that ACE2 overexpression may enhance atherosclerotic plaque stability by antagonizing ACE activity and converting angiotensin II to angiotensin 1-7. METHODS AND RESULTS: Atherosclerotic plaques were induced in the abdominal aorta of 114 rabbits by endothelial injury and atherogenic diet. Gene therapy was performed in group A at week 4 and in group B at week 12, respectively. Each group of rabbits were randomly divided into 3 subgroups which received, respectively, a recombinant ACE2 expressing vector (AdACE2), a control vector AdEGFP and AdACE2+A779, an antagonist of angiotensin 1-7 receptor. Local ACE2 overexpression attenuated the progression of lesions from week 4 to week 8, but not progression of plaque size from week 12 to week 16. In group B rabbits, local ACE2 overexpression resulted in stable plaque compositions, ie, fewer macrophages, less lipid deposition and more collagen contents, higher plaque stability scores, decreased angiotensin II levels, and increased angiotensin 1-7 levels in plaque tissues in the AdACE2 subgroup compared with those in the AdEGFP subgroup. CONCLUSIONS: Overexpression of ACE2 results in stabilized atherosclerotic plaques and the mechanism is probably the conversion of vasoconstrictive angiotensin II to vessel protective angiotensin 1-7.

Donoghue, M., et al. (2000). "A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9." Circ Res **87**(5): E1-9.

ACE2, the first known human homologue of angiotensin-converting enzyme (ACE), was identified from 5' sequencing of a human heart failure ventricle cDNA library. ACE2 has an apparent signal peptide, a single metalloprotease active site, and a transmembrane domain. The metalloprotease catalytic domains of ACE2 and ACE are 42% identical, and comparison of the genomic structures indicates that the two genes arose through duplication. In contrast to the more ubiquitous ACE, ACE2 transcripts are found only in heart, kidney, and testis of 23 human tissues examined. Immunohistochemistry shows ACE2 protein predominantly in the endothelium of coronary and intrarenal vessels and in renal tubular epithelium. Active ACE2 enzyme is secreted from transfected cells by cleavage N-terminal to the transmembrane domain. Recombinant ACE2 hydrolyzes the carboxy terminal leucine from angiotensin I to generate angiotensin 1-9, which is converted to smaller angiotensin peptides by ACE in vitro and by cardiomyocytes in culture. ACE2 can also cleave des-Arg bradykinin and neurotensin but not bradykinin or 15 other vasoactive and hormonal peptides tested. ACE2 is not inhibited by lisinopril or captopril. The organ- and cell-specific expression of ACE2 and its unique cleavage of key vasoactive peptides suggest an essential role for ACE2 in the local renin-angiotensin system of the heart and kidney. The full text of this article is available at http://www. circresaha.org.

Donoghue, M., et al. (2003). "Heart block, ventricular tachycardia, and sudden death in ACE2 transgenic mice with downregulated connexins." J Mol Cell Cardiol **35**(9): 1043-1053.

Angiotensin converting enzyme related carboxypeptidase (ACE2) is a recently discovered homolog of angiotensin converting enzyme with tissue-restricted expression, including heart, and the capacity to cleave angiotensin peptides. We tested the hypothesis that cardiac ACE2 activity contributes to features of ventricular remodeling associated with the renin-angiotensin system by generating transgenic mice with increased cardiac ACE2 expression. These animals had a high incidence of sudden death that correlated with transgene expression levels. Detailed electrophysiology revealed severe, progressive conduction and rhythm disturbances with sustained ventricular tachycardia and terminal ventricular fibrillation. The gap junction proteins connexin40 and

connexin43 were downregulated in the transgenic hearts, indicating that ACE2-mediated gap junction remodeling may account for the observed electrophysiologic disturbances. Spontaneous downregulation of the ACE2 transgene in surviving older animals correlated with restoration of nearly normal conduction, rhythm, and connexin expression.

Doobay, M. F., et al. (2007). "Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system." Am J Physiol Regul Integr Comp Physiol **292**(1): R373-381.

Angiotensin-converting enzyme 2 (ACE2) is a newly discovered carboxy-peptidase responsible for the formation of vasodilatory peptides such as angiotensin-(1-7). We hypothesized that ACE2 is part of the brain renin-angiotensin system, and its expression is regulated by the other elements of this system. ACE2 immunostaining was performed in transgenic mouse brain sections from neuron-specific enolase-AT (1A) (overexpressing AT (1A) receptors), R (+)A (+) (overexpressing angiotensinogen and renin), and control (nontransgenic littermates) mice. Results show that ACE2 staining is widely distributed throughout the brain. Using cell-type-specific antibodies, we observed that ACE2 staining is present in the cytoplasm of neuronal cell bodies but not in glial cells. In the subfornical organ, an area lacking the blood-brain barrier and sensitive to blood-borne angiotensin II, ACE2 was significantly increased in transgenic mice. Interestingly, ACE2 mRNA and protein expression were inversely correlated in the nucleus of tractus solitarius/dorsal motor nucleus of the vagus and the ventrolateral medulla, when comparing transgenic to nontransgenic mice. These results suggest that ACE2 is localized to the cytoplasm of neuronal cells in the brain and that ACE2 levels appear highly regulated by other components of the renin-angiotensin system, confirming its involvement in this system. Moreover, ACE2 expression in brain structures involved in the control of cardiovascular function suggests that the carboxypeptidase may have a role in the central regulation of blood pressure and diseases involving the autonomic nervous system, such as hypertension.

Douglas, G. C., et al. (2004). "The novel angiotensin-converting enzyme (ACE) homolog, ACE2, is selectively expressed by adult Leydig cells of the testis." Endocrinology **145**(10): 4703-4711.

The metallopeptidase angiotensin-converting enzyme (ACE) plays a pivotal role in the cardiovascular system by generating the vasoconstrictor peptide angiotensin II. A homolog of ACE with different substrate specificity, ACE2, has recently been cloned that shows an expression pattern restricted to endothelial cells of the heart and kidney, epithelial cells of the distal tubule of the kidney, and the testis. Although the importance of ACE2 to cardiac function is already evident, its role in the testis remains unknown. In this study, we report the cloning and expression of human testicular ACE2 and confirm that it is identical to the somatic form of the enzyme. ACE2 catalytic activity was present in membrane preparations of whole testes and Leydig cells from adult rats; expression of the protein in Leydig cells was confirmed by Western immunoblot analysis. Using immunohistochemistry, ACE2 expression was confined to the Leydig cells in the rat testis and to Leydig and Sertoli cells in the human testis. Ablation of the Leydig cells in the rat by the specific toxin, ethane dimethane sulfonate, eliminated ACE2-positive cells from the interstitium. Expression of ACE2 in rat Leydig cells was up-regulated during the development of adult-type Leydig cells at puberty and after ethane dimethane sulfonate treatment. Expression of ACE2 activity in the testis was not significantly altered by manipulation of the pituitary-testicular hormonal axis with sc testosterone implants. These data suggest that ACE2 is a constitutive product of adult-type Leydig cells and may participate in the control of testicular function by as yet unknown mechanisms.

Duan, Y., et al. (2019). "Bone Marrow-Derived Cells Restore Functional Integrity of the Gut Epithelial and Vascular Barriers in a Model of Diabetes and ACE2 Deficiency." Circ Res **125**(11): 969-988.

RATIONALE: There is incomplete knowledge of the impact of bone marrow cells on the gut microbiome and gut barrier function. OBJECTIVE: We postulated that diabetes mellitus and systemic ACE2 (angiotensin-converting enzyme 2) deficiency would synergize to adversely impact both the microbiome and gut barrier function. METHODS AND RESULTS: Bacterial 16S rRNA sequencing and metatranscriptomic analysis were performed on fecal samples from wild-type, ACE2(-/y), Akita (type 1 diabetes mellitus), and ACE2(-/y)-Akita mice. Gut barrier integrity was assessed by immunofluorescence, and bone marrow cell extravasation into the small intestine was evaluated by flow cytometry. In the ACE2(-/y)-Akita or Akita mice, the disrupted barrier was associated with reduced levels of myeloid angiogenic cells, but no increase in inflammatory monocytes was observed within the gut parenchyma. Genomic and metatranscriptomic analysis of the microbiome of ACE2(-/y)-Akita mice demonstrated a marked increase in peptidoglycan-producing bacteria. When compared with control cohorts treated with saline, intraperitoneal administration of myeloid angiogenic cells significantly decreased the

microbiome gene expression associated with peptidoglycan biosynthesis and restored epithelial and endothelial gut barrier integrity. Also indicative of diabetic gut barrier dysfunction, increased levels of peptidoglycan and FABP-2 (intestinal fatty acid-binding protein 2) were observed in plasma of human subjects with type 1 diabetes mellitus (n=21) and type 2 diabetes mellitus (n=23) compared with nondiabetic controls (n=23). Using human retinal endothelial cells, we determined that peptidoglycan activates a noncanonical TLR-2 (Toll-like receptor 2) associated MyD88 (myeloid differentiation primary response protein 88)-ARNO (ADP-ribosylation factor nucleotide-binding site opener)-ARF6 (ADP-ribosylation factor 6) signaling cascade, resulting in destabilization of p120-catenin and internalization of VE-cadherin as a mechanism of deleterious impact of peptidoglycan on the endothelium. CONCLUSIONS: We demonstrate for the first time that the defect in gut barrier function and dysbiosis in ACE2(-/y)-Akita mice can be favorably impacted by exogenous administration of myeloid angiogenic cells.

Ejzykowicz, D. E., et al. (2009). "The Aspergillus fumigatus transcription factor Ace2 governs pigment production, conidiation and virulence." Mol Microbiol **72**(1): 155-169.

Aspergillus fumigatus causes serious and frequently fatal infections in immunocompromised patients. To investigate the regulation of virulence of this fungus, we constructed and analysed an A. fumigatus mutant that lacked the transcription factor Ace2, which influences virulence in other fungi. The Deltaace2 mutant had dysmorphic conidiophores, reduced conidia production and abnormal conidial cell wall architecture. This mutant produced an orange pigment when grown on solid media, although its conidia had normal pigmentation. Conidia of the Deltaace2 mutant were larger and had accelerated germination. The resulting germlings were resistant to hydrogen peroxide, but not other stressors. Non-neutropenic mice that were immunosuppressed with cortisone acetate and infected with the Deltaace2 mutant had accelerated mortality, greater pulmonary fungal burden, and increased pulmonary inflammatory responses compared with mice infected with the wild-type or Deltaace2::ace2-complemented strains. The Deltaace2 mutant had reduced ppoC, ecm33 and ags3 mRNA expression. It is known that A. fumigatus mutants with absent or reduced expression of these genes have increased virulence in mice, as well as other phenotypic similarities to the Deltaace2 mutant. Therefore, reduced expression of these genes likely contributes to the increased virulence of the Deltaace2 mutant.

Ender, S. A., et al. (2014). "Expression and function of the ACE2/angiotensin (1-7)/Mas axis in osteosarcoma cell lines U-2 OS and MNNG-HOS." Mol Med Rep **10**(2): 804-810.

The renin-angiotensin-system (RAS), via its classical angiotensin-converting enzyme (ACE)/angiotensin II/angiotensin II type 1 receptor (AT1R)-axis, is associated with proliferation and metastasis of numerous types of solid tumor. AT1R blockers reduce tumor volume and decrease liver and lung metastasis in murine models of osteosarcoma. Expression and function of the alternative ACE2/Ang (1-7)/Mas axis in osteosarcoma is yet to be studied. In the present study, the basic and interleukin (IL)-1beta-stimulated expression of components of this alternative RAS axis were analyzed and the impact of Mas on proliferation and/or migration of U-2 OS and MNNG-HOS osteosarcoma cells was studied. Quantitative polymerase chain reaction revealed that the two cell lines expressed the Ang (17)-generating peptidases ACE2, neutral endopeptidase 24.11 and prolyl-endopeptidase together with the putative receptor for Ang (1-7), Mas. IL-1beta provoked an induction of Mas mRNA and protein expression which was associated with a reduction of proliferation and migration. By contrast, small interfering RNA-mediated knockdown of Mas expression led to increased cell proliferation. In conclusion, osteosarcoma cells express a functional active alternative ACE2/Ang (1-7)/Mas axis. The induction and reinforcement of this axis may be beneficial for the treatment of osteosarcoma by reducing growth and preventing cancer metastasis. These effects may be achieved directly by the administration of Mas agonists or, indirectly, via blocking the classical AngII RAS axis via ACE inhibitors or AT1R antagonists.

Evans, C. E., et al. (2020). "ACE2 activation protects against cognitive decline and reduces amyloid pathology in the Tg2576 mouse model of Alzheimer's disease." Acta Neuropathol **139**(3): 485-502.

Mid-life hypertension and cerebrovascular dysfunction are associated with increased risk of later life dementia, including Alzheimer's disease (AD). The classical renin-angiotensin system (cRAS), a physiological regulator of blood pressure, functions independently within the brain and is overactive in AD. cRAS-targeting anti-hypertensive drugs are associated with reduced incidence of AD, delayed onset of cognitive decline, and reduced levels of Abeta and tau in both animal models and human pathological studies. cRAS activity is moderated by a downstream regulatory RAS pathway (rRAS), which is underactive in AD and is strongly associated with pathological hallmarks in human AD, and cognitive decline in animal models of CNS disease. We now show that

enhancement of brain ACE2 activity, a major effector of rRAS, by intraperitoneal administration of diminazene aceturate (DIZE), an established activator of ACE2, lowered hippocampal Abeta and restored cognition in mid-aged (13-14-month-old) symptomatic Tg2576 mice. We confirmed that the protective effects of DIZE were directly mediated through ACE2 and were associated with reduced hippocampal soluble Abeta42 and IL1-beta levels. DIZE restored hippocampal MasR levels in conjunction with increased NMDA NR2B and downstream ERK signalling expression in hippocampal synaptosomes from Tg2576 mice. Chronic (10 weeks) administration of DIZE to pre-symptomatic 9-10-month-old Tg2576 mice, and acute (10 days) treatment in cognitively impaired 12-13-month-old mice, prevented the development of cognitive impairment. Together these data demonstrate that ACE2 enhancement protects against and reverses amyloid-related hippocampal pathology and cognitive impairment in a preclinical model of AD.

Fan, J., et al. (2015). "Atrial overexpression of angiotensin-converting enzyme 2 improves the canine rapid atrial pacing-induced structural and electrical remodeling. Fan, ACE2 improves atrial substrate remodeling." Basic Res Cardiol **110**(4): 45.

The purpose of this study was to investigate whether atrial overexpression of angiotensin-converting enzyme 2 (ACE2) by homogeneous transmural atrial gene transfer can reverse atrial remodeling and its mechanisms in a canine atrial-pacing model. Twenty-eight mongrel dogs were randomly divided into four groups: Sham-operated, AF-control, gene therapy with adenovirus-enhanced green fluorescent protein (Ad-EGFP) and gene therapy with Ad-ACE2 (Ad-ACE2) (n = 7 per subgroup). AF was induced in all dogs except the Sham-operated group by rapid atrial pacing at 450 beats/min for 2 weeks. Ad-EGFP and Ad-ACE2 group then received epicardial gene painting. Three weeks after gene transfer, all animals except the Sham group underwent rapid atrial pacing for another 3 weeks and then invasive electrophysiological, histological and molecular studies. The Ad-ACE2 group showed an increased ACE2 and Angiotensin-(1-7) expression, and decreased Angiotensin II expression in comparison with Ad-EGFP and AF-control group. ACE2 overexpression attenuated rapid atrial pacing-induced increase in activated extracellular signal-regulated kinases and mitogen-activated protein kinases (MAPKs) levels, and decrease in MAPK phosphatase 1(MKP-1) level, resulting in attenuation of atrial fibrosis collagen protein markers and transforming growth factor-beta1. Additionally, ACE2 overexpression also modulated the tachypacing-induced up-regulation of connexin 40, down-regulation of connexin 43 and Kv4.2, and significantly decreased the inducibility and duration of AF. ACE2 overexpression could shift the renin-angiotensin system balance towards the protective axis, attenuate cardiac fibrosis remodeling associated with up-regulation of MKP-1 and reduction of MAPKs activities, modulate tachypacing-induced ion channels and connexin remodeling, and subsequently reduce the inducibility and duration of AF.

Fan, R., et al. (2017). "Preliminary analysis of the association between methylation of the ACE2 promoter and essential hypertension." Mol Med Rep **15**(6): 3905-3911.

The aim of the present study was to investigate whether methylation of the angiotensin I converting enzyme 2 (ACE2) promoter increases the risk of essential hypertension (EH). A total of 96 patients with EH were recruited and 96 sex and agematched healthy controls. Methylation of 5 CpG dinucleotides in the ACE2 promoter was quantified using bisulfite pyrosequencing. Logistic regression and multiple linear regression were used to adjust for confounding factors and the generalized multifactor dimensionality reduction (GMDR) method was applied to investigate highorder interactions. Methylation of CpG4 (adjusted P=0.020) and CpG5 (adjusted P=0.036) was significantly higher in patients with EH, with frequency 97.56+/-5.65% and 12.75+/-4.15% in EH individuals and 95.73+/-9.11% and 11.47+/-3.67% in healthy controls. GMDR detected significant interaction among the 5 CpG sites (odds ratio=7.33, adjusted P=0.01). Furthermore, receiver operating characteristic curves identified that CpG5 methylation was a significant predictor of EH. Notably, CpG2 methylation was significantly higher in males than in females (adjusted P=0.018). Conversely, CpG5 methylation was significantly lower in males (adjusted P=0.032). These results indicated that aberrant methylation of the ACE2 promoter may be associated with EH risk. In addition, sex may significantly influence ACE2 methylation.

Fan, X., et al. (2007). "Polymorphisms of ACE2 gene are associated with essential hypertension and antihypertensive effects of Captopril in women." Clin Pharmacol Ther **82**(2): 187-196.

ACE2 appears to counterbalance the vasopressor effect of angiotensin I converting enzyme (ACE) in the reninangiotensin system. We hypothesized that ACE2 polymorphisms could confer a high risk of hypertension and have an impact on the antihypertensive response to ACE inhibitors. The hypothesis was tested in two casecontrol studies and a clinical trial of 3,408 untreated hypertensive patients

randomized to Atenolol, Hydrochlorothiazide, Captopril, or Nifedipine treatments for 4 weeks. ACE2 rs2106809 T allele was found to confer a 1.6-fold risk for hypertension in women (95% confidence interval (CI), 1.132.06), whereas when combined with the effect of the ACE DD genotype, the risk was 2.34-fold (95% CI, 1.754.85) in two independent samples. The adjusted diastolic blood pressure response to Captopril was 3.3 mm Hg lower in ACE2 T allele carriers than in CC genotype carriers (P=0.019) in women. We conclude that the ACE2 T allele confers a high risk for hypertension and reduced antihypertensive response to ACE inhibitors.

Fan, X. H., et al. (2009). "Polymorphisms of angiotensin-converting enzyme (ACE) and ACE2 are not associated with orthostatic blood pressure dysregulation in hypertensive patients." Acta Pharmacol Sin **30**(9): 1237-1244.

AIM: The genetic background of orthostatic blood pressure dysregulation remains poorly understood. Since the renin-angiotensin system plays an important role in blood pressure regulation and response to position change, we hypothesized that angiotensin-converting enzyme (ACE) and ACE2 genetic polymorphisms might contribute, at least partially, to orthostatic blood pressure dysregulation in hypertensive patients. METHODS: Two tag single nucleotide polymorphisms (SNPs) of ACE2 and ACE I/D were genotyped in 3630 untreated hypertensive patients and 826 normotensive subjects. Orthostatic hypertension was defined as an increase in systolic blood pressure of 20 mmHg or more and orthostatic hypotension as a drop in blood pressure of 20/10 mmHg or more within three minutes of assumption of upright posture. RESULTS: Female and male patients had similar rates of orthostatic hypertension (16.5% vs 15.3%) and hypotension (22.5% vs 23.8%). No significant differences were detected in the minor allele frequency of ACE2 rs2106809, rs2285666, or ACE I/D in either female or male patients with orthostatic hypertension (15.1%, 22.7%, 19.6%, respectively), hypotension (13.8%, 25%, 16.5%), or normal orthostatic blood pressure response (14.4%, 21.9%, 15.8%) in additive, dominant or recessive models after adjustment for confounders (all P>0.05). The orthostatic changes in systolic and diastolic blood pressure were also comparable among patients carrying different genotypes. Similar results were observed in normotensive subjects. CONCLUSION: These data provide no support for the involvement of ACE or ACE2 in the genetic predisposition to orthostatic hypotension or hypertension.

Fan, Z., et al. (2019). "Hypertension and hypertensive left ventricular hypertrophy are associated with ACE2 genetic polymorphism." Life Sci **225**: 39-45.

AIMS: Renin-angiotensin system modulates cardiac structure independent of blood pressure. The present study aimed at investigating whether single nucleotide polymorphism (SNP) and haplotype of angiotensin converting enzyme 2 (ACE2) could influence blood pressure and the susceptibility to hypertensive left ventricular hypertrophy (LVH). SUBJECTS AND METHODS: A total of 647 patients (347 females and 300 males) with newly diagnosed mild to moderate essential hypertension were enrolled in a blood pressure matched, case-control study. Four ACE2 tagSNPs (rs2074192, rs4646176, rs4646155 and rs2106809) were genotyped and major haplotypes consisting of these four SNPs were reconstructed for all subjects. KEY FINDINGS: In females, minor alleles of ACE2 rs2074192 and rs2106809 respectively conferred a 2.1 and 2.0 fold risk for LVH. ACE2 haplotype TCGT increased the risk for LVH while another haplotype CCGC decreased the risk in females. The covariates-adjusted mean left ventricular mass index was 11% greater in TCGT haplotype carriers than in noncarriers in women. In females, the covariates-adjusted mean systolic blood pressure was 3.4mmHg lower in CCGC haplotype carriers than in noncarriers. In males, the covariates-adjusted mean systolic blood pressure was 2.4mmHg lower in CCGC haplotype carriers than in noncarriers. SIGNIFICANCE: ACE2 tagSNPs rs2074192 and rs2106809 as well as major haplotypes CCGC and TCGT may serve as novel risk markers for LVH in hypertensive patients.

Fang, F., et al. (2013). "Loss of ACE2 exacerbates murine renal ischemia-reperfusion injury." PLoS One **8**(8): e71433.

Ischemia-reperfusion (I/R) is a model of acute kidney injury (AKI) that is characterized by vasoconstriction, oxidative stress, apoptosis and inflammation. Previous studies have shown that activation of the renin-angiotensin system (RAS) may contribute to these processes. Angiotensin converting enzyme 2 (ACE2) metabolizes angiotensin II (Ang II) to angiotensin-(1-7), and recent studies support a beneficial role for ACE2 in models of chronic kidney disease. However, the role of ACE2 in models of AKI has not been fully elucidated. In order to test the hypothesis that ACE2 plays a protective role in AKI we assessed I/R injury in wild-type (WT) mice and ACE2 knock-out (ACE2 KO) mice. ACE2 KO and WT mice exhibited similar histologic injury scores and measures of kidney function at 48 hours after reperfusion. Loss of ACE2 was associated with increased neutrophil, macrophage, and T cell infiltration in the kidney. mRNA levels for pro-

inflammatory cytokines, interleukin-1beta, interleukin-6 and tumour necrosis factor-alpha, as well as chemokines macrophage inflammatory protein 2 and monocyte chemoattractant protein-1, were increased in ACE2 KO mice compared to WT mice. Changes in inflammatory cell infiltrates and cytokine expression were also associated with greater apoptosis and oxidative stress in ACE2 KO mice compared to WT mice. These data demonstrate a protective effect of ACE2 in I/R AKI.

Feng, P., et al. (2020). "Electroacupuncture Improved Chronic Cerebral Hypoperfusion-Induced Anxiety-Like Behavior and Memory Impairments in Spontaneously Hypertensive Rats by Downregulating the ACE/Ang II/AT1R Axis and Upregulating the ACE2/Ang-(1-7)/MasR Axis." Neural Plast **2020**: 9076042.

Electroacupuncture (EA) can effectively alleviate anxiety disorders and memory impairments caused by various neurodegenerative diseases; however, the molecular mechanisms underlying its neuroprotective effects are unclear. Previous studies have shown that the renin-angiotensin system (RAS) comprises of two axes with mutual antagonism: the classical angiotensin converting enzyme/angiotensin II/angiotensin II type 1 receptor (ACE/Ang II/AT1R) axis and the protective angiotensin converting enzyme 2/angiotensin-(1-7)/Mas receptor (ACE2/Ang-(1-7)/MasR) axis. In this study, we observed that chronic cerebral hypoperfusion (CCH) mediated anxiety-like behavior and memory impairments in spontaneously hypertensive rats (SHR) via upregulation of the hippocampal classical axis (ACE/Ang II/AT1R) and the partial hippocampal protective axis (ACE2/Ang-(1-7)). However, Ang II levels were much higher than those of Ang-(1-7), indicating that the ACE/Ang II/AT1R axis plays a dominant role in the comorbidity of CCH and hypertension. Moreover, candesartan cilexetil (Canc) and perindopril (Peril) were used as positive control drugs. We found that EA, Canc, and Peril attenuated CCH-induced anxiety-like behavior and memory impairments in SHR, potentially via downregulation of the hippocampal classical axis (ACE/Ang II/AT1R) and upregulation of the whole hippocampal protective axis (ACE2/Ang-(1-7)/MasR). These results suggest that EA therapy for CCH with hypertension may be mediated by two hippocampal RAS axes.

Feng, W., et al. (2017). "Association of AGTR1 and ACE2 gene polymorphisms with structural atrial fibrillation in a Chinese Han population." Pharmazie **72**(1): 17-21.

The renin-angiotensin system (RAS) is thought to play an important role in atrial fibrillation (AF). The RAS contains the ACE/AngII/AGTR1 axis and the ACE2/Ang (1-7)/MAS axis, which restrict each other via mutual antagonism and regulate myocardial hypertrophy, fibrosis and remodelling. The aim of our study was to investigate the association between single nucleotide polymorphisms (SNPs) in angiotensin-II type-1 receptor (AGTR1) and angiotensin-converting enzyme 2 (ACE2) and structural AF in a Chinese Han population. The SNPs (rs1492100, rs1492099, rs1492097, rs3772616) in AGTR1 and the SNP rs6632677 in ACE2 were compared in 300 structural AF patients (67.61+/-12.56 years) and 300 controls (66.08+/-12.47 years). The genotype frequencies of SNP rs1492099 in AGTR1 in the structural AF cohort vs controls were as follows: GG, 72.7 vs 83.0%; AG 26.0 vs 16.3%; AA 1.3 vs 0.7% (P=0.009). The frequency of the minor allele of SNP rs1492099 in AGTR1 was 14.2% in the structural AF group compared with 8.8% in the controls (t=0.004; odds ratio [OR], 1.727; 95% confidence interval [CI]: 1.154-2.487). In addition, the genotype frequencies of SNP rs6632677 in ACE2 in the structural AF male patients vs male controls were as follows: GG, 70.5 vs 83.1%; CG 26.3 vs 15.6%; and CC 3.2 vs 1.3% (P=0.029). The frequency of the minor allele of SNP rs6632677 in ACE2 was 16.3% in structural AF male patients compared with 9.1% in male controls (P=0.008; OR, 1.954; 95%CI: 1.196-3.192). Furthermore, we found an interaction between the SNP rs6632677 in ACE2 and the SNPs (rs1492100/rs1492099/rs3772616) in AGTR1 in structural AF patients by the multifactor dimensionality reduction (MDR) method. The results indicate that polymorphism rs1492099 in the AGTR1 gene is associated with structural AF in a Chinese Han population. It was hypothesized that the ACE2 gene, which maps to the X chromosome, may be correlated with the risk of structural AF in a Chinese Han male population. Furthermore, we found an interaction between ACE2 and AGTR1 in structural AF patients in a Chinese Han population.

Feng, Y., et al. (2011). "Overexpression of ACE2 produces antitumor effects via inhibition of angiogenesis and tumor cell invasion in vivo and in vitro." Oncol Rep **26**(5): 1157-1164.

Angiotensin II (AngII) is a multifunctional bioactive peptide in the renin-angiotensin system (RAS). Angiotensin-converting enzyme 2 (ACE2) is a newly identified component of RAS. The role of AngII and ACE2 in the metastasis of non-small cell lung cancer (NSCLC) and the effects on matrix metalloproteinases (MMPs) are still unknown. In the present study, the anti-invasive effect and mechanism of ACE2 were investigated in vitro and in vivo. Results of a transwell assay showed that the

overexpression of ACE2 reduces the invasive ability of A549 cells in vitro. According to the results of qRT-PCR and western blot analysis, the inhibitory role of ACE2 was mediated through the down-regulation of MMP-2 and MMP-9. Additionally, we confirmed that the overexpression of ACE2 inhibited cell growth and VEGFa production while simultaneously suppressing ACE and angiotensin II type 1 receptor (AT1R) expression in human lung cancer xenografts. These results suggest that the overexpression of ACE2 may potentially suppress the invasion and angiogenesis of NSCLC.

Ferrario, C. M. (2011). "ACE2: more of Ang-(1-7) or less Ang II?" Curr Opin Nephrol Hypertens **20**(1): 1-6.

PURPOSE OF REVIEW: Previous concepts regarding the pathways involved in the generation of angiotensin II (Ang II) have been challenged by studies showing the existence of a peptide acting as an endogenous antagonist of Ang II. The discovery that angiotensin-(1-7) [Ang-(1-7)] opposes the pressor, proliferative, profibrotic, and prothrombotic actions mediated by Ang II has contributed to the realization that the renin-angiotensin system is composed of two opposing arms: the pressor arm constituted by the enzyme angiotensin-converting enzyme (ACE), Ang II as the product, and the Ang II type 1 (AT1) receptor as the main protein mediating the biological actions of Ang II; the second arm is composed of the monocarboxypeptidase angiotensin-converting enzyme 2 (ACE2), Ang-(1-7) produced through hydrolysis of Ang II, and the Mas receptor as the protein conveying the vasodilator, antiproliferative, antifibrotic, and antithrombotic effects of Ang-(1-7). RECENT FINDINGS: Experimental and clinical studies demonstrate a role for the Ang-(1-7)/ACE2/Mas axis in the evolution of hypertension, the regulation of renal function, and the progression of renal disease including diabetic nephropathy. Additional evidence suggests that a reduction in the expression and activity of this vasodepressor component may be a critical factor in mediating the progression of cardiovascular disease. SUMMARY: Further research on the contribution of the Ang-(1-7)/ACE2/Mas axis to cardiovascular pathology will lead to the development of new pharmacological approaches resulting in the design of molecular or genetic means to increase the expression of ACE2, allow for increased tissue levels of Ang-(1-7), or both.

Ferrario, C. M. and J. Varagic (2010). "The ANG-(1-7)/ACE2/mas axis in the regulation of nephron function." Am J Physiol Renal Physiol **298**(6): F1297-1305.

The study of experimental hypertension and the development of drugs with selective inhibitory effects on the enzymes and receptors constituting the components of the circulating and tissue renin-angiotensin systems have led to newer concepts of how this system participates in both physiology and pathology. Over the last decade, a renewed emphasis on understanding the role of angiotensin-(1-7) and angiotensin-converting enzyme 2 in the regulation of blood pressure and renal function has shed new light on the complexity of the mechanisms by which these components of the renin angiotensin system act in the heart and in the kidneys to exert a negative regulatory influence on angiotensin converting enzyme and angiotensin II. The vasodepressor axis composed of angiotensin-(1-7)/angiotensin-converting enzyme 2/mas receptor emerges as a site for therapeutic interventions within the renin-angiotensin system. This review summarizes the evolving knowledge of the counterregulatory arm of the renin-angiotensin system in the control of nephron function and renal disease.

Ferreira, A. J. and M. K. Raizada (2008). "Are we poised to target ACE2 for the next generation of antihypertensives?" J Mol Med (Berl) **86**(6): 685-690.

Antihypertensive drugs based on the blockade of the renin-angiotensin system (RAS) target classical components of this system, i.e., angiotensin-converting enzyme (ACE) and angiotensin (Ang) II type 1 receptor. These antihypertensives are well-recognized and successful, if prescribed properly, in reducing high blood pressure, but much less effective in preventing and reverting end-organ damage induced by cardiovascular disease (CVD) and hypertension. Thus, new strategies and new drug targets that are more effective must be discovered. Recent identification of a counterregulatory axis of the RAS [ACE2, Ang-(1-7), and Mas receptor] that is potentially important in promoting vasoprotective effects offers a novel target for CVD therapeutics. In this brief review, we will highlight the functional characteristics of this axis with special emphasis on ACE2 and its possible involvement in the pathophysiology of the CVD. In addition, we will present our views on the potential of ACE2 as a new target for the development of innovative antihypertensives by highlighting the development and functional findings obtained with small molecules ACE2 activators.

Fraga-Silva, R. A., et al. (2010). "ACE2 activation promotes antithrombotic activity." Mol Med **16**(5-6): 210-215.

The aim of the present study was to test the hypothesis that the activation of the angiotensin-converting enzyme (ACE)2/angiotensin-(1-7)/Mas receptor axis by use of a novel ACE2 activator (XNT)

would protect against thrombosis. Thrombi were induced in the vena cava of spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, and ACE2 and ACE activity in the thrombus was determined. Real-time thrombus formation was viewed through intravital microscopy of vessels in nude mice. Thrombus weight was 40% greater in the SHR (4.99 +/- 0.39 versus 7.04 +/- 0.66 mg). This weight increase was associated with a 20% decrease in ACE2 activity in the thrombus. In contrast, there were no differences between the WKY and SHR in ACE2 protein and ACE activity in the thrombi. ACE2 inhibition (DX600; 0.1 micromol/L/kg) increased thrombus weight by 30% and XNT treatment (10 mg/kg) resulted in a 30% attenuation of thrombus formation in the SHR. Moreover, XNT reduced platelet attachment to injured vessels, reduced thrombus size, and prolonged the time for complete vessel occlusion in mice. Thus, a decrease in thrombus ACE2 activity is associated with increased thrombus formation in SHR. Furthermore, ACE2 activation attenuates thrombus formation and reduces platelet attachment to vessels. These results suggest that ACE2 could be a novel target for the treatment of thrombogenic diseases.

Frantz, E. D. C., et al. (2017). "High, but not low, exercise volume shifts the balance of renin-angiotensin system toward ACE2/Mas receptor axis in skeletal muscle in obese rats." Am J Physiol Endocrinol Metab **313**(4): E473-E482.

Metabolic syndrome is a cluster of metabolic risk factors that is linked to central obesity, elevated blood pressure, insulin resistance (IR), and dyslipidemia, where the renin-angiotensin system (RAS) may provide a link among them. This study aimed to evaluate volume exercise effects comparing low vs. high volume of chronic aerobic exercise on RAS axes in skeletal muscle in a diet-induced obesity (DIO) rat model. For this, male Wistar-Kyoto rats were fed a standard chow (SC) diet or a high-fat (HF) diet for 32 wk. Animals receiving the HF diet were randomly divided into low exercise volume (LEV, 150 min/wk) and high exercise volume (HEV, 300 min/wk) at the 20th week. After 12 wk of aerobic treadmill training, the body mass and composition, blood pressure, glucose and lipid metabolism, RAS axes, insulin signaling, and inflammatory pathway were performed. HEV slowed the body mass gain, reduced intra-abdominal fat pad and leptin levels, improved total and peripheral body composition and inflammatory cytokine, reduced angiotensin II type 1 receptor expression, and increased Mas receptor protein expression compared with the HF animals. Sedentary groups (SC and HF) presented lower time to exhaustion and maximal velocity compared with the LEV and HEV groups. Both exercise training groups showed reduced resting systolic blood pressure and heart rate, improved glucose tolerance, IR, insulin signaling, and lipid profile. We conclude that the HEV, but not LEV, shifted the balance of RAS toward the ACE2/Mas receptor axis in skeletal muscle, presenting protective effects against the DIO model.

Gaddam, R. R., et al. (2014). "ACE and ACE2 in inflammation: a tale of two enzymes." Inflamm Allergy Drug Targets **13**(4): 224-234.

The renin-angiotensin system (RAS) conceived as a coordinated hormonal cascade plays an important role in controlling multiple functions in many organs and is much more complex than previously thought. The RAS has continued to expand, with the identification of new components, functions and subsystems. Angiotensin-converting enzyme (ACE) and its novel homolog angiotensin converting enzyme 2 (ACE2) are two key enzymes involved in the synthesis of bioactive components of the RAS. The main active peptides of the RAS include angiotensin II (Ang II), Ang III, Ang IV, and angiotensin-(1-7) [Ang-(1-7)] among which Ang II and Ang-(1-7) are much more important in health and disease. The axis formed by ACE2 represents an endogenous counter-regulatory pathway within the RAS, and its actions are opposite to those of the ACE axis. Conventionally the RAS has been considered to be important in the cardiovascular system, metabolism, cell growth and homeostasis. In recent years, a key role of ACE and ACE2 and their peptides has been recognized in the inflammatory process in conditions such as cardiac hypertrophy, pulmonary hypertension, glomerulonephritis, lung injury, sepsis, and acute pancreatitis. Investigations are ongoing to better understand the role of the RAS in inflammation. A comprehensive understanding of the RAS components in inflammation can provide new possibilities for therapeutic approaches against inflammatory diseases. In this review, we discuss our current understanding of the subject, based on recent findings, on the role of ACE and ACE2 in inflammation.

Gallagher, P. E., et al. (2008). "MAP kinase/phosphatase pathway mediates the regulation of ACE2 by angiotensin peptides." Am J Physiol Cell Physiol **295**(5): C1169-1174.

Angiotensin-converting enzyme 2 (ACE2) catalyzes the conversion of the vasoconstrictor angiotensin II (ANG II) to the vasodilatory peptide angiotensin-(1-7) [ANG-(1-7)]. We showed that treatment of hypertensive rats with the AT (1) receptor antagonist olmesartan increased ACE2 mRNA and protein in the thoracic aorta, suggesting that endogenous ANG II tonically reduces the enzyme. We

now report that ANG II downregulates ACE2 activity and mRNA in rat aortic vascular smooth muscle cells (VSMCs) to reduce the conversion of ANG II to ANG-(1-7). Although ANG-(1-7) alone had no effect on the regulation of ACE2 mRNA, the heptapeptide prevented the ANG II-mediated reduction in ACE2 mRNA, an effect blocked by the selective ANG-(1-7) receptor antagonist [d-Ala (7)]-ANG-(1-7). The reduction in ACE2 mRNA by ANG II was also prevented by the mitogen-activated protein (MAP) kinase kinase inhibitor PD98059. Treatment of VSMCs with ANG II increased ERK1/ERK2 activity, which was significantly reduced by pretreatment with ANG-(1-7). Blockade of the ANG II-mediated reduction in ACE2 mRNA and increase in MAP kinase activity by ANG-(1-7) was prevented by pretreatment with sodium vanadate, a tyrosine phosphatase inhibitor, or okadaic acid, a serine-threonine phosphatase inhibitor, suggesting that the heptapeptide activates a MAP kinase phosphatase. This study is the first to show that the MAP kinase-phosphatase pathway is a primary molecular mechanism for regulating ACE2 to maintain the balance between ANG II and ANG-(1-7). The modulatory role of ANG-(1-7) in the regulation of ACE2 by ANG II suggests a complex interplay between the two peptides that is mediated by specific receptors to activate distinct signaling pathways.

Gallagher, P. E., et al. (2008). "Regulation of ACE2 in cardiac myocytes and fibroblasts." Am J Physiol Heart Circ Physiol **295**(6): H2373-2379.

Angiotensin-converting enzyme 2 (ACE2) preferentially forms angiotensin-(1-7) [ANG-(1-7)] from ANG II. We showed that cardiac ACE2 is elevated following treatment of coronary artery-ligated rats with AT1 receptor blockers (ARBs). Cardiac myocytes and fibroblasts were isolated from neonatal rats to determine the molecular mechanisms for the ACE2 upregulation by ARB treatment. ANG II significantly reduced ACE2 activity and downregulated ACE2 mRNA in cardiac myocytes, effects blocked by the ARB losartan, indicating that ANG II regulates ACE2. ANG II also reduced ACE2 mRNA in cardiac fibroblasts; however, no enzyme activity was detected, reflecting the limited expression of ACE2 in these cells. Endothelin-1 (ET-1) also significantly reduced myocyte ACE2 mRNA. The reduction in ACE2 mRNA by ANG II or ET-1 was blocked by inhibitors of mitogen-activated protein kinase kinase 1, suggesting that ANG II or ET-1 activates extracellular signal-regulated kinase (ERK) 1/ERK2 to reduce ACE2. Although ACE2 mRNA was not affected by ANG-(1-7), both the ANG II- and ET-1-mediated reductions in ACE2 mRNA were blocked by the heptapeptide. The ANG-(1-7) modulatory effect was prevented by the ANG-(1-7) receptor antagonist [D-Ala7]-ANG-(1-7), indicating that the ANG-(1-7) response was mediated by a specific AT (1-7) receptor. Myocyte treatment with atrial natriuretic peptide (ANP) also reversed the ACE2 mRNA downregulation by ANG II or ET-1, whereas treatment with ANP alone was ineffective. These results indicate that multiple hypertrophic and anti-hypertropic peptides regulate ACE2 production in myocytes, suggesting that ACE2 expression in the heart is dependent upon the compliment and concentration of regulatory molecules.

Ge, X. Y., et al. (2013). "Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor." Nature **503**(7477): 535-538.

The 2002-3 pandemic caused by severe acute respiratory syndrome coronavirus (SARS-CoV) was one of the most significant public health events in recent history. An ongoing outbreak of Middle East respiratory syndrome coronavirus suggests that this group of viruses remains a key threat and that their distribution is wider than previously recognized. Although bats have been suggested to be the natural reservoirs of both viruses, attempts to isolate the progenitor virus of SARS-CoV from bats have been unsuccessful. Diverse SARS-like coronaviruses (SL-CoVs) have now been reported from bats in China, Europe and Africa, but none is considered a direct progenitor of SARS-CoV because of their phylogenetic disparity from this virus and the inability of their spike proteins to use the SARS-CoV cellular receptor molecule, the human angiotensin converting enzyme II (ACE2). Here we report whole-genome sequences of two novel bat coronaviruses from Chinese horseshoe bats (family: Rhinolophidae) in Yunnan, China: RsSHC014 and Rs3367. These viruses are far more closely related to SARS-CoV than any previously identified bat coronaviruses, particularly in the receptor binding domain of the spike protein. Most importantly, we report the first recorded isolation of a live SL-CoV (bat SL-CoV-WIV1) from bat faecal samples in Vero E6 cells, which has typical coronavirus morphology, 99.9% sequence identity to Rs3367 and uses ACE2 from humans, civets and Chinese horseshoe bats for cell entry. Preliminary in vitro testing indicates that WIV1 also has a broad species tropism. Our results provide the strongest evidence to date that Chinese horseshoe bats are natural reservoirs of SARS-CoV, and that intermediate hosts may not be necessary for direct human infection by some bat SL-CoVs. They also highlight the importance of pathogen-discovery programs targeting high-risk wildlife groups in emerging disease hotspots as a strategy for pandemic preparedness.

Gembardt, F., et al. (2005). "Organ-specific distribution of ACE2 mRNA and correlating peptidase activity in rodents." Peptides **26**(7): 1270-1277.

Biochemical analysis revealed that angiotensin-converting enzyme related carboxy-peptidase (ACE2) cleaves angiotensin (Ang) II to Ang-(1-7), a heptapeptide identified as an endogenous ligand for the G protein-coupled receptor Mas. No data are currently available that systematically describe ACE2 distribution and activity in rodents. Therefore, we analyzed the ACE2 expression in different tissues of mice and rats on mRNA (RNase protection assay) and protein levels (immunohistochemistry, ACE2 activity, western blot). Although ACE2 mRNA in both investigated species showed the highest expression in the ileum, the mouse organ exceeded rat ACE2, as also demonstrated in the kidney and colon. Corresponding to mRNA, ACE2 activity was highest in the ileum and mouse kidney but weak in the rat kidney, which was also confirmed by immunohistochemistry. Contrary to mRNA, we found weak activity in the lung of both species. Our data demonstrate a tissue- and species-specific pattern for ACE2 under physiological conditions.

Ghadhanfar, E., et al. (2017). "The role of ACE2, angiotensin-(1-7) and Mas1 receptor axis in glucocorticoid-induced intrauterine growth restriction." Reprod Biol Endocrinol **15**(1): 97.

BACKGROUND: Plasma and urine levels of the potent vasodilator Ang-(1-7) are elevated in mid and late pregnancy and are correlated with elevated placental angiogenesis, fetal blood flow, and rapid fetal growth. We hypothesized that Ang-(1-7), its receptor (Mas1) and the enzymes involved in Ang-(1-7) production (ACE2 and Membrane metallo-endopeptidase; MME) are down regulated in response to glucocorticoid administration contributing to IUGR. METHODS: Pregnant female Sprague-Dawley rats were injected with dexamethasone (DEX; 0.4 mg/kg/day) starting from 14 day gestation (dg) till sacrifice at 19 or 21 dg while control groups were injected with saline (n = 6/group). The gene and protein expression of ACE2, MME, Ang-(1-7) and Mas1 receptor in the placental labyrinth (LZ) and basal zones (BZ) were studied. RESULTS: DEX administration caused a reduction in LZ weight at 19 and 21 dg ($p < 0.001$). IUGR, as shown by decreased fetal weights, was evident in DEX treated rats at 21 dg ($p < 0.01$). ACE2 gene expression was elevated in the LZ of control placentas at 21 dg ($p < 0.01$) compared to 19 dg and DEX prevented this rise at both gene ($p < 0.01$) and protein levels ($p < 0.05$). In addition, Ang-(1-7) protein expression in LZ was significantly reduced in DEX treated rats at 21 dg ($p < 0.05$). On the other hand, Mas1 and MME were upregulated in LZ at 21 dg in both groups ($p < 0.05$ and $p < 0.001$, respectively). CONCLUSION: The results of this study indicate that a reduced expression of ACE2 and Ang-(1-7) in the placenta by DEX treatment may be responsible for IUGR and consequent disease programming later in life.

Glowacka, I., et al. (2010). "Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63." J Virol **84**(2): 1198-1205.

The human coronaviruses (CoVs) severe acute respiratory syndrome (SARS)-CoV and NL63 employ angiotensin-converting enzyme 2 (ACE2) for cell entry. It was shown that recombinant SARS-CoV spike protein (SARS-S) downregulates ACE2 expression and thereby promotes lung injury. Whether NL63-S exerts a similar activity is yet unknown. We found that recombinant SARS-S bound to ACE2 and induced ACE2 shedding with higher efficiency than NL63-S. Shedding most likely accounted for the previously observed ACE2 downregulation but was dispensable for viral replication. Finally, SARS-CoV but not NL63 replicated efficiently in ACE2-positive Vero cells and reduced ACE2 expression, indicating robust receptor interference in the context of SARS-CoV but not NL63 infection.

Gonzalez, A. A., et al. (2019). "Potassium Intake Prevents the Induction of the Renin-Angiotensin System and Increases Medullary ACE2 and COX-2 in the Kidneys of Angiotensin II-Dependent Hypertensive Rats." Front Pharmacol **10**: 1212.

In angiotensin II (Ang II)-dependent hypertensive rats there is an increased expression of proximal tubule angiotensinogen (AGT), collecting duct renin and angiotensin converting enzyme (ACE), which contributes to intratubular Ang II formation. Ang II acts on Ang II type 1 receptors promoting sodium retention and vasoconstriction. However concurrently, the ACE2-Ang-(1-7) axis and the expression of kallikrein and medullary prostaglandins counteract the effects of Ang II, promoting natriuresis and vasodilation. Human studies demonstrate that dietary potassium (K (+)) intake lowers blood pressure. In this report we evaluate the expression of AGT, ACE, medullary prorenin/renin, ACE2, kallikrein and cyclooxygenase-2 (COX-2) in Ang II-infused rats fed with high K (+) diet (2%) for 14 days. Dietary K (+) enhances diuresis in non-infused and in Ang II-infused rats. The rise in systolic blood pressure in Ang II-infused rats was attenuated by dietary K (+). Ang II-infused rats showed increased renal protein levels of AGT, ACE and medullary prorenin and renin. This effect was attenuated in the Ang II + K (+) group. Ang II infusion decreased ACE2 compared to the control

group; however, K (+) diet prevented this effect in the renal medulla. Furthermore, medullary COX-2 was dramatically induced by K (+) diet in non-infused and in Ang II infused rats. Dietary K (+) greatly increased kallikrein immunostaining in normotensive rats and in Ang II-hypertensive rats. These results indicate that a high K (+) diet attenuates Ang II-dependent hypertension by preventing the induction of ACE, AGT and collecting duct renin and by enhancing medullary COX-2 and ACE2 protein expression in the kidney.

Goru, S. K., et al. (2017). "Diminazene aceturate prevents nephropathy by increasing glomerular ACE2 and AT2 receptor expression in a rat model of type1 diabetes." Br J Pharmacol **174**(18): 3118-3130.

BACKGROUND AND PURPOSE: One of the protective actions of angiotensin converting enzyme-2 (ACE2) is the inactivation of angiotensin II. Expression and activity of ACE2 was reduced in glomeruli of diabetic patients and in animal models of diabetes. Recently the potential role of recombinant ACE2 administration in preventing diabetic nephropathy (DN) has been shown. Here we have tested the effects of the ACE2 activator, diminazene aceturate (DIZE), in a model of DN. EXPERIMENTAL APPROACH: Male Wistar rats were rendered diabetic using a single dose of streptozotocin (55 mg.kg (-1), i.p.). After 4 weeks, diabetic animals were divided into experimental groups and treated with DIZE, at a low dose (5 mg.kg (-1).day (-1)), a high dose (15 mg.kg (-1).day (-1)) and the high dose with of the AT2 receptor antagonist PD123319 (10 mg.kg (-1).day (-1)). At the end of the treatment, kidneys from all the groups were collected and processed separately for glomerular isolation, protein isolation, mRNA extraction and for immunohistochemical studies. KEY RESULTS: Treatment with DIZE restored ACE2 expression in glomeruli and increased expression of AT2 receptors in whole kidney and isolated glomeruli of diabetic animals. DIZE administration reduced angiotensin II levels and increased angiotensin-(1-7) levels in diabetic kidney. However, PD123319 treatment reversed all these actions of DIZE. CONCLUSIONS AND IMPLICATIONS: DIZE treatment reduced diabetes-induced renal damage as shown by reduction of fibrosis and apoptosis. These protective actions of DIZE were blocked by the AT2 receptor antagonist. Taken together, these results suggest that DIZE protected against DN through the ACE2/angiotensin-(1-7)/ AT2 receptor axis.

Goulter, A. B., et al. (2004). "ACE2 gene expression is up-regulated in the human failing heart." BMC Med **2**: 19.

BACKGROUND: ACE2 is a novel homologue of angiotensin converting enzyme (ACE). ACE2 is highly expressed in human heart and animal data suggest that ACE2 is an essential regulator of cardiac function in vivo. Since overactivity of the renin-angiotensin system contributes to the progression of heart failure, this investigation assessed changes in gene expression of ACE2, ACE, AT1 receptor and renin in the human failing heart. METHODS: The sensitive technique of quantitative reverse transcriptase polymerase chain reaction was used to determine the level of mRNA expression of ACE and ACE2 in human ventricular myocardium from donors with non-diseased hearts (n = 9), idiopathic dilated cardiomyopathy (IDC, n = 11) and ischemic cardiomyopathy (ICM, n = 12). Following logarithmic transformation of the data, a one-way analysis of variance was performed for each target gene followed by a Dunnett's test to compare the two disease groups IDC and ICM versus control. RESULTS: As anticipated, ACE mRNA was found to be significantly increased in the failing heart with a 3.1 and 2.4-fold up-regulation found in IDC and ICM relative to non-diseased myocardium. Expression of ACE2 mRNA was also significantly up-regulated in IDC (2.4-fold increase) and ICM (1.8-fold increase) versus non-diseased myocardium. No change in angiotensin AT1 receptor mRNA expression was found in failing myocardium and renin mRNA was not detected. CONCLUSIONS: These data suggest that ACE2 is up-regulated in human IDC and ICM and are consistent with the hypothesis that differential regulation of this enzyme may have important functional consequences in heart failure. This strengthens the hypothesis that ACE2 may be a relevant target for the treatment of heart failure and will hopefully spur further studies to clarify the functional effects in human myocardium of ACE2 derived peptides.

Grobe, J. L., et al. (2007). "ACE2 overexpression inhibits hypoxia-induced collagen production by cardiac fibroblasts." Clin Sci (Lond) **113**(8): 357-364.

Cardiac remodelling is a key risk factor for the development of heart failure in the chronic phase following myocardial infarction. Our previous studies have shown an anti-remodelling role of ACE2 (angiotensin-converting enzyme 2) in vivo during hypertension and that these protective effects are mediated through increased circulating levels of Ang-(1-7) [angiotensin-(1-7)]. In the present study, we have demonstrated that cardiac myocytes have modest ACE2 activity, whereas cardiac fibroblasts do not exhibit any endogenous activity. As fibroblasts are the major cell type found in an infarct zone following a myocardial infarction, we examined the effects of ACE2 gene delivery to cultured cardiac fibroblasts

after acute hypoxic exposure. Cardiac fibroblasts from 5-day-old Sprague-Dawley rat hearts were grown to confluence and transduced with a lentiviral vector containing murine ACE2 cDNA under transcriptional control by the EF1alpha (elongation factor 1alpha) promoter (lenti-ACE2). Transduction of fibroblasts with lenti-ACE2 resulted in a viral dose-dependent increase in ACE2 activity. This was associated with a significant attenuation of both basal and hypoxia/re-oxygenation-induced collagen production by the fibroblasts. Cytokine production, specifically TGFbeta (transforming growth factor beta), by these cells was also significantly attenuated by ACE2 expression. Collectively, these results indicate that: (i) endogenous ACE2 activity is observed in cardiac myocytes, but not in cardiac fibroblasts; (ii) ACE2 overexpression in the cardiac fibroblast attenuates collagen production; and (iii) this prevention is probably mediated by decreased expression of cytokines. We conclude that ACE2 expression, limited to cardiac fibroblasts, may represent a novel paradigm for in vivo therapy following acute ischaemia.

Grobe, N., et al. (2015). "Functional and molecular evidence for expression of the renin angiotensin system and ADAM17-mediated ACE2 shedding in COS7 cells." Am J Physiol Cell Physiol **308**(9): C767-777.

The renin angiotensin system (RAS) plays a vital role in the regulation of the cardiovascular and renal functions. COS7 is a robust and easily transfectable cell line derived from the kidney of the African green monkey, Cercopithecus aethiops. The aims of this study were to 1) demonstrate the presence of an endogenous and functional RAS in COS7, and 2) investigate the role of a disintegrin and metalloproteinase-17 (ADAM17) in the ectodomain shedding of angiotensin converting enzyme-2 (ACE2). Reverse transcription coupled to gene-specific polymerase chain reaction demonstrated expression of ACE, ACE2, angiotensin II type 1 receptor (AT1R), and renin at the transcript levels in total RNA cell extracts. Western blot and immunohistochemistry identified ACE (60 kDa), ACE2 (75 kDa), AT1R (43 kDa), renin (41 kDa), and ADAM17 (130 kDa) in COS7. At the functional level, a sensitive and selective mass spectrometric approach detected endogenous renin, ACE, and ACE2 activities. ANG-(1-7) formation (m/z 899) from the natural substrate ANG II (m/z 1,046) was detected in lysates and media. COS7 cells stably expressing shRNA constructs directed against endogenous ADAM17 showed reduced ACE2 shedding into the media. This is the first study demonstrating endogenous expression of the RAS and ADAM17 in the widely used COS7 cell line and its utility to study ectodomain shedding of ACE2 mediated by ADAM17 in vitro. The transfectable nature of this cell line makes it an attractive cell model for studying the molecular, functional, and pharmacological properties of the renal RAS.

Grzegrzolka, J., et al. (2013). "ACE and ACE2 expression in normal and malignant skin lesions." Folia Histochem Cytobiol **51**(3): 232-238.

The renin-angiotensin system (RAS) is known mainly as a regulator of cardiovascular homeostasis. However, it has also been shown to mediate processes such as proliferation, apoptosis, angiogenesis, and carcinogenesis. Nonmelanoma skin cancers (NMSC) - including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) - are among the most common cancers. The aim of the present study was to determine the immunohistochemical expression of angiotensin-converting enzyme (ACE), angiotensin-converting enzyme 2 (ACE2), and Ki-67 antigen in archival samples of normal skin, actinic keratosis, and malignant skin lesions. Cytoplasmic-nuclear ACE immunoreactivity was observed in 99% of examined cases of both normal skin and cancers. Significantly higher ACE immunoreactivity occurred in normal skin, as compared with BCC and SCC ($p < 0.01$, $p < 0.0001$, respectively). Additionally, ACE immunoreactivity was also significantly higher in BCC, compared with SCC ($p < 0.05$). ACE2 immunoreactivity was noted in basal epidermal layers and in sebaceous gland cells in normal skin, though not in NMSC. These novel observations suggest that ACE and skin RAS may be involved in the pathogenesis of malignant skin lesions.

Guang, C., et al. (2012). "Three key proteases--angiotensin-I-converting enzyme (ACE), ACE2 and renin--within and beyond the renin-angiotensin system." Arch Cardiovasc Dis **105**(6-7): 373-385.

The discovery of angiotensin-I-converting enzyme 2 (ACE2) and a (pro)renin receptor has renewed interest in the physiology of the renin-angiotensin system (RAS). Through the ACE2/angiotensin-(1-7)/Mas counter-regulatory axis, ACE2 balances the vasoconstrictive, proliferative, fibrotic and proinflammatory effects of the ACE/angiotensin II/AT1 axis. The (pro)renin receptor system shows an angiotensin-dependent function related to increased generation of angiotensin I, and an angiotensin-independent aspect related to intracellular signalling. Activation of ACE2 and inhibition of ACE and renin have been at the core of the RAS regulation. The aim of this review is to discuss the biochemistry and biological functions of ACE, ACE2 and renin within and beyond the RAS, and thus provide a perspective for future bioactives from natural plant and/or food resources related to the three proteases.

Guo, J. W., et al. (2010). "[Effects of Panax notoginseng saponins on ACE2 and TNF-alpha in rats with post-myocardial infarction-ventricular remodeling]." Zhong Yao Cai **33**(1): 89-92.

OBJECTIVE: To research the effects of Panax notoginseng saponins (PNS) on angiotensin-converting enzymes 2 (ACE2) and tumor necrosis factor-alpha (TNF-alpha) in rats with post-myocardial infarction ventricular remodeling. METHODS: Models of acute myocardial infarction (AMI) were produced by ligation of left anterior descending coronary artery, 24 hours after operation the rats were randomly divided into control and experiment groups, then respectively administrated with NS, fosinopril and low, middle and high dosage of PNS for four consecutive weeks. To observe effects of PNS on malondialdehyde (MDA), nitric oxide (NO), glutathione peroxidase (GSH-Px), ACE2 and TNF-alpha in rats with post-myocardial infarction ventricular remodeling. RESULTS: Compared with NS group, MDA significantly decreased, the activity of GSH-Px significantly increased ($P < 0.05$ or $P < 0.01$), NO of the high-dose PNS group decreased ($P < 0.05$), Compared with the NS group, ACE2 increased and TNF-a significantly decreased in low-dose PNS group, middle and high-dose groups ($P < 0.05$). CONCLUSION: PNS can stimulate ACE2 to inhibit the expression of TNF-alpha and enhance the antioxidance. PNS can reduce pathological injury of cardiac myocytes in myocardial ischemia and cardiac muscle, which can improve ventricular remodeling.

Guo, Y. J., et al. (2008). "ACE2 overexpression inhibits angiotensin II-induced monocyte chemoattractant protein-1 expression in macrophages." Arch Med Res **39**(2): 149-154.

BACKGROUND: The discovery of angiotensin-converting enzyme 2 (ACE2) has shed light on the potential therapy for cardiovascular disease, owing to its key role in the formation of vasoprotective peptide angiotensin (Ang 1-7) from angiotensin (Ang) II. The aim of this study was to evaluate whether ACE2 overexpression could protect human monocyte cell line (THP-1) macrophages from angiotensin II-induced monocyte chemoattractant protein-1 (MCP-1) formation. METHODS: A truncated form of mouse ACE2 (mACE2) was cloned into adenovirus vector (Ad-ACE2) and transfected into THP-1. We examined expression of MCP-1 by administration of a selected Ang (1-7) antagonist (A779) to show the effect of ACE2 overexpression on MCP-1 level induced by AngII. RESULTS: AngII-induced MCP-1 expression increased obviously at 24 h and at the concentration of 10(-6) M. Transduction of THP-1 with Ad-ACE2 resulted in a viral increase in ACE2 activity. This was associated with a significant attenuation of AngII-induced MCP-1 production by 39.6+/-4.0% in THP-1 (mean+/-SEM, n=3). Moreover, expression of MCP-1 increased by 35.1+/-4.2% in Ad-ACE2 transfected THP-1 after incubation with Ang II and A779 compared to that with AngII alone. Collectively, these results indicated that ACE2 overexpression in the THP-1 attenuates AngII-induced MCP-1 production and that this reduction is likely mediated by increased Ang (1-7) level. CONCLUSIONS: ACE2 overexpression may provide a new therapeutic strategy for atherosclerosis by inhibiting MCP-1 production induced by AngII.

Gupte, M., et al. (2008). "ACE2 is expressed in mouse adipocytes and regulated by a high-fat diet." Am J Physiol Regul Integr Comp Physiol **295**(3): R781-788.

Adipose tissue expresses components of the renin-angiotensin system (RAS). Angiotensin converting enzyme (ACE2), a new component of the RAS, catabolizes the vasoconstrictor peptide ANG II to form the vasodilator angiotensin 1-7 [ANG-(1-7)]. We examined whether adipocytes express ACE2 and its regulation by manipulation of the RAS and by high-fat (HF) feeding. ACE2 mRNA expression increased (threefold) during differentiation of 3T3-L1 adipocytes and was not regulated by manipulation of the RAS. Male C57BL/6 mice were fed low- (LF) or high-fat (HF) diets for 1 wk or 4 mo. At 1 wk of HF feeding, adipose expression of angiotensinogen (twofold) and ACE2 (threefold) increased, but systemic angiotensin peptide concentrations and blood pressure were not altered. At 4 mo of HF feeding, adipose mRNA expression of angiotensinogen (twofold) and ACE2 (threefold) continued to be elevated, and liver angiotensinogen expression increased (twofold). However, adipose tissue from HF mice did not exhibit elevated ACE2 protein or activity. Increased expression of ADAM17, a protease responsible for ACE2 shedding, coincided with reductions in ACE2 activity in 3T3-L1 adipocytes, and an ADAM17 inhibitor decreased media ACE2 activity. Moreover, ADAM17 mRNA expression was increased in adipose tissue from 4-mo HF-fed mice, and plasma ACE2 activity increased. However, HF mice exhibited marked increases in plasma angiotensin peptide concentrations (LF: 2,141 +/- 253; HF: 6,829 +/- 1,075 pg/ml) and elevated blood pressure. These results demonstrate that adipocytes express ACE2 that is dysregulated in HF-fed mice with elevated blood pressure compared with LF controls.

Gurley, S. B., et al. (2006). "Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice." J Clin Invest **116**(8): 2218-2225.

The carboxypeptidase ACE2 is a homologue of angiotensin-converting enzyme (ACE). To clarify the physiological roles of ACE2, we generated mice with targeted disruption of the Ace2 gene. ACE2-deficient mice were viable, fertile, and lacked any gross structural abnormalities. We found normal cardiac dimensions and function in ACE2-deficient animals with mixed or inbred genetic backgrounds. On the C57BL/6 background, ACE2 deficiency was associated with a modest increase in blood pressure, whereas the absence of ACE2 had no effect on baseline blood pressures in 129/SvEv mice. After acute Ang II infusion, plasma concentrations of Ang II increased almost 3-fold higher in ACE2-deficient mice than in controls. In a model of Ang II-dependent hypertension, blood pressures were substantially higher in the ACE2-deficient mice than in WT. Severe hypertension in ACE2-deficient mice was associated with exaggerated accumulation of Ang II in the kidney, as determined by MALDI-TOF mass spectrometry. Although the absence of functional ACE2 causes enhanced susceptibility to Ang II-induced hypertension, we found no evidence for a role of ACE2 in the regulation of cardiac structure or function. Our data suggest that ACE2 is a functional component of the renin-angiotensin system, metabolizing Ang II and thereby contributing to regulation of blood pressure.

Guy, J. L., et al. (2003). "Angiotensin-converting enzyme-2 (ACE2): comparative modeling of the active site, specificity requirements, and chloride dependence." Biochemistry **42**(45): 13185-13192.

Angiotensin-converting enzyme 2 (ACE2), a homologue of ACE, represents a new and potentially important target in cardio-renal disease. A model of the active site of ACE2, based on the crystal structure of testicular ACE, has been developed and indicates that the catalytic mechanism of ACE2 resembles that of ACE. Structural differences exist between the active site of ACE (dipeptidyl carboxypeptidase) and ACE2 (carboxypeptidase) that are responsible for the differences in specificity. The main differences occur in the ligand-binding pockets, particularly at the S2' subsite and in the binding of the peptide carboxy-terminus. The model explains why the classical ACE inhibitor lisinopril is unable to bind to ACE2. On the basis of the ability of ACE2 to cleave a variety of biologically active peptides, a consensus sequence of Pro-X-Pro-hydrophobic/basic for the protease specificity of ACE2 has been defined that is supported by the ACE2 model. The dipeptide, Pro-Phe, completely inhibits ACE2 activity at 180 microM with angiotensin II as the substrate. As with ACE, the chloride dependence of ACE2 is substrate-specific such that the hydrolysis of angiotensin I and the synthetic peptide substrate, Mca-APK (Dnp), are activated in the presence of chloride ions, whereas the cleavage of angiotensin II is inhibited. The ACE2 model is also suggestive of a possible mechanism for chloride activation. The structural insights provided by these analyses for the differences in inhibition pattern and substrate specificity among ACE and its homologue ACE2 and for the chloride dependence of ACE/ACE2 activity are valuable in understanding the function and regulation of ACE2.

Guy, J. L., et al. (2005). "Identification of critical active-site residues in angiotensin-converting enzyme-2 (ACE2) by site-directed mutagenesis." FEBS J **272**(14): 3512-3520.

Angiotensin-converting enzyme-2 (ACE2) may play an important role in cardiorenal disease and it has also been implicated as a cellular receptor for the severe acute respiratory syndrome (SARS) virus. The ACE2 active-site model and its crystal structure, which was solved recently, highlighted key differences between ACE2 and its counterpart angiotensin-converting enzyme (ACE), which are responsible for their differing substrate and inhibitor sensitivities. In this study the role of ACE2 active-site residues was explored by site-directed mutagenesis. Arg273 was found to be critical for substrate binding such that its replacement causes enzyme activity to be abolished. Although both His505 and His345 are involved in catalysis, it is His345 and not His505 that acts as the hydrogen bond donor/acceptor in the formation of the tetrahedral peptide intermediate. The difference in chloride sensitivity between ACE2 and ACE was investigated, and the absence of a second chloride-binding site (CL2) in ACE2 confirmed. Thus ACE2 has only one chloride-binding site (CL1) whereas ACE has two sites. This is the first study to address the differences that exist between ACE2 and ACE at the molecular level. The results can be applied to future studies aimed at unravelling the role of ACE2, relative to ACE, in vivo.

Guy, J. L., et al. (2005). "Membrane-associated zinc peptidase families: comparing ACE and ACE2." Biochim Biophys Acta **1751**(1): 2-8.

In contrast to the relatively ubiquitous angiotensin-converting enzyme (ACE), expression of the mammalian ACE homologue, ACE2, was initially described in the heart, kidney and testis. ACE2 is a type I integral membrane protein with its active site domain exposed to the extracellular surface of endothelial cells and the renal tubular epithelium. Here ACE2 is poised to metabolise circulating peptides which may include angiotensin II, a potent vasoconstrictor and the product of angiotensin I cleavage by ACE. To this end, ACE2 may

counterbalance the effects of ACE within the renin-angiotensin system (RAS). Indeed, ACE2 has been implicated in the regulation of heart and renal function where it is proposed to control the levels of angiotensin II relative to its hypotensive metabolite, angiotensin-(1-7). The recent solution of the structure of ACE2, and ACE, has provided new insight into the substrate and inhibitor profiles of these two key regulators of the RAS. As the complexity of this crucial pathway is unravelled, there is a growing interest in the therapeutic potential of agents that modulate the activity of ACE2.

Haga, S., et al. (2010). "TACE antagonists blocking ACE2 shedding caused by the spike protein of SARS-CoV are candidate antiviral compounds." Antiviral Res **85**(3): 551-555.

Because outbreaks of severe acute respiratory syndrome coronavirus (SARS-CoV) might reemerge, identifying antiviral compounds is of key importance. Previously, we showed that the cellular factor TNF-alpha converting enzyme (TACE), activated by the spike protein of SARS-CoV (SARS-S protein), was positively involved in viral entry, implying that TACE is a possible target for developing antiviral compounds. To demonstrate this possibility, we here tested the effects of TACE inhibitors on viral entry. In vitro and in vivo data revealed that the TACE inhibitor TAPI-2 attenuated entry of both pseudotyped virus expressing the SARS-S protein in a lentiviral vector backbone and infectious SARS-CoV. TAPI-2 blocked both the SARS-S protein-induced shedding of angiotensin-converting enzyme 2 (ACE2), a receptor of SARS-CoV, and TNF-alpha production in lung tissues. Since the downregulation of ACE2 by SARS-S protein was proposed as an etiological event in the severe clinical manifestations, our data suggest that TACE antagonists block SARS-CoV infection and also attenuate its severe clinical outcome.

Haga, S., et al. (2015). "A novel ACE2 activator reduces monocrotaline-induced pulmonary hypertension by suppressing the JAK/STAT and TGF-beta cascades with restored caveolin-1 expression." Exp Lung Res **41**(1): 21-31.

INTRODUCTION: Pulmonary hypertension (PH) is characterized by increased pressure in the pulmonary artery and right ventricular hypertrophy (RVH). Recently, angiotensin-converting enzyme 2 (ACE2), which converts angiotensin (Ang) II into Ang-(1-7), was shown to inhibit experimental PH. Here we identified a novel ACE2 activator and investigated how the compound reduced monocrotaline (MCT)-induced PH. METHODS: To induce PH, Sprague-Dawley rats were injected subcutaneously with MCT, followed by the continuous administration of NCP-2454, an ACE2 activator, using osmotic pumps. Pulmonary arterial compliance was monitored every week until 4 weeks post-injection (wpi). RVH and lung remodeling was evaluated using lung tissue at 4 wpi. RESULTS: NCP-2454 upregulated the production of Ang-(1-7) when incubated with ACE2 and Ang II. Notably, a continuous infusion of NCP-2454 significantly improved pulmonary arterial compliance, right ventricular systolic pressure, and RVH in MCT-treated rats. Interestingly, NCP-2454 increased the relative expression of ACE2 and MAS mRNA in lung tissue, especially in MCT-treated rats. In addition, the compound inhibited the MCT-induced overexpression of transforming growth factor beta, phosphorylation of signal transducer and activator of transcription-3 (STAT3), and interleukin-6 production. The compound also restored the expression of caveolin-1 (Cav-1), which negatively regulates the Janus kinase-STAT signaling cascade. CONCLUSIONS: NCP-2454 prevented MCT-induced PH by suppressing intracellular inflammatory cascades, an upstream molecular change of which is the disruption of Cav-1 expression.

Haga, S., et al. (2008). "Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry." Proc Natl Acad Sci U S A **105**(22): 7809-7814.

Severe acute respiratory syndrome coronavirus (SARS-CoV) is a high-risk infectious pathogen. In the proposed model of respiratory failure, SARS-CoV down-regulates its receptor, angiotensin-converting enzyme 2 (ACE2), but the mechanism involved is unknown. We found that the spike protein of SARS-CoV (SARS-S) induced TNF-alpha-converting enzyme (TACE)-dependent shedding of the ACE2 ectodomain. The modulation of TACE activity by SARS-S depended on the cytoplasmic domain of ACE2, because deletion mutants of ACE2 lacking the carboxyl-terminal region did not induce ACE2 shedding or TNF-alpha production. In contrast, the spike protein of HNL63-CoV (NL63-S), a CoV that uses ACE2 as a receptor and mainly induces the common cold, caused neither of these cellular responses. Intriguingly, viral infection, judged by real-time RT-PCR analysis of SARS-CoV mRNA expression, was significantly attenuated by deletion of the cytoplasmic tail of ACE2 or knock-down of TACE expression by siRNA. These data suggest that cellular signals triggered by the interaction of SARS-CoV with ACE2 are positively involved in viral entry but lead to tissue damage. These findings may lead to the development of anti-SARS-CoV agents.

Hamming, I., et al. (2007). "The emerging role of ACE2 in physiology and disease." J Pathol **212**(1): 1-11.

The renin-angiotensin-aldosterone system (RAAS) is a key regulator of systemic blood pressure and renal function and a key player in renal and cardiovascular disease. However, its (patho)physiological roles and its architecture are more complex than initially anticipated. Novel RAAS components that may add to our understanding have been discovered in recent years. In particular, the human homologue of ACE (ACE2) has added a higher level of complexity to the RAAS. In a short period of time, ACE2 has been cloned, purified, knocked-out, knocked-in; inhibitors have been developed; its 3D structure determined; and new functions have been identified. ACE2 is now implicated in cardiovascular and renal (patho)physiology, diabetes, pregnancy, lung disease and, remarkably, ACE2 serves as a receptor for SARS and NL63 coronaviruses. This review covers available information on the genetic, structural and functional properties of ACE2. Its role in a variety of (patho)physiological conditions and therapeutic options of modulation are discussed.

Hamming, I., et al. (2004). "Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis." J Pathol **203**(2): 631-637.

Severe acute respiratory syndrome (SARS) is an acute infectious disease that spreads mainly via the respiratory route. A distinct coronavirus (SARS-CoV) has been identified as the aetiological agent of SARS. Recently, a metallopeptidase named angiotensin-converting enzyme 2 (ACE2) has been identified as the functional receptor for SARS-CoV. Although ACE2 mRNA is known to be present in virtually all organs, its protein expression is largely unknown. Since identifying the possible route of infection has major implications for understanding the pathogenesis and future treatment strategies for SARS, the present study investigated the localization of ACE2 protein in various human organs (oral and nasal mucosa, nasopharynx, lung, stomach, small intestine, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain). The most remarkable finding was the surface expression of ACE2 protein on lung alveolar epithelial cells and enterocytes of the small intestine. Furthermore, ACE2 was present in arterial and venous endothelial cells and arterial smooth muscle cells in all organs studied. In conclusion, ACE2 is abundantly present in humans in the epithelia of the lung and small intestine, which might provide possible routes of entry for the SARS-CoV. This epithelial expression, together with the presence of ACE2 in vascular endothelium, also provides a first step in understanding the pathogenesis of the main SARS disease manifestations.

Hamming, I., et al. (2008). "Differential regulation of renal angiotensin-converting enzyme (ACE) and ACE2 during ACE inhibition and dietary sodium restriction in healthy rats." Exp Physiol **93**(5): 631-638.

Angiotensin-converting enzyme (ACE) 2 is thought to counterbalance ACE by breakdown of angiotensin (Ang) II and formation of Ang (1-7). Both enzymes are highly expressed in the kidney, but reports on their regulation differ. To enhance our understanding of the regulation of renal ACE and ACE2, we investigated renal ACE and ACE2 expression during conditions of physiological (low-sodium diet) and pharmacological changes (ACE inhibition) in activity of the renin-angiotensin-aldosterone system (RAAS). Healthy rats were treated with vehicle or lisinopril with either a control or a low-sodium diet, and renal ACE2, ACE and plasma angiotensins were studied. During vehicle treatment, low sodium reduced renal ACE mRNA and activity without affecting ACE2 mRNA or activity and plasma Ang (1-7) and Ang II balance. Lisinopril significantly reduced renal ACE activity without affecting renal ACE2 activity. During ACE inhibition, low sodium reduced both ACE and ACE2 mRNA without affecting ACE2 activity or further reducing ACE activity. Measurements of renal neprilysin activity revealed no significant differences between any of the treatment groups. Plasma Ang (1-7) and Ang II balance is positively shifted towards the beneficial vasopeptide Ang (1-7) by the ACE inhibitor lisinopril, especially during a low sodium intake. In conclusion, modulation of the RAAS, by low sodium intake or ACE inhibition, does not affect renal ACE2 despite major variations in renal ACE. Thus, ACE and ACE2 are differentially regulated by low sodium and ACE inhibition. Therefore, we propose that the beneficial effects of ACE inhibitors are predominantly mediated by modulation of ACE and not ACE2. Whether this also applies to renal disease conditions should be investigated in future studies.

Han, D. P., et al. (2006). "Identification of critical determinants on ACE2 for SARS-CoV entry and development of a potent entry inhibitor." Virology **350**(1): 15-25.

Severe acute respiratory syndrome (SARS) is caused by a novel coronavirus, SARS-CoV. Virus entry into cells is mediated through interactions between spike (S) glycoprotein and angiotensin-converting enzyme 2 (ACE2). Alanine scanning mutagenesis analysis was performed to identify determinants on ACE2 critical for SARS-CoV

infection. Results indicated that charged amino acids between residues 22 and 57 were important, K26 and D30, in particular. Peptides representing various regions of ACE2 critical for virus infection were chemically synthesized and evaluated for antiviral activity. Two peptides (a.a. 22-44 and 22-57) exhibited a modest antiviral activity with IC50 of about 50 microM and 6 microM, respectively. One peptide comprised of two discontinuous segments of ACE2 (a.a. 22-44 and 351-357) artificially linked together by glycine, exhibited a potent antiviral activity with IC50 of about 0.1 microM. This novel peptide is a promising candidate as a therapeutic agent against this deadly emerging pathogen.

Hardtner, C., et al. (2013). "High glucose activates the alternative ACE2/Ang-(1-7)/Mas and APN/Ang IV/IRAP RAS axes in pancreatic beta-cells." Int J Mol Med **32**(4): 795-804.

The activation of the classical angiotensin (Ang)-converting enzyme (ACE)/Ang II/Ang II type 1 receptor (AT1R) axis of the renin-angiotensin system (RAS) has been associated with islet dysfunction and insulin resistance. Hyperglycaemia, hypertension and obesity, major components of metabolic syndrome, are all associated with increased systemic and tissue levels of Ang II. Whereas it is well established that Ang II, by binding to AT1R, impairs glucose-stimulated insulin secretion and insulin signaling, the contribution of alternative RAS axes to beta-cell function remains to be fully elucidated. In this study, using the BRIN-BD11 rat insulinoma cell line, we i) examined the basal expression levels of components of classical and alternative RAS axes and ii) investigated the effects of normal (5.5 mM) and elevated (11, 15, 25 mM) glucose concentrations on their expression and/or enzymatic activity by means of reverse transcription quantitative PCR (RT-qPCR), immunoblot analysis and enzymatic activity assays. The results correlated with the insulin production and release. Essential components of all RAS axes were found to be expressed in the BRIN-BD11 cells. Components of the alternative RAS axes, ACE2, neutral endopeptidase 24.11, Mas receptor (Mas), aminopeptidases A (APA) and N (APN) and insulin-regulated aminopeptidase (IRAP) showed an increased expression/activity in response to high glucose. These alterations were paralleled by the glucose-dependent increase in insulin production and release. By contrast, components of the classical RAS axis, ACE, AT1R and Ang II type 2 receptor (AT2R), remained largely unaffected under these conditions. Glucose induced the activation of the alternative ACE2/Ang-(1-7)/Mas and APN/Ang IV/IRAP RAS axes simultaneously with the stimulation of insulin production/release. Our data suggest the existence of a functional link between the local RAS axis and pancreatic beta-cell function; however, further studies are required to confirm this hypothesis.

Harris, R. C. (2012). "Podocyte ACE2 protects against diabetic nephropathy." Kidney Int **82**(3): 255-256.

As new components of the renin-angiotensin system (RAS) are elucidated, our understanding of the complexities of their interactions also advances. Previous studies have determined that podocytes possess a local RAS that can generate angiotensin II. Podocytes have also been shown to express angiotensin-converting enzyme 2 (ACE2), which can decrease angiotensin II levels by generation of angiotensin-(1-7). Nadarajah et al. now show that increased podocyte ACE2 activity can attenuate the development of diabetic nephropathy.

Hashimoto, T., et al. (2012). "ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation." Nature **487**(7408): 477-481.

Malnutrition affects up to one billion people in the world and is a major cause of mortality. In many cases, malnutrition is associated with diarrhoea and intestinal inflammation, further contributing to morbidity and death. The mechanisms by which unbalanced dietary nutrients affect intestinal homeostasis are largely unknown. Here we report that deficiency in murine angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (Ace2), which encodes a key regulatory enzyme of the renin-angiotensin system (RAS), results in highly increased susceptibility to intestinal inflammation induced by epithelial damage. The RAS is known to be involved in acute lung failure, cardiovascular functions and SARS infections. Mechanistically, ACE2 has a RAS-independent function, regulating intestinal amino acid homeostasis, expression of antimicrobial peptides, and the ecology of the gut microbiome. Transplantation of the altered microbiota from Ace2 mutant mice into germ-free wild-type hosts was able to transmit the increased propensity to develop severe colitis. ACE2-dependent changes in epithelial immunity and the gut microbiota can be directly regulated by the dietary amino acid tryptophan. Our results identify ACE2 as a key regulator of dietary amino acid homeostasis, innate immunity, gut microbial ecology, and transmissible susceptibility to colitis. These results provide a molecular explanation for how amino acid malnutrition can cause intestinal inflammation and diarrhoea.

Hayashi, N., et al. (2010). "The counterregulating role of ACE2 and ACE2-mediated angiotensin 1-7

signaling against angiotensin II stimulation in vascular cells." Hypertens Res **33**(11): 1182-1185.

To clarify the role of endogenous angiotensin (Ang)-converting enzyme 2 (ACE2) and its cleavage product, Ang 1-7, in the atherogenic stimulation of vascular cells, we investigated the effect of pharmacological inhibition of ACE2 and Mas, an Ang 1-7 receptor, on cellular responses against Ang II stimulation. We measured extracellular signal-regulated kinase (ERK) 1/2 phosphorylation by western blot, smooth muscle cell (SMC) proliferation by WST assay and the adhesion of monocytes labeled with PKH67 to endothelial cells (ECs) by fluorescence microplate reader. Cells were pretreated with Ang 1-7, olmesartan (Ang II type 1 receptor (AT1) blocker), DX600 (ACE2 inhibitor), -Ala7-Ang1-7 (D-Ala; Mas antagonist), or combinations of treatments before the application of Ang II. Treatment with Ang II increased phosphorylated ERK 1/2 of SMC and EC, proliferation of SMC and adhesion of monocyte to EC, which were blocked by olmesartan. Pretreatment with DX600 either did not accelerate or only slightly accelerated these cellular responses. However, when Ang II signaling through AT1 was reduced by olmesartan, the additional treatment with DX600 significantly blunted some of the effect of olmesartan. Similarly, pretreatment with D-Ala reduced the inhibitory effect of olmesartan in response to Ang II stimulation. Endogenous ACE2 in vascular cells may contribute to counteracting the Ang II-mediated cellular response partly by upregulating the Ang 1-7 signaling through Mas.

He, H. L., et al. (2015). "MSCs modified with ACE2 restore endothelial function following LPS challenge by inhibiting the activation of RAS." J Cell Physiol **230**(3): 691-701.

Angiotensin (Ang) II plays an important role in the process of endothelial dysfunction in acute lung injury (ALI) and is degraded by angiotensin-converting enzyme2 (ACE2). However, treatments that target ACE2 to injured endothelium and promote endothelial repair of ALI are lacking. Mesenchymal stem cells (MSCs) are capable of homing to the injured site and delivering a protective gene. Our study aimed to evaluate the effects of genetically modified MSCs, which overexpress the ACE2 protein in a sustained manner via a lentiviral vector, on Ang II production in endothelium and in vitro repair of lipopolysaccharide (LPS)-induced endothelial injury. We found that the efficiency of lentiviral vector transduction of MSCs was as high as 97.8% and was well maintained over 30 passages. MSCs modified with ACE2 showed a sustained high expression of ACE2 mRNA and protein. The modified MSCs secreted soluble ACE2 protein into the culture medium, which reduced the concentration of Ang II and increased the production of Ang 1-7. MSCs modified with ACE2 were more effective at restoring endothelial function than were unmodified MSCs, as shown by the enhanced survival of endothelial cells; the downregulated production of inflammatory mediators, including ICAM-1, VCAM-1, TNF-alpha, and IL-6; reduced paracellular permeability; and increased expression of VE-cadherin. These data demonstrate that MSCs modified to overexpress the ACE2 gene can produce biologically active ACE2 protein over a sustained period of time and have an enhanced ability to promote endothelial repair after LPS challenge. These results encourage further testing of the beneficial effects of ACE2-modified MSCs in an ALI animal model.

He, L., et al. (2006). "Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS." J Pathol **210**(3): 288-297.

The authors have previously shown that acute lung injury (ALI) produces a wide spectrum of pathological processes in patients who die of severe acute respiratory syndrome (SARS) and that the SARS coronavirus (SARS-CoV) nucleoprotein is detectable in the lungs, and other organs and tissues, in these patients. In the present study, immunohistochemistry (IHC) and in situ hybridization (ISH) assays were used to analyse the expression of angiotensin-converting enzyme 2 (ACE2), SARS-CoV spike (S) protein, and some pro-inflammatory cytokines (PICs) including MCP-1, TGF-beta1, TNF-alpha, IL-1beta, and IL-6 in autopsy tissues from four patients who died of SARS. SARS-CoV S protein and its RNA were only detected in ACE2+ cells in the lungs and other organs, indicating that ACE2-expressing cells are the primary targets for SARS-CoV infection in vivo in humans. High levels of PICs were expressed in the SARS-CoV-infected ACE2+ cells, but not in the uninfected cells. These results suggest that cells infected by SARS-CoV produce elevated levels of PICs which may cause immuno-mediated damage to the lungs and other organs, resulting in ALI and, subsequently, multi-organ dysfunction. Therefore application of PIC antagonists may reduce the severity and mortality of SARS.

Hekmat, A. S., et al. (2019). "Effect of Prolonged Infusion of Alamandine on Cardiovascular Parameters and Cardiac ACE2 Expression in a Rat Model of Renovascular Hypertension." Biol Pharm Bull **42**(6): 960-967.

Alamandine is a new member of the angiotensin family. Here, we studied the mRNA and protein

expression of cardiac angiotensin-converting enzyme 2 (ACE2) in the chronic phase of a rat model of 2-kidney, 1-clip hypertension (2K1C), and the effects of 2-week alamandine infusion on blood pressure, cardiac indices, and ACE2 mRNA and protein expression in the hearts. The rats were subjected to to sham-operation or placement of plexiglass clips around the left renal artery. Alamandine, at a dose of 600 microg/kg/d, was administered for 2 weeks via an osmotic mini-pump. At 18 weeks, after induction of hypertension, blood pressure and cardiac indices of contractility were measured using a Powerlab Physiograph system. The ACE2 mRNA and protein levels were determined using real time-PCR and Western blotting, respectively. In the hypertensive rats, alamandine caused a significant decrease in systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), left ventricular end-diastolic pressure ($p < 0.001$) and, left ventricular systolic pressure ($p < 0.001$) and increase in the maximum rate of pressure change in the left ventricle (dP/dt (max)) ($p < 0.05$). Also, the ACE2 mRNA expression in the heart increased in the hypertensive rats compared to the normotensive rats ($p < 0.05$), and alamandine restored this to normal values, although these changes were only seen at the mRNA and not the protein level. Histological analysis of cardiac tissue confirmed that alamandine decreased cardiac fibrosis and hypertrophy in 2K1C hypertensive rats. Our results indicate that alamandine, which acts as a depressor arm of the renin-angiotensin system, could be developed for treating hypertension.

Herath, C. B., et al. (2009). "Portal pressure responses and angiotensin peptide production in rat liver are determined by relative activity of ACE and ACE2." Am J Physiol Gastrointest Liver Physiol **297**(1): G98-G106.

Angiotensin converting enzyme (ACE) 2 activity and angiotensin-(1-7) [Ang-(1-7)] levels are increased in experimental cirrhosis; however, the pathways of hepatic Ang-(1-7) production have not been studied. This study investigated the role of ACE2, ACE, and neutral endopeptidase (NEP) in the hepatic formation of Ang-(1-7) from angiotensin I (Ang I) and Ang II and their effects on portal resistance. Ang I or Ang II were administered to rat bile duct ligated (BDL) and control livers alone and in combination with the ACE inhibitor lisinopril, the ACE and NEP inhibitor omapatrilat, or the ACE2 inhibitor MLN4760 ($n = 5$ per group). BDL markedly upregulated ACE, ACE2, and NEP. Ang-(1-7) was produced from Ang II in healthy and in BDL livers and was increased following ACE inhibition and decreased by ACE2 inhibition. In contrast, Ang-(1-7) production from Ang I was minimal and not affected by ACE or NEP inhibition. Surprisingly, ACE2 inhibition in BDLs dramatically increased Ang-(1-7) production from Ang I, an effect abolished by ACE2/NEP inhibition. Ang II and Ang I induced greater portal pressure increases in BDL livers than controls. The effects of Ang I were closely correlated with Ang II production and were strongly attenuated by both ACE and ACE/NEP inhibition. These findings show that the major substrate for hepatic production of Ang-(1-7) is Ang II and this is catalyzed by ACE2. Ang I is largely converted to Ang II by ACE, and net conversion of Ang I to Ang-(1-7) is small. NEP has the ability to generate large amounts of Ang-(1-7) in the BDL liver from Ang I only when ACE2 activity is greatly decreased or inhibited.

Herath, C. B., et al. (2007). "Upregulation of hepatic angiotensin-converting enzyme 2 (ACE2) and angiotensin-(1-7) levels in experimental biliary fibrosis." J Hepatol **47**(3): 387-395.

BACKGROUND/AIMS: Angiotensin-converting enzyme 2 (ACE2), its product, angiotensin-(1-7) and its receptor, Mas, may moderate the adverse effects of angiotensin II in liver disease. We examined the expression of these novel components of the renin angiotensin system (RAS) and the production and vasoactive effects of angiotensin-(1-7) in the bile duct ligated (BDL) rat. METHODS: BDL or sham-operated rats were sacrificed at 1, 2, 3 and 4 weeks. Tissue and blood were collected for gene expression, enzyme activity and peptide measurements. In situ perfused livers were used to assess angiotensin peptide production and their effects on portal resistance. RESULTS: Hepatic ACE2 gene and activity ($P<0.0005$), plasma angiotensin-(1-7) ($P<0.0005$) and Mas receptor expression ($P<0.01$) were increased following BDL compared to shams. Perfusion experiments confirmed that BDL livers produced increased angiotensin-(1-7) ($P<0.05$) from angiotensin II and this was augmented ($P<0.01$) by ACE inhibition. Whilst angiotensin II increased vasoconstriction in cirrhotic livers, angiotensin-(1-7) had no effect on portal resistance. CONCLUSIONS: RAS activation in chronic liver injury is associated with upregulation of ACE2, Mas and hepatic conversion of angiotensin II to angiotensin-(1-7) leading to increased circulating angiotensin-(1-7). These results support the presence of an ACE2-angiotensin-(1-7)-Mas axis in liver injury which may counteract the effects of angiotensin II.

Heurich, A., et al. (2014). "TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein." J Virol **88**(2): 1293-1307.

The type II transmembrane serine proteases TMPRSS2 and HAT can cleave and activate the spike protein (S) of the severe acute respiratory syndrome

coronavirus (SARS-CoV) for membrane fusion. In addition, these proteases cleave the viral receptor, the carboxypeptidase angiotensin-converting enzyme 2 (ACE2), and it was proposed that ACE2 cleavage augments viral infectivity. However, no mechanistic insights into this process were obtained and the relevance of ACE2 cleavage for SARS-CoV S protein (SARS-S) activation has not been determined. Here, we show that arginine and lysine residues within ACE2 amino acids 697 to 716 are essential for cleavage by TMPRSS2 and HAT and that ACE2 processing is required for augmentation of SARS-S-driven entry by these proteases. In contrast, ACE2 cleavage was dispensable for activation of the viral S protein. Expression of TMPRSS2 increased cellular uptake of soluble SARS-S, suggesting that protease-dependent augmentation of viral entry might be due to increased uptake of virions into target cells. Finally, TMPRSS2 was found to compete with the metalloprotease ADAM17 for ACE2 processing, but only cleavage by TMPRSS2 resulted in augmented SARS-S-driven entry. Collectively, our results in conjunction with those of previous studies indicate that TMPRSS2 and potentially related proteases promote SARS-CoV entry by two separate mechanisms: ACE2 cleavage, which might promote viral uptake, and SARS-S cleavage, which activates the S protein for membrane fusion. These observations have interesting implications for the development of novel therapeutics. In addition, they should spur efforts to determine whether receptor cleavage promotes entry of other coronaviruses, which use peptidases as entry receptors.

Hoffmann, M., et al. (2020). "SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor." Cell.

The recent emergence of the novel, pathogenic SARS-coronavirus 2 (SARS-CoV-2) in China and its rapid national and international spread pose a global health emergency. Cell entry of coronaviruses depends on binding of the viral spike (S) proteins to cellular receptors and on S protein priming by host cell proteases. Unravelling which cellular factors are used by SARS-CoV-2 for entry might provide insights into viral transmission and reveal therapeutic targets. Here, we demonstrate that SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming. A TMPRSS2 inhibitor approved for clinical use blocked entry and might constitute a treatment option. Finally, we show that the sera from convalescent SARS patients cross-neutralized SARS-2-S-driven entry. Our results reveal important commonalities between SARS-CoV-2 and SARS-CoV infection and identify a potential target for antiviral intervention.

Holappa, M., et al. (2015). "Angiotensin (1-7) and ACE2, "The Hot Spots" of Renin-Angiotensin System, Detected in the Human Aqueous Humor." Open Ophthalmol J **9**: 28-32.

BACKGROUND: The main purpose of the study was to establish whether essential components of the renin-angiotensin system (RAS) exist in the human aqueous humor. METHODS: Forty-five patients >/= 60 (74+/-7) years of age undergoing cataract surgery at Tampere University Hospital were randomly selected for the prospective study. The exclusion criterion was the use of oral antihypertensive medicine acting via renin-angiotensin system. Aqueous humor samples were taken at the beginning of normal cataract extraction. The samples were frozen and stored at -80 degrees C. The concentrations of intraocular endogenous RAS components Ang (1-7), ACE2, and ACE1 were measured using ELISA. RESULTS: Concentration medians of Ang (1-7), ACE2, and ACE1 in the aqueous humor were: Ang (1-7) 4.08 ng/ml, ACE2 2.32 ng/ml and ACE1 0.35 ng/ml. The concentrations were significantly higher in glaucomatous than in non-glaucomatous eyes, ACE1 (p=0.014) and Ang (1-7) (p=0.026) vs non-glaucomatous eyes. CONCLUSIONS: Ang (1-7), ACE2 and ACE1 are found in the human aqueous humor. The observations are consistent with the conception that local tissue-RAS exists in the human eye and it might have a role in the control of intraocular pressure.

Hou, Y., et al. (2010). "Angiotensin-converting enzyme 2 (ACE2) proteins of different bat species confer variable susceptibility to SARS-CoV entry." Arch Virol **155**(10): 1563-1569.

The discovery of SARS-like coronavirus in bats suggests that bats could be the natural reservoir of SARS-CoV. However, previous studies indicated the angiotensin-converting enzyme 2 (ACE2) protein, a known SARS-CoV receptor, from a horseshoe bat was unable to act as a functional receptor for SARS-CoV. Here, we extended our previous study to ACE2 molecules from seven additional bat species and tested their interactions with human SARS-CoV spike protein using both HIV-based pseudotype and live SARS-CoV infection assays. The results show that ACE2s of Myotis daubentoni and Rhinolophus sinicus support viral entry mediated by the SARS-CoV S protein, albeit with different efficiency in comparison to that of the human ACE2. Further, the alteration of several key residues either decreased or enhanced bat ACE2 receptor efficiency, as predicted from a structural modeling study of the different bat ACE2 molecules. These data suggest that M. daubentoni and R. sinicus are likely to be susceptible to SARS-CoV

and may be candidates as the natural host of the SARS-CoV progenitor viruses. Furthermore, our current study also demonstrates that the genetic diversity of ACE2 among bats is greater than that observed among known SARS-CoV susceptible mammals, highlighting the possibility that there are many more uncharacterized bat species that can act as a reservoir of SARS-CoV or its progenitor viruses. This calls for continuation and expansion of field surveillance studies among different bat populations to eventually identify the true natural reservoir of SARS-CoV.

Hsieh, W. Y., et al. (2012). "ACE/ACE2 ratio and MMP-9 activity as potential biomarkers in tuberculous pleural effusions." Int J Biol Sci **8**(8): 1197-1205.

OBJECTIVE: Pleural effusion is common problem, but the rapid and reliable diagnosis for specific pathogenic effusions are lacking. This study aimed to identify the diagnosis based on clinical variables to differentiate pleural tuberculous exudates from other pleural effusions. We also investigated the role of renin-angiotensin system (RAS) and matrix metalloproteinase (MMPs) in the pathogenesis of pleural exudates. EXPERIMENTAL DESIGN: The major components in RAS and extracellular matrix metabolism, including angiotensin converting enzyme (ACE), ACE2, MMP-2 and MMP-9 activities, were measured and compared in the patients with transudative (n = 45) and exudative (n = 80) effusions. The exudative effusions were come from the patients with tuberculosis (n = 20), pneumonia (n = 32), and adenocarcinoma (n = 28). RESULTS: Increased ACE and equivalent ACE2 activities, resulting in a significantly increased ACE/ACE2 ratio in exudates, were detected compared to these values in transudates. MMP-9 activity in exudates was significantly higher than that in transudates. The significant correlation between ACE and ACE2 activity that was found in transudates was not found in exudates. Advanced analyses showed significantly increased ACE and MMP-9 activities, and decreased ACE2 activity in tuberculous pleural effusions compared with those in pneumonia and adenocarcinoma effusions. The results indicate that increased ACE and MMP-9 activities found in the exudates were mainly contributed from a higher level of both enzyme activities in the tuberculous pleural effusions. CONCLUSION: Interplay between ACE and ACE2, essential functions in the RAS, and abnormal regulation of MMP-9 probably play a pivotal role in the development of exudative effusions. Moreover, the ACE/ACE2 ratio combined with MMP-9 activity in pleural fluid may be potential biomarkers for diagnosing tuberculous pleurisy.

Hu, Q., et al. (2017). "BML-111 equilibrated ACE-AngII-AT1R and ACE2-Ang-(1-7)-Mas axis to protect hepatic fibrosis in rats." Prostaglandins Other Lipid Mediat **131**: 75-82.

BACKGROUND: It was recently reported Lipoxins (LXs) had protective effects on fibrous diseases, and renin-angiotensin-aldosterone system (RAAS) had played vital and bidirectional roles in hepatic fibrosis. In this paper, a hepatic fibrosis model, induced by carbon tetrachloride (CCL4) in rats, was used to observe the relations between RAAS and LXs, as well as to further explore the alternative anti-fibrosis mechanisms of LXs. METHODS: The model was evaluated by morphological observations and biochemical assays. The activities and contents of angiotensin converting enzyme (ACE) and angiotensin converting enzyme 2 (ACE2) were examined through assay kits and ELISA. The expression levels of angiotensinII (AngII), Angiotensin II type 1 receptor (AT1R), angiotensin-(1-7) (Ang-1-7), and Mas were all measured using real time PCR, ELISA, and Western blot. RESULTS: The model was established successfully and BML-111 significantly ameliorated CCL4-induced hepatic fibrosis, including reduction inflammation injury, decrease extracellular matrix deposition, and improvement hepatic functions. Furthermore, BML-111 could obviously decrease not only the activities of ACE but also the expression levels of ACE, AngII,and AT1R, which were induced by CCL4. On the other hand, BML-111 could markedly increase the activities of ACE2, besides the expression levels of ACE2, Ang-(1-7) and Mas. More importantly, BOC-2, a lipoxin A4 receptor blocker, could reverse all these phenomena. CONCLUSIONS: Equilibrating ACE-AngII-AT1R axis and ACE2-Ang-(1-7)-Mas axis mediated the protective effect of BML-111 on hepatic fibrosis in rats.

Hu, Y. Y., et al. (2010). "[Effect of irbesartan on ACE2 expression in diabetic rat myocardium]." Nan Fang Yi Ke Da Xue Xue Bao **30**(6): 1336-1338.

OBJECTIVE: To explore the effect of irbesartan on angiotensin-converting enzyme 2 (ACE2) mRNA expression in diabetic rat myocardium. METHODS: Thirty 8-week-old male Wistar rats were randomly divided into control group (n=7), diabetic model group (n=14) and irbesartan group (n=9). Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg), a blood glucose>16.7 mmol/L 72 h after the injection indicated successful establishment of diabetes. Four weeks after the modeling, the rats in irbesartan group were given 50 mg/kg irbesartan. ELISA was used to measure myocardial AngII content in the rats, and myocardial ACE2 mRNA expression was determined by real-time PCR. RESULTS:

Myocardial AngII level in the diabetic model group was significantly higher than that in the control group (P<0.001). Irbesartan administration significantly lowered cardiac AngII levels in the diabetic rats (P<0.001). The rats in irbesartan group showed significantly increased myocardial ACE2 mRNA expression compared with those in the control and diabetic rat groups (P<0.05). CONCLUSION: Irbesartan can increase ACE2 mRNA expression in the myocardium, which might be one of the mechanisms underlying its effect in improving the cardiac function in diabetic rats.

Huang, I. C., et al. (2006). "SARS coronavirus, but not human coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells." J Biol Chem **281**(6): 3198-3203.

Viruses require specific cellular receptors to infect their target cells. Angiotensin-converting enzyme 2 (ACE2) is a cellular receptor for two divergent coronaviruses, SARS coronavirus (SARS-CoV) and human coronavirus NL63 (HCoV-NL63). In addition to hostcell receptors, lysosomal cysteine proteases are required for productive infection by some viruses. Here we show that SARS-CoV, but not HCoV-NL63, utilizes the enzymatic activity of the cysteine protease cathepsin L to infect ACE2-expressing cells. Inhibitors of cathepsin L blocked infection by SARS-CoV and by a retrovirus pseudotyped with the SARS-CoV spike (S) protein but not infection by HCoV-NL63 or a retrovirus pseudotyped with the HCoV-NL63 S protein. Expression of exogenous cathepsin L substantially enhanced infection mediated by the SARS-CoV S protein and by filovirus GP proteins but not by the HCoV-NL63 S protein or the vesicular stomatitis virus G protein. Finally, an inhibitor of endosomal acidification had substantially less effect on infection mediated by the HCoV-NL63 S protein than on that mediated by the SARS-CoV S protein. Our data indicate that two coronaviruses that utilize a common receptor nonetheless enter cells through distinct mechanisms.

Huang, J., et al. (2012). "Polymorphisms of ACE2 are associated with blood pressure response to cold pressor test: the GenSalt study." Am J Hypertens **25**(8): 937-942.

BACKGROUND: Increased blood pressure (BP) reactivity to cold pressor test (CPT) is a risk factor for hypertension. Genetic factors may influence the variation of BP response to CPT among individuals. We explored the association of genetic variants in the apelin system genes (APLN, APLNR and ACE2) and BP response to CPT in a Chinese population. METHODS: A total of 1,998 Han Chinese participants from the Genetic Epidemiology Network of Salt Sensitivity completed a CPT. The percentage changes of BP right after the end of ice-water immersion were used as the measurement of BP responses to CPT. Twenty-two single nucleotide polymorphisms (SNPs) were selected and genotyped, including both tag and potential functional SNPs of the APLN, APLNR, and ACE2 genes. A mixed-effect linear model was used to assess the association between SNPs and BP responses to CPT. RESULTS: In women, three SNPs (rs1514283, rs4646176, and rs879922) of the ACE2 gene were significantly associated with the diastolic BP (DBP) response to CPT in the general and recessive genetic models after adjustment for multiple testing (all false discovery rate $q < 0.05$). There were no significant associations of polymorphisms in APLN and APLNR genes with BP responses to CPT. CONCLUSIONS: Our study identified genetic variants in the ACE2 gene that were significantly associated with DBP responses to cold stress in the Chinese female population. Future studies are warranted to confirm these findings.

Huang, W. K., et al. (2016). "Mutations in Acetylcholinesterase2 (ace2) increase the insensitivity of acetylcholinesterase to fosthiazate in the root-knot nematode Meloidogyne incognita." Sci Rep **6**: 38102.

The root-knot nematode Meloidogyne incognita causes severe damage to continuously cropping vegetables. The control of this nematode relies heavily on organophosphate nematicides in China. Here, we described resistance to the organophosphate nematicide fosthiazate in a greenhouse-collected resistant population (RP) and a laboratory susceptible population (SP) of M. incognita. Fosthiazate was 2.74-fold less toxic to nematodes from RP than that from SP. Quantitative real-time PCR revealed that the acetylcholinesterase2 (ace2) transcription level in the RP was significantly higher than that in the SP. Eighteen nonsynonymous amino acid differences in ace2 were observed between the cDNA fragments of the RP and SP. The acetylcholinesterase (AChE) protein activity in the RP was significantly reduced compared with that in the SP. After knocking down the ace2 gene, the ace2 transcription level was significantly decreased, but no negative impact on the infection of juveniles was observed. The 50% lethal concentration of the RNAi RP population decreased 40%, but the inhibition rate of fosthiazate against AChE activity was significantly increased in RP population. Thus, the increased fosthiazate insensitivity in the M. incognita resistant population was strongly associated with mutations in ace2. These results provide valuable insights into the resistance mechanism of root-knot nematode to organophosphate nematicides.

Huentelman, M. J., et al. (2005). "Protection from angiotensin II-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ACE2 in rats." Exp Physiol **90**(5): 783-790.

Angiotensin converting enzyme 2 (ACE2), a newly discovered member of the renin-angiotensin system (RAS), is a potential therapeutic target for the control of cardiovascular disease owing to its key role in the formation of vasoprotective peptides from angiotensin II. The aim of the present study was to evaluate whether overexpression of ACE2 could protect the heart from angiotensin II-induced hypertrophy and fibrosis. Lentiviral vector encoding mouse ACE2 (lenti-mACE2) or GFP was injected intracardially in 5-day-old Sprague-Dawley rats. This resulted in expression of mACE2 in cardiac tissue for the duration of the study. Infusion of 200 ng kg-1 min-1 angiotensin II for 4 weeks resulted in an 80 mmHg increase in systolic blood pressure, a significant increase in the heart weight to body weight ratio (HW:BW), and marked myocardial fibrosis in control rats. Transduction with lenti-mACE2 resulted in significant attenuation of the increased HW:BW and myocardial fibrosis induced by angiotensin II infusion. These observations demonstrate that ACE2 overexpression results in protective effects on angiotensin II-induced cardiac hypertrophy and fibrosis.

Hui, X. M., et al. (2011). "RNA interference of ace1 and ace2 in Chilo suppressalis reveals their different contributions to motor ability and larval growth." Insect Mol Biol **20**(4): 507-518.

Acetylcholinesterase (AChE, EC 3.1.1.7) is a key enzyme in terminating synaptic transmission. We knocked down the expression of Csace1 or Csace2 using chemically synthesized small interfering RNAs (siRNAs) designed from divergent regions. The mRNA abundance of the two ace genes was reduced to 50-70% of control levels. The enzyme activities were decreased to 40-70%. Silencing of Csace1 or Csace2 resulted in a ~25% mortality rate. Knockdown of Csace1 had major effects on larval growth inhibition and resulted in reduced larval weight and length, malformation and motor disability, whereas silencing of Csace2 had only minor effects. These results suggested that both AChE-1 and AChE-2 have important roles in maintaining life in this insect and indicated that AChE-1 might have nontypical functions in regulating larval growth and motor ability.

Hung, Y. H., et al. (2016). "Alternative Roles of STAT3 and MAPK Signaling Pathways in the MMPs Activation and Progression of Lung Injury Induced by Cigarette Smoke Exposure in ACE2 Knockout Mice." Int J Biol Sci **12**(4): 454-465.

Inflammation-mediated abnormalities in the renin-angiotensin system (RAS) and expression of matrix metalloproteinases (MMPs) are implicated in the pathogenesis of lung injury. Angiotensin converting enzyme II (ACE2), an angiotensin converting enzyme (ACE) homologue that displays antagonist effects on ACE/angiotensin II (Ang II) axis, could also play a protective role against lung diseases. However, the relationship between ACE2 and MMPs activation in lung injury is still largely unclear. The purpose of this study is to investigate whether MMPs activity could be affected by ACE2 and which ACE2 derived signaling pathways could be also involved via using a mouse model with lung injury induced by cigarette smoke (CS) exposure for 1 to 3 weeks. Wild-type (WT; C57BL/6) and ACE2 KO mice (ACE2(-/-)) were utilized to study CS-induced lung injury. Increases in the resting respiratory rate (RRR), pulmonary immunokines, leukocyte infiltration and bronchial hyperplasia were observed in the CS-exposed mice. Compared to WT mice, more serious physiopathological changes were found in ACE2(-/-) mice in the first week of CS exposure. CS exposure increased pulmonary ACE and ACE2 activities in WT mice, and significantly increased ACE in ACE2(-/-) mice. Furthermore, the activity of pulmonary MMPs was decreased in CS-exposed WT mice, whereas this activity was increased in ACE2(-/-) mice. CS exposure increased the pulmonary p-p38, p-JNK and p-ERK1/2 level in all mice. In ACE2(-/-) mice, a significant increase p-STAT3 signaling was detected; however, no effect was observed on the p-STAT3 level in WT mice. Our results support the hypothesis that ACE2 deficiency influences MMPs activation and STAT3 phosphorylation signaling to promote more pulmonary inflammation in the development of lung injury.

Ibrahim, H. S., et al. (2014). "ACE2 activation by xanthenone prevents leptin-induced increases in blood pressure and proteinuria during pregnancy in Sprague-Dawley rats." Reprod Toxicol **49**: 155-161.

This study investigates the effect of ACE2 activation on leptin-induced changes in systolic blood pressure (SBP), proteinuria, endothelial activation and ACE2 expression during pregnancy in Sprague-Dawley rats. Pregnant rats were given subcutaneous injection of either saline, or leptin, or leptin plus xanthenone (ACE2 activator), or xanthenone (XTN) alone. SBP, serum ACE, ACE2, endothelin-1, E-selectin and ICAM-1 levels were estimated; also their gene expressions were determined in the kidney and aorta respectively. Compared to control, SBP was higher in the leptin-only treated group ($P<0.001$) and lower in rats treated with xanthenone alone ($P<0.01$). Proteinuria, markers of endothelial activation were significantly higher than controls in leptin-only treated

rats (P<0.05). ACE2 activity and expression were lower in leptin-only treated rats when compared to controls (P<0.05). It seems, leptin administration during pregnancy significantly increases SBP, proteinuria, endothelial activation, but decreases ACE2 level and expression. These effects are prevented by concurrent administration of xanthenone.

Igase, M., et al. (2005). "Angiotensin II AT1 receptors regulate ACE2 and angiotensin-(1-7) expression in the aorta of spontaneously hypertensive rats." Am J Physiol Heart Circ Physiol **289**(3): H1013-1019.

When increased in vascular tissues, angiotensin-converting enzyme 2 (ACE2), a carboxypeptidase that hydrolyzes angiotensin II to angiotensin-(1-7), may augment the growth inhibitory and vasodilatory effects of the heptapeptide. We investigated the regulation of ACE2 and angiotensin-(1-7) expression in aortas and carotid arteries of 12-wk-old male spontaneously hypertensive rats (SHR) by determining the effect of sustained angiotensin type 1 (AT (1)) receptor blockade with olmesartan (10 mg.kg (-1).day (-1), n = 13) compared with those that received atenolol (30 mg.kg (-1).day (-1), n = 13), hydralazine (10 mg.kg (-1).day (-1), n = 13), or vehicle (n = 21). Systolic blood pressures were approximately 30% lower (P < 0.05) in rats treated for 2 wk with olmesartan compared with vehicle-treated rats. Both atenolol and hydralazine produced similar decreases in systolic blood pressure. ACE2 mRNA in the thoracic aorta of olmesartan-treated rats (n = 8) was fivefold greater (P < 0.05) than that in vehicle-treated rats (n = 16), whereas atenolol (n = 8) or hydralazine (n = 8) had no effect. Immunostaining intensities in rats treated with olmesartan (n = 5) were also associated with increased (P < 0.05) ACE2 and angiotensin-(1-7) in thoracic aorta media compared with vehicle-treated rats. In contrast, immunostaining intensities for both ACE2 and angiotensin-(1-7) were not different from vehicle (n = 5) in carotid arteries of SHR medicated with either atenolol (n = 5) or hydralazine (n = 5). A comparison of vessel wall dimensions showed that olmesartan selectively reduced the thoracic aorta media-to-lumen ratio (P < 0.05) and media thickness (P < 0.05) without an effect on carotid artery morphometry. Compared with vehicle-treated SHR, vascular hypertrophy determined from media and lumen measurements was not changed in SHR given either atenolol or hydralazine. These data represent the first report of ACE2 and angiotensin-(1-7) expression in the aorta and carotid arteries of SHR. Increased ACE2 and angiotensin-(1-7) in association with altered dimensions of the thoracic aorta but not carotid arteries in response to olmesartan treatment provides evidence that this pathway is regulated by AT (1) receptors and may be important in mediating the pressure-independent vascular remodeling effects of angiotensin peptides.

Imai, Y., et al. (2010). "Angiotensin-converting enzyme 2 (ACE2) in disease pathogenesis." Circ J **74**(3): 405-410.

Angiotensin-converting enzyme 2 (ACE2), a first homolog of ACE, regulates the renin-angiotensin system by counterbalancing ACE activity. Accumulating evidence in recent years has demonstrated a physiological and pathological role of ACE2 in the cardiovascular, renal and respiratory systems. For instance, in the acute respiratory distress syndrome (ARDS), ACE, AngII, and AT1R promote the disease pathogenesis, whereas ACE2 and the AT2R protect from ARDS. Importantly, ACE2 has been identified as a key SARS-coronavirus receptor and plays a protective role in SARS pathogenesis. Furthermore, the recent explosion of research into the ACE2 homolog, collectrin, has revealed a new physiological function of ACE2 as an amino acid transporter, which explains the pathogenic role of gene mutations in Hartnup disorder. This review summarizes and discusses the recently unveiled roles for ACE2 in disease pathogenesis.

Imai, Y., et al. (2008). "[Lessons from SARS: a new potential therapy for acute respiratory distress syndrome (ARDS) with angiotensin converting enzyme 2 (ACE2)]." Masui **57**(3): 302-310.

During several months of 2002, severe acute respiratory syndrome (SARS) caused by SARS-coronavirus (SARS-CoV) spread rapidly from China throughout the world causing more than 800 deaths due to the development of acute respiratory distress syndrome (ARDS). Interestingly, a novel homologue of angiotensin converting-enzyme (ACE), termed angiotensin converting enzyme 2 (ACE2) has been identified as a receptor for SARS-CoV. ACE and ACE2 share homology in their catalytic domain and provide different key functions in the renin-angiotensin system. ACE cleaves angiotensin I to generate angiotensin II that is a key effector peptide of the system and exerts multiple biological functions, whereas ACE2 reduces angiotensin II levels and thus is a negative regulator of the system. Importantly, our recent studies using ACE2 knockout mice have demonstrated that ACE2 protects murine lungs from ARDS. Furthermore, SARS-CoV infections and the Spike protein of the SARS-CoV reduce ACE2 expression. Notably, injection of SARS-CoV Spike into mice worsens acute lung failure in vivo that can be attenuated by blocking the renin-angiotensin pathway, suggesting the activation of pulmonary RAS influences the pathogenesis of ARDS and SARS.

Inoue, Y., et al. (2007). "Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted." J Virol **81**(16): 8722-8729.

The penetration of various viruses into host cells is accomplished by hijacking the host endocytosis machinery. In the case of severe acute respiratory syndrome coronavirus (SARS-CoV) infection, viral entry is reported to require a low pH in intracytoplasmic vesicles; however, little is known about how SARS-CoV invades such compartments. Here we demonstrate that SARS-CoV mainly utilizes the clathrin-mediated endocytosis pathway for its entry to target cells by using infectious SARS-CoV, as well as a SARS-CoV pseudovirus packaged in the SARS-CoV envelope. The SARS-CoV entered caveolin-1-negative HepG2 cells, and the entry was significantly inhibited by treatment with chlorpromazine, an inhibitor for clathrin-dependent endocytosis, and by small interfering RNA-mediated gene silencing for the clathrin heavy chain. Furthermore, the SARS-CoV entered COS7 cells transfected with the mutant of ACE2 with the cytoplasmic tail deleted, SARS-CoV receptor, as well as the wild-type ACE2, and their entries were significantly inhibited by treatment with chlorpromazine. In addition, ACE2 translocated into EEA1-positive early endosomes immediately after the virus attachment to ACE2. These results suggest that when SARS-CoV binds ACE2 it is internalized and penetrates early endosomes in a clathrin-dependent manner and that the cytoplasmic tail of ACE2 is not required for the penetration of SARS-CoV.

Iwai, M. and M. Horiuchi (2009). "Devil and angel in the renin-angiotensin system: ACE-angiotensin II-AT1 receptor axis vs. ACE2-angiotensin-(1-7)-Mas receptor axis." Hypertens Res **32**(7): 533-536.

Recent studies have established a new regulatory axis in the renin-angiotensin system (RAS). In this axis, angiotensin (Ang)-(1-7) is finally produced from Ang I or Ang II by the catalytic activity of angiotensin-converting enzyme 2 (ACE2). Ang-(1-7) shows actions different from those of AT (1) receptor stimulation, such as vasodilatation, natriuresis, anti-proliferation and an increase in the bradykinin-NO (nitric oxide) system. As the catalytic efficiency of ACE2 is approximately 400-fold higher with Ang II as a substrate than with Ang I, this axis is possibly acting as a counter-regulatory system against the ACE/Ang II/AT (1) receptor axis. The signaling pathway of the ACE2-Ang-(1-7) axis has not yet been totally and clearly understood. However, a recent report suggests that the Mas oncogene acts as a receptor for Ang-(1-7). Intracellular signaling through Mas is not clear yet. Several factors such as Akt phosphorylation, protein kinase C activation and mitogen-activated protein (MAP) kinase inhibition seem to be involved in this signaling pathway. Further investigations are needed to clarify the regulation and mechanism of action of ACE2 and Ang-(1-7). However, this second axis through ACE2 and Ang-(1-7) in RAS can be an important target for the therapy of cardiovascular and metabolic disorders.

Iwata, M., et al. (2011). "Targeting the ACE2-Ang-(1-7) pathway in cardiac fibroblasts to treat cardiac remodeling and heart failure." J Mol Cell Cardiol **51**(4): 542-547.

Fibroblasts play a pivotal role in cardiac remodeling and the development of heart failure through the deposition of extra-cellular matrix (ECM) proteins and also by affecting cardiomyocyte growth and function. The renin-angiotensin system (RAS) is a key regulator of the cardiovascular system in health and disease and many of its effects involve cardiac fibroblasts. Levels of angiotensin II (Ang II), the main effector molecule of the RAS, are elevated in the failing heart and there is a substantial body of evidence indicating that this peptide contributes to changes in cardiac structure and function which ultimately lead to progressive worsening in heart failure. A pathway involving angiotensin converting enzyme 2 (ACE2) has the capacity to break down Ang II while generating angiotensin-(1-7) (Ang-(1-7)), a heptapeptide, which in contrast to Ang II, has cardioprotective and anti-remodeling effects. Many Ang-(1-7) actions involve cardiac fibroblasts and there is information indicating that it reduces collagen production and also may protect against cardiac hypertrophy. This report describes the effects of ACE2 and Ang-(1-7) that appear to be relevant in cardiac remodeling and heart failure and explores potential therapeutic strategies designed to increase ACE2 activity and Ang-(1-7) levels to treat these conditions. This article is part of a special issue entitled "Key Signaling Molecules in Hypertrophy and Heart Failure."

Jarajapu, Y. P., et al. (2013). "Activation of the ACE2/angiotensin-(1-7)/Mas receptor axis enhances the reparative function of dysfunctional diabetic endothelial progenitors." Diabetes **62**(4): 1258-1269.

We tested the hypothesis that activation of the protective arm of the renin angiotensin system, the angiotensin-converting enzyme 2 (ACE2)/angiotensin-(1-7) [Ang-(1-7)]/Mas receptor axis, corrects the vasoreparative dysfunction typically seen in the CD34(+) cells isolated from diabetic individuals. Peripheral blood CD34(+) cells from patients with diabetes were compared with those of nondiabetic

controls. Ang-(1-7) restored impaired migration and nitric oxide bioavailability/cGMP in response to stromal cell-derived factor and resulted in a decrease in NADPH oxidase activity. The survival and proliferation of CD34(+) cells from diabetic individuals were enhanced by Ang-(1-7) in a Mas/phosphatidylinositol 3-kinase (PI3K)/Akt-dependent manner. ACE2 expression was lower, and ACE2 activators xanthenone and diminazine aceturate were less effective in inducing the migration in cells from patients with diabetes compared with controls. Ang-(1-7) overexpression by lentiviral gene modification restored both the in vitro vasoreparative functions of diabetic cells and the in vivo homing efficiency to areas of ischemia. A cohort of patients who remained free of microvascular complications despite having a history of longstanding inadequate glycemic control had higher expression of ACE2/Mas mRNA than patients with diabetes with microvascular complications matched for age, sex, and glycemic control. Thus, ACE2/Ang-(1-7)\Mas pathway activation corrects existing diabetes-induced CD34(+) cell dysfunction and also confers protection from development of this dysfunction.

Jia, H. (2016). "Pulmonary Angiotensin-Converting Enzyme 2 (ACE2) and Inflammatory Lung Disease." Shock **46**(3): 239-248.

In response to infectious and, in some instances, noninfectious insults, the affected tissues/cells of the host undergo inflammation. However, uncontrolled inflammation could be detrimental to the host, resulting in inflammatory disease, such as inflammatory lung disease. Although the etiology of the disease is well defined, the underling pathogenesis is still incompletely understood. The renin-angiotensin system (RAS), one of the primary cardiovascular regulatory systems, has been proposed to be involved in the pathogenesis of inflammatory lung disease. In particular, the RAS has been implicated as advances in the understanding of the multifunctionality of individual components of the system have been made, and by the fact that the RAS acts not only systemically, but also locally in a variety of tissues, including the lung. Angiotensin-converting enzyme 2 (ACE2), a relatively new member of the RAS, has drawn extensive attention since 2003, because of the findings that ACE2 is the receptor for SARS Corona virus and that maintenance of normal ACE2 levels in the lung is beneficial for the host to combat inflammatory lung disease. Nevertheless, the mechanism through which ACE2 plays a role in inflammatory lung disease has not been clearly identified. In an attempt to summarize current literature findings and progress made in uncovering the role of ACE2 in inflammatory lung disease, this review will focus on recent studies examining pulmonary ACE2 biology, its roles in inflammatory lung disease pathogenesis and possible underlying mechanisms. Finally, we will discuss pulmonary ACE2 as a potential therapeutic target for inflammatory lung disease.

Jia, H. P., et al. (2005). "ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia." J Virol **79**(23): 14614-14621.

Studies of patients with severe acute respiratory syndrome (SARS) demonstrate that the respiratory tract is a major site of SARS-coronavirus (CoV) infection and disease morbidity. We studied host-pathogen interactions using native lung tissue and a model of well-differentiated cultures of primary human airway epithelia. Angiotensin converting enzyme 2 (ACE2), the receptor for both the SARS-CoV and the related human respiratory coronavirus NL63, was expressed in human airway epithelia as well as lung parenchyma. As assessed by immunofluorescence staining and membrane biotinylation, ACE2 protein was more abundantly expressed on the apical than the basolateral surface of polarized airway epithelia. Interestingly, ACE2 expression positively correlated with the differentiation state of epithelia. Undifferentiated cells expressing little ACE2 were poorly infected with SARS-CoV, while well-differentiated cells expressing more ACE2 were readily infected. Expression of ACE2 in poorly differentiated epithelia facilitated SARS spike (S) protein-pseudotyped virus entry. Consistent with the expression pattern of ACE2, the entry of SARS-CoV or a lentivirus pseudotyped with SARS-CoV S protein in differentiated epithelia was more efficient when applied to the apical surface. Furthermore, SARS-CoV replicated in polarized epithelia and preferentially exited via the apical surface. The results indicate that infection of human airway epithelia by SARS coronavirus correlates with the state of cell differentiation and ACE2 expression and localization. These findings have implications for understanding disease pathogenesis associated with SARS-CoV and NL63 infections.

Jiang, T., et al. (2013). "ACE2-Ang-(1-7)-Mas Axis in Brain: A Potential Target for Prevention and Treatment of Ischemic Stroke." Curr Neuropharmacol **11**(2): 209-217.

The renin-angiotensin system (RAS) in brain is a crucial regulator for physiological homeostasis and diseases of cerebrovascular system, such as ischemic stroke. Overactivation of brain Angiotensin-converting enzyme (ACE) - Angiotensin II (Ang II) - Angiotensin II type 1 receptor (AT1R) axis was found to be involved in the progress of hypertension,

atherosclerosis and thrombogenesis, which increased the susceptibility to ischemic stroke. Besides, brain Ang II levels have been revealed to be increased in ischemic tissues after stroke, and contribute to neural damage through elevating oxidative stress levels and inducing inflammatory response in the ischemic hemisphere via AT1R. In recent years, new components of RAS have been discovered, including ACE2, Angiotensin-(1-7) [Ang-(1-7)] and Mas, which constitute ACE2-Ang-(1-7)-Mas axis. ACE2 converts Ang II to Ang-(1-7), and Ang-(1-7) binds with its receptor Mas, exerting benefical effects in cerebrovascular disease. Through interacting with nitric oxide and bradykinin, Ang-(1-7) could attenuate the development of hypertension and the pathologic progress of atherosclerosis. Besides, its antithrombotic activity also prevents thrombogenic events, which may contribute to reduce the risk of ischemic stroke. In addition, after ischemia insult, ACE2-Ang-(1-7)-Mas has been shown to reduce the cerebral infarct size and improve neurological deficits through its antioxidative and anti-inflammatory effects. Taken together, activation of the ACE2-Ang-(1-7)-Mas axis may become a novel therapeutic target in prevention and treatment of ischemia stroke, which deserves further investigations.

Jin, H. Y., et al. (2012). "ACE2 deficiency enhances angiotensin II-mediated aortic profilin-1 expression, inflammation and peroxynitrite production." PLoS One **7**(6): e38502.

Inflammation and oxidative stress play a crucial role in angiotensin (Ang) II-mediated vascular injury. Angiotensin-converting enzyme 2 (ACE2) has recently been identified as a specific Ang II-degrading enzyme but its role in vascular biology remains elusive. We hypothesized that loss of ACE2 would facilitate Ang II-mediated vascular inflammation and peroxynitrite production. 10-week wildtype (WT, Ace2(+/y)) and ACE2 knockout (ACE2KO, Ace2(-/y)) mice received with mini-osmotic pumps with Ang II (1.5 mg.kg (-) (1).d (-) (1)) or saline for 2 weeks. Aortic ACE2 protein was obviously reduced in WT mice in response to Ang II related to increases in profilin-1 protein and plasma levels of Ang II and Ang-(1-7). Loss of ACE2 resulted in greater increases in Ang II-induced mRNA expressions of inflammatory cytokines monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-1beta, and IL-6 without affecting tumor necrosis factor-alpha in aortas of ACE2KO mice. Furthermore, ACE2 deficiency led to greater increases in Ang II-mediated profilin-1 expression, NADPH oxidase activity, and superoxide and peroxynitrite production in the aortas of ACE2KO mice associated with enhanced phosphorylated levels of Akt, p70S6 kinase, extracellular signal-regulated kinases (ERK1/2) and endothelial nitric oxide synthase (eNOS). Interestingly, daily treatment with AT1 receptor blocker irbesartan (50 mg/kg) significantly prevented Ang II-mediated aortic profilin-1 expression, inflammation, and peroxynitrite production in WT mice with enhanced ACE2 levels and the suppression of the Akt-ERK-eNOS signaling pathways. Our findings reveal that ACE2 deficiency worsens Ang II-mediated aortic inflammation and peroxynitrite production associated with the augmentation of profilin-1 expression and the activation of the Akt-ERK-eNOS signaling, suggesting potential therapeutic approaches by enhancing ACE2 action for patients with vascular diseases.

Jin, X. Q., et al. (2011). "[Effect of ACE2 gene transfection on the proliferation of vascular smooth muscle cells in rats]." Zhonghua Yi Xue Za Zhi **91**(2): 125-128.

OBJECTIVE: To investigate the effect of AngII on the proliferation of vascular smooth muscle cell (VSMC) in rats after the transfection of ACE2 gene. METHODS: pm-ACE2 was transfected into the cultured VSMC by Lipofectamine 2000. The normal cell group, AngIIgroup and pcDNA3.1/Hygro (+) transfected + AngII group were taken as controls respectively. After the transfection of ACE2 gene, the cell proliferative effect of AngII on VSMC was investigated by cell counting kit-8 (CCK8) and cell cycle detection by fluorescence activated cell sorter (FACS). RESULTS: The (optical density) OD value of AngIIgroup was obviously higher than that of other groups. And it was obviously lower in the pm-ACE2 + AngII group than the AngII group (0.535 +/- 0.004 vs 0.866 +/- 0.026, $P < 0.05$). Compared with other groups, the G (0)/G (1) stage percentage of VSMC was obviously lower in the AngII group (58.80% +/- 2.00%, $P < 0.05$) while the percentage of S stage was obviously higher (35.90% +/- 1.00%, $P < 0.05$). Compared with the AngII group, the G (0)/G (1) stage percentage of VSMC was obviously higher (63.90% +/- 1.40%, $P < 0.05$) in the pm-ACE2 + AngII group while the percentage of S stage was obviously lower (27.80% +/- 0.46%, $P < 0.05$). CONCLUSION: The over-expression of ACE2 gene can inhibit the proliferation of AngII-induced VSMC.

Johnson, J. A., et al. (2011). "ACE2 improves right ventricular function in a pressure overload model." PLoS One **6**(6): e20828.

BACKGROUND: Right ventricular (RV) dysfunction is a complication of pulmonary hypertension and portends a poor prognosis. Pharmacological therapies targeting RV function in pulmonary hypertension may reduce symptoms, improve hemodynamics, and potentially increase survival. We hypothesize that recombinant human

angiotensin-converting enzyme 2 (rhACE2) will improve RV function in a pressure overload model. RESULTS: rhACE2 administered at 1.8 mg/kg/day improved RV systolic and diastolic function in pulmonary artery banded mice as measured by in vivo hemodynamics. Specifically, rhACE2 increased RV ejection fraction and decreased RV end diastolic pressure and diastolic time constant ($p<0.05$). In addition, rhACE2 decreased RV hypertrophy as measured by RV/LV+S ratio ($p<0.05$). There were no significant negative effects of rhACE2 administration on LV function. rhACE2 had no significant effect on fibrosis as measured by trichrome staining and collagen1alpha1 expression. In pulmonary artery banded mice, rhACE2 increased Mas receptor expression and normalized connexin 37 expression. CONCLUSION: In a mouse RV load-stress model of early heart failure, rhACE2 diminished RV hypertrophy and improved RV systolic and diastolic function in association with a marker of intercellular communication. rhACE2 may be a novel treatment for RV failure.

Kalea, A. Z. and D. Batlle (2010). "Apelin and ACE2 in cardiovascular disease." Curr Opin Investig Drugs **11**(3): 273-282.

Apelin is a peptide that has been identified as the endogenous ligand for the receptor APJ. The apelin/APJ system may be an important factor in the regulation of vascular tone and cardiovascular function. Studies on cultured cells and small animal models have revealed that apelin and APJ are localized in cardiomyocytes and vascular cells. The infusion of apelin affects vascular tone and blood pressure, with both central and peripheral actions. In clinical conditions such as heart failure and atherosclerosis, the gene expression of APJ and apelin, as well as the levels of circulating apelin, may be altered. The only known active homolog of ACE, ACE2, hydrolyzes apelin with similar potency to angiotensin II and, therefore, is responsible for the degradation of both peptides. Emerging data on a potential interaction between the two pathways suggest that the function of apelin/APJ in the vasculature may be relevant to cardiovascular disease, and identifying how this system is regulated could be useful clinically.

Kamel, A. S., et al. (2018). "Stimulation of ACE2/ANG (1-7)/Mas Axis by Diminazene Ameliorates Alzheimer's Disease in the D-Galactose-Ovariectomized Rat Model: Role of PI3K/Akt Pathway." Mol Neurobiol **55**(10): 8188-8202.

Overactivation of angiotensin-converting enzyme/angiotensin 2/angiotensin receptor-1 (ACE/Ang2/AT1) axis provokes amyloid-beta-induced apoptosis and neurodegeneration in Alzheimer's disease (AD). Moreover, activation of AT1 impairs the survival pathway phosphoinositide 3-kinase/protein kinase B (PI3K/Akt). Interestingly, the coupling between ACE2/Ang (1-7)/Mas receptor (MasR) axis and PI3K/Akt activation opposes AT1-induced apoptosis. However, the effect of in vivo stimulation of MasR against AD and its correlation to PI3K/Akt is not yet elucidated. Thus, the present study aimed to investigate the relationship between PI3K/Akt pathway and the activation of ACE2/MasR in the AD model of D-galactose-ovariectomized rats. AD features were induced following 8-week injection of D-galactose (150 mg/kg, i.p.) in ovariectomized female rats. The ACE2 activator dimenazine (15 mg/kg, i.p.) was daily administered for 2 months. DIZE administration boosted the hippocampal expression of ACE2 and Mas receptors while suppressing AT1 receptor. Notably, dimenazine enhanced the expression of phosphorylated survival factors (PI3K, Akt, signal transducer, and activator of transcription-3) and neuroplasticity proteins such as cyclic adenosine monophosphate-responsive element-binding protein and brain-derived neurotrophic factor along with nicotinic and glutamatergic receptors. Such effects were accompanied by suppressing phosphorylated tau and glycogen synthase kinase3beta along with caspase-3, cytochrome-c, nuclear factor kappa B, tumor necrosis factor alpha, and glial fibrillary acidic protein contents. Dimenazine ameliorated the histopathological damage observed in D-galactose-ovariectomized rats and improved their learning and recognition memory in Morris water maze and novel object recognition tests. In conclusion, dimenazine-induced stimulation of ACE2/Ang (1-7)/Mas axis subdues cognitive deficits in AD most probably through activation of PI3K/Akt pathway.

Kamilic, J., et al. (2010). "Renal ACE2 expression and activity is unaltered during established hypertension in adult SHRSP and TGR (mREN2)27." Hypertens Res **33**(2): 123-128.

Differential renal expression of a homolog of the angiotensin-converting enzyme (ACE), that is, ACE2, has been implicated as a genetic basis of polygenetic hypertension in the stroke-prone spontaneously hypertensive rat model. However, data on the role of ACE2 in hypertension are still inconclusive. Therefore, we analyzed kidney ACE2 mRNA, ACE2 protein and ACE2 enzyme activities in the adult polygenetic stroke-prone spontaneously hypertensive rat (SHRSP) and the monogenetic TGR (mREN2)27 rat models, in comparison with their normotensive reference strains, Wistar-Kyoto (WKY) and Spraque-Dawley (SD) rats, respectively. Kidney ACE2 mRNA was studied using quantitative real-time reverse transcriptase-PCR (RT-PCR) in cortex and medulla, whereas protein

expression was scored semiquantitatively in detail in different renal structures using immunohistochemistry. Furthermore, total renal tissue ACE2 activity was measured using a fluorimetric assay that was specified by the ACE2 inhibitor DX600. In SHRSP and homozygous TGR (mREN2)27 rats with established hypertension, kidney ACE2 mRNA, protein and tissue ACE2 activities were not different from their respective WKY and SD reference strain, respectively. In addition, when we looked at renal localization, we found ACE2 protein to be predominantly present in glomeruli and endothelium with weak staining in distal and negative staining in proximal tubuli. Thus, our data challenge previous work that implicates ACE2 as a candidate gene for hypertension in SHRSP by reporting a significant reduction of ACE2 in the kidneys of SHRSP. Taken together, renal ACE2 is not altered in the SHRSP and TGR (mREN2)27 genetic rat models with established hypertension.

Kamran, M., et al. (2004). "Inactivation of transcription factor gene ACE2 in the fungal pathogen Candida glabrata results in hypervirulence." Eukaryot Cell **3**(2): 546-552.

During an infection, the coordinated orchestration of many factors by the invading organism is required for disease to be initiated and to progress. The elucidation of the processes involved is critical to the development of a clear understanding of host-pathogen interactions. For Candida species, the inactivation of many fungal attributes has been shown to result in attenuation. Here we demonstrate that the Candida glabrata homolog of the Saccharomyces cerevisiae transcription factor gene ACE2 encodes a function that mediates virulence in a novel way. Inactivation of C. glabrata ACE2 does not result in attenuation but, conversely, in a strain that is hypervirulent in a murine model of invasive candidiasis. C. glabrata ace2 null mutants cause systemic infections characterized by fungal escape from the vasculature, tissue penetration, proliferation in vivo, and considerable overstimulation of the proinflammatory arm of the innate immune response. Compared to the case with wild-type fungi, mortality occurs much earlier in mice infected with C. glabrata ace2 cells, and furthermore, 200-fold lower doses are required to induce uniformly fatal infections. These data demonstrate that C. glabrata ACE2 encodes a function that plays a critical role in mediating the host-Candida interaction. It is the first virulence-moderating gene to be described for a Candida species.

Kanda, T. and H. Itoh (2012). "[The ACE2/Ang (1-7)/Mas receptor axis in cardiovascular and renal diseases]." Nihon Rinsho **70**(9): 1487-1491.

The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in the regulation of physiological homeostasis and diseases such as hypertension, coronary artery disease and chronic renal failure. In this cascade, the ACE/Ang II/AT1 receptor axis induces pathological effects, such as vasoconstriction, cell proliferation and fibrosis. Recently the ACE2/Ang (1-7)/Mas receptor axis has been recognized as a negative regulator of the RAAS. ACE2 metabolizes Ang II into Ang (1-7), which has opposite properties of Ang II through Mas receptor activation. Both animal and human studies provide strong evidence that the ACE2/Ang (1-7)/Mas receptor axis is protective for end-organ damage. Therefore, the ACE2/Ang (1-7)/Mas receptor axis could be a therapeutic target for coronary artery disease and chronic renal failure.

Kaparianos, A. and E. Argyropoulou (2011). "Local renin-angiotensin II systems, angiotensin-converting enzyme and its homologue ACE2: their potential role in the pathogenesis of chronic obstructive pulmonary diseases, pulmonary hypertension and acute respiratory distress syndrome." Curr Med Chem **18**(23): 3506-3515.

Renin-angiotensin II-aldosterone axis has long been known as a regulator of blood pressure and fluid homeostasis. Yet, local renin-angiotensin II systems have been discovered and novel actions of angiotensin II (AngII) have emerged among which its ability to act as a immunomodulator and profibrotic molecule. The enzyme responsible for its synthesis, Angiotensin-converting-enzyme (ACE), is present in high concentrations in lung tissue. In the present paper, we review data from studies of the past decade that implicate AngII and functional polymorphisms of the ACE gene that increase ACE activity with increased susceptibility for asthma and chronic obstructive pulmonary disease (COPD) and for pulmonary hypertension. Moreover, drugs that inhibit the synthesis of AngII (ACE inhibitors) or that antagonize its actions on its receptors (Angiotensin II receptor blockers -ARBs) have been shown to provide beneficial effects. Another recent discovery reviewed is the presence of a homologue of ACE, ACE2, which cleaves a single amino acid from AngII and forms a heptapeptide with vasodilatory actions, Ang 1-7. The balance between ACE and ACE2 is crucial for controlling AngII levels. ACE and ACE2 also appear to modify the severity of Acute Respiratory Distress Syndrome (ARDS), with ACE2 playing a protective role. Finally, mention is made to the recent discovery of ACE2 as a receptor for the SARS Corona Virus.

Kar, S., et al. (2010). "Exercise training normalizes ACE and ACE2 in the brain of rabbits with

pacing-induced heart failure." J Appl Physiol (1985) **108**(4): 923-932.

Exercise training (EX) normalizes sympathetic outflow and plasma ANG II in chronic heart failure (CHF). The central mechanisms by which EX reduces this sympathoexcitatory state are unclear, but EX may alter components of the brain renin-angiotensin system (RAS). Angiotensin-converting enzyme (ACE) may mediate an increase in sympathetic nerve activity (SNA). ACE2 metabolizes ANG II to ANG-(1-7), which may have antagonistic effects to ANG II. Little is known concerning the regulation of ACE and ACE2 in the brain and the effect of EX on these enzymes, especially in the CHF state. This study aimed to investigate the effects of EX on the regulation of ACE and ACE2 in the brain in an animal model of CHF. We hypothesized that the ratio of ACE to ACE2 would increase in CHF and would be reduced by EX. Experiments were performed on New Zealand White rabbits divided into the following groups: sham, sham + EX, CHF, and CHF + EX (n = 5 rabbits/group). The cortex, cerebellum, medulla, hypothalamus, paraventricular nucleus (PVN), nucleus tractus solitarii (NTS), and rostral ventrolateral medulla (RVLM) were analyzed. ACE protein and mRNA expression in the cerebellum, medulla, hypothalamus, PVN, NTS, and RVLM were significantly upregulated in CHF rabbits (ratio of ACE to GAPDH: 0.3 +/- 0.03 to 0.8 +/- 0.10 in the RVLM, P < 0.05). EX normalized this upregulation compared with CHF (0.8 +/- 0.1 to 0.4 +/- 0.1 in the RVLM). ACE2 protein and mRNA expression decreased in CHF (ratio of ACE2 to GAPDH: 0.3 +/- 0.02 to 0.1 +/- 0.01 in the RVLM). EX increased ACE2 expression compared with CHF (0.1 +/- 0.01 to 0.8 +/- 0.1 in the RVLM). ACE2 was present in the cytoplasm of neurons and ACE in endothelial cells. These data suggest that the activation of the central RAS in animals with CHF involves an imbalance of ACE and ACE2 in regions of the brain that regulate autonomic function and that EX can reverse this imbalance.

Kawabe, Y., et al. (2019). "ACE2 exerts anti-obesity effect via stimulating brown adipose tissue and induction of browning in white adipose tissue." Am J Physiol Endocrinol Metab **317**(6): E1140-E1149.

The angiotensin II (ANG II)-ANG II type 1 receptor (AT1R) axis is a key player in the pathophysiology of obesity. Angiotensin-converting enzyme 2 (ACE2) counteracts the ANG II/AT1R axis via converting ANG II to angiotensin 1-7 (Ang 1-7), which is known to have an anti-obesity effect. In this study, we hypothesized that ACE2 exerts a strong anti-obesity effect by increasing Ang 1-7 levels. We injected intraperitoneally recombinant human ACE2 (rhACE2, 2.0 mg.kg (-1).day (-1)) for 28 days to high-fat diet (HFD)-induced obesity mice. rhACE2 treatment decreased body weight and improved glucose metabolism. Furthermore, rhACE2 increased oxygen consumption and upregulated thermogenesis in HFD-fed mice. In the rhACE2 treatment group, brown adipose tissue (BAT) mass increased, accompanied with ameliorated insulin signaling and increased protein levels of uncoupling protein-1 (UCP-1) and PRD1-BF1-RIZ1 homologous domain containing 16. Importantly, subcutaneous white adipose tissue (sWAT) mass decreased, concomitant with browning, which was established by the increase of UCP-1 expression. The browning is the result of increased H3K27 acetylation via the downregulation of histone deacetylase 3 and increased H3K9 acetylation via upregulation of GCN5 and P300/CBP-associated factor. These results suggest that rhACE2 exerts anti-obesity effects by stimulating BAT and inducing browning in sWAT. ACE2 and the Ang 1-7 axis represent a potential therapeutic approach to prevent the development of obesity.

Kawajiri, M., et al. (2009). "Angiotensin-converting enzyme (ACE) and ACE2 levels in the cerebrospinal fluid of patients with multiple sclerosis." Mult Scler **15**(2): 262-265.

BACKGROUND: We reported a reduction in the levels of angiotensin II in cerebrospinal fluid (CSF) from patients with multiple sclerosis (MS). OBJECTIVE AND METHODS: To clarify the mechanism underlying this reduction, we assayed angiotensin-converting enzyme (ACE) and ACE2 concentrations along with angiotensin II concentrations in CSF samples from 20 patients with MS and 17 controls with non-neurological diseases. RESULTS: ACE levels were significantly elevated in patients with MS compared with controls (48.42 +/- 4.84 vs 44.71 +/- 3.9 pg/mL), whereas ACE2 levels were significantly reduced (2.56 +/- 0.26 vs 2.78 +/- 0.24 pg/mL), acting toward a normalization of angiotensin II levels. CONCLUSION: These results further indicate an alteration of the intrathecal renin-angiotensin system in patients with MS.

Kazemi-Bajestani, S. M., et al. (2012). "Targeting the ACE2 and Apelin Pathways Are Novel Therapies for Heart Failure: Opportunities and Challenges." Cardiol Res Pract **2012**: 823193.

Angiotensin-converting enzyme 2 (ACE2)/Ang II/Ang 1-7 and the apelin/APJ are two important peptide systems which exert diverse effects on the cardiovascular system. ACE2 is a key negative regulator of the renin-angiotensin system (RAS) where it metabolizes angiotensin (Ang) II into Ang 1-7, an endogenous antagonist of Ang II. Both the prolonged activation of RAS and the loss of ACE2 can be

detrimental as they lead to functional deterioration of the heart and progression of cardiac, renal, and vascular diseases. Recombinant human ACE2 in an animal model of ACE2 knockout mice lowers Ang II. These interactions neutralize the pressor and subpressor pathologic effects of Ang II by producing Ang 1-7 levels in vivo, that might be cardiovascular protective. ACE2 hydrolyzes apelin to Ang II and, therefore, is responsible for the degradation of both peptides. Apelin has emerged as a promising peptide biomarker of heart failure. The serum level of apelin in cardiovascular diseases tends to be decreased. Apelin is recognized as an imperative controller of systemic blood pressure and myocardium contractility. Dysregulation of the apelin/APJ system may be involved in the predisposition to cardiovascular diseases, and enhancing apelin action may have important therapeutic effects.

Keidar, S., et al. (2007). "ACE2 of the heart: From angiotensin I to angiotensin (1-7)." Cardiovasc Res **73**(3): 463-469.

Angiotensin II (Ang II), a bioactive peptide of the renin-angiotensin system (RAAS), plays an important role in the development of cardiovascular diseases (CVD). Pharmacological inhibition of angiotensin-converting enzyme (ACE), the Ang II forming enzyme, or specific blockade of Ang II binding to angiotensin type 1 receptor (AT1R) through which it exerts its deleterious effects, were shown to provide some protection against progression of CVD. The ACE-Ang II-AT1R axis has been challenged over the last few years with RAAS components able to counterbalance the effects of the main axis. The ACE homologue ACE2 efficiently hydrolyses Ang II to form Ang (1-7), a peptide that exerts actions opposite to those of Ang II. In contrast to the Ang II axis, the role of the ACE2-Ang (1-7) axis in cardiac function is largely obscure. Ang (1-7) is present in the viable myocardium, and its formation depends on Ang II as a substrate. The expression of this peptide is associated with cardiac remodeling: it is lost in the infarcted area and significantly increased in the border area. Low doses of Ang (1-7) improve cardiac output and antagonize Ang II-induced vasoconstriction. The type of Ang (1-7) biological activity is tissue specific and dose dependent. These findings point to a possible protective role for Ang (1-7) in abating the Ang II-induced actions. The elevated expression of Ang (1-7) in failing heart tissue paralleled the expression of its forming enzyme, ACE2. Several observations and experimental evidence suggest a beneficial role for ACE2 in cardiovascular function. Elevated ACE2 expression at the initial stage of several pathologies which decline with progression of disease might indicate a protective role for ACE2. Genetic manipulation of ACE2 expression, either targeted disruption or overexpression, point to the possible significance of this enzyme in cardiac function. Based on the above, a therapeutic approach that will amplify the ACE2-Ang (1-7) axis could provide further protection against the development of CVD. It turns out that the merits of currently used drugs--ACE inhibitors, AT1R blockers and mineralocorticoid receptor blockers (MRB) - lay beyond their direct effects on suppression of the ACE-Ang II-AT1R axis as they also increase cardiac ACE2 and Ang (1-7) significantly.

Keidar, S., et al. (2007). "ACE2 activity is increased in monocyte-derived macrophages from prehypertensive subjects." Nephrol Dial Transplant **22**(2): 597-601.

BACKGROUND: Hypertension is a major risk factor for cardiovascular disease and the renin-angiotensin-aldosterone system (RAAS) plays a central pathophysiological role in its formation. Angiotensin-converting enzyme (ACE) and its homologue ACE2 control the formation of counteracting effectors, angiotensin II (AngII), a potent vasopressor and Ang-(1-7) which has vasodilatory action. It is therefore hypothesized that the balance of the activities of these two enzymes, ACE and ACE2, could be important for the control of blood pressure (BP). METHODS: Monocyte-derived macrophages were isolated from blood samples of normotensives (NT), prehypertensives (preHTN) and untreated hypertensive (HTN) male patients (n = 28, 18 and 11, respectively). The activities of ACE2 were determined by measuring leucine or phenylalanine released following hydrolysis of Ang I and Ang II, respectively. The activity of ACE was measured using a synthetic substrate. RESULTS: The levels of BP were 112.6 +/- 1.4/74.8 +/- 1.2, 128.3 +/- 0.8/78.1 +/- 1.2 and 151.4 +/- 2.7/99.3 +/- 2.4 mmHg in the NT, preHTN and HTN, respectively ($P < 0.001$). The ACE2-mediated Ang II degrading activity (ACE2-II) was 1201 +/- 241 fmol/min/mg cell protein in NT subjects and was significantly ($P < 0.01$) increased by 2.4-fold in preHTN. ACE2-II activity in HTN and NT was not significantly different. ACE2-mediated Ang I hydrolysis (ACE2-I) was 85-fold lower than the ACE2-II activity. ACE activity in the human monocyte-derived macrophages (HMDM) averaged 21.6 +/- 3.0 mU/mg cell protein and did not differ among the three groups. CONCLUSIONS: PreHTN subjects have higher ACE2-II activity compared with HTN subjects, suggesting a protective role for ACE2 in the early stage of HTN development, probably by accelerated degradation of the vasopressor AngII.

Kim, M. A., et al. (2010). "Effects of ACE2 inhibition in the post-myocardial infarction heart." J Card Fail **16**(9): 777-785.

BACKGROUND: There is evidence that angiotensin-converting enzyme 2 (ACE2) is cardioprotective. To assess this in the post-myocardial infarction (MI) heart, we treated adult male Sprague-Dawley rats with either placebo (PL) or C16, a selective ACE2 inhibitor, after permanent coronary artery ligation or sham operation. METHODS AND RESULTS: Coronary artery ligation resulting in MI between 25% to 50% of the left ventricular (LV) circumference caused substantial cardiac remodeling. Daily C16 administration from postoperative days 2 to 28 at a dose that inhibited myocardial ACE2 activity was associated with a significant increase in MI size and reduction in LV % fractional shortening. Treatment with C16 did not significantly affect post-MI increases in LV end-diastolic dimension but did inhibit increases in wall thickness and fibrosis in non-infarcted LV. On postoperative day 7, C16 had no significant effect on the increased level of apoptosis in the infarct and border zones nor did it significantly affect capillary density surrounding the MI. It did, however, significantly reduce the number of c-kit (+) cells in the border region. CONCLUSIONS: These findings support the notion that ACE2 exerts cardioprotective effects by preserving jeopardized cardiomyocytes in the border zone. The reduction in hypertrophy and fibrosis with C16, however, suggests that ACE2 activity has diverse effects on post-MI remodeling.

Klimas, J., et al. (2015). "Perinatally administered losartan augments renal ACE2 expression but not cardiac or renal Mas receptor in spontaneously hypertensive rats." J Cell Mol Med **19**(8): 1965-1974.

Since the identification of the alternative angiotensin converting enzyme (ACE)2/Ang-(1-7)/Mas receptor axis, renin-angiotensin system (RAS) is a new complex target for a pharmacological intervention. We investigated the expression of RAS components in the heart and kidney during the development of hypertension and its perinatal treatment with losartan in young spontaneously hypertensive rats (SHR). Expressions of RAS genes were studied by the RT-PCR in the left ventricle and kidney of rats: normotensive Wistar, untreated SHR, SHR treated with losartan since perinatal period until week 9 of age (20 mg/kg/day) and SHR treated with losartan only until week 4 of age and discontinued until week 9. In the hypertrophied left ventricle of SHR, cardiac expressions of Ace and Mas were decreased while those of AT1 receptor (Agtr1a) and Ace2 were unchanged. Continuous losartan administration reduced LV weight (0.43 +/- 0.02; $P < 0.05$ versus SHR) but did not influence altered cardiac RAS expression. Increased blood pressure in SHR (149 +/- 2 in SHR versus 109 +/- 2 mmHg in Wistar; $P < 0.05$) was associated with a lower renal expressions of renin, Agtr1a and Mas and with an increase in ACE2. Continuous losartan administration lowered blood pressure to control levels (105 +/- 3 mmHg; $P < 0.05$ versus SHR), however, only renal renin and ACE2 were significantly up-regulated (for both $P < 0.05$ versus SHR). Conclusively, prevention of hypertension and LV hypertrophy development by losartan was unrelated to cardiac or renal expression of Mas. Increased renal Ace2, and its further increase by losartan suggests the influence of locally generated Ang-(1-7) in organ response to the developing hypertension in SHRs.

Koka, V., et al. (2008). "Angiotensin II up-regulates angiotensin I-converting enzyme (ACE), but down-regulates ACE2 via the AT1-ERK/p38 MAP kinase pathway." Am J Pathol **172**(5): 1174-1183.

The recent discovery of the angiotensin II (Ang II)-breakdown enzyme, angiotensin I converting enzyme (ACE) 2, suggests the importance of Ang II degradation in hypertension. The present study explored the signaling mechanism by which ACE2 is regulated under hypertensive conditions. Real-time PCR and immunohistochemistry showed that ACE2 mRNA and protein expression levels were high, whereas ACE expression levels were moderate in both normal kidney and heart. In contrast, patients with hypertension showed marked ACE up-regulation and ACE2 down-regulation in both hypertensive cardiopathy and, particularly, hypertensive nephropathy. The inhibition of ACE2 expression was shown to be associated with ACE up-regulation and activation of extracellular regulated (ERK)1/2 and p38 mitogen-activated protein (MAP) kinases. In vitro, Ang II was able to up-regulate ACE and down-regulate ACE2 in human kidney tubular cells, which were blocked by an angiotensin II (AT)1 receptor antagonist (losartan), but not by an AT2 receptor blocker (PD123319). Furthermore, blockade of ERK1/2 or p38 MAP kinases by either specific inhibitors or a dominant-negative adenovirus was able to abolish Ang II-induced ACE2 down-regulation in human kidney tubular cells. In conclusion, Ang II is able to up-regulate ACE and down-regulate ACE2 expression levels under hypertensive conditions both in vivo and in vitro. The AT1 receptor-mediated ERK/p38 MAP kinase signaling pathway may be a key mechanism by which Ang II down-regulates ACE2 expression, implicating an ACE/ACE2 imbalance in hypertensive cardiovascular and renal damage.

Kong, E. L., et al. (2019). "Spironolactone rescues renal dysfunction in obstructive jaundice rats by upregulating ACE2 expression." J Cell Commun Signal **13**(1): 17-26.

Postoperative acute renal failure in patients with obstructive jaundice is still a serious clinically complication, yet the mechanisms remain unclear. Renin-angiotensin-aldosterone system (RAAS) plays a central role in renal disease progression. Several lines of evidence shows that angiotensin-converting-enzyme-2 (ACE2), a main effector of RAAS acts as a local regulator for renal protection. This study aims to investigate the role of ACE2 and the effect of spironolactone treatment in obstructive jaundice (OJ) rats with renal injury. The rats with obstructive jaundice were established by bile duct ligation. Total bilirubin (TBil), serum creatinine (Scr) and the expression of ACE2 in kidney tissue of obstructive jaundice rats were detected. Comparatively, the expression of ACE2, renin, angiotensin II (AngII), angiotensin-(1-7) [Ang-(1-7)], aldosterone and intercellular adhesion molecule 1 (ICAM-1) in kidney tissues after spironolactone administration were measured by ELISA. Renal necrosis, inflammation and fibrosis induced by OJ were also measured by HE staining and Masson staining. The correlation between the expression of ACE2 and TBil, also the Scr level were investigated. With the time of common bile duct ligation prolonged, the TBil and Scr concentration increased while the expression of ACE2 in OJ rats' kidney tissues decreased. However, after spironolactone intervention, the expressions of ACE2, renin, AngII, Ang-(1-7), aldosterone and ICAM-1 in kidney tissue were changed, moreover, necrotic, inflammatory and fibrotic condition was also decreased. The relationship between the mRNA expression of ACE2 and TBil/Scr was observed to be moderately negatively correlated ($r = -0.516$, $R(2) = 0.292$, $P < 0.01$), ($r = -0.576$, $R(2) = 0.332$, $P < 0.01$), respectively. RAAS exerted an important effect in the renal damage caused by OJ. Spironolactone intervention not only improved the degree of renal fibrosis induced by OJ, but also upregulated the ACE2 expression in the kidney of OJ rats and rescued the renal function.

Kottom, T. J. and A. H. Limper (2013). "The Pneumocystis Ace2 transcription factor regulates cell wall-remodeling genes and organism virulence." J Biol Chem **288**(33): 23893-23902.

Pneumocystis carinii (Pc) beta-glucans are major components of the organism cell wall; yet, the regulation of Pc cell wall genesis and remodeling is not well understood. Ace2 transcription factors, which are present in many fungi, regulate glucanases and other enzymes needed for cell wall remodeling. The cloning and heterologous expression of PcAce2 in ace2Delta Saccharomyces cerevisiae demonstrated that PcAce2 can restore the defective glucanase and endochitinase gene expression of the mutant as well as regulate cell wall beta-glucan biosynthetic genes. Furthermore, when a reconstructed yeast system was used, PcAce2 activated the transcription of the Pneumocystis gsc1 beta-glucan synthetase, confirming the activity of a Pc transcription factor on a native Pneumocystis promoter and gene for the first time. We further observed that Pneumocystis binding to host extracellular matrix proteins and lung epithelial cells induced the phosphorylation (activation) of the PcAce2 transcription factor. Finally, we present a novel method that confirms the role of PcAce2 in modulating organism virulence using ace2Delta Candida glabrata infection in neutropenic mice. Together, these results indicate that the adherence of Pc to lung matrix proteins and epithelial cells leads to the activation of the Ace2 transcription factor, which regulates cell wall degradation and biosynthesis genes that are required for cell wall remodeling.

Kou, Y. L., et al. (2016). "[Protective Effect of Angiotensin Converting Enzyme 2 (ACE2) Against Chronic Intermittent Hypoxia-induced Pulmonary Oxidative Stress Injury in Rats]." Sichuan Da Xue Xue Bao Yi Xue Ban **47**(1): 43-48.

OBJECTIVE: To determine changes of angiotensin converting enzyme 2 (ACE2) in the development of chronic intermittent hypoxia (CIH)-induced pulmonary injury in rats and its mechanism. METHODS: 96 adult male Wistar rats were randomly allocated into four groups: control (UC) group, chronic intermittent hypoxia (CIH) group, normal saline treated CIH (NS) group, and edaravone treated CIH (NE) group. Each group contained four subgroups of rats (n = 6) subjecting to 1 week, 2 weeks, 3 weeks, and 4 weeks experiment, respectively. No intervention was given to rats in the UC group. Rats in the experimental groups were exposed to alternating cycles of nitrogen and compressed air. Rats in the NS and NE groups received daily injection of 0.9% normal saline (3 mg/ kg) and edaravone (3 mg/kg), respectively. Pulmonary sections were taken and stained with hematoxylin-eosin (HE). The levels of malondialdehyde (MDA), ACE2, ACE2 mRNA, and angiotensin II (Ang II) mRNA in the rat homogenate pulmonary samples were measured. RESULTS: Rats in the CIH and NS groups showed high levels of interstitial edema, alveolar atelectasis, and inflammatory cell infiltration in alveolar epithelial cells. The pulmonary injury got worse over time. Rats in the NE group showed later occurrence-and milder pulmonary injury compared with those in the NS

group. Rats in the CIH and NS groups had higher levels of MDA and Ang II mRNA (which increased over time) than those in the UC group ($P < 0.05$). The expression of ACE2 and the level of ACE2 mRNA increased in rats in the CIH group ($P < 0.05$), and peaked at 2 weeks ($P < 0.05$). Rats in the NE group had moderately increased levels of MDA and Ang II mRNA compared with those in the NS group ($P < 0.05$); moderately increased levels of expression of ACE2 and ACE2 mRNA compared with those in the UC and SC groups ($P < 0.05$). The pulmonary level of Ang II mRNA was positively correlated with MDA ($r = 0.782$, $P < 0.01$) in rats in the CIH group. CONCLUSION: CIH can activate oxidation stress and Ang II, which maybe an important mechanism of CIH-induced pulmonary injury.

Kruit, A., et al. (2005). "Angiotensin-converting enzyme 2 (ACE2) haplotypes are associated with pulmonary disease phenotypes in sarcoidosis patients." Sarcoidosis Vasc Diffuse Lung Dis **22**(3): 195-203.

BACKGROUND AND AIM OF THE STUDY: Angiotensin II (Ang II) formation by angiotensin-converting enzyme (ACE) or other enzymes has shown to exhibit profibrotic properties in a variety of fibrotic diseases. A homologue of ACE called ACE2 has been shown to counteract the formation of Ang II. Genetic variation in the components involved in Ang II formation may underlie the progression of pulmonary sarcoidosis. METHOD: Seven ACE2 SNPs, located on the X-chromosome, were investigated using SSP-PCR and haplotypes were constructed. Gender-matched analyses of sarcoidosis patients (80 males/64 females) and controls (110 males/218 females) were performed to correlate disease susceptibility and pulmonary disease phenotypes with ACE2 genotypes and haplotypes. RESULTS: ACE2 SNPs or haplotypes were not associated with susceptibility for sarcoidosis. Haplotype 4 was only present in sarcoid males without parenchymal involvement (frequency: 0.19) and absent in males with parenchymal involvement ($p = 0.006$; pcorr. = 0.05; degrees of freedom (df) = 1; OR = 0). No significant difference was observed between haplotype 4 frequencies in females with (0.08) or without (0.13) parenchymal involvement ($p = 0.5$). Although not significant after correction, analysis of the patient group with fibrosis showed that males with haplotype 5 (0.27) were predominant over those with haplotype 5 of the groups without fibrosis (0.03); $p = 0.01$; pc = 0.08; df = 1; OR = 11.4. Females with fibrosis vs. no fibrosis revealed no difference between haplotype 5 frequencies: 0.05 vs. 0.03; $p = 0.37$; pc = 1; df = 1. CONCLUSION: These results suggest that ACE2 might be involved in the progression of pulmonary sarcoidosis which may depend on gender. Subsequent studies using larger groups are needed to confirm these findings.

Kuan, T. C., et al. (2013). "Angiotensin II downregulates ACE2-mediated enhancement of MMP-2 activity in human cardiofibroblasts." Biochem Cell Biol **91**(6): 435-442.

Angiotensin converting enzyme II (ACE2) is a component of the renin-angiotensin system (RAS) that negatively regulates angiotensin II (Ang II). Ang II, in turn, affects the expression of matrix metalloproteinases (MMPs) to induce heart remodeling. The specific mechanisms by which ACE2 regulates MMP-2, however, remain unclear. The aim of this study was to investigate the regulatory relationships between Ang II, ACE2, and MMP-2. ACE2 expression was upregulated and downregulated in human cardiofibroblasts (HCFs) by lentiviral infection. Effects on MMP-2 activity, shed ACE2 activity, extracellular signal-regulated kinase (ERK) signaling pathway, and ADAM metallopeptidase domain 17 (ADAM17) expression were assessed. ACE2 increased MMP-2 activity, and Ang II inhibited this effect through the Ang II type-1 receptor (AT1R) and ERK1/2 signaling pathway. Ang II also reduced the effect of ACE2 on ERK1/2 levels, the activity of shed ACE2, and adam17 expression in HCFs. Additionally, these Ang II-mediated reductions could be attenuated by AT1R antagonist valsartan. In conclusion, these data help to clarify how ACE2 and Ang II interact to regulate MMP-2 and control tissue remodeling in heart disease.

Kuan, T. C., et al. (2011). "Identifying the regulatory element for human angiotensin-converting enzyme 2 (ACE2) expression in human cardiofibroblasts." Peptides **32**(9): 1832-1839.

Angiotensin-converting enzyme 2 (ACE2) has been proposed as a potential target for cardioprotection in regulating cardiovascular functions, owing to its key role in the formation of the vasoprotective peptides angiotensin-(1-7) from angiotensin II (Ang II). The regulatory mechanism of ace2 expression, however, remains to be explored. In this study, we investigated the regulatory element within the upstream of ace2. The human ace2 promoter region, from position -2069 to +20, was cloned and a series of upstream deletion mutants were constructed and cloned into a luciferase reporter vector. The reporter luciferase activity was analyzed by transient transfection of the constructs into human cardiofibroblasts (HCFs) and an activating domain was identified in the -516/-481 region. Deletion or reversal of this domain within ace2 resulted in a significant decrease in promoter activity. The nuclear proteins isolated from the HCFs formed a DNA-protein complex with double stranded

oligonucleotides of the -516/-481 domain, as detected by electrophoretic mobility shift assay. Site-directed mutagenesis of this region identified a putative protein binding domain and a potential binding site, ATTTGGA, homologous to that of an Ikaros binding domain. This regulatory element was responsible for Ang II stimulation via the Ang II-Ang II type-1 receptor (AT1R) signaling pathway, but was not responsible for pro-inflammatory cytokines TGF-beta1 and TNF-alpha. Our results suggest that the nucleotide sequences -516/-481 of human ace2 may be a binding domain for an as yet unidentified regulatory factor (s) that regulates ace2 expression and is associated with Ang II stimulation.

Kuba, K., et al. (2010). "Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters." Pharmacol Ther **128**(1): 119-128.

Angiotensin-converting enzyme (ACE) 2 is a homolog to the carboxypeptidase ACE, which generates angiotensin II, the main active peptide of renin-angiotensin system (RAS). After the cloning of ACE2 in 2000, three major ACE2 functions have been described so far. First ACE2 has emerged as a potent negative regulator of the RAS counterbalancing the multiple functions of ACE. By targeting angiotensin II ACE2 exhibits a protective role in the cardiovascular system and many other organs. Second ACE2 was identified as an essential receptor for the SARS coronavirus that causes severe acute lung failure. Downregulation of ACE2 strongly contributes to the pathogenesis of severe lung failure. Third, both ACE2 and its homologue Collectrin can associate with amino acid transporters and play essential role in the absorption of amino acids in the kidney and gut. In this review, we will discuss the multiple biological functions of ACE2.

Kuba, K., et al. (2005). "A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury." Nat Med **11**(8): 875-879.

During several months of 2003, a newly identified illness termed severe acute respiratory syndrome (SARS) spread rapidly through the world. A new coronavirus (SARS-CoV) was identified as the SARS pathogen, which triggered severe pneumonia and acute, often lethal, lung failure. Moreover, among infected individuals influenza such as the Spanish flu and the emergence of new respiratory disease viruses have caused high lethality resulting from acute lung failure. In cell lines, angiotensin-converting enzyme 2 (ACE2) has been identified as a potential SARS-CoV receptor. The high lethality of SARS-CoV infections, its enormous economic and social impact, fears of renewed outbreaks as well as the potential misuse of such viruses as biologic weapons make it paramount to understand the pathogenesis of SARS-CoV. Here we provide the first genetic proof that ACE2 is a crucial SARS-CoV receptor in vivo. SARS-CoV infections and the Spike protein of the SARS-CoV reduce ACE2 expression. Notably, injection of SARS-CoV Spike into mice worsens acute lung failure in vivo that can be attenuated by blocking the renin-angiotensin pathway. These results provide a molecular explanation why SARS-CoV infections cause severe and often lethal lung failure and suggest a rational therapy for SARS and possibly other respiratory disease viruses.

Kuba, K., et al. (2006). "Lessons from SARS: control of acute lung failure by the SARS receptor ACE2." J Mol Med (Berl) **84**(10): 814-820.

Angiotensin-converting enzyme 2 (ACE2), a second angiotensin-converting enzyme (ACE), regulates the renin-angiotensin system by counterbalancing ACE activity. Accumulating evidence in recent years has demonstrated a physiological and pathological role of ACE2 in the cardiovascular systems. Recently, it has been shown that severe acute respiratory syndrome (SARS) coronavirus, the cause of SARS, utilizes ACE2 as an essential receptor for cell fusion and in vivo infections in mice. Intriguingly, ACE2 acts as a protective factor in various experimental models of acute lung failure and, therefore, acts not only as a key determinant for SARS virus entry into cells but also contributes to SARS pathogenesis. Here we review the role of ACE2 in disease pathogenesis, including lung diseases and cardiovascular diseases.

Kurischko, C., et al. (2005). "A role for the Saccharomyces cerevisiae regulation of Ace2 and polarized morphogenesis signaling network in cell integrity." Genetics **171**(2): 443-455.

Saccharomyces cerevisiae RAM is a conserved signaling network that regulates maintenance of polarized growth and daughter-cell-specific transcription, the latter of which is critical for septum degradation. Consequently, cells defective in RAM function (designated ramDelta) are round in morphology, form feeble mating projections, and fail to separate following cytokinesis. It was recently demonstrated that RAM genes are essential in strains containing functional SSD1 (SSD1-v), which encodes a protein of unknown function that binds the RAM Cbk1p kinase. Here we investigated the essential function of RAM in SSD1-v strains and identified two functional groups of dosage suppressors for ramDelta lethality. We establish that all ramDelta mutants exhibit cell integrity defects and cell lysis. All dosage

suppressors rescue the lysis but not the cell polarity or cell separation defects of ramDelta cells. One class of dosage suppressors is composed of genes encoding cell wall proteins, indicating that alterations in cell wall structure can rescue the cell lysis in ramDelta cells. Another class of ramDelta dosage suppressors is composed of ZRG8 and SRL1, which encode two unrelated proteins of unknown function. We establish that ZRG8 and SRL1 share similar genetic interactions and phenotypes. Significantly, Zrg8p coprecipitates with Ssd1p, localizes similarly to RAM proteins, and is dependent on RAM for localization. Collectively, these data indicate that RAM and Ssd1p function cooperatively to control cell integrity and suggest that Zrg8p and Srl1p function as nonessential inhibitors of Ssd1p.

Laabs, T. L., et al. (2003). "ACE2 is required for daughter cell-specific G1 delay in Saccharomyces cerevisiae." Proc Natl Acad Sci U S A **100**(18): 10275-10280.

Saccharomyces cerevisiae cells reproduce by budding to yield a mother cell and a smaller daughter cell. Although both mother and daughter begin G1 simultaneously, the mother cell progresses through G1 more rapidly. Daughter cell G1 delay has long been thought to be due to a requirement for attaining a certain critical cell size before passing the commitment point in the cell cycle known as START. We present an alternative model in which the daughter cell-specific Ace2 transcription factor delays G1 in daughter cells. Deletion of ACE2 produces daughter cells that proceed through G1 at the same rate as mother cells, whereas a mutant Ace2 protein that is not restricted to daughter cells delays G1 equally in both mothers and daughters. The differential in G1 length between mothers and daughters requires the Cln3 G1 cyclin, and CLN3-GFP reporter expression is reduced in daughters in an ACE2-dependent manner. Specific daughter delay elements in the CLN3 promoter are required for normal daughter G1 delay, and these elements bind to an unidentified 127-kDa protein. This DNA-binding activity is enhanced by deletion of ACE2. These results support a model in which daughter cell G1 delay is determined not by cell size but by an intrinsic property of the daughter cell generated by asymmetric cell division.

Lai, L., et al. (2017). "MiRNA-30e mediated cardioprotection of ACE2 in rats with Doxorubicin-induced heart failure through inhibiting cardiomyocytes autophagy." Life Sci **169**: 69-75.

OBJECTIVE: miRNAs are a class of small non-coding RNAs that has been proved to be involved in cardioprotection. The present study was to detect role of miR-30e in cardiac-protective action of ACE2 (angiotensin-converting enzyme 2). METHODS: Sprague Dawley rats were divided into 3 groups and received treatment for a total of 6weeks: group1, normal rats; group2, Doxorubicin-induced heart cardiomyopathy (DHC) rats; and group3, rhACE2 (recombinant human ACE2) treated DHC rats. Doxorubicin was discontinuously administered via intraperitoneal injection. Primary cardiomyocytes and H9C2 cell line were used for in vitro experiments. MiR-30a, miR-30c and miR-30e expression were determined using qRT-PCR. Expression of autophagy associated gene expression including Beclin-1 and LC3 II/I were determined using western blot. Cell apoptosis was evaluated using TUNEL assay. RESULTS: Administration of ACE2 suppressed harmful action of Doxorubicin and caused a significant improvement of left ventricular contractility function, upregulation in miR-30 (a, c and e) expression, and inhibition in Beclin-1 expression and LC3-II/I ratio. This was supported by results of Ad-ACE2-incubated primary cardiomyocytes. By manipulating miR-30e expression in H9C2 cells, we observed that miR-30e regulated Beclin-1 expression via inhibiting its 3'UTR activity. MiR-30e mimic treatment resulted in downregulation of Beclin-1 and protected primary cardiomyocytes against apoptosis. Moreover, silencing miR-30e induced cardiomyocytes apoptosis was abrogated by ACE2 overexpresssion. This was further confirmed by in vivo DHC rat experiments that showed that co-injected ACE2 and miR-30 inhibitor reduced cardiac function. CONCLUSION: In summary, administration of ACE2 attenuates Doxorubicin-induced cardiac dysfunction via preservation of cardiomyocytes autophagy in a miR-30e/beclin-1 signal pathway.

Lambert, D. W., et al. (2008). "Calmodulin interacts with angiotensin-converting enzyme-2 (ACE2) and inhibits shedding of its ectodomain." FEBS Lett **582**(2): 385-390.

Angiotensin-converting enzyme-2 (ACE2) is a regulatory protein of the renin-angiotensin system (RAS) and a receptor for the causative agent of severe-acute respiratory syndrome (SARS), the SARS-coronavirus. We have previously shown that ACE2 can be shed from the cell surface in response to phorbol esters by a process involving TNF-alpha converting enzyme (TACE; ADAM17). In this study, we demonstrate that inhibitors of calmodulin also stimulate shedding of the ACE2 ectodomain, a process at least partially mediated by a metalloproteinase. We also show that calmodulin associates with ACE2 and that this interaction is decreased by calmodulin inhibitors.

Lambert, D. W., et al. (2005). "Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2)." J Biol Chem **280**(34): 30113-30119.

Angiotensin-converting enzyme-2 (ACE2) is a critical regulator of heart function and a cellular receptor for the causative agent of severe-acute respiratory syndrome (SARS), SARS-CoV (coronavirus). ACE2 is a type I transmembrane protein, with an extracellular N-terminal domain containing the active site and a short intracellular C-terminal tail. A soluble form of ACE2, lacking its cytosolic and transmembrane domains, has been shown to block binding of the SARS-CoV spike protein to its receptor. In this study, we examined the ability of ACE2 to undergo proteolytic shedding and investigated the mechanisms responsible for this shedding event. We demonstrated that ACE2, heterologously expressed in HEK293 cells and endogenously expressed in Huh7 cells, undergoes metalloproteinase-mediated, phorbol ester-inducible ectodomain shedding. By using inhibitors with differing potency toward different members of the ADAM (a disintegrin and metalloproteinase) family of proteases, we identified ADAM17 as a candidate mediator of stimulated ACE2 shedding. Furthermore, ablation of ADAM17 expression using specific small interfering RNA duplexes reduced regulated ACE2 shedding, whereas overexpression of ADAM17 significantly increased shedding. Taken together, these data provided direct evidence for the involvement of ADAM17 in the regulated ectodomain shedding of ACE2. The identification of ADAM17 as the protease responsible for ACE2 shedding may provide new insight into the physiological roles of ACE2.

Larrinaga, G., et al. (2010). "Angiotensin-converting enzymes (ACE and ACE2) are downregulated in renal tumors." Regul Pept **165**(2-3): 218-223.

The angiotensin-converting enzymes (ACE and ACE2) are highly expressed in renal tubules and play an important role in the regulation of renal function by the intrarenal renin-angiotensin system (iRAS). Dysregulation of these cell-surface peptidases has been associated with renal injury. Most of these studies, however, have focused on non-neoplastic kidney diseases. In the present study, ACE and ACE2 activity and protein and mRNA expression were analysed in a subset of clear-cell (CCRCC) and chromophobe (ChRCC) renal cell carcinomas, and in renal oncocytoma (RO). Enzyme activity was measured by spectrofluorometric (ACE2) and spectrophotometric assays (ACE), and protein and mRNA expression were determined by immunohistochemistry and qRT-PCR assays, respectively. The enzyme activities and immunohistochemistry showed that both enzymes are mainly downregulated in these neoplasms. qRT-PCR studies in CCRCC showed no positive correlation between ACE and ACE2 activity/protein expression and mRNA levels, whereas downregulation of ACE2 mRNA levels was observed in tumors from the distal nephron (ChRCC and RO). These findings suggest a metabolic imbalance in iRAS and a role of this system in renal neoplastic diseases, and point to ACE and ACE2 as potential prognostic/diagnostic markers.

Lavrentyev, E. N. and K. U. Malik (2009). "High glucose-induced Nox1-derived superoxides downregulate PKC-betaII, which subsequently decreases ACE2 expression and ANG (1-7) formation in rat VSMCs." Am J Physiol Heart Circ Physiol **296**(1): H106-118.

In rat diabetic animal models, ANG (1-7) treatment prevents the development of cardiovascular complications. Angiotensin-converting enzyme (ACE)2 is a major ANG (1-7)-generating enzyme in vascular smooth muscle cells (VSMCs), and its expression is decreased by a prolonged exposure to high glucose (HG), which is reflected by lower ANG (1-7) levels. However, the underlying mechanism of its downregulation is unknown and was the subject of this study. Rat aortic VSMCs were maintained in normal glucose (NG) or HG (approximately 4.1 and approximately 23.1 mmol/l, respectively) for up to 72 h. Several PKC and NADPH oxidase inhibitors and short interfering (si)RNAs were used to determine the mechanism of HG-induced ACE2 downregulation. Cell lysates were subjected to Western blot analysis, real-time quantitative PCR, and ANG (1-7) radioimmunodetection. At 72 h of HG exposure, ACE2 mRNA, protein, and ANG (1-7) levels were decreased (0.17 +/- 0.01-, 0.47 +/- 0.03-, and 0.16 +/- 0.01-fold, respectively), and the expression of NADPH oxidase subunit Nox1 was increased (1.70 +/- 0.2-fold). The HG-induced ACE2 decrease was reversed by antioxidants and Nox1 siRNA as well as by inhibitors of glycotoxin formation. ACE2 expression was PKC-betaII dependent, and PKC-betaII protein levels were reduced in the presence of HG (0.32 +/- 0.03-fold); however, the PKC-betaII inhibitor CG-53353 prevented the HG-induced ACE2 loss and Nox1 induction, suggesting a nonspecific effect of the inhibitor. Our data suggest that glycotoxin-induced Nox1 expression is regulated by conventional PKCs. ACE2 expression is PKC-betaII dependent. Nox1-derived superoxides reduce PKC-betaII expression, which lowers ACE2 mRNA and protein levels and consequently decreases ANG (1-7) formation.

Lely, A. T., et al. (2004). "Renal ACE2 expression in human kidney disease." J Pathol **204**(5): 587-593.

Angiotensin-converting enzyme 2 (ACE2) is a recently discovered homologue of angiotensin-converting enzyme (ACE) that is thought to counterbalance ACE. ACE2 cleaves angiotensin I and angiotensin II into the inactive angiotensin 1-9, and the vasodilator and anti-proliferative angiotensin 1-7, respectively. ACE2 is known to be present in human kidney, but no data on renal disease are available to date. Renal biopsies from 58 patients with diverse primary and secondary renal diseases were studied (hypertensive nephropathy n = 5, IgA glomerulopathy n = 8, minimal change nephropathy n = 7, diabetic nephropathy n = 8, focal glomerulosclerosis n = 5, vasculitis n = 7, and membranous glomerulopathy n = 18) in addition to 17 renal transplants and 18 samples from normal renal tissue. Immunohistochemical staining for ACE2 was scored semi-quantitatively. In control kidneys, ACE2 was present in tubular and glomerular epithelium and in vascular smooth muscle cells and the endothelium of interlobular arteries. In all primary and secondary renal diseases, and renal transplants, neo-expression of ACE2 was found in glomerular and peritubular capillary endothelium. There were no differences between the various renal disorders, or between acute and chronic rejection and control transplants. ACE inhibitor treatment did not alter ACE2 expression. In primary and secondary renal disease, and in transplanted kidneys, neo-expression of ACE2 occurs in glomerular and peritubular capillary endothelium. Further studies should elucidate the possible protective mechanisms involved in the de novo expression of ACE2 in renal disease.

Li, S., et al. (2020). "Fugan Wan alleviates hepatic fibrosis by inhibiting ACE/Ang II/AT-1R signaling pathway and enhancing ACE2/Ang 1-7/Mas signaling pathway in hepatic fibrosis rat models." Am J Transl Res **12**(2): 592-601.

Hepatic fibrosis is a repair and healing reaction for chronic injuries of liver. This study aimed to investigate protective effects of Fugan Wan (FGW) on hepatic fibrosis and clarify associated mechanisms. Hepatic fibrosis model was established by administrating dimethyl nitrosamine (DMN) to rats. Rats were divided into control, DMN and FGW groups. Haematoxylin and eosin (HE) staining was conducted to evaluate inflammatory response in hepatic fibrosis tissues. Sirius red staining was used to assess collagen disposition. Quantitative real-time PCR (qRT-PCR) was employed to detect antiotensin-converting enzyme homologue 2 (ACE2), Mas, transforming growth factor beta1 (TGF-beta1) mRNA. Western blot was used to examine collagen I, smooth muscle actin alpha (alpha-SMA), angiotensin type 1 receptor (AT-1R), extra-cellular regulated protein kinase (ERK), phosphorylated ERK (p-ERK), c-Jun and phosphorylated-c-Jun (p-c-Jun) expression. The results indicated that FGW significantly reduced inflammatory response of hepatic fibrosis tissues. FGW significantly decreased collagen deposition compared to that of DMN group ($P < 0.01$). FGW significantly down-regulated alpha-SMA expression compared to that of DMN group ($P < 0.01$). FGW significantly decreased AT-1R levels compared to that of DMN group ($P < 0.01$). Comparing with DMN group, ACE2 and Mas mRNA levels were significantly increased in FGW group ($P < 0.01$). FGW significantly down-regulated p-c-Jun and p-ERK1/2 compared to DMN group ($P < 0.01$). GFW significantly inhibited compared to DMN group ($P < 0.01$). In conclusion, FGW alleviated hepatic fibrosis by inhibiting ACE/Ang II/AT-1R signaling and enhancing ACE2/Ang 1-7/Mas signaling pathway in hepatic fibrosis rat models.

Li, S. M., et al. (2018). "[ACE2 agonist DIZE alleviates lung injury induced by limb ischemia-reperfusion in mice]." Sheng Li Xue Bao **70**(2): 175-183.

This study was aimed to explore the effect of angiotensin converting enzyme 2 (ACE2) agonist diminazene aceturate (DIZE) on acute lung injury (ALI) induced by limb ischemia-reperfusion (LIR) in mice. Male 8-week-old wild-type and hACE2 transgenic ICR mice were randomly divided into 6 groups (6 in each group), including wild-type control (W), wild-type model (WL), wild-type model with DIZE administration (WLD), transgenic control (T), transgenic model (TL), and transgenic model with DIZE administration (TLD) groups. LIR model was established by 4 h reperfusion following 2 h ischemia of bilateral hindlimbs with rubber bands in mice. The WLD and TLD groups were pretreated with DIZE (15 mg/kg, i.p.) for 4 weeks before LIR. At the end of LIR, the mice were sacrificed and lung tissues were sampled. Indexes for evaluating lung injury include organ coefficient and wet/dry weight ratio (W/D), cell count and protein concentration of bronchoalveolar lavage fluid (BALF), as well as morphological change and pathological score were detected. Angiotensin II (Ang II) and Ang (1-7) levels in lung tissue were determined by using ELISA commercial kits. And the protein expressions of angiotensin II type 1 receptor (AT1) and Mas receptor protein in lung tissue were detected by Western blot. The results were as follows: (1) There was obvious lung injury in both the WL and TL groups. The lung injury in the TL group was lighter than that in the WL group. DIZE could

attenuate the lung injury in both the two groups. (2) The WL group showed increased Ang II and decreased Ang (1-7) levels, whereas the TL group did not exhibit any changes of these two proteins. DIZE decreased the level of Ang II in both the WL and TL groups, and increased the level of Ang (1-7) in the WL group. (3) In the WL and TL groups, AT1 and Mas receptor protein expressions were up-regulated. DIZE reversed the change of AT1 protein expression, whereas further increased Mas receptor expression in both the two groups. These results suggest that DIZE may improve the renin-angiotensin system homeostasis by regulating ACE2-Ang (1-7)-Mas axis in local lung tissue and play a protective role in LIR-induced ALI in mice.

Li, W., et al. (2017). "A Human Long Non-Coding RNA ALT1 Controls the Cell Cycle of Vascular Endothelial Cells Via ACE2 and Cyclin D1 Pathway." Cell Physiol Biochem **43**(3): 1152-1167.

BACKGROUND/AIMS: ALT1 is a novel long non-coding RNA derived from the alternatively spliced transcript of the deleted in lymphocytic leukemia 2 (DLEU2). To date, ALT1 biological roles in human vascular endothelial cells have not been reported. METHODS: ALT1 was knocked down by siRNAs. Cell proliferation was analyzed by cck-8. The existence and sequence of human ALT1 were identified by 3' rapid amplification of cDNA ends. The interaction between lncRNA and proteins was analyzed by RNA-Protein pull down assay, RNA immunoprecipitation, and mass spectrometry analysis. RESULTS: ALT1 was expressed in human umbilical vein endothelial cells (HUVECs). The expression of ALT1 was significantly downregulated in contact-inhibited HUVECs and in hypoxia-induced, growth-arrested HUVECs. Knocking down of ALT1 inhibited the proliferation of HUVECs by G0/G1 cell cycle arrest. We observed that angiotensin converting enzyme (ACE2) was a direct target gene of ALT1. Knocking-down of ALT1 or its target gene ACE2 could efficiently decrease the expression of cyclin D1 via the enhanced ubiquitination and degradation, in which HIF-1alpha and protein von Hippel-Lindau (pVHL) might be involved. CONCLUSION: The results suggested the human long non-coding RNA ALT1 is a novel regulator for cell cycle of HUVECs via ACE2 and cyclin D1 pathway.

Li, Y. Y. (2012). "Lack of Association of ACE2 G8790A Gene Mutation with Essential Hypertension in the Chinese Population: A Meta-Analysis Involving 5260 Subjects." Front Physiol **3**: 364.

BACKGROUND: The angiotensin converting enzyme 2 (ACE2) G8790A gene polymorphism has been associated with the susceptibility to essential hypertension (EH), but the results are disputable. OBJECTIVE AND METHODS: To investigate the relationship between the ACE2 G8790A gene polymorphism and EH, eight separate studies with 5260 subjects were meta-analyzed. The pooled odds ratio (OR) and its corresponding 95% confidence interval (CI) were calculated by a random effect model. RESULTS: In the ACE2 G8790A gene polymorphism and EH meta-analysis in a Chinese population, no significant association was found between the ACE2 G8790A gene polymorphism and EH (OR: 1.03, 95% CI: 0.87-1.21, P = 0.76). In the stratified analysis by gender, no significant risk was found among males (OR: 1.06, 95% CI: 0.82-1.36, P = 0.66) or females (OR: 0.98, 95% CI: 0.77-1.24, P = 0.85). Under a dominant model of inheritance in the female subgroup, the pooled OR for the GG/GA + AA value was 1.01 (95% CI: 0.82-1.25, P = 0.92). Under a recessive model of inheritance in the female subgroup, the pooled OR for the AA/AG + GG value was 0.93 (95% CI: 0.50-1.73, P = 0.83). CONCLUSION: The current meta-analysis suggested that the ACE2 G8790A gene polymorphism might not be related to the increased EH risk in the Chinese population.

Liang, B., et al. (2015). "ACE2-Ang (1-7) axis is induced in pressure overloaded rat model." Int J Clin Exp Pathol **8**(2): 1443-1450.

ACE2-Ang (1-7) axis is a key regulator in cardiac hypertrophy, myocardial remodeling and development of heart failure. To investigate how ACE2-Ang (1-7) axis function in pressure-overload-induced heart failure, male SD rats (weighing about 250 g) were used to establish the model of pressure-overload-induced heart failure using aortic stenosis surgery. The level of plasma ACE2, ACE and Ang (1-7) from heart failure group were significantly up-regulated compared with the sham group by ELISA test. The mRNA and protein expression of ACE2 in myocardial tissue from heart failure group also showed remarkably increased. Importantly, we found that the expression of ACE2 and Ang (1-7) were reversed in heart failure group after treatment with AT1 receptor antagonist telmisartan. Compared with heart failure group, the level of plasma ACE2, ACE and Ang (1-7) were significantly decreased in telmisartan treated group. The mRNA and protein expression of ACE2 in cardiac tissue from telmisartan group was also significantly decreased, while Mas mRNA and protein level was increased. Taken together, these studies demonstrated that the expression of ACE2-Ang (1-7) axis was induced in pressure-overload-induced heart failure model, suggesting that ACE2-Ang (1-7) axis may have a protective role in the development of heart failure and may provide a new target for drug development of heart failure.

Liao, K., et al. (2013). "Development of an enzymatic assay for the detection of neutralizing antibodies against therapeutic angiotensin-converting enzyme 2 (ACE2)." J Immunol Methods **389**(1-2): 52-60.

Therapeutic proteins have the potential to elicit immune responses in animals and humans (Mire-Sluis et al., 2004; Yu et al., 2006; Shankar et al., 2008). Contributors to the response could include product related factors such as chemical modifications, impurities that co-purify with product, contaminants, formulation, aggregates, and clinical factors such as dose concentration, dosing frequency, route of drug administration, rate of administration, patient underlying disease, concomitant medication, and genetic status among others (Patten and Schellekens, 2003). Further, an immune response triggered by a therapeutic enzyme may neutralize the endogenous counterpart resulting in a decrease or depletion of the therapeutic and endogenous enzymes imposing safety concerns for patients. Therefore, monitoring of anti-drug antibody (ADA) and neutralizing antibody (NAb) responses to both the recombinant therapeutic enzyme and endogenous enzyme is important during early development and subsequent clinical studies. Testing considerations for NAb detection against therapeutic enzymes have been published mostly for lysosomal storage diseases (Wang et al., 2008). NAb cross-reactivity to the endogenous counterpart has also been characterized (Sominanda et al., 2010). Here, we describe an enzymatic NAb assay which detects neutralizing antibodies to both recombinant and endogenous angiotensin-converting enzyme 2 (ACE2). NAb assay sensitivity was optimized by selecting the assay incubation time as 20 min with an enzyme concentration of 0.5 mug/mL. Four anti-ACE2 antibodies out of a commercial panel of 18 were found to have neutralizing capabilities based upon their ability to abrogate ACE2 enzymatic activity. We demonstrated assay specificity by small peptide inhibitors specific for ACE or ACE2. DX600, an ACE2 specific inhibitor did not cross-react with ACE. Conversely, captopril, an inhibitor of ACE did not inhibit ACE2. The assay specificity for ACE2 neutralizing antibodies was further demonstrated by the lack of reactivity of two species control antibodies and 14 anti-ACE2 antibodies. Moreover, we demonstrated assay specificity to human endogenous ACE2 from human epithelial cells. Three human cell lines (Calu-3, Caco-2, Huh-7) were evaluated for the cell surface expression of ACE2 by flow cytometry and Western blot. Subsequently, whole cell lysates, cell culture supernatant, and live cells were evaluated in the assay. Results demonstrated that Calu-3 had elevated levels of ACE2 compared to Caco-2 or Huh-7. Calu-3 also demonstrated elevated ACE2 enzymatic activity in all three sources and could be inhibited by the ACE2 specific inhibitor DX600 as well as the neutralizing antibodies for the recombinant ACE2. Thus, we describe here a method to detect NAb against a therapeutic enzyme and assess NAb cross-reactivity to the native endogenous enzyme. The approach of method development described here could be applied for the assessment of NAb responses to other enzymatic therapeutics.

Liao, W., et al. (2019). "Egg White-Derived Antihypertensive Peptide IRW (Ile-Arg-Trp) Reduces Blood Pressure in Spontaneously Hypertensive Rats via the ACE2/Ang (1-7)/Mas Receptor Axis." Mol Nutr Food Res **63**(9): e1900063.

SCOPE: It is found in the previous study that egg-white-derived antihypertensive peptide Ile-Arg-Trp (IRW) upregulated angiotensin converting enzyme 2 (ACE2) in spontaneously hypertensive rats (SHRs). The objective of this study is to evaluate the contribution of ACE2 activation by IRW to blood-pressure-lowering activity in vivo. METHODS AND RESULTS: Adult male SHRs (13-15 week old) are assigned into four groups: 1) untreated with saline infusion; 2) IRW administration (15 mg per kg body weight) with saline infusion; 3) Mas receptor (MasR) antagonist A779 (48 microg per kg body weight per h) infusion; 4) A779 infusion and IRW. Animals are implanted with telemetry transmitter first, and then an osmotic pump filled with saline or A779 is implanted. A779/saline is infused for 7 days, continued with an additional 7 days of treatments. Results indicate that blocking MasR abolished the blood-pressure-lowering effect of IRW. Akt/eNOS signaling in aorta is upregulated by IRW treatment but deactivated by A779 infusion. Circulating levels of interleukin 6 and monocyte chemoattractant protein 1, along with cyclooxygenase 2 in aorta are reduced by IRW but restored by A779 infusion. CONCLUSION: IRW reduces blood pressure of SHR via the ACE2/Ang (1-7)/MasR axis. Mechanisms pertaining to IRW as an ACE2 activator in vivo include enhanced endothelium-dependent vasorelaxation and reduced vascular inflammation.

Lieb, W., et al. (2006). "Association of angiotensin-converting enzyme 2 (ACE2) gene polymorphisms with parameters of left ventricular hypertrophy in men. Results of the MONICA Augsburg echocardiographic substudy." J Mol Med (Berl) **84**(1): 88-96.

Angiotensin-converting enzyme (ACE) activity is considered to be of major importance for the conversion of angiotensin (Ang) I to Ang II. Recently, a second ACE, named ACE2, has been identified.

Experimental data provide evidence that ACE2 might be involved in modulating cardiac structure and function. In the present explorative study, we assessed whether polymorphisms in the ACE2 gene are related to echocardiographically determined parameters of left ventricular mass, structure or function in the general population. Five intronic single nucleotide polymorphisms (SNPs) were genotyped using the 5'-exonuclease activity (TaqMan) assay in the echocardiographic substudy of the third MONICA Augsburg survey. As ACE2 is located on the X chromosome, women and men were analysed separately. Four SNPs showed high pairwise linkage disequilibrium (rs4646156, rs879922, rs4240157 and rs233575). The minor alleles of these four SNPs were associated with higher left ventricular mass index (LVMI) and higher septal wall thickness (SWT) in men. Likewise, male carriers of a common haplotype (frequency 29.9%) consisting of the minor alleles of these four SNPs displayed higher values for LVMI and SWT than non-carriers (LVMI: TGGC 98.8+/-1.52 vs non-TGGC 94.8+/-0.99 g/m (2), p=0.027; SWT: TGGC 11.5+/-0.14 vs non-TGGC 11.1+/-0.09 mm, p=0.019). Furthermore, this haplotype was associated with an increased odds ratio (OR) for left ventricular hypertrophy (OR 3.10, p=0.006). In women, similar but less pronounced and consistent trends were observed. No association was observed between any of these SNPs and parameters of left ventricular systolic or diastolic function nor with blood pressure levels. This study provides evidence that genetic variants in the ACE2 gene may be associated with left ventricular mass, SWT and left ventricular hypertrophy in hemizygous men.

Lin, Z., et al. (2015). "[Telmisartan reduces retina vessel endothelial cell apoptosis via upregulating retinal ACE2-Ang-(1-7)-Mas axis in spontaneous hypertensive rats]." Zhonghua Xin Xue Guan Bing Za Zhi **43**(7): 625-630.

OBJECTIVE: To investigate the effects of angiotensin II (Ang II) antagonist telmisartan on retina vessel endothelial cell apoptosis and its impact on the ACE2-Ang-(1-7)-Mas axis in spontaneous hypertensive rats (SHR). METHODS: Thirty-six SHR 16 week-old were randomly divided into 3 groups (n = 12 each): SHR, SHRT (telmisartan 10 mg. kg-1. d-1 by gastric gavage) and SHRTA group (telmisartan 10 mg. kg-1. d-1 by gastric gavage plus intravenous injection of A-779 0.5 mg. kg-1. d-1), twelve WKY rats served as normotensive control group. Systolic blood pressure was measured at pre-treatment and 8 weeks later. After 8 weeks, rats were sacrificed, the expression of ACE2 and Mas in retina were analyzed by qRT-PCR, Western blot and Immunohistochemistry, the Ang-(1-7) concentration in serum was measured by ELISA. Specimens were obtained and stained by hematoxylin and eosin, and the morphology of retina vessel was observed. Apoptosis of vessel endothelial cells were determined by using terminal deoxynucleotidyl transferase mediated dUTP nick end labeling method. RESULTS: The systolic blood pressure of SHR, SHRT and SHRTA groups at baseline were significantly higher than age-matched WKY group (all $P < 0.01$). Eight weeks later, the systolic blood pressure group was significantly lower in SHRT group than in the SHR group ($P < 0.01$), this effect was partly reversed in SHRTA group. The retinal ACE2 mRNA and protein expression was significantly lower in SHR group than in WKY and SHRT groups ($P < 0.01$), which was similar between SHRT group and SHRTA group ($P > 0.05$). The retinal Mas mRNA and protein expression were significantly lower in SHR group compared to WKY and SHRT groups (all $P < 0.01$), which was significantly lower in SHRTA group than in the SHRT group ($P < 0.05$). ELISA results showed that serum Ang-(1-7) protein level was significantly lower in SHR group than in WKY group and SHRT group (both $P < 0.05$), which was lower in SHRTA group compared to SHRT group. Retinal vessel endothelial cell apoptosis was higher in SHR group than in WKY group, which could be reduced by cotreatment with telmisartan and this beneficial effect could be reversed by A-779. CONCLUSION: Telmisartan can reduce retinal vessel endothelial cell apoptosis via upregulating the ACE2-Ang-(1-7)-Mas axis.

Liu, B. C., et al. (2009). "Albumin caused the increasing production of angiotensin II due to the dysregulation of ACE/ACE2 expression in HK2 cells." Clin Chim Acta **403**(1-2): 23-30.

BACKGROUND: Previous studies have proposed that albuminuria is involved in the activation of intrarenal renin angiotensin system (RAS), while its potential mechanism is still unclear. We investigated the influence of albumin on the expression of ACE/ACE2 and generation of Ang II in HK2 cells. METHODS: The mRNA and protein expression of ACE and ACE2 was determined by RT-PCR and western blot respectively. Cellular localization of ACE and ACE2 was shown by laser scanning confocal microscope (LSCM). The concentration of Ang II in the supernatant was detected by radioimmunoassay (RIA). RESULTS: Treatment of HK-2 cells to BSA led to a significant increasing expression of ACE mRNA in dose and time dependent manner. The overexpression of ACE protein induced by BSA was consistent with its mRNA expression. Meanwhile, the mRNA and protein expression of ACE2 upon the stimulation of BSA was significantly downregulated in dose and time dependent manner. BSA could

significantly increase the production of Ang II in the supernatant ($p<0.05$). Captopril, however, attenuated the expression of ACE but increased expression of ACE2 induced by BSA. CONCLUSION: These findings suggested a novel insight on the albuminuria induced activation of intrarenal RAS by upregulation of ACE and downregulation of ACE2.

Liu, C., et al. (2019). "[Establishment of Ace2 knockout mouse model with CRISPR/Cas9 gene targeting technology]." Sheng Li Xue Bao **71**(4): 588-596.

The aim of the study was to establish Ace2 (angiotensin-converting enzyme 2) knockout mouse model with CRISPR/Cas9 gene targeting technology. A vector targeting Ace2 gene knockout was constructed with the primers of single-guide RNA (gRNA), and then transcribed gRNA/Cas9 mRNA was micro-injected into the mouse zygote. The deletion of exons 3 to 18 of Ace2 gene in mice was detected and identified by PCR and gene sequencing. The Ace2 gene knock-out mice were bred and copulated. Ace2 protein and mRNA expression were detected by Western blot and qRT-PCR in F3 progeny knock-out male mice. The gRNA expression vector was successfully constructed and transcribed in vitro, and active gRNA and Cas9 mRNA were injected directly into zygote. The deletion of exons 3 to 18 of Ace2 gene in six positive founder mice as the F0 generation were confirmed by PCR and gene sequencing. Six founder mice were mated with wild-type mice, then achieved F1 generation were mated and produced F2 generation. The female positive mouse of F2 was selected to mate with wild-type mice and produce Ace2(-/Y) mice of F3 generation. Ace2 mRNA and protein were not detected in tissues of these Ace2(-/Y) mice. In conclusion, a mouse model with Ace2 deficiency has been successfully established with CRISPR/Cas9 technique, which shall lay a foundation for future investigation of Ace2.

Liu, C., et al. (2018). "ACE2 polymorphisms associated with cardiovascular risk in Uygurs with type 2 diabetes mellitus." Cardiovasc Diabetol **17**(1): 127.

BACKGROUND: Type 2 diabetes mellitus (T2D), rapidly increasing to epidemic proportions, globally escalates cardiovascular disease risk. Although intensive interventions and comprehensive management of environmental risks factors for T2D are associated with reduced cardiovascular disease, such approaches are limited for individuals with high genetic T2D risk. In this study we investigated possible associations of ACE2 polymorphisms and cardiovascular risks in Uygur patients with T2D. METHODS: 275 Uygur T2D patients and 272 non-diabetic Uygur individuals were enrolled as study participants. 14 ACE2 polymorphisms were genotyped by Matrix-assisted laser desorption ionization time-of-flight mass spectrometry. RESULTS: ACE2 SNP rs1978124, rs2048683, rs2074192, rs233575, rs4240157, rs4646156, rs4646188 and rs879922 were associated with T2D (all $P < 0.05$). The 8 diabetic risk related ACE2 SNPs were further associated with diabetic related cardiovascular complications or events but exhibited heterogeneity as fellows: firstly, almost all diabetic risk related ACE2 SNPs (all $P < 0.05$) were associated with increased SBP except rs1978124 and rs2074192, while rs2074192, rs4646188 and rs879922 were associated elevated DBP (all $P < 0.05$). Secondly, SNP rs4646188 was not correlated with any type of dyslipidemia (TRIG, HDL-C, LDL-C or CHOL), and the other 7 diabetic risk related loci were at least correlated with one type of dyslipidemia (all $P < 0.05$). In particular, rs879922 were simultaneously correlated with four type of dyslipidemia (all $P < 0.05$). Thirdly, ACE2 SNP rs2074192 and rs879922 were associated with carotid arteriosclerosis stenosis (CAS) >/= 50% (both $P < 0.05$). Fourthly, ACE2 SNP rs2074192, rs4240157, rs4646188 and 879922 were associated with increased MAU (all $P < 0.05$). In addition, ACE2 SNP rs2048683, rs4240157, rs4646156, rs4646188 and rs879922 were linked to heavier LVMI (all $P < 0.05$), but only rs4240157, rs4646156 and rs4646188 were associated with lower LVEF (all $P < 0.05$). CONCLUSION: ACE2 SNP rs879922 may be a common genetic loci and optimal genetic susceptibility marker for T2D and T2D related cardiovascular risks in Uygurs.

Liu, C., et al. (2015). "Generation of outbred Ace2 knockout mice by RNA transfection of TALENs displaying colitis reminiscent pathophysiology and inflammation." Transgenic Res **24**(3): 433-446.

The angiotensin I converting enzyme 2 (ACE2) is a key factor in the maintenance of intestinal homeostasis. Dysregulation of homeostasis can lead to inflammation of the colon (colitis), which can cause life-threatening enfeeblement or even cancer. Animal models are valuable surrogates in deciphering the pathology behind such human conditions and for screening of putative therapeutic targets or treatment paradigms. However, development of disease models can be time-consuming and technical demanding, which might hamper their application-value. In this study, we genetically disrupted the mouse Ace2 gene by direct injection of in vitro transcribed mRNA coding for transcription activator-like effector nucleases (TALENs) into the cytoplasm of outbred Kunming mouse zygotes. Consequently, somatic mutations were induced with an efficiency of 57%, of which 39% were frameshift mutations. Moreover, all

modifications were stably transferred during germline transmission. In Ace2-knockout male mice (Ace2(-/y)), we observed severe chemical induced colitis, characterized by considerable weight loss, diarrhea and a shortened colon length. Histologically, Ace2 mutations resulted in the infiltration of leukocytes and the overt damage of the intestinal mucosal barrier. In addition, we detected an increased expression of inflammatory cytokines in the colon tissue of Ace2(-/y) mice. Collectively, the data indicate that high targeting efficiency and heritability can be achieved in an outbred mouse model by zygote injection of TALEN mRNA. Furthermore, the generated Ace2(-/y) mice display phenotypic traits reminiscent of colitis and we anticipate that such mice can be of value in studies of the intestinal microbiome or fecal transplantation.

Liu, C. Q., et al. (2007). "[Effects of ACE2-Ang 1-7-Mas axis on blood vessel]." Sheng Li Ke Xue Jin Zhan **38**(1): 43-48.

The recent identification of angiotensin-converting enzyme 2 (ACE2) and Mas receptor opened new recognition of renin-angiotensin system (RAS). ACE2, a homologue of angiotensin-converting enzyme (ACE) generates angiotensin 1-7 directly through cleaving angiotensin II, or indirectly through angiotensin I in the body. Ang 1-7 exhibits vasodilatory and antiproliferative effects, and these effects were mainly mediated by Mas receptor. So ACE2-angiotensin1-7- Mas axis was considered a negative regulation in renin angiotensin system (RAS), and its significance has been implicated into hypertension and other cardiovascular diseases. The identification of the axis opens a new potential venue for further study and understanding of RAS.

Liu, D., et al. (2016). "Association between circulating levels of ACE2-Ang-(1-7)-MAS axis and ACE2 gene polymorphisms in hypertensive patients." Medicine (Baltimore) **95**(24): e3876.

The angiotensin-converting enzyme 2-angiotensin-(1-7)-MAS axis (ACE2-Ang-[1-7]-MAS axis) plays an important role in the control of blood pressure. Some previous studies indicated that the genetic variants of ACE2 may have a potential to influence this axis. Therefore, the present study aimed at examining the association of ACE2 polymorphisms with circulating ACE2 and Ang-(1-7) levels in patients with essential hypertension.Hypertensive patients who met the inclusion criteria were enrolled in the present study. Three Tag single-nucleotide polymorphisms (rs2106809, rs4646155, and rs879922) in ACE2 gene were genotyped for all participants. Circulating ACE2 and Ang-(1-7) levels were detected by enzyme-linked immunosorbent assay.There were 96 (53.0%) females and 85 (47.0%) males participating in the present study. The circulating Ang-(1-7) levels were significantly greater in female patients carrying the rs2106809 CC or CT genotype compared with those carrying the TT genotype (1321.9 +/- 837.4 or 1077.5 +/- 804.4 pg/mL vs 751.9 +/- 612.4 pg/mL, respectively; P = 0.029, analysis of variance), whereas the circulating Ang-(1-7) levels were comparable among genotypes in male patients. In addition, there was no significant difference in the circulating ACE2 levels among rs2106809 CC, CT, and TT genotype groups in both female and male patients. The circulating ACE2 and Ang-(1-7) levels were related to neither rs4646155 nor rs879922 in female or male patients.In conclusion, the rs2106809 polymorphism of the ACE2 gene may be a determinant of the circulating Ang-(1-7) level in female patients with hypertension, suggesting a genetic association between circulating Ang-(1-7) levels and ACE2 gene polymorphisms in patients with hypertension.

Liu, F. Y., et al. (2015). "[Effect of altitude chronic hypoxia on liver enzymes and its correlation with ACE/ACE2 in yak and migrated cattle]." Zhongguo Ying Yong Sheng Li Xue Za Zhi **31**(3): 272-275.

OBJECTIVE: To investigate the difference of liver enzyme levels and its correlation with serum ACE/ACE2 among yak and cattle on Qinghai-Tibetan plateau, and to further explore the biochemical mechanism of their liver of altitude adaptation. METHODS: The serum samples of yak were collected at 3,000 m, 3,500 m, 4,000 m and 4,300 m respectively, meanwhile the serum samples of migrated cattle on plateau (2,500 m) and lowland cattle (1,300 m) were also collected. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholinesterase (CHE), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), serum lipase (LPS), angiotensin converting enzyme (ACE), angiotensin converting enzyme-2 (ACE2) in serum were measured by using fully automatic blood biochemcal analyzer. We analysed the differences of the above enzymes and its correlation with ACE/ACE2. We used one way analysis of variance (ANOVA). RESULTS: The levels of ALT in 4,000 m group and 4,300 m group of yak increased significantly compared with other groups, there were no statistically significant differences in AST, CHE, GGT, ACE/ACE2 levels of yaks at different altitudes. As compared to lowland cattle, the serum levels of AST and CHE were increased, the level of LPS and ACE was decreased significantly, respectively, and especially, the ratio of ACE/ACE2 of migranted cattle reduced nearly two times. The levels of LPS were significantly correlated to the ratio of ACE/ACE2 in yak (r = 0.357, P < 0.01), and a high

correlation between ALP and ACE/ACE2 in lowland cattle (r = 0.418, P < 0.05), But the biggest contribution rate of the ratio of ACE/ACE2 was only 17.5% for the changes of the levels of liver enzyme. CONCLUSION: The results indicated that with the altitude increased did not significantly influence the changes of liver enzymes' activities in mountainous yaks but not in cattle. However, all above these changes weren't actually correlated to the ratio of ACE/ACE2.

Liu, J., et al. (2018). "Sini decoction alleviates E. coli induced acute lung injury in mice via equilibrating ACE-AngII-AT1R and ACE2-Ang-(1-7)-Mas axis." Life Sci **208**: 139-148.

AIMS: Acute respiratory distress syndrome (ARDS), one of the serious form of acute lung injury (ALI), is the primary cause of death in patients with ALI. Sini decoction (SND) is a widely used Traditional Chinese Medicine (TCM). However, the application of SND in ALI is rarely reported. Previous studies have found that renin-angiotensin-aldosterone system (RAAS) played vital and bidirectional roles in ALI. Therefore, the aim of the present study was to investigate protective effect of SND on ALI model induced by E. coli, as well as to further explore relations between RAAS and SND. MATERIALS AND METHODS: The ALI model was evaluated by morphological observations and biochemical assays. The expression levels of angiotensin converting enzyme (ACE), Angiotensin II type 1 receptor (AT1R) and angiotensin converting enzyme 2 (ACE2) were examined by Western blotting. The expression levels of angiotensinII (AngII) and angiotensin-(1-7) (Ang-(1-7)) were measured through ELISA. MasR, IL-6, IL-1beta and TNFalpha were all measured using qRT-PCR. KEY FINDINGS: SND significantly ameliorated E. coli-induced ALI, including reducing inflammatory factors in lung tissue and the activity of MPO in serum. Furthermore, SND could obviously decrease the expression of ACE, AngII and AT1R, which were induced by E. coli. On the other hand, SND could markedly activate ACE2-Ang-(1-7)-Mas pathway. SIGNIFICANCE: In this paper, we demonstrated that SND alleviates E. coli induced acute lung injury in mice via equilibrating ACE-AngII-AT1R and ACE2-Ang-(1-7)-Mas axis.

Lu, N., et al. (2012). "ACE2 gene polymorphism and essential hypertension: an updated meta-analysis involving 11,051 subjects." Mol Biol Rep **39**(6): 6581-6589.

The polymorphisms of angiotensin-converting enzyme 2 (ACE2) gene have been suggested to be linked to increase risk of essential hypertension in multiple populations. However, the results are still debatable. To assess the association between ACE2 G8970A genetic polymorphism and essential hypertension, we conducted a meta-analysis of case-control studies across different ethnicity. PubMed, Embase, CBM, Wanfang and VIP databases were searched, and a total of 11 separate studies in females and nine separate studies in males met the inclusion criteria. Because ACE2 is on the X chromosome, data for each sex were analyzed separately. The selected studies contained 7,251 (4,472 females/2,779 males) hypertensive patients and 3,800 (2,161 females/1,639 males) normotensive controls. A statistically significant association was observed between the G8970A gene polymorphism and essential hypertension risk in female hypertensive group in the recessive genetic model (AA vs. GG+GA: P = 0.03, OR = 1.15, 95% CI = 1.02-1.30, P (heterogeneity) = 0.40, I (2) = 5%, fixed-effects model). Although no association was shown between the frequency of the A allele and the genetic susceptibility to essential hypertension in all male patients (A Allele: P = 0.38, OR = 1.10, 95% CI = 0.89-1.38, P (heterogeneity) = 0.02, I (2) = 56%, random-effects model), we found that the relationship between carrier of A allele and the essential hypertension risk in Han-Chinese male patients subgroup (A Allele: P = 0.006, OR = 1.21, 95% CI = 1.06-1.38, P (heterogeneity) = 0.10, I (2) = 44%, fixed-effects model). The current meta-analysis provided solid evidence suggesting that ACE2 gene polymorphism G8790A was probably a genetic risk factor for essential hypertension across different ethnic populations in female subjects and in Han-Chinese male subjects.

Luan, J., et al. (2020). "Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection." Biochem Biophys Res Commun.

SARS-CoV-2 causes the recent global COVID-19 public health emergency. ACE2 is the receptor for both SARS-CoV-2 and SARS-CoV. To predict the potential host range of SARS-CoV-2, we analyzed the key residues of ACE2 for recognizing S protein. We found that most of the selected mammals including pets (dog and cat), pangolin and Circetidae mammals remained the most of key residues for association with S protein from SARS-CoV and SARS-CoV-2. The interaction interface between cat/dog/pangolin/Chinese hamster ACE2 and SARS-CoV/SARS-CoV-2 S protein was simulated through homology modeling. We identified that N82 in ACE2 showed a closer contact with SARS-CoV-2 S protein than M82 in human ACE2. Our finding will provide important insights into the host range of SARS-CoV-2 and a new strategy to design an optimized ACE2 for SARS-CoV-2 infection.

Lubel, J. S., et al. (2008). "Angiotensin converting enzyme 2 (ACE2) activity in fetal calf serum: implications for cell culture research." Cytotechnology **58**(3): 119-126.

Cell culture experiments often employ the use of culture media that contain fetal calf serum (FCS). The angiotensin peptides angiotensin II and angiotensin 1-7 have opposing effects with angiotensin converting enzyme 2 (ACE2) being the enzyme predominantly responsible for generating angiotensin 1-7 from angiotensin II. The effect of FCS on angiotensin peptides has not previously been described. We have shown that FCS has ACE2 enzyme activity capable of degrading angiotensin II and generating angiotensin 1-7. Researchers should be aware that FCS possesses ACE2 activity and that heat-treating FCS to 56 degrees C only partially inhibits this enzyme activity, whereas heat-treating to 70 degrees C completely abolishes ACE2 activity.

Lv, Q., et al. (2017). "Effects of Taurine on ACE, ACE2 and HSP70 Expression of Hypothalamic-Pituitary-Adrenal Axis in Stress-Induced Hypertensive Rats." Adv Exp Med Biol **975 Pt 2**: 871-886.

The experiment was to elucidate protective mechanism of taurine against stress-induced hypertension. Thirty two male Wistar rats were randomly divided into four groups. Normal control group and stress control group were intragastrically administered saline; beta-alanine stress group were fed with beta-alanine (200 mg/kg/day) and taurine stress group with taurine (200 mg/kg/day). The hypertensive model was established by giving rats stress for 3 weeks.Results showed that significant expression levels of angiotensin converting enzyme (ACE) in the hypothalamus, pituitary and adrenal were observed in beta-alanine stress group and stress control group ($P < 0.05$), but significant mRNA expression levels of angiotensin-converting enzyme 2 (ACE2) in taurine stress group and normal control group ($P < 0.05$). All the groups showed no significant differences in HSP70 mRNA expression levels in hypothalamus ($P > 0.05$), while taurine stress group exhibited the highest HSP70 mRNA expression levels both in pituitary and in adrenal ($P < 0.05$). It was also found that beta-alanine stress group and stress control group had significantly higher protein expression levels of ACE in hypothalamus, pituitary and adrenal ($P < 0.05$), but significantly lower protein expression of ACE2 compared to taurine stress group and control groups ($P < 0.05$). The results indicated that taurine regulated the hypothalamus pituitary adrenal (HPA) axis of the renin-angiotensin-aldosterone system (RAAS) by inhibiting ACE gene and protein expressions and promoting ACE2 and HSP70 protein expressions, thereby contributing to the prevention of stress-induced hypertension.

Ma, C., et al. (2014). "Relationship between renal injury and the antagonistic roles of angiotensin-converting enzyme (ACE) and ACE2." Genet Mol Res **13**(2): 2333-2342.

Angiotensin-converting enzyme 2 (ACE2), a newly discovered carboxypeptidase in the renin-angiotensin system (RAS), antagonizes ACE activity and plays an active role during tissue injury. Yet the mechanism of its action is not well known. Using a streptozotocin (STZ)-induced renal injury rat model, we investigated the relationship between renal injury and the antagonism between ACE and ACE2. We assayed the levels of urea nitrogen, urine glucose, creatinine, and protein, Ace2, Ace, angiotensin II type 1 receptor (At1) and Mas receptor mRNA, and renal and plasma angiotensin II (Ang II) in STZ-treated and untreated rats. We also used histology and immunohistochemistry to assess glomerular injury and ACE2 glomerular and cortical expression. The amounts of urea nitrogen, urine glucose, creatinine, and protein were significantly higher in STZ-treated rats than in control rats ($P < 0.01$). There were significant pathological changes in the kidney upon STZ-treatment. Ace2 and Ace mRNA levels were significantly higher in STZ-treated rats than in control rats ($P < 0.05$ and $P = 0.05$, respectively). There was no significant difference in the Mas receptor and At1 mRNA levels in the 2 groups, although At1 levels showed an increase upon STZ-treatment. The Ang II level in the renal cortical tissue and plasma of STZ-treated rats was higher than that of control rats ($P < 0.05$). The increase in Ace mRNA levels was higher than that of Ace2 mRNA levels, leading to an elevated Ace/Ace2 ratio. Together, these data suggest that the ACE-Ang II-AT1 axis is the dominant axis in severe kidney injury.

MacCallum, D. M., et al. (2006). "Different consequences of ACE2 and SWI5 gene disruptions for virulence of pathogenic and nonpathogenic yeasts." Infect Immun **74**(9): 5244-5248.

Mutants of Candida albicans, Candida glabrata, and Saccharomyces cerevisiae with disruptions in the ACE2 gene and C. glabrata and S. cerevisiae swi5 disruption mutants were tested for virulence in a murine challenge model of disseminated yeast infection. All mutants showed a clumping phenotype, but clumping was minimized in challenge inocula by inclusion of chitinase in the growth medium. In animals rendered temporarily neutropenic by cyclophosphamide treatment, the C. glabrata ace2 null mutant was confirmed as hypervirulent: it led to early terminal illness and kidney, brain, and lung fungal

burdens substantially and significantly larger than those in controls. The C. glabrata swi5 null mutant did not lead to terminal illness but generated significantly larger brain and lung burdens than those in controls. The C. albicans ace2 null mutant was very slightly attenuated and the S. cerevisiae ace2 and swi5 null mutants were substantially attenuated relative to their parental control strains. The phenotype of aggressive hypervirulence, unique to disruption of the C. glabrata ACE2 gene among the strains tested, was not seen when the C. glabrata ace2 strain was tested in immunologically intact mice. The different effects seen with these mutants rule out the clumping phenotype as the explanation for hypervirulence in the C. glabrata ace2 mutant. The absence of C. glabrata ace2 hypervirulence in healthy mice may be a tool for definitive future study of host-parasite cross talk in microbial opportunism.

Majumder, K., et al. (2015). "Egg ovotransferrin-derived ACE inhibitory peptide IRW increases ACE2 but decreases proinflammatory genes expression in mesenteric artery of spontaneously hypertensive rats." Mol Nutr Food Res **59**(9): 1735-1744.

SCOPE: Egg ovotransferrin-derived angiotensin converting enzyme (ACE) inhibitory peptide IRW was previously shown to reduce blood pressure in spontaneously hypertensive rats through reduced vascular inflammation and increased nitric oxide-mediated vasorelaxation. The main objective of the present study was to investigate the molecular mechanism of this peptide through transcriptome analysis by RNAseq technique. METHODS AND RESULTS: Total RNA was extracted from kidney and mesenteric arteries; the RNAseq libraries (from untreated and IRW-treated groups) were constructed and subjected to sequence using HiSeq 2000 system (Illumina) system. A total of 12 764 and 13 352 genes were detected in kidney and mesenteric arteries, respectively. The differentially expressed (DE) genes between untreated and IRW-treated groups were identified and the functional analysis through ingenuity pathway analysis revealed a greater role of DE genes identified from mesenteric arteries than that of kidney in modulating various cardiovascular functions. Subsequent qPCR analysis further confirmed that IRW significantly increased the expression of ACE-2, ABCB-1, IRF-8, and CDH-1 while significantly decreased the expression ICAM-1 and VCAM-1 in mesenteric arteries. CONCLUSION: Our research showed for the first time that ACE inhibitory peptide IRW could contribute to its antihypertensive activity through increased ACE2 and decreased proinflammatory genes expression.

Mak, K. Y., et al. (2015). "ACE2 Therapy Using Adeno-associated Viral Vector Inhibits Liver Fibrosis in Mice." Mol Ther **23**(9): 1434-1443.

Angiotensin converting enzyme 2 (ACE2) which breaks down profibrotic peptide angiotensin II to antifibrotic peptide angiotensin-(1-7) is a potential therapeutic target in liver fibrosis. We therefore investigated the long-term therapeutic effect of recombinant ACE2 using a liver-specific adeno-associated viral genome 2 serotype 8 vector (rAAV2/8-ACE2) with a liver-specific promoter in three murine models of chronic liver disease, including carbon tetrachloride-induced toxic injury, bile duct ligation-induced cholestatic injury, and methionine- and choline-deficient diet-induced steatotic injury. A single injection of rAAV2/8-ACE2 was administered after liver disease has established. Hepatic fibrosis, gene and protein expression, and the mechanisms that rAAV2/8-ACE2 therapy associated reduction in liver fibrosis were analyzed. Compared with control group, rAAV2/8-ACE2 therapy produced rapid and sustained upregulation of hepatic ACE2, resulting in a profound reduction in fibrosis and profibrotic markers in all diseased models. These changes were accompanied by reduction in hepatic angiotensin II levels with concomitant increases in hepatic angiotensin-(1-7) levels, resulting in significant reductions of NADPH oxidase assembly, oxidative stress and ERK1/2 and p38 phosphorylation. Moreover, rAAV2/8-ACE2 therapy normalized increased intrahepatic vascular tone in fibrotic livers. We conclude that rAAV2/8-ACE2 is an effective liver-targeted, long-term therapy for liver fibrosis and its complications without producing unwanted systemic effects.

Malek, V., et al. (2019). "Simultaneous inhibition of neprilysin and activation of ACE2 prevented diabetic cardiomyopathy." Pharmacol Rep **71**(5): 958-967.

BACKGROUND: Neprilysin inhibitors (NEPi) are assisting the renin-angiotensin system (RAS) inhibitors in halting diabetic cardiomyopathy (DCM). Away from conventional tactic, a recent report revealed the renoprotective potential of NEPi and angiotensin-converting enzyme (ACE2) activator combination therapy against diabetic nephropathy. However, this combination so far not evaluated against DCM, thus the present investigation aiming the same. METHODS: Streptozotocin-induced (55 mg/kg, ip) type 1 diabetic (T1 D) male Wistar rats were treated with either monotherapy of thiorphan (0.1 mg/kg/day, po) or diminazene aceturate (5 mg/kg/day, po), or their combination therapy, for four weeks. After hemodynamic measurements, all the rats' heart and plasma were collected for biochemistry, ELISA, histopathology, and immunoblotting. RESULTS:

Metabolic perturbations and failing cardiac functions associated with diabetes were markedly attenuated by combination therapy. Besides, unfavourable alterations in RAS and natriuretic peptides system (NPS) were corrected by combination therapy. Interestingly, combination therapy significantly increased plasma and heart cGMP levels compared to T1D and monotherapy receiving rats. Moreover, rats receiving combination therapy exhibited significant inhibition of activated NF-KB, TGF-p and apoptotic signalling, and a notable reduction in cardiac fibrosis when compared to Tl D rats. Expressions of posttranslational histone modifications markers; H3K4Me2 and its methyltransferases (SET7/9 and RBBP5) were significantly enhanced in T1D hearts, which were significantly reduced by combination therapy. CONCLUSIONS: The NEPi and ACE2 activator combination therapy effectively prevented DCM by normalising RAS and NPS activities, increasing cGMP, inhibiting inflammatory, pro-fibrotic and apoptotic signalling, and reversing H3K4Me2 and its methyl transferases expressions.

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McBride, H. J., et al. (1999). "Distinct regions of the Swi5 and Ace2 transcription factors are required for specific gene activation." J Biol Chem **274**(30): 21029-21036.

Swi5 and Ace2 are cell cycle-regulated transcription factors that activate expression of early G (1)-specific genes in Saccharomyces cerevisiae. Swi5 and Ace2 have zinc finger DNA-binding domains that are highly conserved, and the two proteins bind to the same DNA sequences in vitro. Despite this similarity in DNA binding, Swi5 and Ace2 activate different genes in vivo, with Swi5 activating the HO gene and Ace2 activating CTS1 expression. In this report we have used chimeric fusions between Swi5 and Ace2 to determine what regions of these proteins are necessary for promoter-specific activation of HO and CTS1. We have identified specific regions of Swi5 and Ace2 that are required for activation of HO and CTS1, respectively. The Swi5 protein binds HO promoter DNA cooperatively with the Pho2 homeodomain protein, and the HO specificity region of Swi5 identified in the chimeric analysis coincides with the region of Swi5 previously identified that interacts with Pho2 in vitro. Swi5 and Ace2 also activate expression of a number of other genes expressed in G (1) phase of the cell cycle, including ASH1, CDC6, EGT2, PCL2, PCL9, RME1, and SIC1. Analysis of the Swi5/Ace2 chimeras shows that distinct regions of Swi5 and Ace2 contribute to the transcriptional activation of some of these other G (1)-regulated genes.

Mehta, S., et al. (2009). "The Med8 mediator subunit interacts with the Rpb4 subunit of RNA polymerase II and Ace2 transcriptional activator in Schizosaccharomyces pombe." FEBS Lett **583**(19): 3115-3120.

Several proteins are involved in separation of cells following division. However, their mutual interactions leading to cell separation is complex and not well understood. To explore the protein network that regulates this process at the transcriptional level in Schizosaccharomyces pombe, we have investigated the role of three proteins Med8, Rpb4 and Ace2. Using genetic and biochemical approaches we demonstrate that Ace2 binds Med8, which in turn interacts with

Rpb4. We have delineated regions of Med8 and Rpb4 involved in their binding. We show that Med8 carboxyl-terminal region is necessary for its interaction with Rpb4 and can partially complement the sep15-598 mutant. Our results suggest that Med8 mediator subunit is involved in transmitting regulatory information from Ace2 to RNA polymerase II via Rpb4.

Mendoza-Torres, E., et al. (2015). "ACE2 and vasoactive peptides: novel players in cardiovascular/renal remodeling and hypertension." Ther Adv Cardiovasc Dis **9**(4): 217-237.

The renin-angiotensin system (RAS) is a key component of cardiovascular physiology and homeostasis due to its influence on the regulation of electrolyte balance, blood pressure, vascular tone and cardiovascular remodeling. Deregulation of this system contributes significantly to the pathophysiology of cardiovascular and renal diseases. Numerous studies have generated new perspectives about a noncanonical and protective RAS pathway that counteracts the proliferative and hypertensive effects of the classical angiotensin-converting enzyme (ACE)/angiotensin (Ang) II/angiotensin type 1 receptor (AT1R) axis. The key components of this pathway are ACE2 and its products, Ang-(1-7) and Ang-(1-9). These two vasoactive peptides act through the Mas receptor (MasR) and AT2R, respectively. The ACE2/Ang-(1-7)/MasR and ACE2/Ang-(1-9)/AT2R axes have opposite effects to those of the ACE/Ang II/AT1R axis, such as decreased proliferation and cardiovascular remodeling, increased production of nitric oxide and vasodilation. A novel peptide from the noncanonical pathway, alamandine, was recently identified in rats, mice and humans. This heptapeptide is generated by catalytic action of ACE2 on Ang A or through a decarboxylation reaction on Ang-(1-7). Alamandine produces the same effects as Ang-(1-7), such as vasodilation and prevention of fibrosis, by interacting with Mas-related GPCR, member D (MrgD). In this article, we review the key roles of ACE2 and the vasoactive peptides Ang-(1-7), Ang-(1-9) and alamandine as counter-regulators of the ACE-Ang II axis as well as the biological properties that allow them to regulate blood pressure and cardiovascular and renal remodeling.

Minato, T., et al. (2020). "B38-CAP is a bacteria-derived ACE2-like enzyme that suppresses hypertension and cardiac dysfunction." Nat Commun **11**(1): 1058.

Angiotensin-converting enzyme 2 (ACE2) is critically involved in cardiovascular physiology and pathology, and is currently clinically evaluated to treat acute lung failure. Here we show that the B38-CAP, a carboxypeptidase derived from Paenibacillus sp. B38, is an ACE2-like enzyme to decrease angiotensin II levels in mice. In protein 3D structure analysis, B38-CAP homolog shares structural similarity to mammalian ACE2 with low sequence identity. In vitro, recombinant B38-CAP protein catalyzed the conversion of angiotensin II to angiotensin 1-7, as well as other known ACE2 target peptides. Treatment with B38-CAP suppressed angiotensin II-induced hypertension, cardiac hypertrophy, and fibrosis in mice. Moreover, B38-CAP inhibited pressure overload-induced pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction in mice. Our data identify the bacterial B38-CAP as an ACE2-like carboxypeptidase, indicating that evolution has shaped a bacterial carboxypeptidase to a human ACE2-like enzyme. Bacterial engineering could be utilized to design improved protein drugs for hypertension and heart failure.

Mineharu, Y., et al. (2006). "Association analysis of common variants of ELN, NOS2A, APOE and ACE2 to intracranial aneurysm." Stroke **37**(5): 1189-1194.

BACKGROUND AND PURPOSE: Previous studies have shown positive evidence of linkage of the intracranial aneurysm (IA) at chromosome 7q11, 17cen, 19q13, and Xp22. These regions contain elastin (ELN), nitric oxide synthetase 2A (NOS2A), apolipoprotein E (APOE), and angiotensin-I converting enzyme 2 (ACE2), which are considered to be promising candidate genes for IA. We aimed to examine the association of single-nucleotide polymorphisms (SNPs) with IA in these candidate genes. METHODS: To identify polymorphisms in NOS2A and ACE2, all exons and exon-intron boundaries were screened by direct sequencing in 30 randomly selected controls. The program tagSNPs was used to select an optimal set of haplotype-tagging SNPs. For ELN and APOE, SNPs were selected from previous reports. These selected SNPs were then genotyped in 362 cases with IA and 332 residential area matched controls. THESIAS software was used to investigate the association of alleles and haplotypes with IA by adjusting with covariates. RESULTS: We genotyped 8 SNPs in ELN, 8 SNPs in NOS2A, 3 epsilon alleles in APOE and 1 SNP in ACE2. No alleles or haplotypes of 4 candidate genes revealed any significant association with IA. CONCLUSIONS: Investigated polymorphisms in this study were not associated with IA.

Mizuiri, S., et al. (2011). "Increased ACE and decreased ACE2 expression in kidneys from patients with IgA nephropathy." Nephron Clin Pract **117**(1): c57-66.

BACKGROUND: Angiotensin-converting enzyme (ACE)2 forms angiotensin-1-7 which may protect kidney in a counterregulatory manner to angiotensin II. Recent studies revealed increased ACE and decreased ACE2 expression in kidneys of patients with diabetic nephropathy. However, these changes may not be specific for diabetic nephropathy. We studied ACE and ACE2 expression in patients with IgA nephropathy. METHODS: Renal ACE and ACE2 expression was assessed by immunohistochemistry and in situ hybridization in 30 patients with IgA nephropathy and 21 healthy controls. Correlation between ACE and ACE2 expression and levels of various biochemical parameters was also assessed. Gene expression was also assessed in minimal change nephrotic syndrome (MCNS) and membranous nephropathy (MN) as disease controls. RESULTS: Reduced ACE2 expression ($p < 0.01$) and increased ACE expression in glomeruli ($p < 0.001$), and reduced ACE2 expression in tubulointerstitium ($p < 0.001$) were observed in patients with IgA nephropathy compared to healthy controls, although the changes in ACE2 mRNA were not statistically significant. Reduced renal ACE2 expression was also found in MN but not in MCNS. Correlation between renal ACE and ACE2 expression and proteinuria was not observed in IgA nephropathy. CONCLUSION: IgA nephropathy is associated with increased ACE and decreased ACE2 expression in kidneys, as in diabetic nephropathy.

Mizuiri, S., et al. (2008). "Expression of ACE and ACE2 in individuals with diabetic kidney disease and healthy controls." Am J Kidney Dis **51**(4): 613-623.

BACKGROUND: Angiotensin-converting enzyme (ACE) 2 (ACE2) is expressed mainly in the heart and kidney and forms angiotensin-1-7 from angiotensin II. ACE2 might act in a counterregulatory manner to ACE. There is little information about renal ACE and ACE2 expression in human diabetic nephropathy. STUDY DESIGN: Cross-sectional study. SETTING & PARTICIPANTS: Kidney tissue from 20 patients with type 2 diabetes and overt nephropathy and 20 healthy kidney donors. PREDICTOR: Diabetes status. OUTCOMES & MEASUREMENTS: Renal expression of ACE and ACE2 assessed by means of immunohistochemistry and in situ hybridization. Correlation between ACE and ACE2 expression and levels of various biochemical parameters. RESULTS: Decreased ACE2 and increased ACE expression in both the tubulointerstitium and glomeruli resulted in a significant ($P < 0.001$) increase in ACE/ACE2 ratio in patients with diabetes with overt nephropathy compared with controls, although ACE messenger RNA in the tubulointerstitium did not significantly increase. ACE/ACE2 ratio correlated positively with values for mean blood pressure, fasting blood glucose, serum creatinine, proteinuria, and hemoglobin A (1c) and inversely with estimated glomerular filtration rate ($P < 0.001$). LIMITATIONS: Inclusion of small number of human renal biopsy specimens with structural distortion of cortical tissue. CONCLUSIONS: The high ACE/ACE2 ratio in kidneys of patients with type 2 diabetes with overt nephropathy may contribute to renal injury.

Mizuiri, S. and Y. Ohashi (2015). "ACE and ACE2 in kidney disease." World J Nephrol **4**(1): 74-82.

Renin angiotensin system (RAS) activation has a significant influence on renal disease progression. The classical angiotensin-converting enzyme (ACE)-angiotensin II (Ang II)-Ang II type 1 (AT1) axis is considered to control the effects of RAS activation on renal disease. However, since its discovery in 2000 ACE2 has also been demonstrated to have a significant impact on the RAS. The synthesis and catabolism of Ang II are regulated via a complex series of interactions, which involve ACE and ACE2. In the kidneys, ACE2 is expressed in the proximal tubules and less strongly in the glomeruli. The synthesis of inactive Ang 1-9 from Ang I and the catabolism of Ang II to produce Ang 1-7 are the main functions of ACE2. Ang 1-7 reduces vasoconstriction, water retention, salt intake, cell proliferation, and reactive oxygen stress, and also has a renoprotective effect. Thus, in the non-classical RAS the ACE2-Ang 1-7-Mas axis counteracts the ACE-Ang II-AT1 axis. This review examines recent human and animal studies about renal ACE and ACE2.

Moon, J. Y. (2011). "ACE2 and Angiotensin-(1-7) in Hypertensive Renal Disease." Electrolyte Blood Press **9**(2): 41-44.

The recently discovered angiotensin-converting enzyme-related carboxypeptidase 2 (ACE2)-[Angiotensin-(1-7) (Ang-(1-7)]-Mas receptor axis has an opposing function to that of the ACE-Angiotensin II (Ang II)-Angiotensin type 1 (AT1) receptor axis. Ang-(1-7) is present in the kidneys at concentrations comparable to those of Ang II and is associated with vasodilation, modulation of sodium and water transport, and stimulation of nitric oxide (NO) synthase. Ang-(1-7) also acts as a physiological antagonist of Ang II by counterbalancing the Ang II-mediated intracellular signaling pathway. In a hypertensive model, increased ACE and decreased ACE2 along with a higher ACE/ACE2 ratio in hypertensive kidneys appeared to favor Ang II generation, leading to hypertensive renal damage. In addition, the administration of a selective Ang-(1-7) receptor blocker or an ACE2 inhibitor was associated

with worsening of hypertension and renal function. Ang-(1-7)-mediated increases in renal blood flow were abolished by blockade of the Mas receptor and by inhibition of prostaglandin release and NO in spontaneously hypertensive rats and in Wistar-Kyoto controls. Further research on the function of the ACE2-Ang-(1-7)-Mas receptor axis could lead to a novel target for inhibiting kidney disease progression.

Moon, J. Y., et al. (2008). "Renal ACE and ACE2 expression in early diabetic rats." Nephron Exp Nephrol **110**(1): e8-e16.

BACKGROUND/AIM: The role of angiotensin-converting enzyme (ACE)-related carboxypeptidase-2 (ACE2) in the regulation of the renin-angiotensin system is not well characterized. This study investigated the changes in the expression of ACE and ACE2 in the kidney in early diabetic rats. METHODS: Streptozotocin-induced diabetic rats were examined. The concentrations of angiotensin II in plasma, urine, and renal cortex were measured by radioimmunoassay. The mRNA expression of ACE, ACE2, angiotensin II type 1 receptor (AT1R), and Mas receptor (MasR) in glomeruli and cortex was assessed using real-time PCR. The glomerular and cortical expression of ACE and ACE2 was assessed by immunohistochemistry. RESULTS: For renal cortical tissue, the angiotensin II level was more intensified in the 8-week diabetic rats. Immunohistochemical experiments showed that ACE was increased, but ACE2 was decreased in the glomeruli of 8-week diabetic rats, while both ACE and ACE2 in the tubules were increased. The AT1R mRNA in the glomeruli was decreased, while the MasR mRNA was increased in 2-week diabetic rats. CONCLUSION: The combined effects of increased ACE and decreased ACE2 in glomeruli may be associated with the activation of the renin-angiotensin system in early diabetic rats, which is known to increase proteinuria.

Moritani, T., et al. (2013). "ACE2 deficiency induced perivascular fibrosis and cardiac hypertrophy during postnatal development in mice." J Am Soc Hypertens **7**(4): 259-266.

In order to investigate the role of angiotensin-converting enzyme 2 (ACE2) in cardiac development, we examined the effects of ACE2 deficiency on postnatal development of the heart using ACE2-knockout (ACE2KO) mice. Heart samples of wild type (WT; C57BL/6J) mice and ACE2KO mice were taken at 1, 4, and 10 weeks of age. In WT mice, expression of ACE2 mRNA increased from 1 week to 10 weeks. A similar increase was observed in immunostaining of ACE2 in the heart, in which ACE2 was strongly expressed in coronary arteries. Compared with WT mice, heart weight was greater in ACE2KO mice at 4 weeks, and coronary artery thickening and perivascular fibrosis were also already enhanced from 4 weeks. Consistent with the increase of fibrosis, cardiac expression of collagen and TIMP was higher, and expression of MMP was lower in ACE2KO mice at 4 weeks. In addition, TGF-beta mRNA was also higher, and lower expression of PPARgamma mRNA was observed at 4 weeks in ACE2KO mice. These results suggest that ACE2 plays an important role in postnatal development of the heart, and that lack of ACE2 enhances coronary artery remodeling with an increase in perivascular fibrosis and cardiac hypertrophy already around the weaning period.

Mossel, E. C., et al. (2005). "Exogenous ACE2 expression allows refractory cell lines to support severe acute respiratory syndrome coronavirus replication." J Virol **79**(6): 3846-3850.

Of 30 cell lines and primary cells examined, productive severe acute respiratory syndrome coronavirus (Urbani strain) (SARS-CoV) infection after low-multiplicity inoculation was detected in only six: three African green monkey kidney epithelial cell lines (Vero, Vero E6, and MA104), a human colon epithelial line (CaCo-2), a porcine kidney epithelial line [PK (15)], and mink lung epithelial cells (Mv 1 Lu). SARS-CoV produced a lytic infection in Vero, Vero E6, and MA104 cells, but there was no visible cytopathic effect in Caco-2, Mv 1 Lu, or PK (15) cells. Multistep growth kinetics were identical in Vero E6 and MA104 cells, with maximum titer reached 24 h postinoculation (hpi). Virus titer was maximal 96 hpi in CaCo-2 cells, and virus was continually produced from infected CaCo-2 cells for at least 6 weeks after infection. CaCo-2 was the only human cell type of 13 tested that supported efficient SARS-CoV replication. Expression of the SARS-CoV receptor, angiotensin-converting enzyme 2 (ACE2), resulted in SARS-CoV replication in all refractory cell lines examined. Titers achieved were variable and dependent upon the method of ACE2 expression.

Motta-Santos, D., et al. (2016). "Effects of ACE2 deficiency on physical performance and physiological adaptations of cardiac and skeletal muscle to exercise." Hypertens Res **39**(7): 506-512.

The renin-angiotensin system (RAS) is related to physiological adaptations induced by exercise. Angiotensin-converting enzyme (ACE) 2 is a major regulator of the RAS in tissues, as it metabolizes angiotensin (Ang) II to Ang-(1-7). The aim of this study was to determine the effects of ACE2 deficiency on physical performance and physiological adaptations induced by voluntary running. Physical performance, body composition and plasma angiotensin levels, as well as tissue morphology and gene expression of

RAS components in the left ventricle (LV) and skeletal muscle (gastrocnemius), were evaluated in ACE2-deficient (ACE2(-/y)) and wild-type (ACE2(+/y)) mice after 6 weeks of voluntary wheel running. ACE2(-/y) mice run less than ACE2(+/y) mice (19+/-4.7 vs. 26+/-12.6 revolutions per day x 100, P<0.01). The ACE2(+/y) group presented a lower fat mass (15+/-1.1%) and higher muscle mass (76.6+/-1.6%) after 6 weeks of voluntary running compared with the sedentary control group (fat mass: 18.3+/-2.1%; muscle mass: 72.7+/-2.2). However, no change in body composition was observed in ACE2(-/y) mice after exercise. Heart and skeletal muscle hypertrophy was observed only in trained ACE2(+/y) mice. Besides a small decrease in Ang I in ACE2(-/y) mice, plasma levels of angiotensin peptides remained unchanged by exercise or ACE2 deficiency. In the LV of trained animals, AT2 gene expression was higher in ACE2(+/y) compared with ACE2(-/y) mice. ACE2 deficiency leads to an increase in AT1 gene expression in skeletal muscle. ACE expression in soleus was increased in all exercised groups. ACE2 deficiency affects physical performance and impairs cardiac and skeletal muscle adaptations to exercise.

Mukerjee, S., et al. (2019). "ACE2 and ADAM17 Interaction Regulates the Activity of Presympathetic Neurons." Hypertension **74**(5): 1181-1191.

Brain renin angiotensin system within the paraventricular nucleus plays a critical role in balancing excitatory and inhibitory inputs to modulate sympathetic output and blood pressure regulation. We previously identified ACE2 and ADAM17 as a compensatory enzyme and a sheddase, respectively, involved in brain renin angiotensin system regulation. Here, we investigated the opposing contribution of ACE2 and ADAM17 to hypothalamic presympathetic activity and ultimately neurogenic hypertension. New mouse models were generated where ACE2 and ADAM17 were selectively knocked down from all neurons (AC-N) or Sim1 neurons (SAT), respectively. Neuronal ACE2 deletion revealed a reduction of inhibitory inputs to AC-N presympathetic neurons relevant to blood pressure regulation. Primary neuron cultures confirmed ACE2 expression on GABAergic neurons synapsing onto excitatory neurons within the hypothalamus but not on glutamatergic neurons. ADAM17 expression was shown to colocalize with angiotensin-II type 1 receptors on Sim1 neurons, and the pressor relevance of this neuronal population was demonstrated by photoactivation. Selective knockdown of ADAM17 was associated with a reduction of FosB gene expression, increased vagal tone, and prevented the acute pressor response to centrally administered angiotensin-II. Chronically, SAT mice exhibited a blunted blood pressure elevation and preserved ACE2 activity during development of salt-sensitive hypertension. Bicuculline injection in those models confirmed the supporting role of ACE2 on GABAergic tone to the paraventricular nucleus. Together, our study demonstrates the contrasting impact of ACE2 and ADAM17 on neuronal excitability of presympathetic neurons within the paraventricular nucleus and the consequences of this mutual regulation in the context of neurogenic hypertension.

Mulhern, S. M., et al. (2006). "Candida albicans transcription factor Ace2 regulates metabolism and is required for filamentation in hypoxic conditions." Eukaryot Cell **5**(12): 2001-2013.

Ace2 transcription factor family genes are found in many fungal genomes and are required for regulation of expression of genes involved in cell separation. We used transcriptional profiling to identify the targets of Ace2 in Candida albicans, and we show that these include several cell wall components, such as glucanases and glycosylphosphatidylinositol-anchored proteins. Expression is downregulated in ace2 deletion mutants in both yeast and hyphal cells. In addition, deleting ace2 results in dramatic changes in expression of metabolic pathways. Expression of glycolytic enzymes is reduced, while expression of respiratory genes (including those involved in the tricarboxylic acid cycle, oxidative phosphorylation, and ATP synthesis) is increased. Similar changes occur in both yeast and hyphal cells. In contrast, genes required for acetyl-coenzyme A and lipid metabolism are upregulated in an ace2 deletion mutant grown predominantly as yeast cells but are downregulated in hyphae. These results suggest that in wild-type strains, Ace2 acts to increase glycolysis and reduce respiration. This is supported by the observation that deleting ace2 results in increased resistance to antimycin A, a drug that inhibits respiration. We also show that Ace2 is required for filamentation in response to low oxygen concentrations (hypoxia). We suggest that filamentation is induced in wild-type cells by reducing respiration (using low oxygen or respiratory drugs) and that mutants with increased respiratory activity fail to undergo filamentation under these conditions.

Munoz, M. C., et al. (2014). "Downregulation of the ACE2/Ang-(1-7)/Mas axis in transgenic mice overexpressing GH." J Endocrinol **221**(2): 215-227.

The renin-angiotensin system (RAS) plays a crucial role in the regulation of physiological homeostasis and diseases such as hypertension, coronary artery disease, and chronic renal failure. In this cascade, the angiotensin-converting enzyme (ACE)/angiotensin II (Ang II)/AT1 receptor axis

induces pathological effects, such as vasoconstriction, cell proliferation, and fibrosis, while the ACE2/Ang-(1-7)/Mas receptor axis is protective for end-organ damage. The altered function of the RAS could be a contributing factor to the cardiac and renal alterations induced by GH excess. To further explore this issue, we evaluated the consequences of chronic GH exposure on the in vivo levels of Ang II, Ang-(1-7), ACE, ACE2, and Mas receptor in the heart and the kidney of GH-transgenic mice (bovine GH (bGH) mice). At the age of 7-8 months, female bGH mice displayed increased systolic blood pressure (SBP), a high degree of both cardiac and renal fibrosis, as well as increased levels of markers of tubular and glomerular damage. Angiotensinogen abundance was increased in the liver and the heart of bGH mice, along with a concomitant increase in cardiac Ang II levels. Importantly, the levels of ACE2, Ang-(1-7), and Mas receptor were markedly decreased in both tissues. In addition, Ang-(1-7) administration reduced SBP to control values in GH-transgenic mice, indicating that the ACE2/Ang-(1-7)/Mas axis is involved in GH-mediated hypertension. The data indicate that the altered expression profile of the ACE2/Ang-(1-7)/Mas axis in the heart and the kidney of bGH mice could contribute to the increased incidence of hypertension, cardiovascular, and renal alterations observed in these animals.

Narayan, S. S., et al. (2019). "Angiotensin converting enzymes ACE and ACE2 in thyroid cancer progression." Neoplasma.

Angiotensin-converting enzymes, ACE and ACE2, play not only a pivotal role in the regulation of blood pressure, but are involved in the processes of pathophysiology, including thyroid dysfunction or progression of several neoplasia such as cancers of skin, lungs, pancreas and leukaemia. However their role in the thyroid carcinogenesis remains unknown. We examined in this study the expression of ACE and ACE2 in thyroid tissues and their possible employment as biomarkers for thyroid cancer progression. Thyroid tissues including 14 goiters (G), 12 follicular adenomas (FA), 10 follicular thyroid carcinomas (FTC), 14 papillary thyroid carcinomas (PTC) and 11 undifferentiated thyroid carcinomas (UTC) were subjected to RT-PCR and protein analyses with primers or antibodies specific for ACE and ACE2, respectively. FA revealed significantly increased ACE as compared to other groups and FTC was significantly higher than UTC. ACE2 was significantly increased in PTC in comparison to G, FA and UTC, and in FTC as compared to G. The ratio ACE/ACE2 decreased while ACE2/ACE increased with the differentiation grade of thyroid carcinoma. ACE was significantly diminished in individuals older than 50. Both ACEs were significantly diminished in M1 patients, ACE2 additionally in higher tumour masses. ACE and ACE2 are regulated within thyroid benign and malignant tissues. As the transcript ratio between both enzymes correlate proportional with the differentiation status of thyroid cancer, ACE and ACE2 may serve as new markers for thyroid carcinoma.

Netland, J., et al. (2008). "Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2." J Virol **82**(15): 7264-7275.

Infection of humans with the severe acute respiratory syndrome coronavirus (SARS-CoV) results in substantial morbidity and mortality, with death resulting primarily from respiratory failure. While the lungs are the major site of infection, the brain is also infected in some patients. Brain infection may result in long-term neurological sequelae, but little is known about the pathogenesis of SARS-CoV in this organ. We previously showed that the brain was a major target organ for infection in mice that are transgenic for the SARS-CoV receptor (human angiotensin-converting enzyme 2). Herein, we use these mice to show that virus enters the brain primarily via the olfactory bulb, and infection results in rapid, transneuronal spread to connected areas of the brain. This extensive neuronal infection is the main cause of death because intracranial inoculation with low doses of virus results in a uniformly lethal disease even though little infection is detected in the lungs. Death of the animal likely results from dysfunction and/or death of infected neurons, especially those located in cardiorespiratory centers in the medulla. Remarkably, the virus induces minimal cellular infiltration in the brain. Our results show that neurons are a highly susceptible target for SARS-CoV and that only the absence of the host cell receptor prevents severe murine brain disease.

Neves, L. A., et al. (2008). "ACE2 and ANG-(1-7) in the rat uterus during early and late gestation." Am J Physiol Regul Integr Comp Physiol **294**(1): R151-161.

The present study was designed to determine ANG peptide content [ANG I, ANG II, ANG-(1-7)], ACE2 mRNA, and the immunocytochemical distribution of ANG-(1-7) and ACE2 in the uteroembryonic unit during early and late gestation in Sprague-Dawley rats and in a rat model of pregnancy-induced hypertension, the reduced uterine perfusion pressure (RUPP) model. At early pregnancy ANG-(1-7) and ACE2 staining were localized in the primary and secondary decidual zone and luminal and glandular epithelial cells. During late gestation, ANG-(1-7) and

ACE2 staining was visualized in the labyrinth placenta and amniotic and yolk sac epithelium. Uterine ANG II concentration at early pregnancy was significantly decreased by 21-55% in the implantation and interimplantation sites compared with virgin rats, whereas ANG-(1-7) levels were maintained at prepregnancy levels. At late gestation, uterine concentrations of ANG I and ANG II were significantly increased (30% and 25%, respectively). In RUPP animals, ANG-(1-7) concentration is significantly reduced in the uterus (181 +/- 16 vs. 372 +/- 74 fmol/g of tissue) and placenta (143 +/- 26 vs. 197 +/- 20 fmol/g of tissue). ACE2 mRNA increased in the uterus of early pregnant compared with virgin rats, yet within the implantation site it was downregulated. At late pregnancy, ACE2 mRNA is elevated by 58% in the uterus and decreased by 59% in RUPP animals. The regulation of ANG-(1-7) and ACE2 in early and late pregnancy supports the hypothesis that ANG-(1-7) and ACE2 may act as a local autocrine/paracrine regulator throughout pregnancy, participating in the early (angiogenesis, apoptosis, and growth) and late (uteroplacental blood flow) events of pregnancy.

Nozato, S., et al. (2019). "Angiotensin 1-7 alleviates aging-associated muscle weakness and bone loss, but is not associated with accelerated aging in ACE2-knockout mice." Clin Sci (Lond) **133**(18): 2005-2018.

The angiotensin-converting enzyme 2 (ACE2)-angiotensin 1-7 (A1-7)-A1-7 receptor (Mas) axis plays a protective role in the renin-angiotensin system (RAS). We recently found that ACE2 knockout (ACE2KO) mice exhibit earlier aging-associated muscle weakness, and that A1-7 alleviates muscle weakness in aging mice. In the present study, we investigated the role of the A1-7-Mas pathway in the effect of ACE2 on physiological aging. Male wild-type, ACE2KO, and Mas knockout (MasKO) mice were subjected to periodical grip strength measurement, followed by administration of A1-7 or vehicle for 4 weeks at 24 months of age. ACE2KO mice exhibited decreased grip strength after 6 months of age, while grip strength of MasKO mice was similar to that of wild-type mice. A1-7 improved grip strength in ACE2KO and wild-type mice, but not in MasKO mice. Muscle fibre size was smaller in ACE2KO mice than that in wild-type and MasKO mice, and increased with A1-7 in ACE2KO and WT mice, but not in MasKO mice. Centrally nucleated fibres (CNFs) and expression of the senescence-associated gene p16INK4a in skeletal muscles were enhanced only in ACE2KO mice and were not altered by A1-7. ACE2KO mice, but not MasKO mice, exhibited thinning of peripheral fat along with increased adipose expression of p16INK4a A1-7 significantly increased bone volume in wild-type and ACE2KO mice, but not in MasKO mice. Our findings suggest that the impact of ACE2 on physiological aging does not depend on the endogenous production of A1-7 by ACE2, while overactivation of the A1-7-Mas pathway could alleviate sarcopenia and osteoporosis in aged mice.

Nunes-Silva, A., et al. (2017). "Physical Exercise and ACE2-Angiotensin-(1-7)-Mas Receptor Axis of the Renin Angiotensin System." Protein Pept Lett **24**(9): 809-816.

BACKGROUND: Many physiological responses of the Renin Angiotensin System (RAS) are associated with two opposite pathways: (1) a classical one formed by angiotensin-converting enzyme (ACE), Angiotensin II (Ang II) and Angiotensin type 1 (AT1) receptor, which is associated to vasoconstriction, cell proliferation, organ hypertrophy, sodium retention and aldosterone release and (2) a counter-regulatory or vasodilator pathway comprising angiotensin-converting enzyme 2 (ACE2), Angiotensin-(1-7) [Ang-(1-7)] and Mas receptor, which is involved in vasodilation, antiproliferation, anti-hypertrophy, cardioprotective and renoprotective actions. OBJECTIVE: This review aimed to bring up-to-date on the interactions between physical exercise and the vasodilator axis of the RAS (ACE2-Ang-(1-7)-Mas receptor axis). We also investigated the relation of acute and chronic exercise with blood pressure regulation and components of the vasodilator axis of the RAS. METHODS: We searched studies with animal models and humans in PUBMED, LILACS and IBECS. RESULTS: Experimental studies showed that physical training can stimulate ACE2-Ang-(1-7)-Mas receptor axis in parallel with the inhibition of ACE-Ang II-AT1 receptor pathway. However, up to now, the interaction between the counter-regulatory RAS axis and physical training is not investigated in humans. CONCLUSION: The activation of ACE2-Ang-(1-7)-Mas receptor axis may have a role in the beneficial effects of physical training in experimental models. Further studies with humans are necessary.

Nunes-Souza, V., et al. (2016). "CD36/Sirtuin 1 Axis Impairment Contributes to Hepatic Steatosis in ACE2-Deficient Mice." Oxid Med Cell Longev **2016**: 6487509.

Background and Aims. Angiotensin converting enzyme 2 (ACE2) is an important component of the renin-angiotensin system. Since angiotensin peptides have been shown to be involved in hepatic steatosis, we aimed to evaluate the hepatic lipid profile in ACE2-deficient (ACE2(-/y)) mice. Methods. Male C57BL/6 and ACE2(-/y) mice were analyzed at the age of 3 and 6 months for alterations in the lipid

profiles of plasma, faeces, and liver and for hepatic steatosis. Results. ACE2(-/y) mice showed lower body weight and white adipose tissue at all ages investigated. Moreover, these mice had lower levels of cholesterol, triglycerides, and nonesterified fatty acids in plasma. Strikingly, ACE2(-/y) mice showed high deposition of lipids in the liver. Expression of CD36, a protein involved in the uptake of triglycerides in liver, was increased in ACE2(-/y) mice. Concurrently, these mice exhibited an increase in hepatic oxidative stress, evidenced by increased lipid peroxidation and expression of uncoupling protein 2, and downregulation of sirtuin 1. ACE2(-/y) mice also showed impairments in glucose metabolism and insulin signaling in the liver. Conclusions. Deletion of ACE2 causes CD36/sirtuin 1 axis impairment and thereby interferes with lipid homeostasis, leading to lipodystrophy and steatosis.

Ocaranza, M. P. and J. E. Jalil (2012). "Protective Role of the ACE2/Ang-(1-9) Axis in Cardiovascular Remodeling." Int J Hypertens **2012**: 594361.

Despite reduction in cardiovascular (CV) events and end-organ damage with the current pharmacologic strategies, CV disease remains the primary cause of death in the world. Pharmacological therapies based on the renin angiotensin system (RAS) blockade are used extensively for the treatment of hypertension, heart failure, and CV remodeling but in spite of their success the prevalence of end-organ damage and residual risk remain still high. Novel approaches must be discovered for a more effective treatment of residual CV remodeling and risk. The ACE2/Ang-(1-9) axis is a new and important target to counterbalance the vasoconstrictive/proliferative RAS axis. Ang-(1-9) is hydrolyzed slower than Ang-(1-7) and is able to bind the Ang II type 2 receptor. We review here the current experimental evidence suggesting that activation of the ACE2/Ang-(1-9) axis protects the heart and vessels (and possibly the kidney) from adverse cardiovascular remodeling in hypertension as well as in heart failure.

Oliveira Andrade, J. M., et al. (2017). "The Angiotensin Converting Enzyme 2 (ACE2), Gut Microbiota, and Cardiovascular Health." Protein Pept Lett **24**(9): 827-832.

BACKGROUND: The renin-angiotensin system (RAS) is an important enzymatic system responsible for the regulation of biological functions, such as the arterial pressure, hydroelectrolytic control, vascular vasodilatation/vasoconstriction and more recently metabolic functions. OBJECTIVES: The aim of the present review is to discuss the associations between the gut microbiome and the renin-angiotensin system and the influence of their intimate relationship on the cardiovascular health. METHODS: A literature review of the main studies published regarding the relationship among the renin-angiotensin system, gut microbiota and cardiovascular health was performed. RESULTS: The association between the ACE2 and gut microbiota has been discussed. It is shown that the ACE2/Ang 1-7 axis modulates the immune response, influencing the microbiota composition, and thus being one of the causes for some diseases physiophatologies, such as diarrhea and intestinal inflammatory disease. CONCLUSION: The association between RAS and gut microbiota seems to have a strong influence on the genesis of cardiovascular diseases, through direct mechanisms, such as nerve stimulation, or indirectly on metabolic parameters, such as weight, adiposity and lipid profile.

Olkowicz, M., et al. (2015). "Perspectives for angiotensin profiling with liquid chromatography/mass spectrometry to evaluate ACE/ACE2 balance in endothelial dysfunction and vascular pathologies." Pharmacol Rep **67**(4): 778-785.

Vascular injury, characterized by endothelial dysfunction, inflammation, structural remodeling, thrombosis and calcification leads to cardiovascular diseases. Angiotensin (Ang) II (1-8) - synthesized mainly by angiotensin converting enzyme (ACE) is the best characterized mediator of the renin-angiotensin system (RAS). This peptide initially identified by its vasoactive properties was found to play a major role in vascular response to insult. However, recent discovery of angiotensin converting enzyme 2 (ACE2) that produces vasoprotective Ang-(1-7) peptide highlighted complexity of the system and suggested that balance between ACE/Ang II and ACE2/Ang-(1-7) is fundamental in maintaining vascular homeostasis and its disorders are associated with cardiovascular pathology. There is therefore a need to develop methods for comprehensive analysis of biologically active Ang peptides and their metabolites of ACE/Ang II and ACE2/Ang-(1-7) axes. Liquid chromatography/mass spectrometry (LC/MS) is an analytical technique that offers potential for specific, simultaneous analysis of Ang peptides. With sensitivity added by application of preconcentration nanochromatography reaching picomolar concentrations, practically all Ang peptides identified so far could be quantified in biological samples. Ang profiling is important not only for understanding their physiological or pathological role but could also serve as an early diagnostic biomarker of endothelial dysfunction and cardiovascular pathology. It could also be used for monitoring the efficacy of the RAS-targeted therapies. Although, the methodology requires further improvements to adopt it for routine application, Ang peptide profiling with targeted

LC/MS analysis might assess functional balance between ACE/Ang II and ACE2/Ang-(1-7) axes, facilitate our understanding of the cardiovascular pathology and enhance biomarker portfolio in cardiovascular diseases.

Oud, B., et al. (2013). "Genome duplication and mutations in ACE2 cause multicellular, fast-sedimenting phenotypes in evolved Saccharomyces cerevisiae." Proc Natl Acad Sci U S A **110**(45): E4223-4231.

Laboratory evolution of the yeast Saccharomyces cerevisiae in bioreactor batch cultures yielded variants that grow as multicellular, fast-sedimenting clusters. Knowledge of the molecular basis of this phenomenon may contribute to the understanding of natural evolution of multicellularity and to manipulating cell sedimentation in laboratory and industrial applications of S. cerevisiae. Multicellular, fast-sedimenting lineages obtained from a haploid S. cerevisiae strain in two independent evolution experiments were analyzed by whole genome resequencing. The two evolved cell lines showed different frameshift mutations in a stretch of eight adenosines in ACE2, which encodes a transcriptional regulator involved in cell cycle control and mother-daughter cell separation. Introduction of the two ace2 mutant alleles into the haploid parental strain led to slow-sedimenting cell clusters that consisted of just a few cells, thus representing only a partial reconstruction of the evolved phenotype. In addition to single-nucleotide mutations, a whole-genome duplication event had occurred in both evolved multicellular strains. Construction of a diploid reference strain with two mutant ace2 alleles led to complete reconstruction of the multicellular-fast sedimenting phenotype. This study shows that whole-genome duplication and a frameshift mutation in ACE2 are sufficient to generate a fast-sedimenting, multicellular phenotype in S. cerevisiae. The nature of the ace2 mutations and their occurrence in two independent evolution experiments encompassing fewer than 500 generations of selective growth suggest that switching between unicellular and multicellular phenotypes may be relevant for competitiveness of S. cerevisiae in natural environments.

Oudit, G. Y., et al. (2003). "The role of ACE2 in cardiovascular physiology." Trends Cardiovasc Med **13**(3): 93-101.

The renin-angiotensin system (RAS) is critically involved in cardiovascular and renal function and in disease conditions, and has been shown to be a far more complex system than initially thought. A recently discovered homologue of angiotensin-converting enzyme (ACE)--ACE2--appears to negatively regulate the RAS. ACE2 cleaves Ang I and Ang II into the inactive Ang 1-9 and Ang 1-7, respectively. ACE2 is highly expressed in kidney and heart and is especially confined to the endothelium. With quantitative trait locus (QTL) mapping, ACE2 was defined as a QTL on the X chromosome in rat models of hypertension. In these animal models, kidney ACE2 messenger RNA and protein expression were markedly reduced, making ACE2 a candidate gene for this QTL. Targeted disruption of ACE2 in mice failed to elicit hypertension, but resulted in severe impairment in myocardial contractility with increased angiotensin II levels. Genetic ablation of ACE in the ACE2 null mice rescued the cardiac phenotype. These genetic data show that ACE2 is an essential regulator of heart function in vivo. Basal renal morphology and function were not altered by the inactivation of ACE2. The novel role of ACE2 in hydrolyzing several other peptides-such as the apelin peptides, opioids, and kinin metabolites-raises the possibility that peptide systems other than angiotensin and its derivatives also may have an important role in regulating cardiovascular and renal function.

Oudit, G. Y., et al. (2009). "SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS." Eur J Clin Invest **39**(7): 618-625.

BACKGROUND: Angiotensin converting enzyme 2 (ACE2), a monocarboxylase that degrades angiotensin II to angiotensin 1-7, is also the functional receptor for severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and is highly expressed in the lungs and heart. Patients with SARS also suffered from cardiac disease including arrhythmias, sudden cardiac death, and systolic and diastolic dysfunction. MATERIALS AND METHODS: We studied mice infected with the human strain of the SARS-CoV and encephalomyocarditis virus and examined ACE2 mRNA and protein expression. Autopsy heart samples from patients who succumbed to the SARS crisis in Toronto (Canada) were used to investigate the impact of SARS on myocardial structure, inflammation and ACE2 protein expression. RESULTS: Pulmonary infection with the human SARS-CoV in mice led to an ACE2-dependent myocardial infection with a marked decrease in ACE2 expression confirming a critical role of ACE2 in mediating SARS-CoV infection in the heart. The SARS-CoV viral RNA was detected in 35% (7/20) of autopsied human heart samples obtained from patients who succumbed to the SARS crisis during the Toronto SARS outbreak. Macrophage-specific staining showed a marked increase in macrophage infiltration with evidence of myocardial damage in patients who had SARS-CoV in their hearts. The presence of SARS-CoV in the heart was also associated with marked reductions in ACE2 protein

expression. CONCLUSIONS: Our data show that SARS-CoV can mediate myocardial inflammation and damage associated with down-regulation of myocardial ACE2 system, which may be responsible for the myocardial dysfunction and adverse cardiac outcomes in patients with SARS.

Oudit, G. Y., et al. (2007). "Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ACE2 null mice." Cardiovasc Res **75**(1): 29-39.

OBJECTIVES: The peptidase action of angiotensin converting enzyme 2 (ACE2) allows it to function as a negative regulator of the renin-angiotensin system. Current pharmacotherapies for human heart failure, such as ACE inhibitors and angiotensin and aldosterone receptor blockers, increase the activity of ACE2 in the heart. In this study, we investigate the mechanism for the age-dependent cardiomyopathy in ACE2 null mice. METHODS AND RESULTS: Ace2(-/y) mutant mice develop a progressive age-dependent dilated cardiomyopathy with increased oxidative stress, neutrophilic infiltration, inflammatory cytokine and collagenase levels, mitogen-activated protein kinase (MAPK) activation and pathological hypertrophy. The angiotensin II receptor-1 (AT1) blocker, irbesartan, prevented the dilated cardiomyopathy in aged Ace2(-/y) mutant mice, confirming a critical role of angiotensin II (Ang II)-mediated stimulation of AT1 receptors. Ang II activation of AT1 receptors triggers G-protein-coupled receptor (GPCR)-activated phosphoinositide 3-kinase gamma (PI3Kgamma) and its downstream pathways. We showed that p110gamma, the catalytic subunit of PI3Kgamma, is a key mediator of NADPH oxidase activation in response to Ang II. The double mutant mice (Ace2(-/y)/p110gamma (-/-)) exhibited marked reductions in oxidative stress, neutrophilic infiltration, and pathological hypertrophy resulting in myocardial protection, suggesting that PI3Kgamma plays a critical role in Ang II-mediated cardiomyopathy. CONCLUSIONS: Our findings demonstrate that the age-dependent cardiomyopathy in ACE2 null mice is related to increased Ang II-mediated oxidative stress and neutrophilic infiltration via AT1 receptors. Our combination of genetic and pharmacological approaches defines a critical role of ACE2 in the suppression of Ang II-mediated heart failure.

Oudit, G. Y., et al. (2010). "Human recombinant ACE2 reduces the progression of diabetic nephropathy." Diabetes **59**(2): 529-538.

OBJECTIVE: Diabetic nephropathy is one of the most common causes of end-stage renal failure. Inhibition of ACE2 function accelerates diabetic kidney injury, whereas renal ACE2 is downregulated in diabetic nephropathy. We examined the ability of human recombinant ACE2 (hrACE2) to slow the progression of diabetic kidney injury. RESEARCH DESIGN AND METHODS: Male 12-week-old diabetic Akita mice (Ins2(WT/C96Y)) and control C57BL/6J mice (Ins2(WT/WT)) were injected daily with placebo or with rhACE2 (2 mg/kg, i.p.) for 4 weeks. Albumin excretion, gene expression, histomorphometry, NADPH oxidase activity, and peptide levels were examined. The effect of hrACE2 on high glucose and angiotensin II (ANG II)-induced changes was also examined in cultured mesangial cells. RESULTS: Treatment with hrACE2 increased plasma ACE2 activity, normalized blood pressure, and reduced the urinary albumin excretion in Akita Ins2(WT/C96Y) mice in association with a decreased glomerular mesangial matrix expansion and normalization of increased alpha-smooth muscle actin and collagen III expression. Human recombinant ACE2 increased ANG 1-7 levels, lowered ANG II levels, and reduced NADPH oxidase activity. mRNA levels for p47(phox) and NOX2 and protein levels for protein kinase Calpha (PKCalpha) and PKCbeta1 were also normalized by treatment with hrACE2. In vitro, hrACE2 attenuated both high glucose and ANG II-induced oxidative stress and NADPH oxidase activity. CONCLUSIONS: Treatment with hrACE2 attenuates diabetic kidney injury in the Akita mouse in association with a reduction in blood pressure and a decrease in NADPH oxidase activity. In vitro studies show that the protective effect of hrACE2 is due to reduction in ANG II and an increase in ANG 1-7 signaling.

Pan, X., et al. (2018). "FGF21 Prevents Angiotensin II-Induced Hypertension and Vascular Dysfunction by Activation of ACE2/Angiotensin-(1-7) Axis in Mice." Cell Metab **27**(6): 1323-1337 e1325.

Fibroblast growth factor 21 (FGF21) is a metabolic hormone with pleiotropic effects on glucose and lipid metabolism and insulin sensitivity. However, the role of FGF21 in hypertension remains elusive. Here we show that FGF21 deficiency significantly exacerbates angiotensin II-induced hypertension and vascular dysfunction, whereas such negative effects are reversed by replenishment of FGF21. Mechanistically, FGF21 acts on adipocytes and renal cells to promote induction of angiotensin-converting enzyme 2 (ACE2), which in turn converts angiotensin II to angiotensin-(1-7), then inhibits hypertension and reverses vascular damage. In addition, ACE2 deficiency strikingly abrogates these beneficial effects of FGF21 in mice, including alleviation of angiotensin II-associated hypertension and vascular damage. Otherwise, pharmaceutical inhibition of angiotensin-

(1-7) attenuates the protective effect of FGF21 on angiotensin II-induced vascular dysfunction, but not on hypertension. Thus, FGF21 protects against angiotensin II-induced hypertension and vascular impairment by activation of the ACE2/angiotensin-(1-7) axis via fine-tuning the multi-organ crosstalk between liver, adipose tissue, kidney, and blood vessels.

Pan, Y., et al. (2010). "Down-regulated transcriptional level of Ace1 combined with mutations in Ace1 and Ace2 of Aphis gossypii are related with omethoate resistance." Chem Biol Interact **188**(3): 553-557.

The degree of insecticide resistance, acetylcholinesterase (AChE) activity kinetics, AChE gene expression and the cDNA sequence of AChE gene were investigated in resistant and relatively susceptible strains of the cotton aphids, Aphis gossypii (Glover). The resistant strain (ORR) exhibited 53.28-fold resistance to omethoate compared to the relatively susceptible strain (OSS) in cotton aphids. AChE activity, V (max) and K (m) were significantly lower in the ORR strain than in the OSS strain (0.13-, 0.04- and 0.31-fold, respectively). Based on analysis of IC (50) indices, enzyme inhibition experiments showed that AChE from the ORR strain was 7.99-, 4.12-, 4.27-, 8.71- and 4.57-fold insensitive to inhibition by eserine, omethoate, paraoxon, paraoxon-methyl and malaoxon than the OSS strain. Sequence analysis indicated that there were no amino acid substitutions in AChEI (Ace1) and AChEII (Ace2) between the OSS and ORR strain. However, when compared with the 81-171B strain (GenBank No. AF502081), we detected two site mutations (S146N and L532P) in Ace1 with high frequency in both the ORR and OSS strains. One conserved mutation (S431F) in Ace2 was also found in both strains when compared with the 171B strain (GenBank No. AJ748114). Measurements of the levels of gene expression for Ace1 and Ace2 in ORR and OSS, as determined by real-time quantitative PCRs, revealed that the relative transcription levels of Ace1 and Ace2 were 0.26- and 1.07-fold, respectively, in the ORR strain as compared to the OSS strain. These results indicate that the altered AChE sensitivity brought about by a decreased transcriptional level of Ace1 mRNA and combined with the site mutants in both Ace1 and Ace2 might be related to omethoate resistance in cotton aphids.

Pan, Y., et al. (2018). "Association of ACE2 polymorphisms with susceptibility to essential hypertension and dyslipidemia in Xinjiang, China." Lipids Health Dis **17**(1): 241.

BACKGROUND: Cardiovascular benefits by reversing environmental risks factors for essential hypertension (EH) and dyslipidemia could be weaken by high genetic risk. We investigated possible associations between ACE2 polymorphisms and dyslipidemia in patients with EH. METHODS: Four hundred and two hypertensive patients were enrolled in an EH group and 233 normotensive individuals were enrolled as control group from the Xinjiang region of China. Fourteen ACE2 polymorphisms were genotyped by Matrix-assisted laser desorption ionization time-of-flight mass spectrometry. RESULTS: Participants carrying T allele (TT + CT) of rs2074192 (P = 0.006), rs4646155 (P = 0.030) and rs4646188 (P < 0.001), C allele (CT + CT or CC + CG) of rs4240157 (P = 0.012), rs4830542 (P = 0.020) and rs879922 (P < 0.001) and TT genotype of rs2106809 (P = 0.012) were associated with EH. Meanwhile,ACE2 SNPs also exhibited association with dyslipidemia but exhibited obvious heterogeneity. rs1978124 (TT + CT, P = 0.009), rs2106809 (TT, P = 0.045), rs233575 (CC + CT, P = 0.018), rs4646188 (CC, P = 0.011) and rs879922 (CC + CG, P = 0.003) were association with increased LDL-C (>/=1.8 mmol/L). rs2106809 (CC + CT, P < 0.001), rs2285666(TT + CT, P = 0.017), rs4646142(CC + CG, P = 0.044), rs4646155(TT + CT, P < 0.001) and rs4646188(TT + CT, P = 0.033) were association with decreased HDL-C (< 1.0 mmol/L). rs2074192 (TT + CT, P = 0.012), rs4240157 (CC + CT, P = 0.027), rs4646156 (AA+AT, P = 0.007), rs4646188 (TT + CT, P = 0.005), rs4830542 (CC + CT, P = 0.047) and rs879922 (CC + CG, P = 0.001) were association with increased TC (>/=5.2 mmol/L). rs2106809 (P = 0.034) and rs4646188 (P = 0.013) were associated with hypertriglyceridemia. Further, ischemic stroke was more prevalent with rs4240157 (CC + CT, P = 0.043), rs4646188 (CC + CT, P = 0.013) and rs4830542 (CC + CT, P = 0.037). In addition, rs2048683 and rs6632677 were not association with EH, dyslipidemia and ischemic stroke. CONCLUSION: The ACE2 rs4646188 variant may be a potential and optimal genetic susceptibility marker for EH, dyslipidemia and its related ischemic stroke.

Pang, X. F., et al. (2015). "Attenuation of myocardial fibrosis with curcumin is mediated by modulating expression of angiotensin II AT1/AT2 receptors and ACE2 in rats." Drug Des Devel Ther **9**: 6043-6054.

Curcumin is known to improve cardiac function by balancing degradation and synthesis of collagens after myocardial infarction. This study tested the hypothesis that inhibition of myocardial fibrosis by curcumin is associated with modulating expression of angiotensin II (Ang II) receptors and angiotensin-converting enzyme 2 (ACE2). Male Sprague Dawley rats were subjected to Ang II infusion (500 ng/kg/min)

using osmotic minipumps for 2 and 4 weeks, respectively, and curcumin (150 mg/kg/day) was fed by gastric gavage during Ang II infusion. Compared to the animals with Ang II infusion, curcumin significantly decreased the mean arterial blood pressure during the course of the observation. The protein level of the Ang II type 1 (AT1) receptor was reduced, and the Ang II type 2 (AT2) receptor was up-regulated, evidenced by an increased ratio of the AT2 receptor over the AT1 receptor in the curcumin group (1.2+/-0.02%) vs in the Ang II group (0.7+/-0.03%, P<0.05). These changes were coincident with less locally expressed AT1 receptor and enhanced AT2 receptor in the intracardiac vessels and intermyocardium. Along with these modulations, curcumin significantly decreased the populations of macrophages and alpha smooth muscle actin-expressing myofibroblasts, which were accompanied by reduced expression of transforming growth factor beta 1 and phosphorylated-Smad2/3. Collagen I synthesis was inhibited, and tissue fibrosis was attenuated, as demonstrated by less extensive collagen-rich fibrosis. Furthermore, curcumin increased protein level of ACE2 and enhanced its expression in the intermyocardium relative to the Ang II group. These results suggest that curcumin could be considered as an add-on therapeutic agent in the treatment of fibrosis-derived heart failure patient who is intolerant of ACE inhibitor therapy.

Park, S. E., et al. (2013). "High urinary ACE2 concentrations are associated with severity of glucose intolerance and microalbuminuria." Eur J Endocrinol **168**(2): 203-210.

OBJECTIVE: Angiotensin-converting enzyme 2 (ACE2) plays an important role in glucose metabolism and renal function. However, the relationship between ACE2 and hyperglycemia or microalbuminuria has not been established in humans. We investigated whether urinary ACE2 levels are associated with abnormal glucose homeostasis and urinary albumin excretion. METHODS: We developed an ELISA for quantifying ACE2 in urine. The ELISA was used to measure urinary ACE2 levels in 621 subjects with: normal glucose tolerance (NGT; n=77); impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (n=132); and type 2 diabetes mellitus (T2DM, n=412). Insulin resistance was assessed by homeostasis model assessment for insulin resistance (HOMA-IR) index and urinary albumin excretion by urine albumin-to-creatinine ratio (ACR). Other biochemical and anthropometric parameters were measured. RESULTS: Urinary ACE2 levels were significantly higher in insulin-resistant subjects with IFG, IGT, and T2DM than in the NGT group (P<0.001). Urinary ACE2 concentrations appeared to correlate with HOMA-IR, fasting blood glucose, triglyceride, high-sensitivity C-reactive protein, serum creatinine, urinary ACR, and systolic blood pressure (all P<0.05). After adjustment for impaired renal function and other metabolic parameters, urinary ACE2 concentration was still associated with a higher risk for T2DM (OR 1.80, 95% CI 1.05-3.08, P=0.02). In addition, urinary ACE2 levels were highly predictive of microalbuminuria after adjusting for clinical risk factors (OR 2.68, 95% CI 1.55-4.64, P<0.001). CONCLUSION: Our data suggest that the urinary ACE2 level is closely associated with T2DM and is an independent risk factor for microalbuminuria.

Parnell, E. J., et al. (2014). "The Rts1 regulatory subunit of PP2A phosphatase controls expression of the HO endonuclease via localization of the Ace2 transcription factor." J Biol Chem **289**(51): 35431-35437.

The RTS1 gene encodes a subunit of the PP2A phosphatase that regulates cell cycle progression. Ace2 and Swi5 are cell cycle-regulated transcription factors, and we recently showed that phosphorylation of Ace2 and Swi5 is altered in an rts1 mutant. Here we examine expression of Ace2 and Swi5 target genes and find that an rts1 mutation markedly reduces expression of the HO gene. The decreased HO expression in an rts1 mutant is significantly restored by an additional ace2 mutation, a surprising result because HO is normally activated by Swi5 but not by Ace2. Ace2 normally accumulates only in daughter cells, and only activates transcription in daughters. However, in an rts1 mutant, Ace2 is present in both mother and daughter cells. One of the genes activated by Ace2 is ASH1, a protein that normally accumulates mostly in daughter cells; Ash1 is a transcriptional repressor, and it blocks HO expression in daughters. We show that in the rts1 mutant, Ace2 accumulation in mother cells results in Ash1 expression in mothers, and the Ash1 can now repress HO expression in mothers.

Patel, S. K., et al. (2014). "From gene to protein-experimental and clinical studies of ACE2 in blood pressure control and arterial hypertension." Front Physiol **5**: 227.

Hypertension is a major risk factor for stroke, coronary events, heart and renal failure, and the renin-angiotensin system (RAS) plays a major role in its pathogenesis. Within the RAS, angiotensin converting enzyme (ACE) converts angiotensin (Ang) I into the vasoconstrictor Ang II. An "alternate" arm of the RAS now exists in which ACE2 counterbalances the effects of the classic RAS through degradation of Ang II, and generation of the vasodilator Ang 1-7. ACE2 is highly expressed in the heart, blood vessels, and kidney. The

catalytically active ectodomain of ACE2 undergoes shedding, resulting in ACE2 in the circulation. The ACE2 gene maps to a quantitative trait locus on the X chromosome in three strains of genetically hypertensive rats, suggesting that ACE2 may be a candidate gene for hypertension. It is hypothesized that disruption of tissue ACE/ACE2 balance results in changes in blood pressure, with increased ACE2 expression protecting against increased blood pressure, and ACE2 deficiency contributing to hypertension. Experimental hypertension studies have measured ACE2 in either the heart or kidney and/or plasma, and have reported that deletion or inhibition of ACE2 leads to hypertension, whilst enhancing ACE2 protects against the development of hypertension, hence increasing ACE2 may be a therapeutic option for the management of high blood pressure in man. There have been relatively few studies of ACE2, either at the gene or the circulating level in patients with hypertension. Plasma ACE2 activity is low in healthy subjects, but elevated in patients with cardiovascular risk factors or cardiovascular disease. Genetic studies have investigated ACE2 gene polymorphisms with either hypertension or blood pressure, and have produced largely inconsistent findings. This review discusses the evidence regarding ACE2 in experimental hypertension models and the association between circulating ACE2 activity and ACE2 polymorphisms with blood pressure and arterial hypertension in man.

Patel, S. K., et al. (2012). "Association of ACE2 genetic variants with blood pressure, left ventricular mass, and cardiac function in Caucasians with type 2 diabetes." Am J Hypertens **25**(2): 216-222.

BACKGROUND: Cardiovascular disease is common in diabetes, and is associated with activation of the renin-angiotensin system (RAS). Angiotensin-converting enzyme (ACE)2 is a recently described member of the RAS, and this study investigated whether ACE2 polymorphisms are associated with hypertension, left ventricular (LV) mass, and cardiac function in type 2 diabetes. METHODS: Variants in ACE2 (rs1978124, rs2074192, rs4240157, rs4646156, rs4646188) were examined in 503 Caucasian subjects with type 2 diabetes. As ACE2 is located on the X chromosome, analyses were performed separately for men and women. Hypertension was defined by a history of hypertension, and/or antihypertensive medications or blood pressure (BP) >130/80 mm Hg. LV mass and systolic function (ejection fraction) were assessed by transthoracic echocardiography. RESULTS: In men, hypertension was more prevalent with the ACE2 rs2074192 C allele (P = 0.023), rs4240157 G allele (P = 0.016) and rs4646188 T allele (P = 0.006). In men, the rs1978124 A allele was associated with a significantly lower ejection fraction compared to the G allele (62.3 +/- 13.3 vs. 67.2 +/- 10.9%, P = 0.002). This association remained significant after covariate adjustment for age, body mass index, hypertension, antihypertensive treatment, and BP. In women, the prevalence of hypertension was higher (P = 0.009) with the rs4240157 G allele, and the rs1978124 A allele was associated with significantly higher LV mass (P = 0.008). CONCLUSIONS: In Caucasians with type 2 diabetes, genetic variation in ACE2 is associated with hypertension and reduced systolic function in men, and hypertension and increased LV mass in women.

Patel, V. B., et al. (2016). "ACE2/Ang 1-7 axis: A critical regulator of epicardial adipose tissue inflammation and cardiac dysfunction in obesity." Adipocyte **5**(3): 306-311.

Obesity is characterized by an excessive fat accumulation in adipose tissues leading to weight gain and is increasing in prevalence and is strongly associated with metabolic and cardiovascular disorders. The renin-angiotensin system (RAS) has emerged as a key pathogenic mechanism for these disorders; activated RAS and angiotensin (Ang) II production results in worsening of cardiovascular diseases and angiotensin converting enzyme 2 (ACE2) negatively regulates RAS by metabolizing Ang II into Ang 1-7. ACE2 is expressed in the adipocytes and its expression is upregulated in response to high fat diet induced obesity in mice. Loss of ACE2 results in heart failure with preserved ejection fraction which is mediated in part by epicardial adipose tissue inflammation. Angiotensin 1-7 reduces the obesity associated cardiac dysfunction predominantly via its role in adiponectin expression and attenuation of epicardial adipose tissue inflammation. Human heart disease is also linked with inflammed epicardial adipose tissue. Here, we discuss the important interpretation of the novel of ACE2/Ang 1-7 pathway in obesity associated cardiac dysfunction.

Patel, V. B., et al. (2014). "Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: a positive feedback mechanism in the RAS." J Mol Cell Cardiol **66**: 167-176.

Angiotensin converting enzyme (ACE) 2 is a key negative regulator of the renin-angiotensin system where it metabolizes angiotensin (Ang) II into Ang 1-7. We hypothesize that Ang II suppresses ACE2 by increasing TNF-alpha converting enzyme (TACE) activity and ACE2 cleavage. Ang II infusion (1.5 mg/kg/day) in wild-type mice for 2 weeks resulted in substantial decrease in myocardial ACE2 protein levels and activity with corresponding increase in plasma ACE2 activity, prevented by AT1R blockade.

Ang II resulted in AT1R-mediated increase in myocardial TACE expression and activity, and membrane translocation of TACE. Ang II treatment in Huh7 cells exhibited AT1R-dependent metalloproteinase mediated shedding of ACE2 while transfection with siTACE prevented shedding of ACE2; cardiomyocyte-specific deletion of TACE also prevented shedding of ACE2. Reactive oxygen species played a key role since p47(phox)KO mice were resistant to Ang II-induced TACE phosphorylation and activation with preservation of myocardial ACE2 which dampened Ang II-induced cardiac dysfunction and hypertrophy. In conclusion, Ang II induces ACE2 shedding by promoting TACE activity as a positive feedback mechanism whereby Ang II facilitates the loss of its negative regulator, ACE2. In HF, elevated plasma ACE2 activity likely represents loss of the protective effects of ACE2 in the heart.

Patel, V. B., et al. (2017). "Recombinant Human ACE2 and the Angiotensin 1-7 Axis as Potential New Therapies for Heart Failure." Can J Cardiol **33**(7): 943-946.

Heart failure (HF) is a common cause of death and disability and a major economic burden in industrialized nations. Heart disease remains the leading cause of death in North America, with ischemic and hypertensive heart disease as the leading cause of HF. Various basic and clinical studies have established the role of an activated renin-angiotensin (Ang) system and Ang II generation in the progression of HF. Inhibition of an activated renin-Ang system using Ang-converting enzyme inhibitors, Ang II type 1 receptor blockers, and mineralocorticoid receptors antagonists have shown clinical benefits in patients with HF, although, largely limited to HF with reduced ejection fraction (HF-rEF). In contrast, there is no approved pharmacotherapy for HF with preserved ejection fraction (HF-pEF). Ang-converting enzyme (ACE) 2 (ACE2) is a homolog of ACE, which, being a monocarboxypeptidase converts Ang II into Ang 1-7 and is downregulated in HF. Various preclinical studies have shown a potent cardioprotective role of ACE2/Ang 1-7 axis in HF, which counter-regulates the ACE/Ang II/Ang II type 1 receptor axis. Importantly, ACE2 and Ang 1-7 show substantial benefit in preclinical models of HF-pEF and HF-rEF. Improvement in endothelial dysfunction, suppression of tissue inflammation and myocardial fibrosis, correction of metabolic dysfunction, and reversal of pathological hypertrophy are the key beneficial effects seen when ACE2 or Ang 1-7 action are enhanced. Clinical benefit of recombinant human ACE2 and Ang 1-7 need to be evaluated in patients with HF-rEF and HF-pEF.

Patel, V. B., et al. (2016). "ACE2 Deficiency Worsens Epicardial Adipose Tissue Inflammation and Cardiac Dysfunction in Response to Diet-Induced Obesity." Diabetes **65**(1): 85-95.

Obesity is increasing in prevalence and is strongly associated with metabolic and cardiovascular disorders. The renin-angiotensin system (RAS) has emerged as a key pathogenic mechanism for these disorders; angiotensin (Ang)-converting enzyme 2 (ACE2) negatively regulates RAS by metabolizing Ang II into Ang 1-7. We studied the role of ACE2 in obesity-mediated cardiac dysfunction. ACE2 null (ACE2KO) and wild-type (WT) mice were fed a high-fat diet (HFD) or a control diet and studied at 6 months of age. Loss of ACE2 resulted in decreased weight gain but increased glucose intolerance, epicardial adipose tissue (EAT) inflammation, and polarization of macrophages into a proinflammatory phenotype in response to HFD. Similarly, human EAT in patients with obesity and heart failure displayed a proinflammatory macrophage phenotype. Exacerbated EAT inflammation in ACE2KO-HFD mice was associated with decreased myocardial adiponectin, decreased phosphorylation of AMPK, increased cardiac steatosis and lipotoxicity, and myocardial insulin resistance, which worsened heart function. Ang 1-7 (24 microg/kg/h) administered to ACE2KO-HFD mice resulted in ameliorated EAT inflammation and reduced cardiac steatosis and lipotoxicity, resulting in normalization of heart failure. In conclusion, ACE2 plays a novel role in heart disease associated with obesity wherein ACE2 negatively regulates obesity-induced EAT inflammation and cardiac insulin resistance.

Patel, V. B., et al. (2014). "Role of angiotensin-converting enzyme 2 (ACE2) in diabetic cardiovascular complications." Clin Sci (Lond) **126**(7): 471-482.

Diabetes mellitus results in severe cardiovascular complications, and heart disease and failure remain the major causes of death in patients with diabetes. Given the increasing global tide of obesity and diabetes, the clinical burden of diabetes-induced cardiovascular disease is reaching epidemic proportions. Therefore urgent actions are needed to stem the tide of diabetes which entails new prevention and treatment tools. Clinical and pharmacological studies have demonstrated that AngII (angiotensin II), the major effector peptide of the RAS (renin-angiotensin system), is a critical promoter of insulin resistance and diabetes mellitus. The role of RAS and AngII has been implicated in the progression of diabetic cardiovascular complications and AT1R (AngII type 1 receptor) blockers and ACE (angiotensin-converting enzyme) inhibitors have shown clinical benefits.

ACE2, the recently discovered homologue of ACE, is a monocarboxypeptidase which converts AngII into Ang-(1-7) [angiotensin-(1-7)] which, by virtue of its actions on the MasR (Mas receptor), opposes the effects of AngII. In animal models of diabetes, an early increase in ACE2 expression and activity occurs, whereas ACE2 mRNA and protein levels have been found to decrease in older STZ (streptozotocin)-induced diabetic rats. Using the Akita mouse model of Type 1 diabetes, we have recently shown that loss of ACE2 disrupts the balance of the RAS in a diabetic state and leads to AngII/AT1R-dependent systolic dysfunction and impaired vascular function. In the present review, we will discuss the role of the RAS in the pathophysiology and treatment of diabetes and its complications with particular emphasis on potential benefits of the ACE2/Ang-(1-7)/MasR axis activation.

Patel, V. B., et al. (2015). "Antagonism of angiotensin 1-7 prevents the therapeutic effects of recombinant human ACE2." J Mol Med (Berl) **93**(9): 1003-1013.

UNLABELLED: Activation of the angiotensin 1-7/Mas receptor (MasR) axis counteracts angiotensin II (Ang II)-mediated cardiovascular disease. Recombinant human angiotensin-converting enzyme 2 (rhACE2) generates Ang 1-7 from Ang II. We hypothesized that the therapeutic effects of rhACE2 are dependent on Ang 1-7 action. Wild type male C57BL/6 mice (10-12 weeks old) were infused with Ang II (1.5 mg/kg/d) and treated with rhACE2 (2 mg/kg/d). The Ang 1-7 antagonist, A779 (200 ng/kg/min), was administered to a parallel group of mice. rhACE2 prevented Ang II-induced hypertrophy and diastolic dysfunction while A779 prevented these beneficial effects and precipitated systolic dysfunction. rhACE2 effectively antagonized Ang II-mediated myocardial fibrosis which was dependent on the action of Ang 1-7. Myocardial oxidative stress and matrix metalloproteinase 2 activity was further increased by Ang 1-7 inhibition even in the presence of rhACE2. Activation of Akt and endothelial nitric oxide synthase (eNOS) by rhACE2 were suppressed by the antagonism of Ang 1-7 while the activation of pathological signaling pathways was maintained. Blocking Ang 1-7 action prevents the therapeutic effects of rhACE2 in the setting of elevated Ang II culminating in systolic dysfunction. These results highlight a key cardioprotective role of Ang 1-7, and increased Ang 1-7 action represents a potential therapeutic strategy for cardiovascular diseases. KEY MESSAGES: Activation of the renin-angiotensin system (RAS) plays a key pathogenic role in cardiovascular disease. ACE2, a monocarboxypeptidase, negatively regulates pathological effects of Ang II. Antagonizing Ang 1-7 prevents the therapeutic effects of recombinant human ACE2. Our results highlight a key protective role of Ang 1-7 in cardiovascular disease.

Patel, V. B., et al. (2016). "Role of the ACE2/Angiotensin 1-7 Axis of the Renin-Angiotensin System in Heart Failure." Circ Res **118**(8): 1313-1326.

Heart failure (HF) remains the most common cause of death and disability, and a major economic burden, in industrialized nations. Physiological, pharmacological, and clinical studies have demonstrated that activation of the renin-angiotensin system is a key mediator of HF progression. Angiotensin-converting enzyme 2 (ACE2), a homolog of ACE, is a monocarboxypeptidase that converts angiotensin II into angiotensin 1-7 (Ang 1-7) which, by virtue of its actions on the Mas receptor, opposes the molecular and cellular effects of angiotensin II. ACE2 is widely expressed in cardiomyocytes, cardiofibroblasts, and coronary endothelial cells. Recent preclinical translational studies confirmed a critical counter-regulatory role of ACE2/Ang 1-7 axis on the activated renin-angiotensin system that results in HF with preserved ejection fraction. Although loss of ACE2 enhances susceptibility to HF, increasing ACE2 level prevents and reverses the HF phenotype. ACE2 and Ang 1-7 have emerged as a key protective pathway against HF with reduced and preserved ejection fraction. Recombinant human ACE2 has been tested in phase I and II clinical trials without adverse effects while lowering and increasing plasma angiotensin II and Ang 1-7 levels, respectively. This review discusses the transcriptional and post-transcriptional regulation of ACE2 and the role of the ACE2/Ang 1-7 axis in cardiac physiology and in the pathophysiology of HF. The pharmacological and therapeutic potential of enhancing ACE2/Ang 1-7 action as a novel therapy for HF is highlighted.

Pedersen, K. B., et al. (2013). "The transcription factor HNF1alpha induces expression of angiotensin-converting enzyme 2 (ACE2) in pancreatic islets from evolutionarily conserved promoter motifs." Biochim Biophys Acta **1829**(11): 1225-1235.

Pancreatic angiotensin-converting enzyme 2 (ACE2) has previously been shown to be critical for maintaining glycemia and beta-cell function. Efforts to maintain or increase ACE2 expression in pancreatic beta-cells might therefore have therapeutic potential for treating diabetes. In our study, we investigated the transcriptional role of hepatocyte nuclear factor 1alpha (HNF1alpha) and hepatocyte nuclear factor 1beta (HNF1beta) in induction of ACE2 expression in insulin-secreting cells. A deficient allele of HNF1alpha or HNF1beta causes maturity-onset diabetes of the young (MODY) types 3 and 5,

respectively, in humans. We found that ACE2 is primarily transcribed from the proximal part of the ACE2 promoter in the pancreas. In the proximal part of the human ACE2 promoter, we further identified three functional HNF1 binding sites, as they have binding affinity for HNF1alpha and HNF1beta and are required for induction of promoter activity by HNF1beta in insulinoma cells. These three sites are well-conserved among mammalian species. Both HNF1alpha and HNF1beta induce expression of ACE2 mRNA and lead to elevated levels of ACE2 protein and ACE2 enzymatic activity in insulinoma cells. Furthermore, HNF1alpha dose-dependently increases ACE2 expression in primary pancreatic islet cells. We conclude that HNF1alpha can induce the expression of ACE2 in pancreatic islet cells via evolutionarily conserved HNF1 binding sites in the ACE2 promoter. Potential therapeutics aimed at counteracting functional HNF1alpha depletion in diabetes and MODY3 will thus have ACE2 induction in pancreatic islets as a likely beneficial effect.

Pedersen, K. B., et al. (2015). "Dynamics of ADAM17-Mediated Shedding of ACE2 Applied to Pancreatic Islets of Male db/db Mice." Endocrinology **156**(12): 4411-4425.

Angiotensin-converting enzyme 2 (ACE2) gene therapy aimed at counteracting pancreatic ACE2 depletion improves glucose regulation in two diabetic mouse models: db/db mice and angiotensin II-infused mice. A disintegrin and metalloproteinase 17 (ADAM17) can cause shedding of ACE2 from the cell membrane. The aim of our studies was to determine whether ADAM17 depletes ACE2 levels in pancreatic islets and beta-cells. Dynamics of ADAM17-mediated ACE2 shedding were investigated in 832/13 insulinoma cells. Within a wide range of ACE2 expression levels, including the level observed in mouse pancreatic islets, overexpression of ADAM17 increases shed ACE2 and decreases cellular ACE2 levels. We provide a mathematical description of shed and cellular ACE2 activities as a function of the ADAM17 activity. The effect of ADAM17 on the cellular ACE2 content was relatively modest with an absolute control strength value less than 0.25 and approaching 0 at low ADAM17 activities. Although we found that ADAM17 and ACE2 are both expressed in pancreatic islets, the beta-cell is not the major cell type expressing ACE2 in islets. During diabetes progression in 8-, 12-, and 15-week-old db/db mice, ACE2 mRNA and ACE2 activity levels in pancreatic islets were not decreased over time nor significantly decreased compared with nondiabetic db/m mice. Levels of ADAM17 mRNA and ADAM17 activity were also not significantly changed. Inhibiting basal ADAM17 activity in mouse islets failed to affect ACE2 levels. We conclude that whereas ADAM17 has the ability to shed ACE2, ADAM17 does not deplete ACE2 from pancreatic islets in diabetic db/db mice.

Perlot, T. and J. M. Penninger (2013). "ACE2 - from the renin-angiotensin system to gut microbiota and malnutrition." Microbes Infect **15**(13): 866-873.

The renin-angiotensin system (RAS) is a complex network that regulates blood pressure, electrolyte and fluid homeostasis, as well as the function of several organs. Angiotensin-converting enzyme 2 (ACE2) was identified as an enzyme that negatively regulates the RAS by converting Ang II, the main bioactive molecule of the RAS, to Ang 1-7. Thus, ACE2 counteracts the role of angiotensin-converting enzyme (ACE) which generates Ang II from Ang I. ACE and ACE2 have been implicated in several pathologies such as cardiovascular and renal disease or acute lung injury. In addition, ACE2 has functions independent of the RAS: ACE2 is the receptor for the SARS coronavirus and ACE2 is essential for expression of neutral amino acid transporters in the gut. In this context, ACE2 modulates innate immunity and influences the composition of the gut microbiota, which can explain diarrhea and intestinal inflammation observed in Hartnup disorder, Pellagra, or under conditions of severe malnutrition. Here we review and discuss the diverse functions of ACE2 and its relevance to human pathologies.

Pernomian, L., et al. (2014). "Counter-regulatory effects played by the ACE - Ang II - AT1 and ACE2 - Ang-(1-7) - Mas axes on the reactive oxygen species-mediated control of vascular function: perspectives to pharmacological approaches in controlling vascular complications." Vasa **43**(6): 404-414.

The Renin-Angiotensin system plays an important role in the regulation of systemic blood pressure as well as in fluid and electrolyte balance. It is divided into two described axes, the ACE - Ang II - AT1 receptor, with Ang II as the main mediator, and the ACE2 - Ang-(1-7) - Mas receptor, with Ang-(1-7) responsible for the main effects. The main vascular effect induced by Ang II is contraction, while Ang-(1-7) includes relaxation in several vascular beds. Ang II also activates several cytokines that are important in the genesis of vascular inflammation and hypertrophy. In this context, Ang-(1-7) seems to have a protective role. Both AT1 and Mas receptors modulate, in different ways, the generation of, which are involved in the control of vascular tone and the genesis of vascular dysfunction triggered by several diseases, including diabetes mellitus, arterial hypertension and atherosclerosis. Thereby, this review presents an overview of the modulation played by the whole Renin-Angiotensin system on the reactive oxygen

species-mediated control of vascular tone and the oxidative stress-elicited vascular dysfunction.

Pinheiro, D. S., et al. (2019). "The combination of ACE I/D and ACE2 G8790A polymorphisms revels susceptibility to hypertension: A genetic association study in Brazilian patients." PLoS One **14**(8): e0221248.

BACKGROUND: Systemic arterial hypertension (SAH) is a multifactorial condition that already affects one third of the worldwide population. The identification of candidate genes for hypertension is a challenge for the next years. Nevertheless, the small contribution of each individual genetic factor to the disease brings the necessity of evaluate genes in an integrative manner and taking into consideration the physiological interaction of functions. Angiotensin I-converting enzymes, ACE and ACE2, are key regulators of blood pressure that have counterbalance roles by acting on vasoactive peptides from Renin-Angiotensin-Aldosterone System (RAAS). Insertion/deletion (I/D) polymorphism of ACE gene and single nucleotide polymorphism G8790A of ACE2 gene have been associated with susceptibility to SAH, but the literature is controversial. We proposed to evaluate these two polymorphisms jointly exploring the combined effects of ACE and ACE2 genotypes on SAH susceptibility, an approach that have not been done yet for ACE and ACE2 polymorphisms. METHODS AND FINDINGS: This genetic association study included 117 hypertensive (mean age 59.7 years) patients and 123 normotensive and diabetes-free controls (mean age 57.5 years). ACE and ACE2 polymorphisms were genotyped by SYBR Green real-time PCR and RFLP-PCR, respectively. Crude and adjusted odds ratio (OR) values were calculated to estimate the susceptibility to SAH development. It was obtained homogeneity regarding distribution by sex, age range, smoking, alcohol consumption and body mass index (BMI) between case and control groups. No-association was verified for each gene individually, but the combination of ACE and ACE2 polymorphisms on female gender revealed a significative association for DD/G_ carriers who had a 3-fold increased risk to SAH development ($p = 0.03$), with a stronger susceptibility on DD/GG carriers (7-fold increased risk, $p = 0.01$). The D allele of ACE showed association with altered levels of lipid profile variables on case group (VLDL-cholesterol, $p = 0.01$) and DD genotype in all individuals analysis (triglycerides, $p = 0.01$ and VLDL-cholesterol, $p = 0.01$). CONCLUSION: These findings indicate that the combination of ACE and ACE2 polymorphisms effects may play a role in SAH predisposition been the DD/G_ genotype the susceptibility profile. This result allowed us to raise the hypothesis that an increased activity of ACE (prohypertensive effects) in conjunction with reduced ACE2 activity (antihypertensive effects) could be the underlining mechanism. The association of ACE D allele with lipid alterations indicate that this can be a marker of poor prognostic on SAH evolution and contribute to CVD development. Although these preliminary findings must be confirmed by further researches with larger sample size, we could observe that the integrative analysis of ACE and ACE2 can be an informative tool in hypertension understanding that needs to be explored in new studies.

Poglitsch, M., et al. (2012). "Recombinant Expression and Characterization of Human and Murine ACE2: Species-Specific Activation of the Alternative Renin-Angiotensin-System." Int J Hypertens **2012**: 428950.

Angiotensin-converting enzyme 2 (ACE2) is a monocarboxypeptidase of the renin-angiotensin-system (RAS) which is known to cleave several substrates among vasoactive peptides. Its preferred substrate is Angiotensin II, which is tightly involved in the regulation of important physiological functions including fluid homeostasis and blood pressure. Ang 1-7, the main enzymatic product of ACE2, became increasingly important in the literature in recent years, as it was reported to counteract hypertensive and fibrotic actions of Angiotensin II via the MAS receptor. The functional connection of ACE2, Ang 1-7, and the MAS receptor is also referred to as the alternative axis of the RAS. In the present paper, we describe the recombinant expression and purification of human and murine ACE2 (rhACE2 and rmACE2). Furthermore, we determined the conversion rates of rhACE2 and rmACE2 for different natural peptide substrates in plasma samples and discovered species-specific differences in substrate specificities, probably leading to functional differences in the alternative axis of the RAS. In particular, conversion rates of Ang 1-10 to Ang 1-9 were found to be substantially different when applying rhACE2 or rmACE2 in vitro. In contrast to rhACE2, rm ACE2 is substantially less potent in transformation of Ang 1-10 to Ang 1-9.

Portnoy, T., et al. (2011). "Differential regulation of the cellulase transcription factors XYR1, ACE2, and ACE1 in Trichoderma reesei strains producing high and low levels of cellulase." Eukaryot Cell **10**(2): 262-271.

Due to its capacity to produce large amounts of cellulases, Trichoderma reesei is increasingly being investigated for second-generation biofuel production from lignocellulosic biomass. The induction mechanisms of T. reesei cellulases have been described recently, but the regulation of the genes

involved in their transcription has not been studied thoroughly. Here we report the regulation of expression of the two activator genes xyr1 and ace2, and the corepressor gene ace1, during the induction of cellulase biosynthesis by the inducer lactose in T. reesei QM 9414, a strain producing low levels of cellulase (low producer). We show that all three genes are induced by lactose. xyr1 was also induced by d-galactose, but this induction was independent of d-galactose metabolism. Moreover, ace1 was carbon catabolite repressed, whereas full induction of xyr1 and ace2 in fact required CRE1. Significant differences in these regulatory patterns were observed in the high-producer strain RUT C30 and the hyperproducer strain T. reesei CL847. These observations suggest that a strongly elevated basal transcription level of xyr1 and reduced upregulation of ace1 by lactose may have been important for generating the hyperproducer strain and that thus, these genes are major control elements of cellulase production.

Prabakaran, P., et al. (2004). "A model of the ACE2 structure and function as a SARS-CoV receptor." Biochem Biophys Res Commun **314**(1): 235-241.

The angiotensin-converting enzyme 2 (ACE2) is an important regulator of the renin-angiotensin system and was very recently identified as a functional receptor for the SARS virus. The ACE2 sequence is similar (sequence identities 43% and 35%, and similarities 61% and 55%, respectively) to those of the testis-specific form of ACE (tACE) and the Drosophila homolog of ACE (AnCE). The high level of sequence similarity allowed us to build a robust homology model of the ACE2 structure with a root-mean-square deviation from the aligned crystal structures of tACE and AnCE less than 0.5A. A prominent feature of the model is a deep channel on the top of the molecule that contains the catalytic site. Negatively charged ridges surrounding the channel may provide a possible binding site for the positively charged receptor-binding domain (RBD) of the S-glycoprotein, which we recently identified [Biochem. Biophys. Res. Commun. 312 (2003) 1159]. Several distinct patches of hydrophobic residues at the ACE2 surface were noted at close proximity to the charged ridges that could contribute to binding. These results suggest a possible binding region for the SARS-CoV S-glycoprotein on ACE2 and could help in the design of experiments to further elucidate the structure and function of ACE2.

Prata, L. O., et al. (2017). "Original Research: ACE2 activator associated with physical exercise potentiates the reduction of pulmonary fibrosis." Exp Biol Med (Maywood) **242**(1): 8-21.

The interstitial lung diseases are poorly understood and there are currently no studies evaluating the association of physical exercise with an ACE2 activator (DIZE) as a possible treatment for this group of diseases. We evaluate the effects of pharmacological treatment with an angiotensin-converting enzyme 2 activator drug, associated with exercise, on the pulmonary lesions induced by bleomycin. From the 96 male Balb/c mice used in the experiment, only 49 received 8 U/kg of bleomycin (BLM, intratracheally). The mice were divided into control (C) and bleomycin (BLM) groups, sedentary and trained (C-SED, C-EXE, BLM-SED, BLM-EXE), control and bleomycin and also sedentary and trained treated with diminazene (C-SED/E, C-EXE/E, BLM-SED/E, BLM-EXE/E). The animals were trained five days/week, 1 h/day with 60% of the maximum load obtained in a functional capacity test, for four weeks. Diminazene groups were treated (1 mg/kg, by gavage) daily until the end of the experiment. The lungs were collected 48 h after the training program, set in buffered formalin and investigated by Gomori's trichrome, immunohistochemistry of collagen type I, TGF-beta1, beta-prolyl-4-hydroxylase, MMP-1 and -2. The BLM-EXE/E group obtained a significant increase in functional capacity, reduced amount of fibrosis and type I collagen, decreased expression of TGF-beta1 and beta-prolyl-4-hydroxylase and an increase of metalloproteinase -1, -2 when compared with the other groups. The present research shows, for the first time, that exercise training associated with the activation of ACE2 potentially reduces pulmonary fibrosis.

Prieto, M. C., et al. (2011). "Reciprocal changes in renal ACE/ANG II and ACE2/ANG 1-7 are associated with enhanced collecting duct renin in Goldblatt hypertensive rats." Am J Physiol Renal Physiol **300**(3): F749-755.

Alterations in the balance between ANG II/ACE and ANG 1-7/ACE2 in ANG II-dependent hypertension could reduce the generation of ANG 1-7 and contribute further to increased intrarenal ANG II. Upregulation of collecting duct (CD) renin may lead to increased ANG II formation during ANG II-dependent hypertension, thus contributing to this imbalance. We measured ANG I, ANG II, and ANG 1-7 contents, angiotensin-converting enzyme (ACE) and ACE2 gene expression, and renin activity in the renal cortex and medulla in the clipped kidneys (CK) and nonclipped kidneys (NCK) of 2K1C rats. After 3 wk of unilateral renal clipping, systolic blood pressure and plasma renin activity increased in 2K1C rats (n = 11) compared with sham rats (n = 9). Renal medullary

angiotensin peptide levels were increased in 2K1C rats [ANG I: (CK = 171 +/- 4; NCK = 251 +/- 8 vs. sham = 55 +/- 3 pg/g protein; P < 0.05); ANG II: (CK = 558 +/- 79; NCK = 328 +/- 18 vs. sham = 94 +/- 7 pg/g protein; P < 0.001)]; and ANG 1-7 levels decreased (CK = 18 +/- 2; NCK = 19 +/- 2 pg/g vs. sham = 63 +/- 10 pg/g; P < 0.001). In renal medullas of both kidneys of 2K1C rats, ACE mRNA levels and activity increased but ACE2 decreased. In further studies, we compared renal ACE and ACE2 mRNA levels and their activities from chronic ANG II-infused (n = 6) and sham-operated rats (n = 5). Although the ACE mRNA levels did not differ between ANG II rats and sham rats, the ANG II rats exhibited greater ACE activity and reduced ACE2 mRNA levels and activity. Renal medullary renin activity was similar in the CK and NCK of 2K1C rats but higher compared with sham. Thus, the differential regulation of ACE and ACE2 along with the upregulation of CD renin in both the CK and NCK in 2K1C hypertensive rats indicates that they are independent of perfusion pressure and contribute to the altered content of intrarenal ANG II and ANG 1-7.

Qaradakhi, T., et al. (2020). "The potential actions of angiotensin-converting enzyme II (ACE2) activator diminazene aceturate (DIZE) in various diseases." Clin Exp Pharmacol Physiol.

The renin angiotensin system (RAS) regulates fluid balance, blood pressure and maintains vascular tone. The potent vasoconstrictor angiotensin II (Ang II) produced by angiotensin-converting enzyme (ACE) comprises the classical RAS. The non-classical RAS involves the conversion of Ang II via ACE2 into the vasodilator Ang (1-7) to counterbalance the effects of Ang II. Furthermore, ACE2 converts AngA into another vasodilator named alamandine. The over activation of the classical RAS (increased vasoconstriction) and depletion of the non-classical RAS (decreased vasodilation) results in vascular dysfunction. Vascular dysfunction is the leading cause of atherosclerosis and cardiovascular disease (CVD). Additionally, local RAS is expressed in various tissues and regulates cellular functions. RAS dysregulation is involved in other several diseases such as inflammation, renal dysfunction and even cancer growth. An approach in restoring vascular dysfunction and other pathological diseases is to either increase the activity of ACE2 or reduce the effect of the classical RAS by counterbalancing Ang II effects. The antitrypanosomal agent, diminazene aceturate (DIZE), is one approach in activating ACE2. DIZE has been shown to exert beneficial effects in CVD experimental models of hypertension, myocardial infarction, type 1 diabetes and atherosclerosis. Thus, this review focuses on DIZE and its effect in several tissues such as blood vessels, cardiac, renal, immune and cancer cells.

Qin, S., et al. (2013). "[Expression and significance of ACE2-Ang-(1-7)-Mas axis in the endometrium of patients with polycystic ovary syndrome]." Zhonghua Yi Xue Za Zhi **93**(25): 1989-1992.

OBJECTIVE: To explore the expression of renin-angiotensin system component (ACE-AngII-AT1/AT2 & ACE2-Ang-(1-7)-Mas) in endometrium of polycystic ovary syndrome (PCOS) patients and normal controls. METHODS: Thirty cases of normal endometrium in proliferative and secretory phases respectively were selected for the protein levels of AngII, AT1, AT2, ACE2, Ang-(1-7) and Mas through immunohistochemistry. And the specimens of proliferative endometrium from 15 PCOS patients and 15 normal controls respectively were prepared for the expressions of AT1, AT2, ACE2 and Mas mRNA through relative quantitative -polymerase chain reaction (RQ-PCR). The histological phases of endometria were confirmed by hematoxylin & eosin staining under microscope. RESULTS: The expressions of AngII, AT1R, AT2R, ACE2, Ang-(1-7) and Mas showed periodical changes in endometrium throughout normal menstrual cycles and shared a similar trend. The expression was more pronounced in epithelial cells than that in stromal cells while it was also more dramatic in secretory phase than proliferative phase; The mRNA expressions of AT1, AT2, ACE2 and MAS were higher in PCOS endometrium than those in normal controls. Statistically significant differences existed between two groups (P < 0.01). CONCLUSIONS: There is local existence of RAS in endometrium; Increased expressions of AT1 mRNA, AT2 mRNA, ACE2 mRNA and Mas mRNA in endometrium of PCOS may influence endometrial development and play a role in the pathological process of PCOS.

Qin, X. T., et al. (2008). "[Effect of atorvastatin on ACE2 expression in pressure overload induced cardiac hypertrophy in rats]." Zhong Nan Da Xue Xue Bao Yi Xue Ban **33**(5): 438-442.

OBJECTIVE: To investigate the effect of atorvastatin on the expression of angiotensin converting enzyme 2 (ACE2) mRNA and its protein in hypertrophic myocardium in rats. METHODS: Suprarenal abdominal aortic coarctation was performed to create the pressure overload induced left ventricular hypertrophy model in rats. RESULTS: Rats were randomly divided into 5 groups: (1) normal control group (Group A); (2) normal control group treated with atorvastatin [(5 mg/(kg.dd), Group B]; (3) sham group (Group C); (4) atorvastatin given orally by

gastric gavage for 4 weeks [5 mg/(kg.dd),Group D]; (5) vehicle group (Group E). Stained pathological section was observed under light microscope to measure cardiomyocyte diameter transversa and collagen volume fraction. ACE2 mRNA and its protein expression were detected by real-time RT-PCR and Western blot. Compared with Group A,B, and C, the left ventricular mass index, cardiomyocyte diameter transversa and collagen volume fraction in Group E increased statistically (P< 0.01), ACE2 mRNA and its protein expression also elevated remarkably (P< 0.01). Compared with Group E, the above mentioned indexes in Group D reduced significantly (P< 0.01). CONCLUSION: ACE2 mRNA and its protein expression increase significantly in hypertrophic myocardium in rats; atorvastatin can attenuate cardiac hypertrophy due to pressure overload in rats effectively, and part of this anti-hypertrophy effect may be attributed to decrease ACE2 mRNA and protein expression.

Qiu, Y., et al. (2014). "Angiotensin-converting enzyme 2 (ACE2) activator diminazene aceturate ameliorates endotoxin-induced uveitis in mice." Invest Ophthalmol Vis Sci **55**(6): 3809-3818.

PURPOSE: Uveitis is a common cause of vision loss. The renin angiotensin system (RAS), which plays a vital role in cardiovascular system, is a potent mediator of inflammation and has been implicated in the pathogenesis of uveitis. A newly identified axis of RAS, ACE2/Ang-(1-7)/Mas, has emerged as a novel target because it counteracts the deleterious effect of angiotensin II. The purpose of this study was to investigate the effect of endogenous ACE2 activation in preventing endotoxin-induced uveitis (EIU) in mice. METHODS: ACE2 activator diminazene aceturate (DIZE) was administered both systemically and locally. For systemic administration, female BALB/c mice received intraperitoneal injection of DIZE (60 mg/kg body weight [BW]) for 2 days prior to lipopolysaccharide (LPS) intravitreal injection (125 ng) to induce uveitis. For local study, DIZE was given at 0.5, 0.1, and 0 mg/mL as eyedrops six times per day for 2 days before LPS injection. The anterior segment of the mice was examined at 12, 24, 48, and 72 hours after LPS injection, and clinical scores were determined at the same time. Morphology and infiltrating inflammatory cells were evaluated after 24 hours. The mRNA levels of inflammatory cytokines were analyzed by real-time RT-PCR. ACE2 activity was determined using a self-quenching fluorescent substrate. RESULTS: At 24 hours, the clinical score of mice treated with DIZE systemically was significantly lower (mean, approximately 1.75) than the saline vehicle group (mean, approximately 4) (P < 0.001). Histological examination showed 63.4% reduction of infiltrating inflammatory cells in the anterior segment and 57.4% reduction in the posterior segment of DIZE-treated eyes. The number of CD45(+) inflammatory cells in the vitreous of the DIZE-treated group was decreased (43.3%) compared to the vehicle group (P < 0.01). The mRNA levels of inflammatory cytokines were significantly reduced in the DIZE-treated group (P < 0.01, P < 0.001). The number of infiltrating inflammatory cells was also significantly reduced in eyes that received topical administration of DIZE: 73.8% reduction in the 0.5 mg/mL group and 51.7% reduction in the 0.1mg/mL group compared to the control group. DIZE treatment resulted in significantly increased ACE2 activity in the retina (P < 0.001). CONCLUSIONS: Endogenous ACE2 activation by DIZE has a preventive effect on LPS-induced ocular inflammation in the EIU mouse model. These results support the notions that RAS plays a role in modulating ocular immune response and that enhancing ACE2 provides a novel therapeutic strategy for uveitis.

Qiu, Y., et al. (2020). "Predicting the angiotensin converting enzyme 2 (ACE2) utilizing capability as the receptor of SARS-CoV-2." Microbes Infect.

SARS-CoV-2, the newly identified human coronavirus causing severe pneumonia epidemic, was probably originated from Chinese horseshoe bats. However, direct transmission of the virus from bats to humans is unlikely due to lack of direct contact, implying the existence of unknown intermediate hosts. Angiotensin converting enzyme 2 (ACE2) is the receptor of SARS-CoV-2, but only ACE2s of certain species can be utilized by SARS-CoV-2. Here, we evaluated and ranked the receptor-utilizing capability of ACE2s from various species by phylogenetic clustering and sequence alignment with the currently known ACE2s utilized by SARS-CoV-2. As a result, we predicted that SARS-CoV-2 tends to utilize ACE2s of various mammals, except murines, and some birds, such as pigeon. This prediction may help to screen the intermediate hosts of SARS-CoV-2.

Rabelo, L. A., et al. (2011). "ACE2-angiotensin-(1-7)-Mas axis and oxidative stress in cardiovascular disease." Hypertens Res **34**(2): 154-160.

The renin-angiotensin-aldosterone system (RAAS) is a pivotal regulator of physiological homeostasis and diseases of the cardiovascular system. Recently, new factors have been discovered, such as angiotensin-converting enzyme 2 (ACE2), angiotensin-(1-7) and Mas. This newly defined ACE2-angiotensin-(1-7)-Mas axis was shown to have a critical role in the vasculature and in the heart, exerting mainly protective effects. One important mechanism of the classic and the new RAAS regulate

vascular function is through the regulation of redox signaling. Angiotensin II is a classic prooxidant peptide that increases superoxide production through the activation of NAD (P)H oxidases. This review summarizes the current knowledge about the ACE2-angiotensin-(1-7)-Mas axis and redox signaling in the context of cardiovascular regulation and disease. By interacting with its receptor Mas, angiotensin-(1-7) induces the release of nitric oxide from endothelial cells and thereby counteracts the effects of angiotensin II. ACE2 converts angiotensin II to angiotensin-(1-7) and, thus, is a pivotal regulator of the local effects of the RAAS on the vessel wall. Taken together, the ACE2-angiotensin-(1-7)-Mas axis emerges as a novel therapeutic target in the context of cardiovascular and metabolic diseases.

Rabelo, L. A., et al. (2016). "Genetic Deletion of ACE2 Induces Vascular Dysfunction in C57BL/6 Mice: Role of Nitric Oxide Imbalance and Oxidative Stress." PLoS One **11**(4): e0150255.

Accumulating evidence indicates that angiotensin-converting enzyme 2 (ACE2) plays a critical role in cardiovascular homeostasis, and its altered expression is associated with major cardiac and vascular disorders. The aim of this study was to evaluate the regulation of vascular function and assess the vascular redox balance in ACE2-deficient (ACE2-/y) animals. Experiments were performed in 20-22 week-old C57BL/6 and ACE2-/y male mice. Evaluation of endothelium-dependent and -independent relaxation revealed an impairment of in vitro and in vivo vascular function in ACE2-/y mice. Drastic reduction in eNOS expression at both protein and mRNA levels, and a decrease in *NO concentrations were observed in aortas of ACE2-/y mice in comparison to controls. Consistently, these mice presented a lower plasma and urine nitrite concentration, confirming reduced *NO availability in ACE2-deficient animals. Lipid peroxidation was significantly increased and superoxide dismutase activity was decreased in aorta homogenates of ACE2-/y mice, indicating impaired antioxidant capacity. Taken together, our data indicate, that ACE2 regulates vascular function by modulating nitric oxide release and oxidative stress. In conclusion, we elucidate mechanisms by which ACE2 is involved in the maintenance of vascular homeostasis. Furthermore, these findings provide insights into the role of the renin-angiotensin system in both vascular and systemic redox balance.

Raizada, M. K. and A. J. Ferreira (2007). "ACE2: a new target for cardiovascular disease therapeutics." J Cardiovasc Pharmacol **50**(2): 112-119.

The discovery of angiotensin-converting enzyme 2 (ACE2) in 2000 is an important event in the renin-angiotensin system (RAS) story. This enzyme, an homolog of ACE, hydrolyzes angiotensin (Ang) I to produce Ang-(1-9), which is subsequently converted into Ang-(1-7) by a neutral endopeptidase and ACE. ACE2 releases Ang-(1-7) more efficiently than its catalysis of Ang-(1-9) by cleavage of Pro (7)-Phe (8) bound in Ang II. Thus, the major biologically active product of ACE2 is Ang-(1-7), which is considered to be a beneficial peptide of the RAS cascade in the cardiovascular system. This enzyme has 42% identity with the catalytic domain of ACE, is present in most cardiovascular-relevant tissues, and is an ectoenzyme as ACE. Despite these similarities, ACE2 is distinct from ACE. Since it is a monocarboxypeptidase, it has only 1 catalytic site and is insensitive to ACE inhibitors. As a result, ACE2 is a central enzyme in balancing vasoconstrictor and proliferative actions of Ang II with vasodilatory and antiproliferative effects of Ang-(1-7). In this review, we will summarize the role of ACE2 in the cardiovascular system and discuss the importance of ACE2-Ang-(1-7) axis in the control of normal cardiovascular physiology and ACE2 as a potential target in the development of novel therapeutic agents for cardiovascular diseases.

Rajasekar, A., et al. (2007). "Role of Serratia marcescens ACE2 on diesel degradation and its influence on corrosion." J Ind Microbiol Biotechnol **34**(9): 589-598.

A facultative anaerobic species Serratia marcescens ACE2 isolated from the corrosion products of diesel transporting pipeline in North West, India was identified by 16S rDNA sequence analysis. The role of Serratia marcesens ACE2 on biodegradation of diesel and its influence on the corrosion of API 5LX steel has been elucidated. The degrading strain ACE2 is involved in the process of corrosion of steel API 5LX and also utilizes the diesel as an organic source. The quantitative biodegradation efficiency (BE) of diesel was 58%, calculated by gas-chromatography-mass spectrum analysis. On the basis of gas-chromatography-mass spectrum (GC-MS), Fourier Transform infrared spectroscopy (FTIR) and X-ray diffractometer (XRD), the involvement of Serratia marcescens on degradation and corrosion has been investigated. This basic study will be useful for the development of new approaches for detection, monitoring and control of microbial corrosion.

Ramchand, J., et al. (2020). "Plasma ACE2 Activity Predicts Mortality in Aortic Stenosis and Is Associated With Severe Myocardial Fibrosis." JACC Cardiovasc Imaging **13**(3): 655-664.

OBJECTIVES: This study investigated the relationship between plasma angiotensin-converting enzyme 2 (ACE2) activity levels and the severity of stenosis and myocardial remodeling in patients with aortic stenosis (AS) and determined if plasma ACE2 levels offered incremental prognostic usefulness to predict all-cause mortality. BACKGROUND: ACE2 is an integral membrane protein that degrades angiotensin II and has an emerging role as a circulating biomarker of cardiovascular disease. METHODS: Plasma ACE2 activity was measured in 127 patients with AS; a subgroup had myocardial tissue collected at the time of aortic valve replacement. RESULTS: The median plasma ACE2 activity was 34.0 pmol/ml/min, and levels correlated with increased valvular calcification ($p = 0.023$) and the left ventricular (LV) mass index ($r = 0.34$; $p < 0.001$). Patients with above-median plasma ACE2 had higher LV end-diastolic volume (57 ml/m (2) vs. 48 ml/m (2); $p = 0.021$). Over a median follow-up of 5 years, elevated plasma ACE2 activity was an independent predictor of all-cause mortality after adjustment for relevant clinical, imaging, and biochemical parameters (HR: 2.28; 95% CI: 1.03 to 5.06; $p = 0.042$), including brain natriuretic peptide activation (integrated discrimination improvement: 0.08; $p < 0.001$). In 22 patients with plasma and tissue, increased circulating ACE2 was associated with reduced myocardial ACE2 gene expression (0.7-fold; $p = 0.033$) and severe myocardial fibrosis ($p = 0.027$). CONCLUSIONS: In patients with AS, elevated plasma ACE2 was a marker of myocardial structural abnormalities and an independent predictor of mortality with incremental value over traditional prognostic markers. Loss of ACE2 from the myocardium was associated with increased fibrosis and higher circulating ACE2 levels.

Raz, A., et al. (2007). "[The importance of ACE2 in regulating the cardiovascular system]." Harefuah **146**(9): 703-706, 733.

A recently discovered homologue of the angiotensin-converting enzyme, ACE2, insensitive to ACE inhibitors, was found in rodents and humans. ACE2 is expressed mainly in the vasculature, heart and kidney. ACE2 removes a single amino acid of the carboxy terminal of peptides. In the renin-angiotensin-aldosterone system (RAAS), it is responsible for the conversion of angiotensin I (Ang I) and angiotensin II (Ang II) to Ang 1-9 and Ang 1-7, respectively. While ACE forms Ang II, a potent vasoconstrictor, ACE2 degrades this peptide to form Ang 1-7 which has an opposite action. Therefore, ACE2 counteracts ACE in the balance of vasopressor/vasodilator as well as heart and kidney function. The importance of ACE2 in physiological and pathophysiological conditions is unclear and is currently being studied.

Reich, H. N., et al. (2008). "Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease." Kidney Int **74**(12): 1610-1616.

Angiotensin converting enzyme (ACE) generates angiotensin II from angiotensin I, which plays a critical role in the pathophysiology of diabetic nephropathy. However, ACE2 generates angiotensin 1-7, which may protect the kidney by attenuating the effects of angiotensin II, since deletion of the Ace2 gene leads to glomerulosclerosis in mice, and pharmacologic inhibition of ACE2 exacerbates experimental diabetic nephropathy. We measured ACE2 and ACE expression in renal biopsies of patients with kidney disease due to type 2 diabetes to determine if the expression pattern is specific to diabetic nephropathy. ACE2 and ACE mRNA levels were measured by real-time PCR in laser microdissected renal biopsies from 13 diabetic and 8 control patients. ACE2 mRNA was significantly reduced by more than half in both the glomeruli and proximal tubules of the diabetic patients compared to controls, but ACE mRNA was increased in both compartments. There was a significant parallel decrease in ACE2 protein expression, determined by immunohistochemistry, in proximal tubules, a pattern not found in 12 patients with focal glomerulosclerosis or 10 patients with chronic allograft nephropathy. Our results suggest that the kidney disease of patients with type 2 diabetes is associated with a reduction in ACE2 gene and protein expression and this may contribute to the progression of renal injury.

Ren, X., et al. (2006). "Analysis of ACE2 in polarized epithelial cells: surface expression and function as receptor for severe acute respiratory syndrome-associated coronavirus." J Gen Virol **87**(Pt 6): 1691-1695.

The primary target of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) is epithelial cells in the respiratory and intestinal tract. The cellular receptor for SARS-CoV, angiotensin-converting enzyme 2 (ACE2), has been shown to be localized on the apical plasma membrane of polarized respiratory epithelial cells and to mediate infection from the apical side of these cells. Here, these results were confirmed and extended by including a colon carcinoma cell line (Caco-2), a lung carcinoma cell line (Calu-3) and Vero E6 cells in our analysis. All three cell types expressed human ACE2 on the apical membrane domain and were infected via this route, as determined with vesicular stomatitis virus pseudotypes containing the S protein of SARS-CoV. In a histological analysis of the respiratory tract, ACE2 was detected in the trachea, main bronchus and alveoli,

and occasionally also in the small bronchi. These data will help us to understand the pathogenesis of SARS-CoV infection.

Revuelta, L., et al. (2009). "RNAi of ace1 and ace2 in Blattella germanica reveals their differential contribution to acetylcholinesterase activity and sensitivity to insecticides." Insect Biochem Mol Biol **39**(12): 913-919.

Cyclorrhapha insect genomes contain a single acetylcholinesterase (AChE) gene while other insects contain at least two ace genes (ace1 and ace2). In this study we tested the hypothesis that the two ace paralogous from Blattella germanica have different contributions to AChE activity, using RNA interference (RNAi) to knockdown each one individually. Paralogous-specific depletion of Bgace transcripts was evident in ganglia of injected cockroaches, although the effects at the protein level were less pronounced. Using spectrophotometric and zymogram measurements, we obtained evidence that BgAChE1 represents 65-75% of the total AChE activity in nerve tissue demonstrating that ace1 encodes a predominant AChE. A significant increase in sensitivity of Bgace1-interfered cockroaches was observed after 48 h of exposure to chlorpyrifos. In contrast, Bgace2 knockdown had a negligible effect on mortality to this organophosphate. These results point out a key role, qualitative and/or quantitative, of AChE1 as target of organophosphate insecticides in this species. Silencing the expression of Bgace1 but not Bgace2 also produced an increased mortality in insects when synergized with lambda-cyhalothrin, a situation which resembles the synergistic effects observed between organophosphates and pyrethroids. Gene silencing of ace genes by RNAi offers an exciting approach for examining a possible functional differentiation in ace paralogous.

Rice, G. I., et al. (2004). "Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism." Biochem J **383**(Pt 1): 45-51.

In the RAS (renin-angiotensin system), Ang I (angiotensin I) is cleaved by ACE (angiotensin-converting enzyme) to form Ang II (angiotensin II), which has effects on blood pressure, fluid and electrolyte homoeostasis. We have examined the kinetics of angiotensin peptide cleavage by full-length human ACE, the separate N- and C-domains of ACE, the homologue of ACE, ACE2, and NEP (neprilysin). The activity of the enzyme preparations was determined by active-site titrations using competitive tight-binding inhibitors and fluorogenic substrates. Ang I was effectively cleaved by NEP to Ang (1-7) (kcat/K (m) of 6.2x10(5) M (-1) x s (-1)), but was a poor substrate for ACE2 (kcat/K (m) of 3.3x10(4) M (-1) x s (-1)). Ang (1-9) was a better substrate for NEP than ACE (kcat/K (m) of 3.7x10(5) M (-1) x s (-1) compared with kcat/K (m) of 6.8x10(4) M (-1) x s (-1)). Ang II was cleaved efficiently by ACE2 to Ang (1-7) (kcat/K (m) of 2.2x10(6) M (-1) x s (-1)) and was cleaved by NEP (kcat/K (m) of 2.2x10(5) M (-1) x s (-1)) to several degradation products. In contrast with a previous report, Ang (1-7), like Ang I and Ang (1-9), was cleaved with a similar efficiency by both the N- and C-domains of ACE (kcat/K (m) of 3.6x10(5) M (-1) x s (-1) compared with kcat/K (m) of 3.3x10(5) M (-1) x s (-1)). The two active sites of ACE exhibited negative co-operativity when either Ang I or Ang (1-7) was the substrate. In addition, a range of ACE inhibitors failed to inhibit ACE2. These kinetic data highlight that the flux of peptides through the RAS is complex, with the levels of ACE, ACE2 and NEP dictating whether vasoconstriction or vasodilation will predominate.

Riera, M., et al. (2016). "Paricalcitol modulates ACE2 shedding and renal ADAM17 in NOD mice beyond proteinuria." Am J Physiol Renal Physiol **310**(6): F534-546.

Circulating and renal activity of angiotensin-converting enzyme 2 (ACE2) is increased in non-obese diabetic (NOD) mice. Because paricalcitol has been reported to protect against diabetic nephropathy, we investigated the role of paricalcitol in modulating ACE2 in these mice. In addition, renal ADAM17, a metalloprotease implied in ACE2 shedding, was assessed. NOD female and non-diabetic control mice were studied for 21 days after diabetes onset and divided into various treatment groups. Diabetic animals received either vehicle; 0.4 or 0.8 mug/kg paricalcitol, aliskiren, or a combination of paricalcitol and aliskiren. We then studied the effect of paricalcitol on ACE2 expression in proximal tubular epithelial cells. Paricalcitol alone or in combination with aliskiren resulted in significantly reduced circulating ACE2 activity in NOD mice but there were no changes in urinary albumin excretion. Serum renin activity was significantly decreased in mice that received aliskiren but no effect was found when paricalcitol was used alone. Renal content of ADAM17 was significantly decreased in animals that received a high dose of paricalcitol. Renal and circulating oxidative stress (quantified by plasma H2O2 levels and immunolocalization of nitrotyrosine) were reduced in high-dose paricalcitol-treated mice compared with non-treated diabetic mice. In culture, paricalcitol incubation resulted in a significant increase in ACE2 expression compared with nontreated cells. In NOD mice with type 1 diabetes, paricalcitol modulates ACE2 activity, ADAM17, and oxidative stress renal

content independently from the glycemic profile and urinary albumin excretion. In tubular cells, paricalcitol may modulate ACE2 by blocking its shedding. In the early stage of diabetic nephropathy, paricalcitol treatment counterbalances the effect of diabetes on circulating ACE2 activity. Our results suggest that additional use of paricalcitol may be beneficial in treating patients with diabetes under standard therapeutic strategies.

Riera, M., et al. (2014). "Effect of insulin on ACE2 activity and kidney function in the non-obese diabetic mouse." PLoS One **9**(1): e84683.

We studied the non-obese diabetic (NOD) mice model because it develops autoimmune diabetes that resembles human type 1 diabetes. In diabetic mice, urinary albumin excretion (UAE) was ten-fold increased at an "early stage" of diabetes, and twenty-fold increased at a "later stage" (21 and 40 days, respectively after diabetes diagnosis) as compared to non-obese resistant controls. In NOD Diabetic mice, glomerular enlargement, increased glomerular filtration rate (GFR) and increased blood pressure were observed in the early stage. In the late stage, NOD Diabetic mice developed mesangial expansion and reduced podocyte number. Circulating and urine ACE2 activity were markedly increased both, early and late in Diabetic mice. Insulin administration prevented albuminuria, markedly reduced GFR, blood pressure, and glomerular enlargement in the early stage; and prevented mesangial expansion and the reduced podocyte number in the late stage of diabetes. The increase in serum and urine ACE2 activity was normalized by insulin administration at the early and late stages of diabetes in Diabetic mice. We conclude that the Diabetic mice develops features of early kidney disease, including albuminuria and a marked increase in GFR. ACE2 activity is increased starting at an early stage in both serum and urine. Moreover, these alterations can be completely prevented by the chronic administration of insulin.

Riquelme, C., et al. (2014). "ACE2 is augmented in dystrophic skeletal muscle and plays a role in decreasing associated fibrosis." PLoS One **9**(4): e93449.

Duchenne muscular dystrophy (DMD) is the most common inherited neuromuscular disease and is characterized by absence of the cytoskeletal protein dystrophin, muscle wasting, and fibrosis. We previously demonstrated that systemic infusion or oral administration of angiotensin-(1-7) (Ang-(1-7)), a peptide with opposing effects to angiotensin II, normalized skeletal muscle architecture, decreased local fibrosis, and improved muscle function in mdx mice, a dystrophic model for DMD. In this study, we investigated the presence, activity, and localization of ACE2, the enzyme responsible for Ang-(1-7) production, in wild type (wt) and mdx skeletal muscle and in a model of induced chronic damage in wt mice. All dystrophic muscles studied showed higher ACE2 activity than wt muscle. Immunolocalization studies indicated that ACE2 was localized mainly at the sarcolemma and, to a lesser extent, associated with interstitial cells. Similar results were observed in the model of chronic damage in the tibialis anterior (TA) muscle. Furthermore, we evaluated the effect of ACE2 overexpression in mdx TA muscle using an adenovirus containing human ACE2 sequence and showed that expression of ACE2 reduced the fibrosis associated with TA dystrophic muscles. Moreover, we observed fewer inflammatory cells infiltrating the mdx muscle. Finally, mdx gastrocnemius muscles from mice infused with Ang-(1-7), which decreases fibrosis, contain less ACE2 associated with the muscle. This is the first evidence supporting ACE2 as an important therapeutic target to improve the dystrophic skeletal muscle phenotype.

Riviere, G., et al. (2005). "Angiotensin-converting enzyme 2 (ACE2) and ACE activities display tissue-specific sensitivity to undernutrition-programmed hypertension in the adult rat." Hypertension **46**(5): 1169-1174.

Human epidemiological studies have shown that low birth weight is associated with hypertension in adulthood. Rodent models of intrauterine growth retardation (IUGR) support these findings because offspring from undernourished dams develop hypertension. Angiotensin-converting enzyme 2 (ACE2) is a newly described renin-angiotensin system (RAS) component that competes with ACE for angiotensin peptide hydrolysis and therefore may modulate blood pressure. However, ACE2 potential participation in hypertension programming remains unknown, although RAS alterations were reported in IUGR models. Hence, we first investigated the tissue distribution of ACE2 and ACE in the rat and then whether hypertension programming differentially affects both enzymes. Using multiplex RT-PCR and in situ hybridization, we show that ACE2 mRNA is widely expressed and coregionalized with ACE. Moreover, tissues involved in blood pressure homeostasis (lung, heart, and kidney) express high levels of both enzymes. Enzymatic assays reveal that ACE2 and ACE are coactive in these tissues. Adult (4-month-old) offspring from 70% food-restricted dams throughout gestation (FR30 rats) present mild hypertension, impaired renal morphology, as well as elevated plasma angiotensin II and aldosterone, suggesting alterations of the systemic RAS. In FR30 rats, we show that ACE2 and ACE activities are

increased only in the lung, whereas their mRNA expression is not significantly altered, showing that the enzymes display tissue-specific sensitivity to programming. Our results indicate that ACE2 and ACE are coexpressed in numerous rat tissues and that their increased activity in the lung of FR30 rats may participate in hypertension programming.

Roca-Ho, H., et al. (2017). "Characterization of ACE and ACE2 Expression within Different Organs of the NOD Mouse." Int J Mol Sci **18**(3).

Renin angiotensin system (RAS) is known to play a key role in several diseases such as diabetes, and renal and cardiovascular pathologies. Its blockade has been demonstrated to delay chronic kidney disease progression and cardiovascular damage in diabetic patients. In this sense, since local RAS has been described, the aim of this study is to characterize angiotensin converting enzyme (ACE) and ACE2 activities, as well as protein expression, in several tissues of the non-obese diabetic (NOD) mice model. After 21 or 40 days of diabetes onset, mouse serums and tissues were analyzed for ACE and ACE2 enzyme activities and protein expression. ACE and ACE2 enzyme activities were detected in different tissues. Their expressions vary depending on the studied tissue. Thus, whereas ACE activity was highly expressed in lungs, ACE2 activity was highly expressed in pancreas among the studied tissues. Interestingly, we also observed that diabetes up-regulates ACE mainly in serum, lung, heart, and liver, and ACE2 mainly in serum, liver, and pancreas. In conclusion, we found a marked serum and pulmonary alteration in ACE activity of diabetic mice, suggesting a common regulation. The increase of ACE2 activity within the circulation in diabetic mice may be ascribed to a compensatory mechanism of RAS.

Rodrigues Prestes, T. R., et al. (2017). "The Anti-Inflammatory Potential of ACE2/Angiotensin-(1-7)/Mas Receptor Axis: Evidence from Basic and Clinical Research." Curr Drug Targets **18**(11): 1301-1313.

BACKGROUND: The renin angiotensin system (RAS) plays an important role in inflammation and fibrosis. The classical axis of the RAS, formed by angiotensin converting en-zyme (ACE), angiotensin II (Ang II) and angiotensin receptor type 1 (AT1), activates several cell functions and molecular signaling pathways related to tissue injury, inflammation and fibrosis. In sharp contrast, the RAS axis composed by angiotensin converting enzyme 2 (ACE2), angiotensin-(1-7) and Mas receptor exerts opposite effects in relation to inflammatory response and tissue fibrosis. OBJECTIVE: In this review, we have the aim to summarize recent findings on the anti-inflammatory and anti-fibrogenic role of ACE2/Ang-(1-7)/Mas axis in the context of basic research, experimental human dis-eases and clinical studies. RESULTS: Several studies showed that ACE2/Angiotensin-(1-7)/Mas axis reduces cytokine release and inhibits signaling pathways of tissue fibrosis in experimental models of human diseases including atherosclerosis, cerebral ischemia, obesity, chronic kidney disease, liver diseases and asthma. On the other hand, very few data was provided by clinical studies. CONCLUSION: Experimental studies clearly support the anti-inflammatory and anti-fibrotic effects of ACE2/ Ang-(1-7)/Mas axis. Clinical studies, especially phase III and IV trials, will be necessary to establish the therapeutic role of ACE2/Ang-(1-7)/Mas axis in controlling inflammation in different human diseases.

Rushworth, C. A., et al. (2008). "Residues affecting the chloride regulation and substrate selectivity of the angiotensin-converting enzymes (ACE and ACE2) identified by site-directed mutagenesis." FEBS J **275**(23): 6033-6042.

Angiotensin-converting enzyme (ACE) and its homologue angiotensin-converting enzyme 2 (ACE2) are critical counter-regulatory enzymes of the renin-angiotensin system, and have been implicated in cardiac function, renal disease, diabetes, atherosclerosis and acute lung injury. Both ACE and ACE2 have catalytic activity that is chloride sensitive and is caused by the presence of the CL1 and CL2 chloride-binding sites in ACE and the CL1 site in ACE2. The chloride regulation of activity is also substrate dependent. Site-directed mutagenesis was employed to elucidate which of the CL1 and CL2 site residues are responsible for chloride sensitivity. The CL1 site residues Arg186, Trp279 and Arg489 of testicular ACE and the equivalent ACE2 residues Arg169, Trp271 and Lys481 were found to be critical to chloride sensitivity. Arg522 of testicular ACE was also confirmed to be vital to the chloride regulation mediated by the CL2 site. In addition, Arg514 of ACE2 was identified as a residue critical to substrate selectivity, with the R514Q mutant, relative to the wild-type, possessing a fourfold greater selectivity for the formation of the vasodilator angiotensin-(1-7) from the vasoconstrictor angiotensin II. The enhancement of angiotensin II cleavage by R514Q ACE2 was a result of a 2.5-fold increase in V (max) compared with the wild-type. Inhibition of ACE2 was also found to be chloride sensitive, as for testicular ACE, with residues Arg169 and Arg514 of ACE2 identified as influencing the potency of the ACE2-specific inhibitor MLN-4760. Consequently, important insights into the chloride sensitivity, substrate selectivity and inhibition of testicular ACE and ACE2 were elucidated.

Salem, E. S., et al. (2014). "Insulin treatment attenuates renal ADAM17 and ACE2 shedding in diabetic Akita mice." Am J Physiol Renal Physiol **306**(6): F629-639.

Angiotensin-converting enzyme 2 (ACE2) is located in several tissues and is highly expressed in renal proximal tubules, where it degrades the vasoconstrictor angiotensin II (ANG II) to ANG-(1-7). Accumulating evidence supports protective roles of ACE2 in several disease states, including diabetic nephropathy. A disintegrin and metalloprotease (ADAM) 17 is involved in the shedding of several transmembrane proteins, including ACE2. Our previous studies showed increased renal ACE2, ADAM17 expression, and urinary ACE2 in type 2 diabetic mice (Chodavarapu H, Grobe N, Somineni HK, Salem ES, Madhu M, Elased KM. PLoS One 8: e62833, 2013). The aim of the present study was to determine the effect of insulin on ACE2 shedding and ADAM17 in type 1 diabetic Akita mice. Results demonstrate increased renal ACE2 and ADAM17 expression and increased urinary ACE2 fragments (approximately 70 kDa) and albumin excretion in diabetic Akita mice. Immunostaining revealed colocalization of ACE2 with ADAM17 in renal tubules. Renal proximal tubular cells treated with ADAM17 inhibitor showed reduced ACE2 shedding into the media, confirming ADAM17-mediated shedding of ACE2. Treatment of Akita mice with insulin implants for 20 wk normalized hyperglycemia and decreased urinary ACE2 and albumin excretion. Insulin also normalized renal ACE2 and ADAM17 but had no effect on tissue inhibitor of metalloproteinase 3 (TIMP3) protein expression. There was a positive linear correlation between urinary ACE2 and albuminuria, blood glucose, plasma creatinine, glucagon, and triglycerides. This is the first report showing an association between hyperglycemia, cardiovascular risk factors, and increased shedding of urinary ACE2 in diabetic Akita mice. Urinary ACE2 could be used as a biomarker for diabetic nephropathy and as an index of intrarenal ACE2 status.

Sampson, A. K., et al. (2012). "Postnatal ontogeny of angiotensin receptors and ACE2 in male and female rats." Gend Med **9**(1): 21-32.

BACKGROUND: Sex differences in the expression of the angiotensin (Ang) II receptors and angiotensin-converting enzyme 2 (ACE2) have been hypothesized to be a potential mechanism contributing to sex-specific differences in arterial pressure. Currently, sex differences in the expression of the angiotensin receptors and ACE2 remain undefined. OBJECTIVES: The aim of this study was to define the postnatal ontogeny of mRNA expression, from birth to adulthood, of the Ang II and Ang-(1-7) receptors and ACE2 in male and female rats. METHODS: Kidney and heart tissue was collected from male and female Sprague Dawley rats and snap-frozen at postnatal days (PNDs) 1, 30, 42, 70, and 110 (adult), and real-time polymerase chain reaction was performed to determine relative expression of the Ang II and Ang-(1-7) receptors (AT (1a)R, AT (1b)R, AT (2)R, and MasR) and ACE2. RESULTS: All these components of the renin-angiotensin system (RAS) were detected in the kidney and left ventricle, although expression levels differed significantly between the sexes and across organs. Gene expression of most components of the RAS was high at birth and decreased with age in both sexes, except for ACE2 expression, which increased in the left ventricle with age (P (Age) < 0.001). Low levels of AT (2)R were observed in the ventricles in both sexes as adults. Most notably, AT (2)R expression was greatest in female kidneys and lowest in male kidneys compared with the left ventricle (P (Age*Sex) < 0.05). Interestingly, MasR expression in the female kidney was similar to the level of AT (2)R expression. Left ventricular MasR expression was greater than AT (2)R expression in both sexes but was not different between the sexes. The highest level of ACE2 expression was observed in adult female kidneys (P (AS) < 0.05). CONCLUSIONS: The enhanced mRNA expression of the vasodilatory arm of the renal RAS (ACE2, AT (2)R) in females observed in the present study may contribute to sex differences in the regulation of arterial pressure and the incidence of cardiovascular disease in women.

Samuel, P., et al. (2012). "High Na intake increases renal angiotensin II levels and reduces expression of the ACE2-AT (2)R-MasR axis in obese Zucker rats." Am J Physiol Renal Physiol **303**(3): F412-419.

High sodium intake is known to regulate the renal renin-angiotensin system (RAS) and is a risk factor for the pathogenesis of obesity-related hypertension. The complex nature of the RAS reveals that its various components may have opposing effects on natriuresis and blood pressure regulation. We hypothesized that high sodium intake differentially regulates and shifts a balance between opposing components of the renal RAS, namely, angiotensin-converting enzyme (ACE)-ANG II-type 1 ANG II receptor (AT (1)R) vs. AT (2)-ACE2-angiotensinogen (Ang) (1-7)-Mas receptor (MasR), in obesity. In the present study, we evaluated protein and/or mRNA expression of angiotensinogen, renin, AT (1A/B)R, ACE, AT (2)R, ACE2, and MasR in the kidney cortex following 2 wk of a 8% high-sodium (HS) diet in lean and obese Zucker rats. The expression data showed that the relative expression pattern of ACE and AT

(1B)R increased, renin decreased, and ACE2, AT (2)R, and MasR remained unaltered in HS-fed lean rats. On the other hand, HS intake in obese rats caused an increase in the cortical expression of ACE, a decrease in ACE2, AT (2)R, and MasR, and no changes in renin and AT (1)R. The cortical levels of ANG II increased by threefold in obese rats on HS compared with obese rats on normal salt (NS), which was not different than in lean rats. The HS intake elevated mean arterial pressure in obese rats (27 mmHg) more than in lean rats (16 mmHg). This study suggests that HS intake causes a pronounced increase in ANG II levels and a reduction in the expression of the ACE2-AT (2)R-MasR axis in the kidney cortex of obese rats. We conclude that such changes may lead to the potentially unopposed function of AT (1)R, with its various cellular and physiological roles, including the contribution to the pathogenesis of obesity-related hypertension.

Santos, R. A. S., et al. (2018). "The ACE2/Angiotensin-(1-7)/MAS Axis of the Renin-Angiotensin System: Focus on Angiotensin-(1-7)." Physiol Rev **98**(1): 505-553.

The renin-angiotensin system (RAS) is a key player in the control of the cardiovascular system and hydroelectrolyte balance, with an influence on organs and functions throughout the body. The classical view of this system saw it as a sequence of many enzymatic steps that culminate in the production of a single biologically active metabolite, the octapeptide angiotensin (ANG) II, by the angiotensin converting enzyme (ACE). The past two decades have revealed new functions for some of the intermediate products, beyond their roles as substrates along the classical route. They may be processed in alternative ways by enzymes such as the ACE homolog ACE2. One effect is to establish a second axis through ACE2/ANG-(1-7)/MAS, whose end point is the metabolite ANG-(1-7). ACE2 and other enzymes can form ANG-(1-7) directly or indirectly from either the decapeptide ANG I or from ANG II. In many cases, this second axis appears to counteract or modulate the effects of the classical axis. ANG-(1-7) itself acts on the receptor MAS to influence a range of mechanisms in the heart, kidney, brain, and other tissues. This review highlights the current knowledge about the roles of ANG-(1-7) in physiology and disease, with particular emphasis on the brain.

Saputo, S., et al. (2014). "Efg1 directly regulates ACE2 expression to mediate cross talk between the cAMP/PKA and RAM pathways during Candida albicans morphogenesis." Eukaryot Cell **13**(9): 1169-1180.

The cyclic AMP/protein kinase A (cAMP/PKA) and regulation of Ace2 and morphogenesis (RAM) pathways are important regulators of the yeast-to-hypha transition in Candida albicans that interact genetically during this process. To further understand this interaction, we have characterized the expression of ACE2 during morphogenesis. In normoxic, planktonic conditions, ACE2 expression is very low in stationary-phase cells at both the mRNA and protein levels. Upon shifting to Spider medium, ACE2/Ace2p levels increase. Although Ace2 is not absolutely required for hypha formation, ace2Delta/Delta mutants show delayed hypha formation in Spider medium (but not others) and morphological changes to the hyphal tip and lateral yeast. We also show that Efg1 directly binds the promoter of Ace2 in stationary phase, and ACE2 levels are increased in strains lacking Efg1 and the protein kinase A proteins Tpk1 and Tpk2, indicating that the PKA pathway directly regulates ACE2 expression. ACE2 expression is positively regulated by Tec1 and Brg1, which bind the promoters of ACE2 in hyphal cells but not in the yeast phase. Under embedded conditions, Efg1 is dispensable for filamentation and Ace2 is required. We have found that ACE2 expression is much higher in embedded cells than in planktonic cells, providing a potential rationale for this observation. Taken together, our observations indicate that the PKA pathway directly regulates the RAM pathway under specific conditions and are consistent with a model where the two pathways carry out similar functions that depend on the specific environmental context.

Sato, T., et al. (2013). "Apelin is a positive regulator of ACE2 in failing hearts." J Clin Invest **123**(12): 5203-5211.

Angiotensin converting enzyme 2 (ACE2) is a negative regulator of the renin-angiotensin system (RAS), catalyzing the conversion of Angiotensin II to Angiotensin 1-7. Apelin is a second catalytic substrate for ACE2 and functions as an inotropic and cardioprotective peptide. While an antagonistic relationship between the RAS and apelin has been proposed, such functional interplay remains elusive. Here we found that ACE2 was downregulated in apelin-deficient mice. Pharmacological or genetic inhibition of angiotensin II type 1 receptor (AT1R) rescued the impaired contractility and hypertrophy of apelin mutant mice, which was accompanied by restored ACE2 levels. Importantly, treatment with angiotensin 1-7 rescued hypertrophy and heart dysfunctions of apelin-knockout mice. Moreover, apelin, via activation of its receptor, APJ, increased ACE2 promoter activity in vitro and upregulated ACE2 expression in failing hearts in vivo. Apelin treatment also increased cardiac contractility and

ACE2 levels in AT1R-deficient mice. These data demonstrate that ACE2 couples the RAS to the apelin system, adding a conceptual framework for the apelin-ACE2-angiotensin 1-7 axis as a therapeutic target for cardiovascular diseases.

Shenoy, V., et al. (2011). "ACE2, a promising therapeutic target for pulmonary hypertension." Curr Opin Pharmacol **11**(2): 150-155.

Pulmonary arterial hypertension (PAH) is a chronic lung disease with poor diagnosis and limited therapeutic options. The currently available therapies are ineffective in improving the quality of life and reducing mortality rates. There exists a clear unmet medical need to treat this disease, which necessitates the discovery of novel therapeutic targets/agents for safe and successful therapy. An altered renin-angiotensin system (RAS) has been implicated as a causative factor in the pathogenesis of PAH. Angiotensin II (Ang II), a key effector peptide of the RAS, can exert deleterious effects on the pulmonary vasculature resulting in vasoconstriction, proliferation, and inflammation, all of which contribute to PAH development. Recently, a new member of the RAS, angiotensin converting enzyme 2 (ACE2), was discovered. This enzyme functions as a negative regulator of the angiotensin system by metabolizing Ang II to a putative protective peptide, angiotensin-(1-7). ACE2 is abundantly expressed in the lung tissue and emerging evidence suggests a beneficial role for this enzyme against lung diseases. In this review, we focus on ACE2 in relation to pulmonary hypertension and provide proof of principle for its therapeutic role in PAH.

Shil, P. K., et al. (2014). "Oral delivery of ACE2/Ang-(1-7) bioencapsulated in plant cells protects against experimental uveitis and autoimmune uveoretinitis." Mol Ther **22**(12): 2069-2082.

Hyperactivity of the renin-angiotensin system (RAS) resulting in elevated Angiotensin II (Ang II) contributes to all stages of inflammatory responses including ocular inflammation. The discovery of angiotensin-converting enzyme 2 (ACE2) has established a protective axis of RAS involving ACE2/Ang-(1-7)/Mas that counteracts the proinflammatory and hypertrophic effects of the deleterious ACE/AngII/AT1R axis. Here we investigated the hypothesis that enhancing the systemic and local activity of the protective axis of the RAS by oral delivery of ACE2 and Ang-(1-7) bioencapsulated in plant cells would confer protection against ocular inflammation. Both ACE2 and Ang-(1-7), fused with the non-toxic cholera toxin subunit B (CTB) were expressed in plant chloroplasts. Increased levels of ACE2 and Ang-(1-7) were observed in circulation and retina after oral administration of CTB-ACE2 and Ang-(1-7) expressing plant cells. Oral feeding of mice with bioencapsulated ACE2/Ang-(1-7) significantly reduced endotoxin-induced uveitis (EIU) in mice. Treatment with bioencapsulated ACE2/Ang-(1-7) also dramatically decreased cellular infiltration, retinal vasculitis, damage and folding in experimental autoimmune uveoretinitis (EAU). Thus, enhancing the protective axis of RAS by oral delivery of ACE2/Ang-(1-7) bioencapsulated in plant cells provide an innovative, highly efficient and cost-effective therapeutic strategy for ocular inflammatory diseases.

Shiota, A., et al. (2010). "Loss of ACE2 accelerates time-dependent glomerular and tubulointerstitial damage in streptozotocin-induced diabetic mice." Hypertens Res **33**(4): 298-307.

As angiotensin-converting enzyme-2 (ACE2) was identified as a negative regulator of the renin-angiotensin system, there have been many reports concerning its role in several tissues, including the kidney. However, the role of ACE2 during the development of diabetic nephropathy remains undetermined, as previous reports did not necessarily support a protective role against renal injury. Thus, we performed detailed observations of kidneys in ACE2-knockout (ACE2-KO) mice at early (4 weeks) and advanced (18 weeks) stages of diabetes. ACE2-KO and wild-type C57BL/6 mice were rendered diabetic by intraperitoneal injection of streptozotocin. Diabetic ACE2-KO mice showed earlier onset and more severe progression of albuminuria than those did wild-type mice. The elevation of serum creatinine and urea nitrogen levels at 18 weeks of diabetes was more prominent in ACE2-KO mice. Periodic acid-Schiff-stained cross-section of diabetic ACE2-KO mice showed a more severe time-dependent increase in glomerular/tubulointerstitial damage than did that of wild-type mice, confirmed by the immunostaining of alpha-smooth muscle actin, collagen IV and F4-80 antigen. Glomeruli of diabetic ACE2-KO mice showed earlier and more severe decrease in the expression of nephrin, whose degradation is involved in the onset of albuminuria, and more potent increase of vascular endothelial growth factor expression. In addition, treatment with AT1 receptor blocker olmesartan significantly, but not totally, ameliorated the functional and morphological deterioration of diabetic nephropathy in ACE2-KO mice. These results suggest that ACE2 might continuously protect from both glomerular and tubulointerstitial injury during the development of diabetic nephropathy. The renal-protective effect of ACE2 might involve more than just suppressing angiotensin II-mediated AT1 receptor signaling.

Shoemaker, R., et al. (2019). "Adipocyte deficiency of ACE2 increases systolic blood pressures of obese female C57BL/6 mice." Biol Sex Differ **10**(1): 45.

BACKGROUND: Obesity increases the risk for hypertension in both sexes, but the prevalence of hypertension is lower in females than in males until menopause, despite a higher prevalence of obesity in females. We previously demonstrated that angiotensin-converting enzyme 2 (ACE2), which cleaves the vasoconstrictor, angiotensin II (AngII), to generate the vasodilator, angiotensin-(1-7) (Ang-(1-7)), contributes to sex differences in obesity-hypertension. ACE2 expression in adipose tissue was influenced by obesity in a sex-specific manner, with elevated ACE2 expression in obese female mice. Moreover, estrogen stimulated adipose ACE2 expression and reduced obesity-hypertension in females. In this study, we hypothesized that deficiency of adipocyte ACE2 contributes to obesity-hypertension of females. METHODS: We generated a mouse model of adipocyte ACE2 deficiency. Male and female mice with adipocyte ACE2 deficiency or littermate controls were fed a low (LF) or a high fat (HF) diet for 16 weeks and blood pressure was quantified by radiotelemetry. HF-fed mice of each sex and genotype were challenged by an acute AngII injection, and blood pressure response was quantified. To translate these findings to humans, we performed a proof-of-principle study in obese transwomen in which systemic angiotensin peptides and blood pressure were quantified prior to and after 12 weeks of gender-affirming 17beta-estradiol hormone therapy. RESULTS: Adipocyte ACE2 deficiency had no effect on the development of obesity in either sex. HF feeding increased systolic blood pressures (SBP) of wild-type male and female mice compared to LF-fed controls. Adipocyte ACE2 deficiency augmented obesity-induced elevations in SBP in females, but not in males. Obese female, but not obese male mice with adipocyte ACE2 deficiency, had an augmented SBP response to acute AngII challenge. In humans, plasma 17beta-estradiol concentrations increased in obese transwomen administered 17beta-estradiol and correlated positively with plasma Ang-(1-7)/AngII balance, and negatively to SBP after 12 weeks of 17beta-estradiol administration. CONCLUSIONS: Adipocyte ACE2 protects female mice from obesity-hypertension, and reduces the blood pressure response to systemic AngII. In obese transwomen undergoing gender-affirming hormone therapy, 17beta-estradiol administration may regulate blood pressure via the Ang-(1-7)/AngII balance.

Shoemaker, R., et al. (2015). "ACE2 deficiency reduces beta-cell mass and impairs beta-cell proliferation in obese C57BL/6 mice." Am J Physiol Endocrinol Metab **309**(7): E621-631.

Drugs that inhibit the renin-angiotensin system (RAS) decrease the onset of type 2 diabetes (T2D). Pancreatic islets express RAS components, including angiotensin-converting enzyme 2 (ACE2), which cleaves angiotensin II (Ang II) to angiotensin-(1-7) [Ang-(1-7)]. Overexpression of ACE2 in pancreas of diabetic mice improved glucose homeostasis. The purpose of this study was to determine if deficiency of endogenous ACE2 contributes to islet dysfunction and T2D. We hypothesized that ACE2 deficiency potentiates the decline in beta-cell function and augments the development of diet-induced T2D. Male Ace2(+/y) or Ace2(-/y) mice were fed a low-fat (LF) or high-fat (HF) diet for 1 or 4 mo. A subset of 1-mo HF-fed mice were infused with Sal (Sal), losartan (Los), or Ang-(1-7). At 4 mo, while both genotypes of HF-fed mice developed a similar level of insulin resistance, adaptive hyperinsulinemia was reduced in Ace2(-/y) vs. Ace2(+/y) mice. Similarly, in vivo glucose-stimulated insulin secretion (GSIS) was reduced in 1-mo HF-fed Ace2(-/y) compared with Ace2(+/y) mice, resulting in augmented hyperglycemia. The average islet area was significantly smaller in both LF- and HF-fed Ace2(-/y) vs. Ace2(+/y) mice. Additionally, beta-cell mass and proliferation were reduced significantly in HF-fed Ace2(-/y) vs. Ace2(+/y) mice. Neither infusion of Los nor Ang-(1-7) was able to correct impaired in vivo GSIS of HF-fed ACE2-deficient mice. These results demonstrate a critical role for endogenous ACE2 in the adaptive beta-cell hyperinsulinemic response to HF feeding through regulation of beta-cell proliferation and growth.

Simoes e Silva, A. C., et al. (2013). "ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis." Br J Pharmacol **169**(3): 477-492.

Recent advances have improved our understanding of the renin-angiotensin system (RAS). These have included the recognition that angiotensin (Ang)-(1-7) is a biologically active product of the RAS cascade. The identification of the ACE homologue ACE2, which forms Ang-(1-7) from Ang II, and the GPCR Mas as an Ang-(1-7) receptor have provided the necessary biochemical and molecular background and tools to study the biological significance of Ang-(1-7). Most available evidence supports a counter-regulatory role for Ang-(1-7) by opposing many actions of Ang II on AT (1) receptors, especially vasoconstriction and proliferation. Many studies have now shown that Ang-(1-7) by acting via Mas receptor exerts inhibitory effects on inflammation and on vascular and cellular growth mechanisms. Ang-(1-7)

has also been shown to reduce key signalling pathways and molecules thought to be relevant for fibrogenesis. Here, we review recent findings related to the function of the ACE2/Ang-(1-7)/Mas axis and focus on the role of this axis in modifying processes associated with acute and chronic inflammation, including leukocyte influx, fibrogenesis and proliferation of certain cell types. More attention will be given to the involvement of the ACE2/Ang-(1-7)/Mas axis in the context of renal disease because of the known relevance of the RAS for the function of this organ and for the regulation of kidney inflammation and fibrosis. Taken together, this knowledge may help in paving the way for the development of novel treatments for chronic inflammatory and renal diseases.

Simoes, E. S. A. C. and M. M. Teixeira (2016). "ACE inhibition, ACE2 and angiotensin-(1-7) axis in kidney and cardiac inflammation and fibrosis." Pharmacol Res **107**: 154-162.

The Renin Angiotensin System (RAS) is a pivotal physiological regulator of heart and kidney homeostasis, but also plays an important role in the pathophysiology of heart and kidney diseases. Recently, new components of the RAS have been discovered, including angiotensin converting enzyme 2 (ACE2), Angiotensin (Ang)-(1-7), Mas receptor, Ang-(1-9) and Alamandine. These new components of RAS are formed by the hydrolysis of Ang I and Ang II and, in general, counteract the effects of Ang II. In experimental models of heart and renal diseases, Ang-(1-7), Ang-(1-9) and Alamandine produced vasodilation, inhibition of cell growth, anti-thrombotic, anti-inflammatory and anti-fibrotic effects. Recent pharmacological strategies have been proposed to potentiate the effects or to enhance the formation of Ang-(1-7) and Ang-(1-9), including ACE2 activators, Ang-(1-7) in hydroxypropyl beta-cyclodextrin, cyclized form of Ang-(1-7) and nonpeptide synthetic Mas receptor agonists. Here, we review the role and effects of ACE2, ACE2 activators, Ang-(1-7) and synthetic Mas receptor agonists in the control of inflammation and fibrosis in cardiovascular and renal diseases and as counter-regulators of the ACE-Ang II-AT1 axis. We briefly comment on the therapeutic potential of the novel members of RAS, Ang-(1-9) and alamandine, and the interactions between classical RAS inhibitors and new players in heart and kidney diseases.

Singer, D. and S. M. Camargo (2011). "Collectrin and ACE2 in renal and intestinal amino acid transport." Channels (Austin) **5**(5): 410-423.

Neutral amino acid transporters of the SLC6 family are expressed at the apical membrane of kidney and/or small intestine, where they (re-)absorb amino acids into the body. In this review we present the results concerning the dependence of their apical expression with their association to partner proteins. We will in particular focus on the situation of B0AT1 and B0AT3, that associate with members of the renin-angiotensin system (RAS), namely Tmem27 and angiotensin-converting enzyme 2 (ACE2), in a tissue specific manner. The role of this association in relation to the formation of a functional unit related to Na+ or amino acid transport will be assessed. We will conclude with some remarks concerning the relevance of this association to Hartnup disorder, where some mutations have been shown to differentially interact with the partner proteins.

Singer, D., et al. (2012). "Defective intestinal amino acid absorption in Ace2 null mice." Am J Physiol Gastrointest Liver Physiol **303**(6): G686-695.

Mutations in the main intestinal and kidney luminal neutral amino acid transporter B (0)AT1 (Slc6a19) lead to Hartnup disorder, a condition that is characterized by neutral aminoaciduria and in some cases pellagra-like symptoms. These latter symptoms caused by low-niacin are thought to result from defective intestinal absorption of its precursor L-tryptophan. Since Ace2 is necessary for intestinal B (0)AT1 expression, we tested the impact of intestinal B (0)AT1 absence in ace2 null mice. Their weight gain following weaning was decreased, and Na (+)-dependent uptake of B (0)AT1 substrates measured in everted intestinal rings was defective. Additionally, high-affinity Na (+)-dependent transport of L-proline, presumably via SIT1 (Slc6a20), was absent, whereas glucose uptake via SGLT1 (Slc5a1) was not affected. Measurements of small intestine luminal amino acid content following gavage showed that more L-tryptophan than other B (0)AT1 substrates reach the ileum in wild-type mice, which is in line with its known lower apparent affinity. In ace2 null mice, the absorption defect was confirmed by a severalfold increase of L-tryptophan and of other neutral amino acids reaching the ileum lumen. Furthermore, plasma and muscle levels of glycine and L-tryptophan were significantly decreased in ace2 null mice, with other neutral amino acids displaying a similar trend. A low-protein/low-niacin diet challenge led to differential changes in plasma amino acid levels in both wild-type and ace2 null mice, but only in ace2 null mice to a stop in weight gain. Despite the combination of low-niacin with a low-protein diet, plasma niacin concentrations remained normal in ace2 null mice and no pellagra symptoms, such as photosensitive skin rash or ataxia, were observed. In summary, mice lacking Ace2-dependent intestinal amino acid transport display no total niacin deficiency nor clear pellagra symptoms,

even under a low-protein and low-niacin diet, despite gross amino acid homeostasis alterations.

Singh, N., et al. (2015). "ACE2/Ang-(1-7)/Mas axis stimulates vascular repair-relevant functions of CD34+ cells." Am J Physiol Heart Circ Physiol **309**(10): H1697-1707.

CD34(+) stem/progenitor cells have been identified as a promising cell population for the autologous cell-based therapies in patients with cardiovascular disease. The counter-regulatory axes of renin angiotensin system, angiotensin converting enzyme (ACE)/Ang II/angiotensin type 1 (AT1) receptor and ACE2/Ang-(1-7)/Mas receptor, play an important role in the cardiovascular repair. This study evaluated the expression and vascular repair-relevant functions of these two pathways in human CD34(+) cells. CD34(+) cells were isolated from peripheral blood mononuclear cells (MNCs), obtained from healthy volunteers. Expression of ACE, ACE2, AT1, and angiotensin type 2 and Mas receptors were determined. Effects of Ang II, Ang-(1-7), Norleu (3)-Ang-(1-7), and ACE2 activators, xanthenone (XNT) and diminazene aceturate (DIZE) on proliferation, migration, and adhesion of CD34(+) cells were evaluated. ACE2 and Mas were relatively highly expressed in CD34(+) cells compared with MNCs. Ang-(1-7) or its analog, Norleu (3)-Ang-(1-7), stimulated proliferation of CD34(+) cells that was associated with decrease in phosphatase and tensin homologue deleted on chromosome 10 levels and was inhibited by triciribin, an AKT inhibitor. Migration of CD34(+) cells was enhanced by Ang-(1-7) or Norleu (3)-Ang-(1-7) that was decreased by a Rho-kinase inhibitor, Y-27632. In the presence of Ang II, XNT or DIZE enhanced proliferation and migration that were blocked by DX-600, an ACE2 inhibitor. Treatment of MNCs with Ang II, before the isolation of CD34(+) cells, attenuated the proliferation and migration to stromal derived factor-1alpha. This attenuation was reversed by apocynin, an NADPH oxidase inhibitor. Adhesion of MNCs or CD34(+) cells to fibronectin was enhanced by Ang II and was unaffected by Ang-(1-7). This study suggests that ACE2/Ang-(1-7)/Mas pathway stimulates functions of CD34(+) cells that are cardiovascular protective, whereas Ang II attenuates these functions by acting on MNCs. These findings imply that activation of ACE2/Ang-(1-7)/Mas axis is a promising approach for enhancing reparative outcomes of cell-based therapies.

Singh, Y., et al. (2018). "Embarking Effect of ACE2-Angiotensin 1-7/Mas Receptor Axis in Benign Prostate Hyperplasia." Crit Rev Eukaryot Gene Expr **28**(2): 115-124.

The proliferative cell process that causes prostate enlargement, obstruction of the bladder outlet, and lower urinary tract symptoms (LUTS) is known as benign prostatic hyperplasia (BPH). The prevalence of BPH worldwide is approximately 10%, 20%, 50%, and 80% for men in their 30s, 40s, 60s, and 70s, respectively. Recent data have revealed that overactivation of the renin angiotensin aldosterone system increases the level of bioactive peptide hormone angiotensin II, which downregulates the ACE2-angiotensin 1-7/Mas receptor axis path and upregulates angiotensin receptor type 1-mediated signaling, which finally leads to a proliferation of cellular elements in the prostate. We have hypothesized the mechanism that reverses the downregulation of the ACE2-angiotensin 1-7/Mas receptor axis path and the upregulation of angiotensin receptor type 1-mediated signaling. Thus, we posit that ACE2, Ang-(1-7), and the Mas receptor could be novel therapeutic targets for treating BPH/LUTS.

Sluimer, J. C., et al. (2008). "Angiotensin-converting enzyme 2 (ACE2) expression and activity in human carotid atherosclerotic lesions." J Pathol **215**(3): 273-279.

Angiotensin-converting enzyme (ACE)2 is a recently identified homologue of ACE. As ACE2 inactivates the pro-atherogenic angiotensin II, we hypothesize that ACE2 may play a protective role in atherogenesis. The spatiotemporal localization of ACE2 mRNA and protein in human vasculature and a possible association with atherogenesis were investigated using molecular histology (in situ hybridization, immunohistochemistry). Also, the ACE: ACE2 balance was investigated using enzymatic assays. ACE2 mRNA was expressed in early and advanced human carotid atherosclerotic lesions. In addition, ACE2 protein was present in human veins, non-diseased mammary arteries and atherosclerotic carotid arteries and expressed in endothelial cells, smooth muscle cells and macrophages. Quantitative analysis of immunoreactivity showed that total vessel wall expression of ACE and ACE2 was similar during all stages of atherosclerosis. The observed ACE2 protein was enzymatically active and activity was lower in the stable advanced atherosclerotic lesions, compared to early and ruptured atherosclerotic lesions. These results suggest a differential regulation of ACE2 activity during the progression of atherosclerosis and suggest that this novel molecule of the renin-angiotensin system may play a role in the pathogenesis of atherosclerosis.

Smith, R. G. and P. A. Berry (2011). "Evaluation of the differences between the SRTM and satellite radar altimetry height measurements and the approach

taken for the ACE2 GDEM in areas of large disagreement." J Environ Monit **13**(6): 1646-1652.

The new ACE2 (Altimeter Corrected Elevations 2) Global Digital Elevation Model (GDEM) which has recently been released aims to provide the most accurate GDEM to date. ACE2 was created by synergistically merging the SRTM and altimetry datasets. The comprehensive comparison carried out between the two datasets yielded a myriad of information, with the areas of disagreement providing as much valuable information as the areas of agreement. Analysis of the comparison dataset revealed that certain topographic features displayed consistent differences between the two datasets. The largest differences globally are present over the rainforests, particularly the two largest, around the Amazon and the Congo. The differences range between 10 m and 40 m; these differences can be attributed to the height of the rainforest canopy, as the SRTM returned height values from somewhere within the uppermost reaches of the vegetation whereas the altimeter was able to penetrate through and return true ground heights. The second major class of terrain feature to demonstrate coherent differences are desert regions; here, different deserts present different characteristics. The final area of interest is that of Wetlands; these are areas of special significance because even a slight misrepresentation of the heights can have wide ranging effects in modelling wetland areas. These examples illustrate the valuable additional information content gleaned from the synergistic global combination of the two datasets.

Sodhi, C. P., et al. (2019). "A Dynamic Variation of Pulmonary ACE2 Is Required to Modulate Neutrophilic Inflammation in Response to Pseudomonas aeruginosa Lung Infection in Mice." J Immunol **203**(11): 3000-3012.

Angiotensin-converting enzyme 2 (ACE2) is a potent negative regulator capable of restraining overactivation of the renin-angiotensin system, which contributes to exuberant inflammation after bacterial infection. However, the mechanism through which ACE2 modulates this inflammatory response is not well understood. Accumulating evidence indicates that infectious insults perturb ACE2 activity, allowing for uncontrolled inflammation. In the current study, we demonstrate that pulmonary ACE2 levels are dynamically varied during bacterial lung infection, and the fluctuation is critical in determining the severity of bacterial pneumonia. Specifically, we found that a pre-existing and persistent deficiency of active ACE2 led to excessive neutrophil accumulation in mouse lungs subjected to bacterial infection, resulting in a hyperinflammatory response and lung damage. In contrast, pre-existing and persistent increased ACE2 activity reduces neutrophil infiltration and compromises host defense, leading to overwhelming bacterial infection. Further, we found that the interruption of pulmonary ACE2 restitution in the model of bacterial lung infection delays the recovery process from neutrophilic lung inflammation. We observed the beneficial effects of recombinant ACE2 when administered to bacterially infected mouse lungs following an initial inflammatory response. In seeking to elucidate the mechanisms involved, we discovered that ACE2 inhibits neutrophil infiltration and lung inflammation by limiting IL-17 signaling by reducing the activity of the STAT3 pathway. The results suggest that the alteration of active ACE2 is not only a consequence of bacterial lung infection but also a critical component of host defense through modulation of the innate immune response to bacterial lung infection by regulating neutrophil influx.

Sodhi, C. P., et al. (2018). "Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg (9) bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration." Am J Physiol Lung Cell Mol Physiol **314**(1): L17-L31.

Angiotensin-converting enzyme 2 (ACE2) is a terminal carboxypeptidase with important functions in the renin-angiotensin system and plays a critical role in inflammatory lung diseases. ACE2 cleaves single-terminal residues from several bioactive peptides such as angiotensin II. However, few of its substrates in the respiratory tract have been identified, and the mechanism underlying the role of ACE2 in inflammatory lung disease has not been fully characterized. In an effort to identify biological targets of ACE2 in the lung, we tested its effects on des-Arg (9) bradykinin (DABK) in airway epithelial cells on the basis of the hypothesis that DABK is a biological substrate of ACE2 in the lung and ACE2 plays an important role in the pathogenesis of acute lung inflammation partly through modulating DABK/bradykinin receptor B1 (BKB1R) axis signaling. We found that loss of ACE2 function in mouse lung in the setting of endotoxin inhalation led to activation of the DABK/BKB1R axis, release of proinflammatory chemokines such as C-X-C motif chemokine 5 (CXCL5), macrophage inflammatory protein-2 (MIP2), C-X-C motif chemokine 1 (KC), and TNF-alpha from airway epithelia, increased neutrophil infiltration, and exaggerated lung inflammation and injury. These results indicate that a reduction in pulmonary ACE2 activity contributes to the pathogenesis of lung inflammation, in part because of an impaired ability to inhibit DABK/BKB1R axis-mediated signaling, resulting in more prompt onset of neutrophil infiltration and more severe inflammation in the lung. Our study identifies a biological substrate

of ACE2 within the airways, as well as a potential new therapeutic target for inflammatory diseases.

Soler, M. J., et al. (2008). "Pharmacologic modulation of ACE2 expression." Curr Hypertens Rep **10**(5): 410-414.

Angiotensin-converting enzyme 2 (ACE2) is an enzymatically active homologue of angiotensin-converting enzyme that degrades angiotensin I, angiotensin II, and other peptides. Recent studies have shown that under pathologic conditions, ACE2 expression in the kidney is altered. In this review, we briefly summarize recent studies dealing with pharmacologic interventions that modulate ACE2 expression. ACE2 amplification may have a potential therapeutic role for kidney disease and hypertension.

Soler, M. J., et al. (2019). "ACE2 and ACE in acute and chronic rejection after human heart transplantation." Int J Cardiol **275**: 59-64.

OBJECTIVES: The authors sought to evaluate cardiac activity of angiotensin-converting enzyme (ACE) and ACE2 after heart transplantation (HT) and its relation with acute rejection (AR) and chronic allograft vasculopathy (CAV). BACKGROUND: The renin-angiotensin system is altered in heart failure and HT. However, ACE and ACE2 activities in post-HT acute and chronic rejection have not been previously studied. METHODS: HT patients (n=45) were included when appropriate serial endomyocardial biopsies (EMB) and coronary angiography were available for analysis. In 21 patients, three post-HT time points were selected for CAV study in EMB tissue: basal (0-3wks), second (2-3months) and third (4-5months). At 10years post-HT, CAV was evaluated by coronary angiography (CA) and patients were grouped by degree of CAV: 0-1, non-CAV (n=15) and 2-3, CAV (n=6). For the AR study, 28 HT patients with evidence of one EMB rejection at grade 3 and two EMB grade 1A and/or 1B rejections were selected. RESULTS: Post-HT, ACE2 activity was increased in the CAV group, compared to non-CAV. Patients with AR showed increased ACE, but not ACE2, activity. CONCLUSIONS: Our results suggest that early post-HT cardiac ACE2 activity may have an important role in CAV development. In contrast, ACE activity was increased in AR. The renin-angiotensin system seems to be altered after HT and strategies to balance the system may be useful.

Soler, M. J., et al. (2013). "ACE2 alterations in kidney disease." Nephrol Dial Transplant **28**(11): 2687-2697.

Angiotensin-converting enzyme 2 (ACE2) is a monocarboxypeptidase that degrades angiotensin (Ang) II to Ang-(1-7). ACE2 is highly expressed within the kidneys, it is largely localized in tubular epithelial cells and less prominently in glomerular epithelial cells and in the renal vasculature. ACE2 activity has been shown to be altered in diabetic kidney disease, hypertensive renal disease and in different models of kidney injury. There is often a dissociation between tubular and glomerular ACE2 expression, particularly in diabetic kidney disease where ACE2 expression is increased at the tubular level but decreased at the glomerular level. In this review, we will discuss alterations in circulating and renal ACE2 recently described in different renal pathologies and disease models as well as their possible significance.

Soler, M. J., et al. (2007). "ACE2 inhibition worsens glomerular injury in association with increased ACE expression in streptozotocin-induced diabetic mice." Kidney Int **72**(5): 614-623.

Angiotensin converting enzyme 2 (ACE2) is localized to the glomerular epithelial cells. Since ACE2 promotes the degradation of angiotensin II, a decrease in ACE2 activity could lead to the development of glomerular injury. We gave a specific ACE2 inhibitor, MLN-4760, for 4 weeks to mice rendered diabetic with streptozotocin. The urinary albumin/creatinine ratio was increased along with expansion of the glomerular matrix in diabetic mice treated with the inhibitor compared to the vehicle-treated mice. Glomerular staining of ACE was increased in the diabetic group and was further significantly increased in the diabetic group treated with MLN-4760. In renal vessels, ACE expression was also increased in the diabetic mice and, again, further increased in those diabetic mice treated with the ACE2 inhibitor. Our study shows that chronic pharmacologic ACE2 inhibition worsens glomerular injury in streptozotocin-induced diabetic mice in association with increased ACE expression.

Soler, M. J., et al. (2009). "Localization of ACE2 in the renal vasculature: amplification by angiotensin II type 1 receptor blockade using telmisartan." Am J Physiol Renal Physiol **296**(2): F398-405.

Angiotensin-converting enzyme (ACE)2 is a carboxypeptidase that degrades angiotensin II and other peptides. In the kidney, ACE2 localization within the glomerulus and tubules is cell specific. This study was aimed to investigate the localization of ACE2 within the renal vasculature. We also studied the effect of the administration of a specific angiotensin II type 1 receptor blocker, telmisartan, on ACE2 expression in the renal vasculature. ACE2 and ACE were localized in renal arterioles using confocal microscopy and specific cell markers. Quantitative measurements of ACE2 and ACE mRNA were estimated in kidney arterioles isolated by laser capture

microdissection using real-time PCR. In kidney arterioles, ACE was localized in the endothelial layer, whereas ACE2 was localized in the tunica media. In mice treated with telmisartan (2 mg.kg (-1).day (-1)) for 2 wk, ACE2 expression was increased by immunostaining, whereas ACE expression was decreased. This was reflected in a decrease in the ACE/ACE2 ratio compared with vehicle-treated controls (0.53 +/- 0.14 vs. 7.59 +/- 2.72, P = 0.027, respectively). In kidney arterioles isolated by laser capture microdissection, the ACE/ACE2 mRNA ratio was also decreased compared with control mice (1.21 +/- 0.31 vs. 4.63 +/- 0.86, P = 0.044, respectively). In conclusion, in kidney arterioles ACE2 is preferentially localized in the tunica media, and its expression is increased after administration of the angiotensin II type 1 receptor blocker, telmisartan. Amplification of ACE2 in the renal vasculature may contribute to the therapeutic action of telmisartan by increasing angiotensin II degradation.

Song, W., et al. (2018). "Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2." PLoS Pathog **14**(8): e1007236.

The trimeric SARS coronavirus (SARS-CoV) surface spike (S) glycoprotein consisting of three S1-S2 heterodimers binds the cellular receptor angiotensin-converting enzyme 2 (ACE2) and mediates fusion of the viral and cellular membranes through a pre- to postfusion conformation transition. Here, we report the structure of the SARS-CoV S glycoprotein in complex with its host cell receptor ACE2 revealed by cryo-electron microscopy (cryo-EM). The complex structure shows that only one receptor-binding domain of the trimeric S glycoprotein binds ACE2 and adopts a protruding "up" conformation. In addition, we studied the structures of the SARS-CoV S glycoprotein and its complexes with ACE2 in different in vitro conditions, which may mimic different conformational states of the S glycoprotein during virus entry. Disassociation of the S1-ACE2 complex from some of the prefusion spikes was observed and characterized. We also characterized the rosette-like structures of the clustered SARS-CoV S2 trimers in the postfusion state observed on electron micrographs. Structural comparisons suggested that the SARS-CoV S glycoprotein retains a prefusion architecture after trypsin cleavage into the S1 and S2 subunits and acidic pH treatment. However, binding to the receptor opens up the receptor-binding domain of S1, which could promote the release of the S1-ACE2 complex and S1 monomers from the prefusion spike and trigger the pre- to postfusion conformational transition.

Soro-Paavonen, A., et al. (2012). "Circulating ACE2 activity is increased in patients with type 1 diabetes and vascular complications." J Hypertens **30**(2): 375-383.

OBJECTIVE: Angiotensin-converting enzyme 2 (ACE2) is a homolog of ACE that counterbalances the actions of angiotensin (AT)II and promotes vasodilatation. Circulating ACE2 activity is increased in diabetes in experimental models. The role of ACE2 in human pathophysiology is unknown. We examined whether ACE2 activity is altered in patients with type 1 diabetes (T1D), with and without diabetic nephropathy. METHODS: Quantitative ACE2 activity in serum was measured by a fluorometric assay in 859 patients with T1D in the Finnish Diabetic Nephropathy (FinnDiane) study and in 204 healthy controls. Pulse-wave analysis with augmentation index (AIx) measurement was performed in 319 patients with T1D and 114 controls. RESULTS: ACE2 activity was increased in men with T1D and microalbuminuria (30.2 +/- 1.5 ngE/ml) when compared to patients without albuminuria (27.0 +/- 0.5 ngE/ml, P < 0.05) or controls (25.6 +/- 0.8 ngE/ml, P < 0.05). ACE2 activity was increased in male and female patients who were on ACE inhibitor (ACEi) treatment, also independently of albuminuria. Male and female patients with coronary heart disease (CHD) had significantly increased ACE2 activity (35.5 +/- 2.5 vs. 27.0 +/- 0.5 ngE/ml, P < 0.001 among male T1D patients vs. male controls). ACE2 activity correlated positively with systolic blood pressure (rs = 0.175, P < 0.001), AIx (rs = 0.191, P = 0.010) and diabetes duration (rs = 0.198, P < 0.001), and negatively with estimated glomerular filtration rate (rs = -0.109, P = 0.016) among male T1D patients. CONCLUSIONS: ACE2 activity increases with increasing vascular tone and when the patient with T1D has microvascular or macrovascular disease, indicating that ACE2 may participate as a compensatory mechanism in the regulation of vascular and renal function in patients with T1D.

Sriramula, S., et al. (2011). "ACE2 overexpression in the paraventricular nucleus attenuates angiotensin II-induced hypertension." Cardiovasc Res **92**(3): 401-408.

AIMS: Angiotensin II (Ang II) has been shown to have both central and peripheral effects in mediating hypertension, for which the hypothalamic paraventricular nucleus (PVN) is an important brain cardio-regulatory centre. Angiotensin-converting enzyme 2 (ACE2) has been identified as a negative regulator of the pro-hypertensive actions of Ang II. Recent findings from our laboratory suggest that Ang II infusion decreases ACE2 expression in the PVN. In the present study, we hypothesized that ACE2

overexpression in the PVN will have beneficial effects in counteracting Ang II-induced hypertension. METHODS AND RESULTS: Male Sprague-Dawley rats were used in this study. Bilateral microinjection of an adenovirus encoding hACE2 (Ad-ACE2) into the PVN was used to overexpress ACE2 within this region. Mean arterial pressure measured by radiotelemetry was significantly increased after 14 days in Ang II-infused (200 ng/kg/min) rats vs. saline-infused controls (162.9 +/- 3.6 vs. 102.3 +/- 1.5 mmHg). Bilateral PVN microinjection of Ad-ACE2 attenuated this Ang II-induced hypertension (130.2 +/- 5.7 vs. 162.9 +/- 3.6 mmHg). ACE2 overexpression also significantly decreased AT (1)R and ACE expression and increased AT (2)R and Mas expression in the PVN. Additionally, ACE2 overexpression in the PVN attenuated the Ang II-induced increase in the expression of the pro-inflammatory cytokines tumour necrosis factor-alpha, interleukin (IL)-1beta and IL-6 in the PVN. CONCLUSION: Our findings suggest that attenuation of pro-inflammatory cytokines in the PVN in combination with the shift of the renin-angiotensin system towards the anti-hypertensive axis (ACE2/Ang-(1-7)/Mas) may be responsible for the overall beneficial effects of ACE2 overexpression in the PVN on the Ang II-induced hypertensive response.

Sriramula, S., et al. (2017). "Determining the Enzymatic Activity of Angiotensin-Converting Enzyme 2 (ACE2) in Brain Tissue and Cerebrospinal Fluid Using a Quenched Fluorescent Substrate." Methods Mol Biol **1527**: 117-126.

Angiotensin-converting enzyme 2 (ACE2) is a component of the renin-angiotensin system (RAS) which plays an important role in the regulation of blood pressure and volume homeostasis. Accumulating evidence shows alterations in ACE2 expression and activity in several hypertensive animal models, as well as in patients with hypertension. In order to assess the role of brain ACE2 in hypertension, a specific ACE2 assay is required. Based on a quenched fluorescent substrate, we describe an easy-to-use method for determining ACE2 activity in brain tissue and cerebrospinal fluid. The method can further be adapted for other tissues, plasma, cell extracts, and cell culture supernatants.

Stead, D., et al. (2005). "Proteomic changes associated with inactivation of the Candida glabrata ACE2 virulence-moderating gene." Proteomics **5**(7): 1838-1848.

Inactivation of the gene encoding the transcriptional activator Ace2 in the fungal pathogen Candida glabrata results in an almost 200-fold increase in virulence characterised by acute mortality and a massive over-stimulation of the pro-inflammatory arm of the innate immune system. In this study we have adopted a proteomics approach to identify cellular functions regulated by C. glabrata Ace2 that might contribute to this increase in virulence. A two-dimensional polyacrylamide gel electrophoresis map of the C. glabrata proteome was constructed. We identified a total of 123 proteins, 61 of which displayed reproducible and statistically significant alterations in their levels following inactivation of ACE2. Of these, the levels of 32 proteins were elevated, and 29 were reduced in ace2 cells. These data show that Ace2 influences metabolism, protein synthesis, folding and targeting, and aspects of cell growth and polarisation. Some of these functions are likely to contribute to the effects of Ace2 upon the virulence of C. glabrata.

Stead, D. A., et al. (2010). "Impact of the transcriptional regulator, Ace2, on the Candida glabrata secretome." Proteomics **10**(2): 212-223.

Candida glabrata is a major fungal pathogen of humans, and the virulence of C. glabrata is increased by inactivation of the transcription factor, Ace2. Our previous examination of the effects of Ace2 inactivation upon the intracellular proteome suggested that the hypervirulence of C. glabrata ace2 mutants might be caused by differences in the secretome. Therefore in this study we have characterised the C. glabrata secretome and examined the effects of Ace2 inactivation upon this extracellular proteome. We have identified 31 distinct proteins in the secretome of wild-type C. glabrata cells by MS/MS of proteins that were precipitated from the growth medium and enriched by affinity chromatography on concanavalin A. Most of these proteins are predicted to be cell wall proteins, cell wall modifying enzymes and aspartyl proteinases. The endochitinase Cts1 and the endoglucanase Egt2 were not detected in the C. glabrata secretome following Ace2 inactivation. This can account for the cell separation defect of C. glabrata ace2 cells. Ace2 inactivation also resulted in the detection of new proteins in the C. glabrata secretome. The release of such proteins might contribute to the hypervirulence of ace2 cells.

Stoll, D., et al. (2019). "Both aldosterone and spironolactone can modulate the intracellular ACE/ANG II/AT1 and ACE2/ANG (1-7)/MAS receptor axes in human mesangial cells." Physiol Rep **7**(11): e14105.

The kidney is an important target of the renin-ANG-aldosterone system (RAAS). To date, several studies have demonstrated the existence of a local RAAS in various tissues, including the renal tissue. The mineralocorticoid aldosterone is known to play a critical role in the classical RAAS; however, its effect

on mesangial cells (MCs) remains to be elucidated. Based on this, our aim was to investigate whether aldosterone stimulation can modulate the intracellular RAAS of immortalized human MCs by evaluating ANG-converting enzyme (ACE)/ANG II/ANG II receptor type 1 (AT1) and ANG-converting enzyme 2 (ACE2)/ANG (1-7)/MAS receptor axes. To realise this, protein expression, enzyme activity, and immunofluorescence were performed under aldosterone stimulation and in the presence of the mineralocorticoid receptor (MR) antagonist spironolactone (SPI). We observed that high doses of aldosterone increase ACE activity. The effect of aldosterone on the catalytic activity of ACE was completely abolished with the pretreatment of SPI suggesting that the aldosterone-induced cell injuries through ANG II release were attenuated. Aldosterone treatment also decreased the expression of MAS receptor, but did not alter the expression or the catalytic activity of ACE 2 and ANG (1-7) levels. Spironolactone modulated the localization of ANG II and AT1 receptor and decreased ANG (1-7) and MAS receptor levels. Our data suggest that both aldosterone and the MR receptor antagonist can modulate both of these axes and that spironolactone can protect MCs from the damage induced by aldosterone.

Stricker, A. R., et al. (2008). "Role of Ace2 (Activator of Cellulases 2) within the xyn2 transcriptosome of Hypocrea jecorina." Fungal Genet Biol **45**(4): 436-445.

Ace2 (Activator of Cellulases 2)-encoding gene was deleted from and retransformed in the H. jecorinaQM9414 genome. Comparison of xylanase activity and xyn2 transcription of the corresponding strains after cultivation on inducing compounds (xylan, xylobiose) revealed a faster initial inducibility in the Deltaace2-strain, but final levels of xyn2 transcript and xylanase activity of the parental strain could not be reached. This suggests a role for Ace2 in the regulation of xyn2 induction mechanisms, moreover Ace2 is responsible for the basal level of xyn2 transcription. Furthermore, a palindrome in the xyn2 promoter consisting of a GGGTAA- and a CCAGCC-element was identified. Both Xyr1 and Ace2 are able to bind the complete motif, the latter also only to one part of it. Phosphorylation as well as dimerization are prerequisites for binding of Ace2 to the xyn2 promoter. Finally, the impact of Ace2 on xyr1 transcription could be demonstrated under inducing conditions.

Struck, A. W., et al. (2012). "A hexapeptide of the receptor-binding domain of SARS corona virus spike protein blocks viral entry into host cells via the human receptor ACE2." Antiviral Res **94**(3): 288-296.

In vitro infection of Vero E6 cells by SARS coronavirus (SARS-CoV) is blocked by hexapeptide Tyr-Lys-Tyr-Arg-Tyr-Leu. The peptide also inhibits proliferation of coronavirus NL63. On human cells both viruses utilize angiotensin-converting enzyme 2 (ACE2) as entry receptor. Blocking the viral entry is specific as alpha virus Sindbis shows no reduction in infectivity. Peptide (438)YKYRYL (443) is part of the receptor-binding domain (RBD) of the spike protein of SARS-CoV. Peptide libraries were screened by surface plasmon resonance (SPR) to identify RBD binding epitopes. (438)YKYRYL (443) carries the dominant binding epitope and binds to ACE2 with K (D)=46 muM. The binding mode was further characterized by saturation transfer difference (STD) NMR spectroscopy and molecular dynamic simulations. Based on this information the peptide can be used as lead structure to design potential entry inhibitors against SARS-CoV and related viruses.

Suarez, M. B., et al. (2015). "Regulation of Ace2-dependent genes requires components of the PBF complex in Schizosaccharomyces pombe." Cell Cycle **14**(19): 3124-3137.

The division cycle of unicellular yeasts is completed with the activation of a cell separation program that results in the dissolution of the septum assembled during cytokinesis between the 2 daughter cells, allowing them to become independent entities. Expression of the eng1(+) and agn1(+) genes, encoding the hydrolytic enzymes responsible for septum degradation, is activated at the end of each cell cycle by the transcription factor Ace2. Periodic ace2(+) expression is regulated by the transcriptional complex PBF (PCB Binding Factor), composed of the forkhead-like proteins Sep1 and Fkh2 and the MADS box-like protein Mbx1. In this report, we show that Ace2-dependent genes contain several combinations of motifs for Ace2 and PBF binding in their promoters. Thus, Ace2, Fkh2 and Sep1 were found to bind in vivo to the eng1(+) promoter. Ace2 binding was coincident with maximum level of eng1(+) expression, whereas Fkh2 binding was maximal when mRNA levels were low, supporting the notion that they play opposing roles. In addition, we found that the expression of eng1(+) and agn1(+) was differentially affected by mutations in PBF components. Interestingly, agn1(+) was a major target of Mbx1, since its ectopic expression resulted in the suppression of Mbx1 deletion phenotypes. Our results reveal a complex regulation system through which the transcription factors Ace2, Fkh2, Sep1 and Mbx1 in combination control the expression of the genes involved in separation at the end of the cell division cycle.

Takeda, M., et al. (2013). "Loss of ACE2 exaggerates high-calorie diet-induced insulin resistance by reduction of GLUT4 in mice." Diabetes **62**(1): 223-233.

ACE type 2 (ACE2) functions as a negative regulator of the renin-angiotensin system by cleaving angiotensin II (AII) into angiotensin 1-7 (A1-7). This study assessed the role of endogenous ACE2 in maintaining insulin sensitivity. Twelve-week-old male ACE2 knockout (ACE2KO) mice had normal insulin sensitivities when fed a standard diet. AII infusion or a high-fat, high-sucrose (HFHS) diet impaired glucose tolerance and insulin sensitivity more severely in ACE2KO mice than in their wild-type (WT) littermates. The strain difference in glucose tolerance was not eliminated by an AII receptor type 1 (AT1) blocker but was eradicated by A1-7 or an AT1 blocker combined with the A1-7 inhibitor (A779). The expression of GLUT4 and a transcriptional factor, myocyte enhancer factor (MEF) 2A, was dramatically reduced in the skeletal muscles of the standard diet-fed ACE2KO mice. The expression of GLUT4 and MEF2A was increased by A1-7 in ACE2KO mice and decreased by A779 in WT mice. A1-7 enhanced upregulation of MEF2A and GLUT4 during differentiation of myoblast cells. In conclusion, ACE2 protects against high-calorie diet-induced insulin resistance in mice. This mechanism may involve the transcriptional regulation of GLUT4 via an A1-7-dependent pathway.

Tanno, T., et al. (2016). "Olmesartan Inhibits Cardiac Hypertrophy in Mice Overexpressing Renin Independently of Blood Pressure: Its Beneficial Effects on ACE2/Ang (1-7)/Mas Axis and NADPH Oxidase Expression." J Cardiovasc Pharmacol **67**(6): 503-509.

Enhanced renin-angiotensin activity causes hypertension and cardiac hypertrophy. The angiotensin (Ang)-converting enzyme (ACE)2/Ang (1-7)/Mas axis pathway functions against Ang II type 1 receptor (AT1R) signaling. We investigated whether olmesartan (Olm), an AT1R blocker, inhibits cardiac hypertrophy independently of blood pressure, and evaluated the potential mechanisms. The 3- to 4-month-old male mice overexpressing renin in the liver (Ren-Tg) were given Olm (5 mg/kg/d) and hydralazine (Hyd) (3.5 mg/kg/d) orally for 2 months. Systolic blood pressure was higher in the Ren-Tg mice than in wild-type littermates. Olm and Hyd treatments lowered systolic blood pressure to the same degree. However, cardiac hypertrophy, evaluated by echocardiography, heart weight, cross-sectional area of cardiomyocytes, and gene expression, was inhibited by only Olm treatment, but not by Hyd. Olm treatment reversed decreased gene expressions of ACE2 and Mas receptor of Ren-Tg mice and inhibited enhanced NADPH oxidase (Nox)4 expression and reactive oxygen species, whereas Hyd treatment had no influence on them. These findings indicate that Olm treatment inhibits cardiac hypertrophy independently of blood pressure, not only through its original AT1R blockade but partly through enhancement of ACE2/Ang (1-7)/Mas axis and suppression of Nox4 expression.

Thatcher, S. E., et al. (2012). "Deficiency of ACE2 in Bone-Marrow-Derived Cells Increases Expression of TNF-alpha in Adipose Stromal Cells and Augments Glucose Intolerance in Obese C57BL/6 Mice." Int J Hypertens **2012**: 762094.

Deficiency of ACE2 in macrophages has been suggested to promote the development of an inflammatory M1 macrophage phenotype. We evaluated effects of ACE2 deficiency in bone-marrow-derived stem cells on adipose inflammation and glucose tolerance in C57BL/6 mice fed a high fat (HF) diet. ACE2 activity was increased in the stromal vascular fraction (SVF) isolated from visceral, but not subcutaneous adipose tissue of HF-fed mice. Deficiency of ACE2 in bone marrow cells significantly increased mRNA abundance of F4/80 and TNF-alpha in the SVF isolated from visceral adipose tissue of HF-fed chimeric mice, supporting increased presence of inflammatory macrophages in adipose tissue. Moreover, deficiency of ACE2 in bone marrow cells modestly augmented glucose intolerance in HF-fed chimeric mice and increased blood levels of glycosylated hemoglobin. In summary, ACE2 deficiency in bone marrow cells promotes inflammation in adipose tissue and augments obesity-induced glucose intolerance.

Thomas, M. C., et al. (2010). "Genetic Ace2 deficiency accentuates vascular inflammation and atherosclerosis in the ApoE knockout mouse." Circ Res **107**(7): 888-897.

RATIONALE: Angiotensin-converting enzyme (ACE)2 opposes the actions of angiotensin (Ang) II by degrading it to Ang 1-7. OBJECTIVE: Given the important role of Ang II/Ang 1-7 in atherogenesis, we investigated the impact of ACE2 deficiency on the development of atherosclerosis. METHODS AND RESULTS: C57Bl6, Ace2 knockout (KO), apolipoprotein E (ApoE) KO and ApoE/Ace2 double KO mice were followed until 30 weeks of age. Plaque accumulation was increased in ApoE/Ace2 double KO mice when compared to ApoE KO mice. This was associated with increased expression of adhesion molecules and inflammatory cytokines, including interleukin-6, monocyte chemoattractant protein-1, and vascular cell adhesion molecule-1, and an early

increase in white cell adhesion across the whole aortae on dynamic flow assay. In the absence of a proatherosclerotic (ApoE KO) genotype, ACE2 deficiency was also associated with increased expression of these markers, suggesting that these differences were not an epiphenomenon. ACE inhibition prevented increases of these markers and atherogenesis in ApoE/ACE2 double KO mice. Bone marrow macrophages isolated from Ace2 KO mice showed increased proinflammatory responsiveness to lipopolysaccharide and Ang II when compared to macrophages isolated from C57Bl6 mice. Endothelial cells isolated from Ace2 KO mice also showed increased basal activation and elevated inflammatory responsiveness to TNF-alpha. Similarly, selective inhibition of ACE2 with MLN-4760 also resulted in a proinflammatory phenotype with a physiological response similar to that observed with exogenous Ang II (10(-7) mol/L). CONCLUSIONS: Genetic Ace2 deficiency is associated with upregulation of putative mediators of atherogenesis and enhances responsiveness to proinflammatory stimuli. In atherosclerosis-prone ApoE KO mice, these changes potentially contribute to increased plaque accumulation. These findings emphasize the potential utility of ACE2 repletion as a strategy to reduce atherosclerosis.

Tikellis, C., et al. (2008). "ACE2 deficiency modifies renoprotection afforded by ACE inhibition in experimental diabetes." Diabetes **57**(4): 1018-1025.

OBJECTIVE: The degradation of angiotensin (Ang) II by ACE2, leading to the formation of Ang 1-7, is an important step in the renin-angiotensin system (RAS) and one that is significantly altered in the diabetic kidney. This study examines the role of ACE2 in early renal changes associated with diabetes and the influence of ACE2 deficiency on ACE inhibitor-mediated renoprotection. RESEARCH DESIGN AND METHODS: Diabetes was induced by streptozotocin in male c57bl6 mice and ACE2 knockout (KO) mice. After 5 weeks of study, animals were randomized to receive the ACE inhibitor perindopril (2 mg x kg (-1) x day (-1)). Wild-type mice were further randomized to receive the selective ACE2 inhibitor MLN-4760 (10 mg x kg (-1) x day (-1)) and followed for an additional 5 weeks. Markers of renal function and injury were then assessed. RESULTS: Induction of diabetes in wild-type mice was associated with a reduction in renal ACE2 expression and decreased Ang 1-7. In diabetic mice receiving MLN-4760 and in ACE2 KO mice, diabetes-associated albuminuria was enhanced, associated with an increase in blood pressure. However, renal hypertrophy and fibrogenesis were reduced in diabetic mice with ACE2 deficiency, and hyperfiltration was attenuated. Diabetic wild-type mice treated with an ACE inhibitor experienced a reduction in albuminuria and blood pressure. These responses were attenuated in both diabetic ACE2 KO mice and diabetic mice receiving MLN-4760. However, other renoprotective and antifibrotic actions of ACE inhibition in diabetes were preserved in ACE2-deficient mice. CONCLUSIONS: The expression of ACE2 is significantly modified by diabetes, which impacts both pathogenesis of kidney disease and responsiveness to RAS blockade. These data indicate that ACE2 is a complex and site-specific modulator of diabetic kidney disease.

Tikellis, C., et al. (2006). "Developmental expression of ACE2 in the SHR kidney: a role in hypertension?" Kidney Int **70**(1): 34-41.

The abnormal development of the intrarenal renin-angiotensin system (RAS) is thought contribute to adult-onset hypertension in the spontaneously hypertensive rat (SHR). Angiotensin-converting enzyme 2 (ACE2) is a novel enzyme with complementary actions to that of ACE. Recent studies have shown that ACE2 expression is reduced in the adult SHR. However, its regulation in pre-hypertensive animals is unknown. In this study, we examine the developmental expression of ACE2 in the rodent kidney and its temporal expression, as it relates to the development of hypertension in the SHR model. Kidneys from SHR and normotensive Wistar Kyoto (WKY) rats (n=8-12/group) at birth, 6 weeks of age, and adulthood (80 days) were examined. Gene expression and activity of ACE2 were determined by real-time reverse transcription-polymerase chain reaction and quenched fluorescence assays, respectively. Renal expression was localized by in situ hybridization and immunohistochemistry. The expression and ACE2 activity are significantly increased in the SHR kidney at birth. With the onset of hypertension, the tubular expression of ACE2 falls in SHR compared to WKY and remains reduced in the adult SHR kidney. Glomerular expression is paradoxically increased in the SHR glomerulus. The overall developmental pattern of ACE2 expression in the SHR kidney is also modified, with declining expression over the course of renal development. The developmental pattern of ACE2 expression in the SHR kidney is altered before the onset of hypertension, consistent with the key role of the RAS in the pathogenesis of adult-onset hypertension. Further research is required to distinguish the contribution of these changes to the development and progression of hypertension in this model.

Tikellis, C., et al. (2012). "Interaction of diabetes and ACE2 in the pathogenesis of cardiovascular

disease in experimental diabetes." Clin Sci (Lond) **123**(8): 519-529.

Local and systemic AngII (angiotensin II) levels are regulated by ACE2 (angiotensin-converting enzyme 2), which is reduced in diabetic tissues. In the present study, we examine the effect of ACE2 deficiency on the early cardiac and vascular changes associated with experimental diabetes. Streptozotocin diabetes was induced in male C57BL6 mice and Ace2-KO (knockout) mice, and markers of RAS (renin-angiotensin system) activity, cardiac function and injury were assessed after 10 weeks. In a second protocol, diabetes was induced in male ApoE (apolipoprotein E)-KO mice and ApoE/Ace2-double-KO mice, and plaque accumulation and markers of atherogenesis assessed after 20 weeks. The induction of diabetes in wild-type mice led to reduced ACE2 expression and activity in the heart, elevated circulating AngII levels and reduced cardiac Ang-(1-7) [angiotensin-(1-7)] levels. This was associated structurally with thinning of the LV (left ventricular) wall and mild ventricular dilatation, and histologically with increased cardiomyocyte apoptosis on TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) staining and compensatory hypertrophy denoted by an increased cardiomyocyte cross-sectional area. By contrast Ace2-KO mice failed to increase circulating AngII concentration, experienced a paradoxical fall in cardiac AngII levels and no change in Ang-(1-7) following the onset of diabetes. At the same time the major phenotypic differences between Ace2-deficient and Ace2-replete mice with respect to BP (blood pressure) and cardiac hypertrophy were eliminated following the induction of diabetes. Consistent with findings in the heart, the accelerated atherosclerosis that was observed in diabetic ApoE-KO mice was not seen in diabetic ApoE/Ace2-KO mice, which experienced no further increase in plaque accumulation or expression in key adhesion molecules beyond that seen in ApoE/Ace2-KO mice. These results point to the potential role of ACE2 deficiency in regulating the tissue and circulating levels of AngII and their sequelae in the context of diabetes, as well as the preservation or augmentation of ACE2 expression or activity as a potential therapeutic target for the prevention of CVD (cardiovascular disease) in diabetes.

Tikellis, C. and M. C. Thomas (2012). "Angiotensin-Converting Enzyme 2 (ACE2) Is a Key Modulator of the Renin Angiotensin System in Health and Disease." Int J Pept **2012**: 256294.

Angiotensin-converting enzyme 2 (ACE2) shares some homology with angiotensin-converting enzyme (ACE) but is not inhibited by ACE inhibitors. The main role of ACE2 is the degradation of Ang II resulting in the formation of angiotensin 1-7 (Ang 1-7) which opposes the actions of Ang II. Increased Ang II levels are thought to upregulate ACE2 activity, and in ACE2 deficient mice Ang II levels are approximately double that of wild-type mice, whilst Ang 1-7 levels are almost undetectable. Thus, ACE2 plays a crucial role in the RAS because it opposes the actions of Ang II. Consequently, it has a beneficial role in many diseases such as hypertension, diabetes, and cardiovascular disease where its expression is decreased. Not surprisingly, current therapeutic strategies for ACE2 involve augmenting its expression using ACE2 adenoviruses, recombinant ACE2 or compounds in these diseases thereby affording some organ protection.

Tikoo, K., et al. (2015). "Tissue specific up regulation of ACE2 in rabbit model of atherosclerosis by atorvastatin: role of epigenetic histone modifications." Biochem Pharmacol **93**(3): 343-351.

Growing body of evidence points out the crucial role of ACE2 in preventing atherosclerosis. However, data on how atherosclerosis affects ACE2 expression in heart and kidney remains unknown. Atherosclerosis was induced by feeding New Zealand White rabbits with high cholesterol diet (HCD - 2%) for 12 weeks and atorvastatin was administered (5mg/kg/day p.o) in last 3 weeks. ACE2 mRNA and protein expression was assessed by Western blotting and real time PCR. HCD fed rabbits developed atherosclerosis as confirmed by increase in plasma total cholesterol, LDL and triglycerides as well as formation atherosclerotic plaques in arch of aorta. The ACE2 protein but not mRNA expression was reduced in heart and kidney of HCD rabbits. Interestingly, atorvastatin increased the ACE2 protein expression in heart and kidney of HCD rabbits. However, atorvastatin increased ACE2 mRNA in heart but not in kidney of HCD rabbits. Atorvastatin increased the occupancy of histone H3 acetylation (H3-Ac) mark on ACE2 promoter region in heart of HCD rabbits indicating direct or indirect epigenetic up-regulation of ACE2 by atorvastatin. Further, atorvastatin suppressed Ang II-induced contractile responses and enhanced AT2 receptor mediated relaxant responses in atherosclerotic aorta. We propose that atherosclerosis is associated with reduced ACE2 expression in heart and kidney. We also show an unexplored potential of atorvastatin to up-regulate ACE2 via epigenetic histone modifications. Our data suggest a novel way of replenishing ACE2 expression for preventing not only atherosclerosis but also other cardiovascular disorders.

To, K. F. and A. W. Lo (2004). "Exploring the pathogenesis of severe acute respiratory syndrome (SARS): the tissue distribution of the coronavirus

(SARS-CoV) and its putative receptor, angiotensin-converting enzyme 2 (ACE2)." J Pathol **203**(3): 740-743.

Severe acute respiratory syndrome (SARS) is an emerging infectious disease associated with a new coronavirus, SARS-CoV. Pulmonary involvement is the dominant clinical feature but extra-pulmonary manifestations are also common. Factors that account for the wide spectrum of organ system involvement and disease severity are poorly understood and the pathogenesis of SARS-CoV infection remains unclear. Angiotensin converting enzyme 2 (ACE2) has recently been identified as the functional cellular receptor for SARS-CoV. Studies of the tissue and cellular distribution of SARS-CoV, and ACE2 protein expression, reveal new insights into the pathogenesis of this deadly disease. ACE2 is expressed at high level in the primary target cells of SARS-CoV, namely pneumocytes and surface enterocytes of the small intestine. Despite the fact that SARS-CoV can infect the lung and intestine, the tissue responses in these two organs are different. All other tissues and cell types expressing ACE2 may be potential targets of SARS-CoV infection. Remarkably, endothelial cells, which express ACE2 to a high level, have not been shown to be infected by SARS-CoV. There is also evidence that cell types without detectable ACE2 expression may also be infected by the virus. Furthermore, studies in a new human cell culture model have indicated that the presence of ACE2 alone is not sufficient for maintaining viral infection. Therefore, other virus receptors or co-receptors may be required in different tissues. Moreover, the interaction between SARS-CoV and the immunological or lymphoid system remains to be defined. It is clear that we are only at the dawn of our understanding of the pathogenesis of SARS. As our knowledge of the pathogenic mechanisms improves, a more rational approach to therapeutic and vaccine development can be designed in order to combat this new and fatal human disease.

Turner, A. J., et al. (2004). "ACE2: from vasopeptidase to SARS virus receptor." Trends Pharmacol Sci **25**(6): 291-294.

The zinc metallopeptidase angiotensin-converting enzyme 2 (ACE2) is the only known human homologue of the key regulator of blood pressure angiotensin-converting enzyme (ACE). Since its discovery in 2000, ACE2 has been implicated in heart function, hypertension and diabetes, with its effects being mediated, in part, through its ability to convert angiotensin II to angiotensin-(1-7). Unexpectedly, ACE2 also serves as the cellular entry point for the severe acute respiratory syndrome (SARS) virus and the enzyme is therefore a prime target for pharmacological intervention on several disease fronts.

Turner, A. J., et al. (2002). "ACEH/ACE2 is a novel mammalian metallocarboxypeptidase and a homologue of angiotensin-converting enzyme insensitive to ACE inhibitors." Can J Physiol Pharmacol **80**(4): 346-353.

A human zinc metalloprotease (termed ACEH or ACE2) with considerable homology to angiotensin-converting enzyme (ACE) (EC 3.4.15.1) has been identified and subsequently cloned and functionally expressed. The translated protein contains an N-terminal signal sequence, a single catalytic domain with zinc-binding motif (HEMGH), a transmembrane region, and a small C-terminal cytosolic domain. Unlike somatic ACE, ACEH functions as a carboxypeptidase when acting on angiotensin I and angiotensin II or other peptide substrates. ACEH may function in conjunction with ACE and neprilysin in novel pathways of angiotensin metabolism of physiological significance. In contrast with ACE, ACEH does not hydrolyse bradykinin and is not inhibited by typical ACE inhibitors. ACEH is unique among mammalian carboxypeptidases in containing an HEXXH zinc motif but, in this respect, resembles a bacterial enzyme, Thermus aquaticus (Taq) carboxypeptidase (EC 3.4.17.19). Collectrin, a developmentally regulated renal protein, is homologous with the C-terminal region of ACEH but has no similarity with ACE and no catalytic domain. Thus, the ACEH protein may have evolved as a chimera of a single ACE-like domain and a collectrin domain. The collectrin domain may regulate tissue response to injury whereas the catalytic domain is involved in peptide processing events.

Tyrankiewicz, U., et al. (2018). "Activation pattern of ACE2/Ang-(1-7) and ACE/Ang II pathway in course of heart failure assessed by multiparametric MRI in vivo in Tgalphaq*44 mice." J Appl Physiol (1985) **124**(1): 52-65.

Here, we analyzed systemic (plasma) and local (heart/aorta) changes in ACE/ACE-2 balance in Tgalphaq*44 mice in course of heart failure (HF). Tgalphaq*44 mice with cardiomyocyte-specific Galphaq overexpression and late onset of HF were analyzed at different age for angiotensin pattern in plasma, heart, and aorta using liquid chromatography/mass spectrometry, for progression of HF by in vivo magnetic resonance imaging under isoflurane anesthesia, and for physical activity by voluntary wheel running. Six-month-old Tgalphaq*44 mice displayed decreased ventricle radial strains and impaired left atrial function. At 8-10 mo, Tgalphaq*44 mice showed impaired systolic performance and reduced voluntary wheel running but exhibited preserved inotropic reserve. At 12 mo, Tgalphaq*44

mice demonstrated a severe impairment of basal cardiac performance and modestly compromised inotropic reserve with reduced voluntary wheel running. Angiotensin analysis in plasma revealed an increase in concentration of angiotensin-(1-7) in 6- to 10-mo-old Tgalphaq*44 mice. However, in 12- to 14-mo-old Tgalphaq*44 mice, increased angiotensin II was noted with a concomitant increase in Ang III, Ang IV, angiotensin A, and angiotensin-(1-10). The pattern of changes in the heart and aorta was also compatible with activation of ACE2, followed by activation of the ACE pathway. In conclusion, mice with cardiomyocyte Galphaq protein overexpression develop HF that is associated with activation of the systemic and the local ACE/Ang II pathway. However, it is counterbalanced by a prominent ACE2/Ang-(1-7) activation, possibly allowing to delay decompensation. NEW & NOTEWORTHY Changes in ACE/ACE-2 balance were analyzed based on measurements of a panel of nine angiotensins in plasma, heart, and aorta of Tgalphaq*44 mice in relation to progression of heart failure (HF) characterized by multiparametric MRI and exercise performance. The early stage of HF was associated with upregulation of the ACE2/angiotensin-(1-7) pathway, whereas the end-stage HF was associated with downregulation of ACE2/angiotensin-(1-7) and upregulation of the ACE/Ang II pathway. ACE/ACE-2 balance seems to determine the decompensation of HF in this model.

Uri, K., et al. (2016). "Circulating ACE2 activity correlates with cardiovascular disease development." J Renin Angiotensin Aldosterone Syst **17**(4).

It was shown recently that angiotensin-converting enzyme activity is limited by endogenous inhibition in vivo, highlighting the importance of angiotensin II (ACE2) elimination. The potential contribution of the ACE2 to cardiovascular disease progression was addressed. Serum ACE2 activities were measured in different clinical states (healthy, n=45; hypertensive, n=239; heart failure (HF) with reduced ejection fraction (HFrEF) n=141 and HF with preserved ejection fraction (HFpEF) n=47). ACE2 activity was significantly higher in hypertensive patients (24.8+/-0.8 U/ml) than that in healthy volunteers (16.2+/-0.8 U/ml, p=0.01). ACE2 activity further increased in HFrEF patients (43.9+/-2.1 U/ml, p=0.001) but not in HFpEF patients (24.6+/-1.9 U/ml) when compared with hypertensive patients. Serum ACE2 activity negatively correlated with left ventricular systolic function in HFrEF, but not in hypertensive, HFpEF or healthy populations. Serum ACE2 activity had a fair diagnostic value to differentiate HFpEF from HFrEF patients in this study. Serum ACE2 activity correlates with cardiovascular disease development: it increases when hypertension develops and further increases when the cardiovascular disease further progresses to systolic dysfunction, suggesting that ACE2 metabolism plays a role in these processes. In contrast, serum ACE2 activity does not change when hypertension progresses to HFpEF, suggesting a different pathomechanism for HFpEF, and proposing a biomarker-based identification of these HF forms.

Uri, K., et al. (2014). "New perspectives in the renin-angiotensin-aldosterone system (RAAS) IV: circulating ACE2 as a biomarker of systolic dysfunction in human hypertension and heart failure." PLoS One **9**(4): e87845.

BACKGROUND: Growing evidence exists for soluble Angiotensin Converting Enzyme-2 (sACE2) as a biomarker in definitive heart failure (HF), but there is little information about changes in sACE2 activity in hypertension with imminent heart failure and in reverse remodeling. METHODS, FINDINGS: Patients with systolic HF (NYHAII-IV, enrolled for cardiac resynchronisation therapy, CRT, n = 100) were compared to hypertensive patients (n = 239) and to a healthy cohort (n = 45) with preserved ejection fraction (EF>50%) in a single center prospective clinical study. The status of the heart failure patients were checked before and after CRT. Biochemical (ACE and sACE2 activity, ACE concentration) and echocardiographic parameters (EF, left ventricular end-diastolic (EDD) and end-systolic diameter (ESD) and dP/dt) were measured. sACE2 activity negatively correlated with EF and positively with ESD and EDD in all patient's populations, while it was independent in the healthy cohort. sACE2 activity was already increased in the hypertensive group, where signs for imminent heart failure (slightly decreased EF and barely increased NT-proBNP levels) were detected. sACE2 activities further increased in patients with definitive heart failure (EF<50%), while sACE2 activities decreased with the improvement of the heart failure after CRT (reverse remodeling). Serum angiotensin converting enzyme (ACE) concentrations were lower in the diseased populations, but did not show a strong correlation with the echocardiographic parameters. CONCLUSIONS: Soluble ACE2 activity appears to be biomarker in heart failure, and in hypertension, where heart failure may be imminent. Our data suggest that sACE2 is involved in the pathomechanism of hypertension and HF.

Valdes, G., et al. (2013). "Utero-placental expression of angiotensin-(1-7) and ACE2 in the pregnant guinea-pig." Reprod Biol Endocrinol **11**: 5.

BACKGROUND: In humans, trophoblast invasion, vascular remodeling and placental development are critical to determine the fate of

pregnancy. Since guinea-pigs (GP) and humans share common pregnancy features including extensive trophoblast invasion, transformation of the uterine spiral arteries and a haemomonochorial placenta, the GP animal model was deemed suitable to extend our knowledge on the spatio-temporal immunoreactive expression of the vasodilator arpeptide of the renin-angiotensin system, angiotensin-(1-7) [Ang-(1-7)] and its main generating enzyme, angiotensin converting enzyme 2 (ACE2). METHODS: Utero-placental units were collected in days 15, 20, 40 and 60 of a 64-67 day long pregnancy in 25 Pirbright GP. Ang-(1-7) and ACE2 expression in utero-placental units were evaluated by immunohistochemistry. RESULTS: Ang-(1-7) and ACE2 were detected in the endothelium and syncytiotrophoblast of the labyrinthine placenta, interlobium, subplacenta, giant cells, syncytial sprouts, syncytial streamers, and myometrium throughout pregnancy. In late pregnancy, perivascular or intramural trophoblasts in spiral and mesometrial arteries expressed both factors. Immunoreactive Ang-(1-7) and ACE2 were present in decidua and in the vascular smooth muscle of spiral, myometrial and mesometrial arteries, which also express kallikrein (Kal), the bradykinin receptor 2 (B2R), vascular endothelial growth factor (VEGF) and its type 2 receptor (KDR), but no endothelial nitric oxide synthase (eNOS). In addition, the signal of Ang-(1-7) and ACE2 was especially remarkable in giant cells, which also show Kal, B2R. eNOS, VEGF and KDR. CONCLUSIONS: The spatio-temporal expression of Ang-(1-7) and ACE2 in GP, similar to that of humans, supports a relevant evolutionary conserved function of Ang-(1-7) and ACE2 in decidualization, trophoblast invasion, vascular remodeling and placental flow regulation, as well as the validity of the GP model to understand the local adaptations of pregnancy. It also integrates Ang-(1-7) to the utero-placental vasodilatory network. However, its antiangiogenic effect may counterbalance the proangiogenic activity of some of the other vasodilator components.

Valdes, G., et al. (2006). "Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies." Placenta **27**(2-3): 200-207.

This work was designed to study the expression of the vasodilator peptide angiotensin-(1-7) [Ang-(1-7)] and its generating enzyme (ACE2) in the uteroplacental interface. Placentas were obtained from 11 early pregnancy failures (5 miscarriages and 6 ectopic pregnancies), 15 normotensive, and 10 preeclamptic gestations. In placental villi, the main sites of immunocytochemical expression of Ang-(1-7) and ACE2 were the syncytiotrophoblast, cytotrophoblast, endothelium and vascular smooth muscle of primary and secondary villi. Syncitial Ang-(1-7) expression in samples obtained from miscarriages and ectopic pregnancies was increased compared to normal term pregnancy [2.0 (2.0-2.25 for the 25 and 75% interquartile range) vs 1.3 (1.0-1.9), $p<0.01$]. In the maternal stroma, Ang-(1-7) and ACE2 were expressed in the invading and intravascular trophoblast and in decidual cells in all 3 groups. Ang-(1-7) and ACE2 staining was also found in arterial and venous endothelium and smooth muscle of the umbilical cord. The expression of Ang-(1-7) and ACE2 was similar in samples obtained from normal term or preeclamptic pregnancies, except for increased expression of ACE2 in umbilical arterial endothelium in preeclampsia [0.5 (0.5-0.8) vs 0.0 (0.0-0.0), $p<0.01$]. The uteroplacental location of Ang-(1-7) and ACE2 in pregnancy suggests an autocrine function of Ang-(1-7) in the vasoactive regulation that characterizes placentation and established pregnancy.

van Wijlick, L., et al. (2016). "Candida albicans responds to glycostructure damage by Ace2-mediated feedback regulation of Cek1 signaling." Mol Microbiol **102**(5): 827-849.

Candida albicans uses the Cek1 MAPK pathway to restore cells from damage of its cell wall glycostructures. Defective protein N- or O-glycosylation activates Cek1 and the transcription factor Ace2 as its downstream target, to upregulate genes encoding protein O-mannosyltransferases (Pmt proteins). In unstressed cells, Cek1-Ace2 activity blocks expression of PMT1, which is de-repressed by tunicamycin. Genomic binding targets of Ace2 included ZCF21, which was upregulated by Ace2 and found to repress PMT1 transcription in unstressed cells. Surprisingly, genes encoding components of the Cek1 pathway including MSB2, CST20, HST7, CEK1 and ACE2 were also identified as Ace2 targets indicating Ace2-mediated transcriptional amplification of pathway genes under N-glycosylation stress. In this condition, physical interaction of the Ace2 protein with the upstream MAPKKK Cst20 was detected. Cst20-GFP showed stress-induced import from the cytoplasm into the nucleus and phosphorylation of Ace2. Interestingly, forced nuclear localization of Cst20 inhibited Cek1-Ace2 signaling, while forced cytoplasmic localization of Cst20 retained full signaling activity, suggesting that nuclear Cst20 downregulates the Cek1 pathway. Collectively, the results indicate that Ace2 is a versatile multifunctional transcriptional regulator, which activates glycostress responses of C. albicans by both positive forward and negative feedback regulation of Cek1 signaling.

Vangjeli, C., et al. (2011). "A polymorphism in ACE2 is associated with a lower risk for fatal

cardiovascular events in females: the MORGAM project." J Renin Angiotensin Aldosterone Syst **12**(4): 504-509.

Angiotensin II, a vasoconstrictor and the main effector molecule of the renin-angiotensin system, is known to influence inflammation, thrombosis, low-density lipoprotein oxidation and growth factors, all of which contribute to cardiovascular disease. The associations of polymorphisms in the angiotensin-converting enzyme 2 (ACE2) gene with cardiovascular risk have not been fully determined. Single nucleotide polymorphisms (SNPs) in ACE2 were genotyped in participants of the prospective MORGAM study (n = 5092) from five cohorts: ATBC, FINRISK, Northern Sweden, PRIME/Belfast and PRIME/France. Using a case-cohort design, associations were sought between SNPs and haplotypes with cardiovascular events during follow-up (Cox proportional hazards model). The comparison group were a subset of all MORGAM participants who were selected to ensure similar age and sex distributions among the cases and controls. The A allele of the rs2285666 SNP (HR = 0.3, p = 0.04) was significantly associated with the risk of cardiovascular death in female subjects. These findings complement those found in other studies of SNPs in the ACE2 gene in relation to cardiovascular disease risk. As females carry two copies of the ACE2 gene, and given its plausible biological role in cardiovascular disease risk, further studies of ACE2 should be prioritized.

Varagic, J., et al. (2014). "ACE2: angiotensin II/angiotensin-(1-7) balance in cardiac and renal injury." Curr Hypertens Rep **16**(3): 420.

Our current recognition of the renin-angiotensin system is more convoluted than originally thought due to the discovery of multiple novel enzymes, peptides, and receptors inherent in this interactive biochemical cascade. Over the last decade, angiotensin-converting enzyme 2 (ACE2) has emerged as a key player in the pathophysiology of hypertension and cardiovascular and renal disease due to its pivotal role in metabolizing vasoconstrictive/hypertrophic/proliferative angiotensin II into favorable angiotensin-(1-7). This review addresses the considerable advancement in research on the role of tissue ACE2 in the development and progression of hypertension and cardiac and renal injury. We summarize the results from recent clinical and experimental studies suggesting that serum or urine soluble ACE2 may serve as a novel biomarker or independent risk factor relevant for diagnosis and prognosis of cardiorenal disease. We also review recent proceedings on novel therapeutic approaches to enhance ACE2/angiotensin-(1-7) axis.

Vasku, A., et al. (2013). "ACE2 gene polymorphisms and invasively measured central pulse pressure in cardiac patients indicated for coronarography." J Renin Angiotensin Aldosterone Syst **14**(3): 220-226.

BACKGROUND AND AIM: The objective of this research was to determine whether invasively measured central pulse pressure (PP) in patients indicated for coronarography is associated with two common polymorphisms in the ACE2 region (rs4646156 and rs4646174). METHODS: A total of 307 patients were enrolled in the study. The genotyping of both SNPs from peripheral blood samples was carried out using 5'exonuclease (Taqman (R)) chemistry on the ABI Prism (R) 7000 system (Applied Biosystems, Foster City, CA, USA). RESULTS: In both polymorphisms, the associations with central PP were found to be highly significant when all five possible genotypes in the population had been compared (p = 0.0001). In men, there was a higher incidence of previous myocardial infarction in G0 genotype carriers of rs54646174 (OR ratio = 7; p = 0.005). The AA genotype of rs4646156 had a 7.81x higher risk of severe angina pectoris in women (OR = 7.81, p = 0.05). A significant difference in allelic frequency of ACE2rs4646174 was found between women with and without significant stenoses of the circumflex branch of the left coronary artery. CONCLUSION: More research into the role of ACE2 genetic variability in PP regulations is necessary for more detailed physiological and pathophysiological comprehension of PP regulation.

Velkoska, E., et al. (2010). "Reduction in renal ACE2 expression in subtotal nephrectomy in rats is ameliorated with ACE inhibition." Clin Sci (Lond) **118**(4): 269-279.

Alterations within the RAS (renin-angiotensin system) are pivotal for the development of renal disease. ACE2 (angiotensin-converting enzyme 2) is expressed in the kidney and converts the vasoconstrictor AngII (angiotensin II) into Ang-(1-7), a peptide with vasodilatory and anti-fibrotic actions. Although the expression of ACE2 in the diabetic kidney has been well studied, little is known about its expression in non-diabetic renal disease. In the present study, we assessed ACE2 in rats with acute kidney injury induced by STNx (subtotal nephrectomy). STNx and Control rats received vehicle or ramipril (1 mg. kg (-1) of body weight. day (-1), and renal ACE, ACE2 and mas receptor gene and protein expression were measured 10 days later. STNx rats were characterized by polyuria, proteinuria, hypertension and elevated plasma ACE2 activity (all P<0.01) and plasma Ang-(1-7) (P<0.05) compared with Control rats. There was increased cortical ACE binding and

medullary mas receptor expression (P<0.05), but reduced cortical and medullary ACE2 activity in the remnant kidney (P<0.05 and P<0.001 respectively) compared with Control rats. In STNx rats, ramipril reduced blood pressure (P<0.01), polyuria (P<0.05)and plasma ACE2 (P<0.01), increased plasma Ang-(1-7) (P<0.001), and inhibited renal ACE (P<0.001). Ramipril increased both cortical and medullary ACE2 activity (P<0.01), but reduced medullary mas receptor expression (P<0.05). In conclusion, our results show that ACE2 activity is reduced in kidney injury and that ACE inhibition produced beneficial effects in association with increased renal ACE2 activity. As ACE2 both degrades AngII and generates the vasodilator Ang-(1-7), a decrease in renal ACE2 activity, as observed in the present study, has the potential to contribute to the progression of kidney disease.

Velkoska, E., et al. (2015). "Short-term treatment with diminazene aceturate ameliorates the reduction in kidney ACE2 activity in rats with subtotal nephrectomy." PLoS One **10**(3): e0118758.

Angiotensin converting enzyme (ACE) 2 is an important modulator of the renin angiotensin system (RAS) through its role to degrade angiotensin (Ang) II. Depletion of kidney ACE2 occurs following kidney injury due to renal mass reduction and may contribute to progressive kidney disease. This study assessed the effect of diminazine aceturate (DIZE), which has been described as an ACE2 activator, on kidney ACE2 mRNA and activity in rats with kidney injury due to subtotal nephrectomy (STNx). Sprague Dawley rats were divided into Control groups or underwent STNx; rats then received vehicle or the DIZE (s.c. 15 mg/kg/day) for 2 weeks. STNx led to hypertension (P<0.01), kidney hypertrophy (P<0.001) and impaired kidney function (P<0.001) compared to Control rats. STNx was associated with increased kidney cortical ACE activity, and reduced ACE2 mRNA in the cortex (P<0.01), with reduced cortical and medullary ACE2 activity (P<0.05), and increased urinary ACE2 excretion (P<0.05) compared to Control rats. Urinary ACE2 activity correlated positively with urinary protein excretion (P<0.001), and negatively with creatinine clearance (P=0.04). In STNx rats, DIZE had no effect on blood pressure or kidney function, but was associated with reduced cortical ACE activity (P<0.01), increased cortical ACE2 mRNA (P<0.05) and increased cortical and medullary ACE2 activity (P<0.05). The precise in vivo mechanism of action of DIZE is not clear, and its effects to increase ACE2 activity may be secondary to an increase in ACE2 mRNA abundance. In ex vivo studies, DIZE did not increase ACE2 activity in either Control or STNx kidney cortical membranes. It is not yet known if chronic administration of DIZE has long-term benefits to slow the progression of kidney disease.

Verma, A., et al. (2012). "ACE2 and Ang-(1-7) confer protection against development of diabetic retinopathy." Mol Ther **20**(1): 28-36.

Despite evidence that hyperactivity of the vasodeleterious axis (ACE/angiotensin II (Ang II)/AT1 receptor) of the renin-angiotensin system (RAS) is associated with the pathogenesis of diabetic retinopathy (DR) use of the inhibitors of this axis has met with limited success in the control of this pathophysiology. We investigated the hypothesis that enhancing the local activity of the recently established protective axis of the RAS, ACE2/Ang-(1-7), using adeno-associated virus (AAV)-mediated gene delivery of ACE2 or Ang-(1-7) would confer protection against diabetes-induced retinopathy. Genes expressing ACE2 and Ang-(1-7) were cloned in AAV vector. The effects of ocular AAV-ACE2/Ang-(1-7) gene transfer on DR in diabetic eNOS (-/-) mice and Sprague-Dawley (SD) rats were examined. Diabetes was associated with approximately tenfold and greater than threefold increases in the ratios of ACE/ACE2 and AT1R/Mas mRNA levels in the retina respectively. Intraocular administration of AAV-ACE2/Ang-(1-7) resulted in significant reduction in diabetes-induced retinal vascular leakage, acellular capillaries, infiltrating inflammatory cells and oxidative damage in both diabetic mice and rats. Our results demonstrate that DR is associated with impaired balance of retinal RAS. Increased expression of ACE2/Ang-(1-7) overcomes this imbalance and confers protection against DR. Thus, strategies enhancing the protective ACE2/Ang-(1-7) axis of RAS in the eye could serve as a novel therapeutic target for DR.

Verma, A., et al. (2019). "Expression of Human ACE2 in Lactobacillus and Beneficial Effects in Diabetic Retinopathy in Mice." Mol Ther Methods Clin Dev **14**: 161-170.

The angiotensin converting enzyme 2 (ACE2) catalyzes the degradation of Angiotensin II (Ang II) to generate Angiotensin-(1-7), which reduces inflammation and oxidative stress stimulated by Ang II. ACE2 has been shown to be protective in cardiovascular and metabolic diseases including diabetes and its complications. However, the challenge for its clinical application is large-scale production of high-quality ACE2 with sufficient target tissue bioavailability. We developed an expression and delivery system based on the use of probiotic species Lactobacillus paracasei (LP) to serve as a live vector for oral delivery of human ACE2. We show that codon-optimized ACE2 can be efficiently expressed in LP. Mice treated with the recombinant LP expressing

the secreted ACE2 in fusion with the non-toxic subunit B of cholera toxin, which acts as a carrier to facilitate transmucosal transport, showed increased ACE2 activities in serum and tissues. ACE2-LP administration reduced the number of acellular capillaries, blocked retinal ganglion cell loss, and decreased retinal inflammatory cytokine expression in two mouse models of diabetic retinopathy. These results provide proof of concept for feasibility of using engineered probiotic species as live vector for delivery of human ACE2 with enhanced tissue bioavailability for treating diabetic retinopathy, as well as other diabetic complications.

Vuille-dit-Bille, R. N., et al. (2015). "Human intestine luminal ACE2 and amino acid transporter expression increased by ACE-inhibitors." Amino Acids **47**(4): 693-705.

Sodium-dependent neutral amino acid transporter B (0)AT1 (SLC6A19) and imino acid (proline) transporter SIT1 (SLC6A20) are expressed at the luminal membrane of small intestine enterocytes and proximal tubule kidney cells where they exert key functions for amino acid (re)absorption as documented by their role in Hartnup disorder and iminoglycinuria, respectively. Expression of B (0)AT1 was shown in rodent intestine to depend on the presence of the carboxypeptidase angiotensin-converting enzyme 2 (ACE2). This enzyme belongs to the renin-angiotensin system and its expression is induced by treatment with ACE-inhibitors (ACEIs) or angiotensin II AT1 receptor blockers (ARBs) in many rodent tissues. We show here in the Xenopus laevis oocyte expression system that human ACE2 also functionally interacts with SIT1. To investigate in human intestine the potential effect of ACEIs or ARBs on ACE2, we analysed intestinal biopsies taken during routine gastroduodenoscopy and ileocolonoscopy from 46 patients of which 9 were under ACEI and 13 ARB treatment. Analysis of transcript expression by real-time PCR and of proteins by immunofluorescence showed a co-localization of SIT1 and B (0)AT1 with ACE2 in the brush-border membrane of human small intestine enterocytes and a distinct axial expression pattern of the tested gene products along the intestine. Patients treated with ACEIs displayed in comparison with untreated controls increased intestinal mRNA levels of ACE2, peptide transporter PEPT1 (SLC15A1) and AA transporters B (0)AT1 and PAT1 (SLC36A1). This study unravels in human intestine the localization and distribution of intestinal transporters involved in amino acid absorption and suggests that ACEIs impact on their expression.

Wakahara, S., et al. (2007). "Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 ratio." Endocrinology **148**(5): 2453-2457.

Angiotensin-converting enzyme (ACE) 2, a newly emerging component of the renin-angiotensin system, is presumed to be a counterregulator against ACE in generating and degrading angiotensin II. It remains to be elucidated how mRNA levels of these two genes are quantitatively regulated in the kidney and also what kind of clinicopathological characteristics could influence the gene expressions in humans. Seventy-eight cases of biopsy-proven renal conditions were examined in detail. Total RNA from a small part of each renal cortical biopsy specimen was reverse transcribed, and the resultant cDNA was amplified for ACE, ACE2, and glyceraldehyde-3-phosphate dehydrogenase with a real-time PCR system. Then we investigated the relationship between clinicopathological variables and mRNA levels adjusted for glyceraldehyde-3-phosphate dehydrogenase. Statistically significant correlation was not observed between any clinicopathological variables and either of the gene expressions by pairwise comparison. However, a strong correlation was observed between the gene expressions of ACE and those of ACE2. Moreover, the ACE to ACE2 ratio was significantly higher in subjects with hypertension (HT) than that in subjects without HT. Whereas parameters of renal function, e.g. urinary protein excretion (UPE) and creatinine clearance (Ccr), are not significantly related to the ACE to ACE2 ratio as a whole, the HT status may reflect disease-induced deterioration of renal function. That is, UPE and Ccr of subjects with HT are significantly different from those without HT, in which a significant correlation is also observed between UPE and Ccr. Finally, stepwise regression analysis further revealed that only the HT status is an independent confounding determinant of the ACE to ACE2 ratio among the variables tested. Our data suggest that ACE2 might play an important role in maintaining a balanced status of local renin-angiotensin system synergistically with ACE by counterregulatory effects confounded by the presence of hypertension. Thus, ACE2 may exert pivotal effects on cardiovascular and renal conditions.

Wang, G., et al. (2011). "Expression of ACE and ACE2 in patients with hypertensive nephrosclerosis." Kidney Blood Press Res **34**(3): 141-149.

BACKGROUND: The interplay between intrarenal angiotensin-converting enzyme (ACE) and type 2 ACE (ACE2) might play important roles in the pathogenesis of hypertensive nephrosclerosis (HTN), but human data are limited. METHODS: Renal biopsy specimens of 41 patients with HTN and 10 transplant donors as controls (CTL) were studied. The

glomerular and tubulointerstitial mRNA expression of ACE and ACE2 was measured by laser microdissection and real-time quantitative polymerase chain reaction. The corresponding protein level was determined by immunohistochemistry. RESULTS: Neither the glomerular nor tubulointerstitial mRNA expression of ACE or ACE2 correlated with the corresponding protein level by immunohistochemistry. The tubulointerstitial levels of ACE and ACE2 were significantly lower in HTN than CTL, while the glomerular ACE and ACE2 levels were similar between the groups. The tubulointersitial ACE and ACE2 levels significantly correlated with the estimated glomerular filtration rate (GFR) and inversely with the degree of histological damage. The glomerular ACE and ACE2 levels significantly correlated with the rate of GFR decline. The ratio of glomerular ACE and ACE2 level correlated with the estimated GFR and the degree of glomerulosclerosis. CONCLUSION: Our results suggest that intrarenal ACE and ACE2 may play an important role in the pathogenesis and progression of HTN. Studies based on the mRNA expression of ACE and ACE2 should be cautiously interpreted.

Wang, G., et al. (2008). "Urinary mRNA expression of ACE and ACE2 in human type 2 diabetic nephropathy." Diabetologia **51**(6): 1062-1067.

AIMS/HYPOTHESIS: The interplay of ACE and type 2 ACE (ACE2) has been recognised as playing an important role in the tissue renin-angiotensin system within the kidney. In the present study, we measured urinary mRNA expression of ACE and ACE2 in patients with type 2 diabetic nephropathy. METHODS: We studied 50 patients with diabetic nephropathy: 26 were being treated by ACE inhibitor (ACEI) alone (ACEI group), the other 24 by ACEI and angiotensin-receptor blocker (ARB) (ACEI+ARB group). mRNA expression of ACE and ACE2 was measured by real-time quantitative RT-PCR at 0 and 12 weeks. All patients were then followed for 56 weeks. RESULTS: Proteinuria correlated significantly with urinary ACE (r=0.454, p=0.001) and ACE2 expression (r=0.651, p<0.001). Urinary ACE2 expression correlated with estimated GFR (r= -0.289, p=0.042). In the ACEI group, there was a significant inverse correlation between the rate of GFR decline and urinary ACE2 expression at baseline (r= -0.423, p=0.031) as well as at 12 weeks (r= -0.395, p=0.046). In contrast, there was no significant correlation between the rate of GFR decline and urinary ACE2 expression at baseline or at 12 weeks in the ACEI+ARB group. The rate of GFR decline did not correlate with the baseline urinary ACE expression of either group. CONCLUSION/INTERPRETATION: There was a relationship between urinary mRNA expression of ACE2 and the degree of proteinuria. The physiological implication and possibility of clinical application of quantifying urinary ACE2 expression require further study.

Wang, G., et al. (2009). "Discrepancy between intrarenal messenger RNA and protein expression of ACE and ACE2 in human diabetic nephropathy." Am J Nephrol **29**(6): 524-531.

BACKGROUND: The intrarenal angiotensin-converting enzyme (ACE) and type 2 ACE (ACE2) play important roles in the pathogenesis of diabetic nephropathy, but human data are limited. We studied glomerular and tubulointerstitial mRNA and the protein expression of ACE and ACE2 in patients with diabetic nephropathy. METHODS: We studied renal biopsy specimens of 22 patients with diabetic nephropathy and 11 transplant donors as normal controls. Intrarenal mRNA expression of ACE and ACE2 was measured by laser microdissection and real-time quantitative polymerase chain reaction; expression at the protein level was determined by immunostaining. RESULTS: Glomerular and tubulointerstitial mRNA expression levels of ACE and ACE2 were significantly higher in patients with diabetic nephropathy than in normal controls ($p < 0.001$ for all comparisons). Glomerular ACE and ACE2 protein levels of patients with diabetic nephropathy were significantly higher than those of kidney donors (4.90 +/- 2.55% vs. 2.64 +/- 0.98%, $p = 0.022$, and 7.40 +/- 3.36% vs. 4.37 +/- 2.36%, $p = 0.017$, respectively). The tubulointerstitial ACE at the protein level, however, was similar between diabetic patients and controls (8.76 +/- 4.18% vs. 10.44 +/- 6.61%, $p = 0.453$), and the tubulointerstitial ACE2 at the protein level was significantly lower in diabetic nephropathy (16.48 +/- 7.68% vs. 23.23 +/- 7.65%, $p = 0.025$). CONCLUSION: The mRNA expression of ACE and ACE2 increased in both the glomerular and tubulointerstitial area of diabetic nephropathy. However, the tubulointerstitial ACE expression at the protein level remained unchanged, while that of ACE2 actually decreased. Our results suggest a posttranscriptional modulation of tubulointerstitial ACE and ACE2 expression. Experimental data of intrarenal mRNA expression of ACE and ACE2 should be interpreted with caution.

Wang, J., et al. (2020). "Exosome-Mediated Transfer of ACE2 (Angiotensin-Converting Enzyme 2) from Endothelial Progenitor Cells Promotes Survival and Function of Endothelial Cell." Oxid Med Cell Longev **2020**: 4213541.

Angiotensin-converting enzyme 2 (ACE2) is an emerging cardiovascular protective target that mediates the metabolism of angiotensin (Ang) II into

Ang (1-7). Our group has demonstrated that ACE2 overexpression enhances the function of endothelial progenitor cells (EPCs). Here, we investigated whether ACE2-primed EPCs (ACE2-EPCs) can protect cerebral microvascular endothelial cells (ECs) against injury and dysfunction in an in vitro model, with focusing on their exosomal and cytokine paracrine effects on endothelial mitochondria. Human EPCs were transfected with lentivirus containing null or human ACE2 cDNA (denoted as Null-EPCs and ACE2-EPCs, respectively). Their conditioned culture media, w/wo depletion of exosomes (ACE2-EPC-CM (EX-), Null-EPC-CM (EX-), ACE2-EPC-CM, and Null-EPC-CM), were used for coculture experiments. EC injury and dysfunction model was induced by Ang II before coculture. Apoptosis, angiogenic ability, mitochondrion functions (ROS production, membrane potential, fragmentation), and gene expressions (ACE2, Nox2, and Nox4) of ECs were analyzed. The supernatant was collected for measuring the levels of ACE2, Ang II/Ang-(1-7), and growth factors (VEGF and IGF). Our results showed that (1) ACE2-EPC-CM had higher levels of ACE2, Ang (1-7), VEGF, and IGF than that of Null-EPC-CM. (2) Ang II-injured ECs displayed an increase of apoptotic rate and reduction in tube formation and migration abilities, which were associated with ACE2 downregulation, Ang II/Ang (1-7) imbalance, Nox2/Nox4 upregulation, ROS overproduction, an increase of mitochondrion fragmentation, and a decrease of membrane potential. (3) ACE2-EPC-CM had better protective effects than Null-EPC-CM on Ang II-injured ECs, which were associated with the improvements on ACE2 expression, Ang II/Ang (1-7) balance, and mitochondrial functions. (4) ACE2-EPC-CM (EX-) and Null-EPC-CM (EX-) showed reduced effects as compared to ACE2-EPCs-CM and Null-EPCs-CM. In conclusion, our data demonstrate that ACE2 overexpression can enhance the protective effects of EPCs on ECs injury, majorly through the exosomal effects on mitochondrial function.

Wang, J., et al. (2017). "The ACE2-Ang (1-7)-Mas receptor axis attenuates cardiac remodeling and fibrosis in post-myocardial infarction." Mol Med Rep **16**(2): 1973-1981.

Myocardial remodeling serves an important role in the pathophysiology of coronary heart disease. The angiotensin-converting enzyme (ACE)2-angiotensin-(1-7) [Ang (17)]Mas receptor (MasR) axis is a key regulator in myocardial remodeling and development of heart failure. To investigate how ACE2Ang (17)MasR axis function on myocardial remodeling and cardiac fibrosis in postmyocardial infarction (MI), male SpragueDawley rats (weight, 200+/-20 g) were used to establish the model of myocardial infarction by ligating the left coronary artery. The present study suggests that telmisartan (Tel) and olmesartan (Olm) (5 mg/kg/d) can inhibit myocardial remodeling of postmyocardial infarction through the ACE2Ang (17)MasR pathway. Administration of Tel or Olm was demonstrated to significantly inhibit collagen deposition using Masson staining. In addition, telmisartan and olmesartan was indicated to antagonize angiotensin II (Ang II) and upregulate ACE2, MasR, Ang (17) expression in myocardial tissue using immunoassay and ELISA test, and the effect of Olm was more marked than that of Tel at the same dosage. Simultaneously, compared with the MI or Sham group, the mRNA and protein expression of ACE2, Ang II and MasR in myocardial tissue demonstrated a remarkable increase in the Olm group, when compared with the Tel group. Taken together, our data demonstrated that ACE2Ang (17)MasR axis may present a potential protective role in the development of myocardial remodeling and may provide a new target for drug development of cardiac fibrosis. In conclusion, Olm is superior to Tel in inhibiting myocardial local Ang II level reducing myocardial collagen deposition and improving myocardial remodeling by upregulating the expression of ACE2, Ang (17) and MasR.

Wang, J., et al. (2015). "The ACE2-angiotensin-(1-7)-Mas axis protects against pancreatic cell damage in cell culture." Pancreas **44**(2): 266-272.

OBJECTIVE: Angiotensin-converting enzyme 2 (ACE2), its product angiotensin-(1-7), and its receptor Mas have been shown to moderate the adverse effects of the ACE-angiotensin II-AT1 axis in many diseases. The aim of this study was to determine whether the ACE2-Ang-(1-7)-Mas axis could have similar effects in a cell culture model of pancreatic damage. METHODS: AR42J cells were stimulated with 10 nmol/L cerulein to simulate acute pancreatitis. ACE2, Ang-(1-7), Mas receptor, and PI3K/AKT pathway were measured by quantitative real-time polymerase chain reaction and Western blot analysis. RESULTS: ACE2 and Mas receptor protein levels in AR42J cells were significantly increased ($P < 0.05$) between 30 minutes and 6 hours postdisease induction compared with the control group. Mas receptor gene expression was significantly increased ($P < 0.05$) at 2 hours postdisease induction, and Ang-(1-7) was increased at 6 hours. Treatment with Ang-(1-7) in AR42J cells increased IL-10, decreased IL-6 and IL-8, and reduced the damage to pancreatic cells. Levels of IL-6 and IL-8 in AR42J cell culture were increased significantly after treatment with A779. Moreover, Ang-(1-7) increased the concentration of PI3K/AKT pathway and eNOSin AR42J cells. CONCLUSIONS: ACE2-angiotensin-(1-7)-Mas axis significantly inhibits pancreatitis in

response to decreased inflammatory factors by the activation of endothelial nitric oxide synthase and NO signaling pathways.

Wang, L., et al. (2016). "Crosstalk between ACE2 and PLGF regulates vascular permeability during acute lung injury." Am J Transl Res **8**(2): 1246-1252.

Angiotensin converting enzyme 2 (ACE2) treatment suppresses the severity of acute lung injury (ALI), through antagonizing hydrolyzing angiotensin II (AngII) and the ALI-induced apoptosis of pulmonary endothelial cells. Nevertheless, the effects of ACE2 on vessel permeability and its relationship with placental growth factor (PLGF) remain ill-defined. In the current study, we examined the relationship between ACE2 and PLGF in ALI model in mice. We used a previously published bleomycin method to induce ALI in mice, and treated the mice with ACE2. We analyzed the levels of PLGF in these mice. The mouse lung vessel permeability was determined by a fluorescence pharmacokinetic assay following i.v. injection of 62.5 microg/kg Visudyne. PLGF pump or soluble Flt-1 (sFlt-1) pump was given to augment or suppress PLGF effects, respectively. The long-term effects on lung function were determined by measurement of lung resistance using methacholine. We found that ACE2 treatment did not alter PLGF levels in lung, but antagonized the effects of PLGF on increases of lung vessel permeability. Ectogenic PLGF abolished the antagonizing effects of ACE2 on the vessel permeability against PLGF. On the other hand, suppression of PLGF signaling mimicked the effects of ACE2 on the vessel permeability against PLGF. The suppression of vessel permeability resulted in improvement of lung function after ALI. Thus, ACE2 may antagonize the PLGF-mediated increases in lung vessel permeability during ALI, resulting in improvement of lung function after ALI.

Wang, L., et al. (2015). "The ACE2/Ang-(1-7)/Mas Axis Regulates the Development of Pancreatic Endocrine Cells in Mouse Embryos." PLoS One **10**(6): e0128216.

Angiotensin-converting enzyme 2 (ACE2), its product Angiotensin-(1-7) [Ang-(1-7)], and Ang-(1-7) receptor Mas, have been shown to regulate organogenesis during embryonic development in various species. However, it is not known whether a local ACE2/Ang-(1-7)/Mas axis is present in the fetal pancreas. It is hypothesized that there is a local ACE2/Ang-(1-7)/Mas axis in the embryonic pancreas in mice that is involved in regulating islet cell development. To address this issue, the endogenous expression profile of axis constituents in embryonic mouse pancreata was examined. Involvement of the ACE2 axis in the regulation of pancreatic development was also examined. The present experiments showed in an in vivo animal model that endogenous expression levels of ACE2 and the Mas receptor were upregulated in mouse pancreata in late embryogenesis, peaking on embryonic day E16.5, when it reached 3 folds compared to that seen at E12.5. Consistently, endogenous expression of Ang-(1-7) also peaked at E16.5. Treatment with the ACE2 inhibitor DX600 did not alter islet development. However, prenatal treatment with A779, a Mas receptor antagonist, reduced the beta-cell to alpha-cell ratio in neonatal islets, impaired islet insulin secretory function, and impaired the pups' glucose tolerance. In ex vivo pancreas explant cultures, A779 again decreased the beta-cell to alpha-cell ratio, apparently through its effects on beta-cell proliferation (reduced proliferation shown with Ki67 staining), and also decreased Insulin and Ngn3 mRNA expression. Furthermore, treatment of explant cultures with Ang-(1-7) increased mRNA levels of Insulin and pancreatic progenitor marker Ngn3, as well as Nox4, the ROS generation enzyme; these stimulatory effects were attenuated by co-treatment with A779, suggesting that Ang-(1-7), via Mas receptor signaling, may promote differentiation of pancreatic progenitors into insulin-producing cells via modulation of reactive oxygen species. These data together suggest that a Mas receptor-mediated mechanism may stimulate pancreatic cell development.

Wang, L. P., et al. (2016). "Protective role of ACE2-Ang-(1-7)-Mas in myocardial fibrosis by downregulating KCa3.1 channel via ERK1/2 pathway." Pflugers Arch **468**(11-12): 2041-2051.

The intermediate-conductance Ca (2+)-activated K (+) (KCa3.1) channel plays a vital role in myocardial fibrosis induced by angiotensin (Ang) II. However, as the antagonists of Ang II, the effect of angiotensin-converting enzyme 2 (ACE2)-angiotensin-(1-7)-Mas axis on KCa3.1 channel during myocardial fibrosis remains unknown. This study was designed to explore the function of KCa3.1 channel in the cardioprotective role of ACE2-Ang-(1-7)-Mas. Wild-type (WT) mice, hACE2 transgenic mice (Tg), and ACE2 deficiency mice (ACE2(-/-)) were administrated with Ang II by osmotic mini-pumps. As the activator of ACE2, diminazene aceturate (DIZE) inhibited increase of blood pressure, collagen deposition, and KCa3.1 protein expression in myocardium of WT mice induced by Ang II. In Tg and ACE2(-/-) mice, besides the elevation of blood pressure, Ang II induced transformation of cardiac fibroblast into myofibroblast and resulted in augmentation of hydroxyproline concentration and collagen deposition, as well as KCa3.1 protein expression, but the changes in ACE2(-

/-) mice were more obvious than those in Tg mice. Mas antagonist A779 reduced blood pressure, myocardium fibrosis, and myocardium KCa3.1 protein expression by Ang II in Tg mice, but activation of KCa3.1 with SKA-31 in Tg mice promoted the pro-fibrogenic effects of Ang II. Respectively, in ACE2(-/-) mice, TRAM-34, the KCa3.1 blocker, and Ang-(1-7) inhibited increase of blood pressure, collagen deposition, and KCa3.1 protein expression by Ang II. Moreover, DIZE and Ang-(1-7) depressed p-ERK1/2/t-ERK increases by Ang II in WT mice, and after blockage of ERK1/2 pathway with PD98059, the KCa3.1 protein expression was reduced in WT mice. In conclusion, the present study demonstrates that ACE2-Ang-(1-7)-Mas protects the myocardium from hypertension-induced injury, which is related to its inhibiting effect on KCa3.1 channels through ERK1/2 pathway. Our results reveal that KCa3.1 channel is likely to be a critical target on the ACE2-Ang-(1-7)-Mas axis for its protective role in myocardial fibrosis and changes of KCa3.1 induced by homeostasis of ACE-Ang II-AT1 axis and ACE2-Ang-(1-7)-Mas axis may be a new therapeutic target in myocardial fibrosis.

Wang, Q. Y., et al. (2015). "[Effect of Astragali Radix in improving early renal damage in metabolic syndrome rats through ACE2/Mas pathway]." Zhongguo Zhong Yao Za Zhi **40**(21): 4245-4250.

To study the expression of angiotensin converting enzyme 2 (ACE2) and angiotensin (Ang) 1-7 specific receptor Mas protain in renal blood vessels of metabolic syndrome (MS) rats and its anti-oxidative effect. A total of 80 male SD rats were divided into four groups: the normal control group (NC, the same volume of normal saline), the MS group (high fat diet), the MS + Astragali Radix group (MS + HQ, 6 g x kg (-1) x d (-1) in gavage) and the MS + Valsartan group (MS + XST, 30 mg x kg (-1) x d (-1) in gavage). After four weeks of intervention, their general indexes, biochemical indexes and blood pressure were measured; plasma and renal tissue Ang II, malondialdehyde (MDA) and superoxide demutase (SOD) levels were measured with radioimmunoassay. The protein expressions of Mas receptor, AT1R, ACE and ACE2 were detected by western blot analysis. According to the result, compared with the NC group, the MS group and the MS + HQ group showed significant increases in systolic and diastolic pressures, body weight, fasting glucose, fasting insulin, triglycerides, free fatty acid and Ang II level of MS rats ($P < 0.05$). The MS + XST group showed notable decreases in systolic and diastolic pressures than that of the MS group. The MS group showed significant increases in the SOD activity and NO level and decrease in the MDA level after being intervened with Astragali Radix. ACE and AT1R protein expressions in renal tissues of the MS group were higher than that in the NC group, but with lower ACE2 and -Mas receptor expressions (all $P < 0.05$). Compared with the MS group, the MS + HQ group showed significant increase in Mas receptor expression in renal tissues, whereas the MS + XST group showed notable decrease in AT1R (all $P < 0.05$). In conclusion, Astragali Radix can increase the Mas receptor expressions in renal tissues, decrease ACE expression and change local Ang II, MDA, NO and SOD in kidneys, so as to protect early damages in renal tissues.

Wang, S., et al. (2008). "Endocytosis of the receptor-binding domain of SARS-CoV spike protein together with virus receptor ACE2." Virus Res **136**(1-2): 8-15.

Cell entry of severe acute respiratory syndrome coronavirus (SARS-CoV) is mediated by the viral spike (S) protein. Amino acids 319-510 on the S protein have been mapped as the receptor-binding domain (RBD), which mediates binding to the SARS-CoV receptor angiotensin converting enzyme 2 (ACE2) on SARS-CoV susceptible cells. In this study, we expressed a fusion protein containing the human codon-optimized RBD of the SARS-CoV spike protein linked to the Fc portion of human IgG1 (named RBD-Fc) in HEK293 cells. The RBD-Fc protein was purified by affinity chromatography. The flow cytometry assay showed that the purified RBD-Fc protein could bind to ACE2. We demonstrated that the RBD spike protein alone could be internalized into SARS-CoV susceptible cells together with ACE2. We also showed that the removal of N-glycans from the RBD spike protein did not abolish this phenomenon. Our discoveries may have some implications for the development of the SARS vaccine.

Wang, W., et al. (2012). "Role of ACE2 in diastolic and systolic heart failure." Heart Fail Rev **17**(4-5): 683-691.

A novel angiotensin-converting enzyme (ACE) homolog, named ACE2, is a monocarboxypeptidase which metabolizes several peptides. ACE2 degrades Angiotensin (Ang) II, a peptide with vasoconstrictive/proliferative effects, to generate Ang-(1-7), which acting through its receptor Mas exerts vasodilatory/anti-proliferative actions. In addition, as ACE2 is a multifunctional enzyme and its actions on other vasoactive peptides can also contribute to its vasoactive effects including the apelin-13 and apelin-17 peptides. The discovery of ACE2 corroborates the establishment of two counter-regulatory arms within the renin-angiotensin system. The first one is formed by the classical pathway involving the ACE-Ang II-AT (1) receptor axis and the second arm is constituted by the ACE2-Ang 1-7/Mas receptor axis. Loss of

ACE2 enhances the adverse pathological remodeling susceptibility to pressure-overload and myocardial infarction. ACE2 is also a negative regulator of Ang II-induced myocardial hypertrophy, fibrosis, and diastolic dysfunction. The ACE2-Ang 1-7/Mas axis may represent new possibilities for developing novel therapeutic strategies for the treatment of hypertension and cardiovascular diseases. In this review, we will summarize the biochemical and pathophysiological aspects of ACE2 with a focus on its role in diastolic and systolic heart failure.

Wang, W., et al. (2014). "Heterozygote loss of ACE2 is sufficient to increase the susceptibility to heart disease." J Mol Med (Berl) **92**(8): 847-858.

UNLABELLED: Angiotensin-converting enzyme 2 (ACE2) metabolizes Ang II into Ang 1-7 thereby negatively regulating the renin-angiotensin system. However, heart disease in humans and in animal models is associated with only a partial loss of ACE2. ACE2 is an X-linked gene; and as such, we tested the clinical relevance of a partial loss of ACE2 by using female ACE2(+/+) (wildtype) and ACE2(+/-) (heterozygote) mice. Pressure overload in ACE2(+/-) mice resulted in greater LV dilation and worsening systolic and diastolic dysfunction. These changes were associated with increased myocardial fibrosis, hypertrophy, and upregulation of pathological gene expression. In response to Ang II infusion, there was increased NADPH oxidase activity and myocardial fibrosis resulting in the worsening of Ang II-induced diastolic dysfunction with a preserved systolic function. Ang II-mediated cellular effects in cultured adult ACE2(+/-) cardiomyocytes and cardiofibroblasts were exacerbated. Ang II-mediated pathological signaling worsened in ACE2(+/-) hearts characterized by an increase in the phosphorylation of ERK1/2 and JNK1/2 and STAT-3 pathways. The ACE2(+/-) mice showed an exacerbated pressor response with increased vascular fibrosis and stiffness. Vascular superoxide and nitrotyrosine levels were increased in ACE2(+/-) vessels consistent with increased vascular oxidative stress. These changes occurred with increased renal fibrosis and superoxide production. Partial heterozygote loss of ACE2 is sufficient to increase the susceptibility to heart disease secondary to pressure overload and Ang II infusion. KEY MESSAGE: Heart disease in humans with idiopathic dilated cardiomyopathy is associated with a partial loss of ACE2. Heterozygote female ACE2 mutant mice showed enhanced susceptibility to pressure overload-induced heart disease. Heterozygote female ACE2 mutant mice showed enhanced susceptibility to Ang II-induced heart and vascular diseases. Partial loss of ACE2 is sufficient to enhance the susceptibility to heart disease.

Wang, Y., et al. (2012). "Severe acute pancreatitis is associated with upregulation of the ACE2-angiotensin-(1-7)-Mas axis and promotes increased circulating angiotensin-(1-7)." Pancreatology **12**(5): 451-457.

BACKGROUND/OBJECTIVES: Angiotensin-converting enzyme 2 (ACE2), its product angiotensin-(1-7) and its receptor Mas may counteract the adverse effects of the ACE-angiotensin receptor II-AT (1) axis in many diseases. We examined the expression of these novel components of the rennin-angiotensin system in an experimental mouse model of severe acute pancreatitis (SAP). METHODS: SAP was induced by six intraperitoneal injections of caerulein, and mice were sacrificed at 2, 12, 24, 48 and 72 h post disease-induction (normal control group mice were sacrificed at 2 h post disease-induction). Tissue and blood were collected for biochemical detection, gene and protein expression by qRT-PCR and western blot analysis, enzyme-linked immunosorbent assay and immunohistology detection. RESULTS: Pancreatic ACE2 gene and protein expression, plasma and pancreatic angiotensin-(1-7) levels and Mas receptor gene and protein expression were significantly increased ($p < 0.05$) following SAP induction compared with the normal control group. CONCLUSIONS: Severe acute pancreatitis is associated with upregulation of the ACE2-angiotensin-(1-7)-Mas axis and promotes increased circulating angiotensin-(1-7). These results support the presence of an ACE2-angiotensin-(1-7)-Mas axis in pancreatitis.

Wang, Z. J., et al. (2017). "[Role of mesenteric lymph drainage in the balance of ACE/ACE2 in murine myocardium following hemorrhagic shock]." Zhongguo Ying Yong Sheng Li Xue Za Zhi **33**(1): 61-65.

OBJECTIVE: To observe the change of angiotensin converting enzyme (ACE) and ACE2 in the murine myocardium followed hemorrhagic shock and the role of post-hemorrhagic shock mesenteric lymph (PHSML) drainage. METHODS: Twenty-four male mice were ran-domly divided into control, sham, shock, and shock + drainage groups. A hemorrhagic shock model was established and then fluid resuscita-tion was performed to the mice in the shock and shock + drainage groups, and the PHMSL was drained in the shock + drainage group after fluid resuscitation. After 6 h of resuscitation in the shock and shock + drainage groups or corresponding time in the sham group, or after anesthesia in the control group, the myocardial tissues were harvested for the determination of the mRNA expressions of ACE, ACE2, angiotensin (Ang) type 1 receptor (AT1R), and Mas related G protein coupled receptor (Mas1R) using the method of qRT-

PCR, and the levels of Ang and Ang (1-7) using the method of ELISA. RESULTS: In the myocardial tissue of shock group, the ACE and AT1R mRNA expressions and Ang level were significantly increased than those of the control and sham groups, the ACE2 and Mas1R mRNA expressions were significantly de-creased than that of the control and sham groups, the Ang (1-7) level was decreased compared with the control group, the ratios of ACE/ACE2, Ang /Ang (1-7), and AT1R/Mas1R in the shock group were significantly increased than the control and sham groups. Meanwhile, PHSML drainage obviously suppressed the effects of hemorrhagic shock on these indices. CONCLUSIONS: Hemorrhagic shock up-regulated the ACE-Ang -AT1R axis, down-regulated the ACE2-Ang (1-7)-Mas1R axis, and induced the unbalance of ACE and ACE2 in myocardial tis-sue. PHSML drainage decreased the ACE-Ang -AT1R axis and increased the ACE2-Ang (1-7)-Mas1R axis, resulted in the balance of ACE and ACE2.

Warner, F. J., et al. (2005). "Angiotensin-converting enzyme 2 (ACE2), but not ACE, is preferentially localized to the apical surface of polarized kidney cells." J Biol Chem **280**(47): 39353-39362.

Angiotensin-converting enzyme-2 (ACE2) is a homologue of angiotensin-I converting enzyme (ACE), the central enzyme of the renin-angiotensin system (RAS). ACE2 is abundant in human kidney and heart and has been implicated in renal and cardiac function through its ability to hydrolyze Angiotensin II. Although ACE2 and ACE are both type I integral membrane proteins and share 61% protein sequence similarity, they display distinct modes of enzyme action and tissue distribution. This study characterized ACE2 at the plasma membrane of non-polarized Chinese hamster ovary (CHO) cells and polarized Madin-Darby canine kidney (MDCKII) epithelial cells and compared its cellular localization to its related enzyme, ACE, using indirect immunofluorescence, cell-surface biotinylation, Western analysis, and enzyme activity assays. This study shows ACE2 and ACE are both cell-surface proteins distributed evenly to detergent-soluble regions of the plasma membrane in CHO cells. However, in polarized MDCKII cells under steady-state conditions the two enzymes are differentially expressed. ACE2 is localized predominantly to the apical surface (approximately 92%) where it is proteolytically cleaved within its ectodomain to release a soluble form. Comparatively, ACE is present on both the apical (approximately 55%) and basolateral membranes (approximately 45%) where it is also secreted but differentially; the ectodomain cleavage of ACE is 2.5-fold greater from the apical surface than the basolateral surface. These studies suggest that both ACE2 and ACE are ectoenzymes that have distinct localization and secretion patterns that determine their role on the cell surface in kidney epithelium and in urine.

Wong, D. W., et al. (2007). "Loss of angiotensin-converting enzyme-2 (Ace2) accelerates diabetic kidney injury." Am J Pathol **171**(2): 438-451.

Diabetic nephropathy is one of the most common causes of end-stage renal failure, but the factors responsible for the development of diabetic nephropathy have not been fully elucidated. We examined the effect of deletion of the angiotensin-convert-ing enzyme 2 (Ace2) gene on diabetic kidney injury. Ace2(-/-) mice were crossed with Akita mice (Ins2(WT/C96Y)), a model of type 1 diabetes mellitus, and four groups of mice were studied at 3 months of age: Ace2(+/y)Ins2(WT/WT), Ace2(-/y)Ins2(WT/WT), Ace2(+/y) Ins2(WT/C96Y), and Ace2(-/y)Ins2(WT/C96Y). Ace2(-/y) Ins2(WT/C96Y) mice exhibited a twofold increase in the urinary albumin excretion rate compared with Ace2(+/y)Ins2(WT/C96Y) mice despite similar blood glucose levels. Ace2(-/y)Ins2(WT/C96Y) mice were the only group to exhibit increased mesangial matrix scores and glomerular basement membrane thicknesses compared with Ace2(+/y)Ins2(WT/WT) mice, accompanied by increased fibronectin and alpha-smooth muscle actin immunostaining in the glomeruli of Ace2(-/y) Ins2(WT/C96Y) mice. There were no differences in blood pressure or heart function to account for the exacerbation of kidney injury. Although kidney levels of angiotensin (Ang) II were not increased in the diabetic mice, treatment with an Ang II receptor blocker reduced urinary albumin excretion rate in Ace2(-/y)Ins2(WT/C96Y) mice, suggesting that acceleration of kidney injury in these mice is Ang II-mediated. We conclude that ACE2 plays a protective role in the diabetic kidney, and ACE2 is an important determinant of diabetic nephropathy.

Wong, T. P., et al. (2012). "Upregulation of ACE2-ANG-(1-7)-Mas axis in jejunal enterocytes of type 1 diabetic rats: implications for glucose transport." Am J Physiol Endocrinol Metab **303**(5): E669-681.

The inhibitory effects of the angiotensin-converting enzyme (ACE)-ANG II-angiotensin type 1 (AT (1)) receptor axis on jejunal glucose uptake and the reduced expression of this system in type 1 diabetes mellitus (T1DM) have been documented previously. The ACE2-ANG-(1-7)-Mas receptor axis is thought to oppose the actions of the ACE-ANG II-AT (1) receptor axis in heart, liver, and kidney. However, the possible involvement of the ACE2-

ANG-(1-7)-Mas receptor system on enhanced jejunal glucose transport in T1DM has yet to be determined. Rat everted jejunum and Caco-2 cells were used to determine the effects of ANG-(1-7) on glucose uptake and to study the ACE2-ANG-(1-7)-Mas receptor signaling pathway. Expression of target gene and protein in jejunal enterocytes and human Caco-2 cells were quantified using real-time PCR and Western blotting. T1DM increased jejunal protein and mRNA expression of ACE2 (by 59 and 173%, respectively) and Mas receptor (by 55 and 100%, respectively) in jejunum. One millimolar ANG-(1-7) reduced glucose uptake in jejunum and Caco-2 cells by 30.6 and 30.3%, respectively, effects that were abolished following addition of 1 muM A-779 (a Mas receptor blocker) or 1 muM GF-109203X (protein kinase C inhibitor) to incubation buffer for jejunum or Caco-2 cells, respectively. Finally, intravenous treatment of animals with ANG-(1-7) significantly improved oral glucose tolerance in T1DM but not control animals. In conclusion, enhanced activity of the ACE2-ANG-(1-7)-Mas receptor axis in jejunal enterocytes is likely to moderate the T1DM-induced increase in jejunal glucose uptake resulting from downregulation of the ACE-ANG II-AT (1) receptor axis. Therefore, altered activity of both ACE and ACE2 systems during diabetes will determine the overall rate of glucose transport across the jejunal epithelium.

Wosten-van Asperen, R. M., et al. (2011). "Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist." J Pathol **225**(4): 618-627.

Acute respiratory distress syndrome (ARDS) is a devastating clinical syndrome. Angiotensin-converting enzyme (ACE) and its effector peptide angiotensin (Ang) II have been implicated in the pathogenesis of ARDS. A counter-regulatory enzyme of ACE, ie ACE2 that degrades Ang II to Ang-(1-7), offers a promising novel treatment modality for this syndrome. As the involvement of ACE and ACE2 in ARDS is still unclear, this study investigated the role of these two enzymes in an animal model of ARDS. ARDS was induced in rats by intratracheal administration of LPS followed by mechanical ventilation. During ventilation, animals were treated with saline (placebo), losartan (Ang II receptor antagonist), or with a protease-resistant, cyclic form of Ang-(1-7) [cAng-(1-7)]. In bronchoalveolar lavage fluid (BALF) of ventilated LPS-exposed animals, ACE activity was enhanced, whereas ACE2 activity was reduced. This was matched by enhanced BALF levels of Ang II and reduced levels of Ang-(1-7). Therapeutic intervention with cAng-(1-7) attenuated the inflammatory mediator response, markedly decreased lung injury scores, and improved lung function, as evidenced by increased oxygenation. These data indicate that ARDS develops, in part, due to reduced pulmonary levels of Ang-(1-7) and that repletion of this peptide halts the development of ARDS.

Wu, H. T., et al. (2018). "Loss of angiotensin converting enzyme II (ACE2) accelerates the development of liver injury induced by thioacetamide." Exp Anim **67**(1): 41-49.

Angiotensin converting enzyme II (ACE2), an angiotensin converting enzyme (ACE) homologue that displays antagonist effects on ACE/angiotensin II (Ang II) axis in renin-angiotensin system (RAS), could play a protective role against liver damages. The purpose of this study is to investigate whether inflammation-mediated liver injury could be affected by ACE2 derived pathways in the RAS. Eight-weeks-old wild-type (WT; C57BL/6) and Ace2 KO (hemizygous Ace2(-/y)) male mice were used to induce liver fibrosis by thioacetamide (TAA) administration (0, 100, and 200 mg/kg BW). The mice administrated with TAA could be successfully induced liver fibrosis in a TAA-dose dependent manner. Compared to WT mice, the results show that Ace2 KO mice have high sensitive, and developed more serious reaction of hepatic inflammation and fibrosis by TAA administration. The physiological and pathological examinations demonstrated higher serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels, infiltration of white blood cells and fibrotic lesions within liver in the Ace2 KO mice. The severe liver damage of Ace2 KO mice were also confirmed by the evidence of higher expression of hepatic inflammation-related genes (IL-6 and Tnf) and fibrosis-related genes (Col1a1, Timp1 and Mmp9). Ace2 gene deficiency could lead to a severe inflammation and collagen remodeling in the liver administrated by TAA, and the responses lead the pathogenesis of liver fibrosis. Our studies provided the main messages and favorable study directions of relationship of Ace2 and liver disease.

Wu, S. J., et al. (2014). "[Differential expression in ACE2, Ang (1-7) and Mas receptor during progression of liver fibrosis in a rat model]." Zhonghua Gan Zang Bing Za Zhi **22**(2): 118-121.

OBJECTIVE: To investigate the changes in expression of the ACE2/Ang (1-7)/Mas receptor axis' components that occur during progression of liver fibrosis using a rat model system. METHODS: Thirty-six adult male Wistar rats, were randomly assigned to groups of normal control (n = 6; no manipulation) and liver fibrosis (n = 30; given a subcutaneous injection of 40% chronic carbon tetrachloride (CCl4)). At post-

injection days 15, 30, 45, 60 and 75, 1 control rat and 6 modeled rats were sacrificed for analysis. Histopathological analysis of liver tissue was performed with hematoxylin-eosin and rapid Masson staining. Protein expression level of Ang (1-7) was determined by enzyme-linked immunosorbent assay, and of ACE2 and Mas receptor was evaluated by immunohistochemistry. Real-time PCR was used to measure the mRNA expression levels of ACE2 and Mas receptor. RESULTS: The expression levels of ACE2, Ang (1-7) and Mas receptor showed a statistically significant upward trend that followed the progression of fibrosis up to post-injection day 60 (P less than 0.01), but the significant increase was not seen from day 60 to day 75. CONCLUSION: Each component of the ACE2/Ang (1-7)/Mas receptor axis shows differential expression during the development of liver fibrosis and may contribute to disease progression.

Wu, X., et al. (2018). "The Association Between ACE2 Gene Polymorphism and the Stroke Recurrence in Chinese Population." J Stroke Cerebrovasc Dis **27**(10): 2770-2780.

OBJECTIVES: The angiotensin-converting enzyme 2 (ACE2) is closely associated with cardiovascular disease and cerebrovascular disease. Most studies on ACE2 gene polymorphism focused on its relations with cardiovascular disease, but there was a lack of research on its relations with stroke. Our study aimed to explore the association between 4 single-nucleotidepolymorphisms (SNPs) of ACE2 gene polymorphism and stroke recurrence. DESIGN AND PARTICIPANTS: In our study, the case group included 125 stroke patients with recurrence and the control group included 153 patients without recurrence. Four SNPs (rs2106809, rs2285666, rs879922, and rs2074192) were genotyped by Ligase detection reaction. The association between stroke recurrence and SNPs were analyzed by multivariate logistic regression. RESULTS: We find no association between ACE2 gene polymorphism and stroke recurrence. Haplotype A-G-C may associate with the stoke recurrence of male patients. The recurrence risk of male stroke patients with hypertension history and rs2285666-C allele is 2.82 times as high as that of those without hypertension history but with T allele. Among male stroke patients with hypertension history, the recurrence risk of those with rs2285666-C allele is 2.38 times as high as those with T allele; and the recurrence risk of those with rs2106809-A allele is 2.12 times as high as those with G allele. But those recurrence risks lose their statistical significance after adjustment for other factors. CONCLUSIONS: We find no influence of ACE2 gene polymorphism on stroke recurrence and only find possible interaction between hypertension history and the ACE2 gene in male stroke patients.

Wysocki, J., et al. (2013). "Regulation of urinary ACE2 in diabetic mice." Am J Physiol Renal Physiol **305**(4): F600-611.

Angiotensin-converting enzyme-2 (ACE2) enhances the degradation of ANG II and its expression is altered in diabetic kidneys, but the regulation of this enzyme in the urine is unknown. Urinary ACE2 was studied in the db/db model of type 2 diabetes and stretozotocin (STZ)-induced type 1 diabetes during several physiological and pharmacological interventions. ACE2 activity in db/db mice was increased in the serum and to a much greater extent in the urine compared with db/m controls. Neither a specific ANG II blocker, telmisartan, nor an ACE inhibitor, captopril, altered the levels of urinary ACE2 in db/db or db/m control mice. High-salt diet (8%) increased whereas low-salt diet (0.1%) decreased urinary ACE2 activity in the urine of db/db mice. In STZ mice, urinary ACE2 was also increased, and insulin decreased it partly but significantly after several weeks of administration. The increase in urinary ACE2 activity in db/db mice reflected an increase in enzymatically active protein with two bands identified of molecular size at 110 and 75 kDa and was associated with an increase in kidney cortex ACE2 protein at 110 kDa but not at 75 kDa. ACE2 activity was increased in isolated tubular preparations but not in glomeruli from db/db mice. Administration of soluble recombinant ACE2 to db/m and db/db mice resulted in a marked increase in serum ACE2 activity, but no gain in ACE2 activity was detectable in the urine, further demonstrating that urinary ACE2 is of kidney origin. Increased urinary ACE2 was associated with more efficient degradation of exogenous ANG II (10(-9) M) in urine from db/db compared with that from db/m mice. Urinary ACE2 could be a potential biomarker of increased metabolism of ANG II in diabetic kidney disease.

Wysocki, J., et al. (2014). "ACE2 deficiency increases NADPH-mediated oxidative stress in the kidney." Physiol Rep **2**(3): e00264.

Abstract Angiotensin-converting enzyme 2 (ACE2) is highly expressed in the kidney and hydrolyzes angiotensin II (Ang II) to Ang (1-7). Since Ang II is a strong activator of oxidative stress, we reasoned that ACE2 could be involved in the regulation of renal oxidative stress by governing the levels of Ang II. We, therefore, assessed levels of oxidative stress in kidney cortex of ACE2 knockout and wild-type littermate mice under baseline conditions. We found multiple markers of increased oxidative stress in ACE2KO mice. NADPH oxidase

activity was increased in kidney cortex from ACE2KO mice as compared to WT (227 +/- 24% vs.100 +/- 19%, P < 0.001). However, kidney catalase and superoxide dismutase activities were not different between groups. Exogenous Ang II was degraded less efficiently by kidneys from ACE2KO mice than WT mice, and administration of an AT1R blocker (losartan 30 mg/kg/day) resulted in normalization of NADPH oxidase activity in the ACE2KO. These findings suggest that an AT1R-dependent mechanism contributes to increased ROS observed in the ACE2KO. This study demonstrates that genetic deficiency of ACE2 activity in mice fosters oxidative stress in the kidney in the absence of overt hypertension and is associated with reduced kidney capacity to hydrolyze Ang II. ACE2KO mice serve as a novel in vivo model to examine the role of overactivity of NADPH oxidase in kidney function.

Wysocki, J., et al. (2006). "ACE and ACE2 activity in diabetic mice." Diabetes **55**(7): 2132-2139.

ACE-related carboxypeptidase (ACE2) may counterbalance the angiotensin (ANG) II-promoting effects of ACE in tissues where both enzymes are found. Alterations in renal ACE and ACE2 expression have been described in experimental models of diabetes, but ACE2 activity was not assessed in previous studies. We developed a microplate-based fluorometric method for the concurrent determination of ACE and ACE2 activity in tissue samples. Enzymatic activity (relative fluorescence unit [RFU]. microg protein (-1). h (-1)) was examined in ACE and ACE2 knockout mice and in two rodent models of diabetes, the db/db and streptozotocin (STZ)-induced diabetic mice. In kidney cortex, preparations consisting mainly of proximal tubules and cortical collecting tubules, ACE2 activity had a strong positive correlation with ACE2 protein expression (90-kDa band) in both knockout models and their respective wild-type littermates (r = 0.94, P < 0.01). ACE activity, likewise, had a strong positive correlation with renal cortex ACE protein expression (170-kDa band) (r = 0.838, P < 0.005). In renal cortex, ACE2 activity was increased in both models of diabetes (46.7 +/- 4.4 vs. 22.0 +/- 4.7 in db/db and db/m, respectively, P < 0.01, and 22.1 +/- 2.8 vs. 13.1 +/- 1.5 in STZ-induced diabetic versus untreated mice, respectively, P < 0.05). ACE2 mRNA levels in renal cortex from db/db and STZ-induced diabetic mice, by contrast, were not significantly different from their respective controls. In cardiac tissue, ACE2 activity was lower than in renal cortex, and there were no significant differences between diabetic and control mice (db/db 2.03 +/- 0.23 vs. db/m 1.85 +/- 0.10; STZ-induced diabetic 0.42 +/- 0.04 vs. untreated 0.52 +/- 0.07 mice). ACE2 activity in renal cortex correlated positively with ACE2 protein in db/db and db/m mice (r = 0.666, P < 0.005) as well as in STZ-induced diabetic and control mice (r = 0.621, P < 0.05) but not with ACE2 mRNA (r = -0.468 and r = -0.522, respectively). We conclude that in renal cortex from diabetic mice, ACE2 expression is increased at the posttranscriptional level. The availability of an assay for concurrent measurement of ACE and ACE2 activity should be helpful in the evaluation of kidney-specific alterations in the balance of these two carboxypeptidases, which are involved in the control of local ANG II formation and degradation.

Yamaleyeva, L. M., et al. (2015). "Uterine artery dysfunction in pregnant ACE2 knockout mice is associated with placental hypoxia and reduced umbilical blood flow velocity." Am J Physiol Endocrinol Metab **309**(1): E84-94.

Angiotensin-converting enzyme 2 (ACE2) knockout is associated with reduced fetal weight at late gestation; however, whether uteroplacental vascular and/or hemodynamic disturbances underlie this growth-restricted phenotype is unknown. Uterine artery reactivity and flow velocities, umbilical flow velocities, trophoblast invasion, and placental hypoxia were determined in ACE2 knockout (KO) and C57Bl/6 wild-type (WT) mice at day 14 of gestation. Although systolic blood pressure was higher in pregnant ACE2 KO vs. WT mice (102.3 +/- 5.1 vs. 85.1 +/- 1.9 mmHg, n = 5-6), the magnitude of difference was similar to that observed in nonpregnant ACE2 KO vs. WT mice. Maternal urinary protein excretion, serum creatinine, and kidney or heart weights were not different in ACE2 KO vs. WT. Fetal weight and pup-to-placental weight ratio were lower in ACE2 KO vs. WT mice. A higher sensitivity to Ang II [pD2 8.64 +/- 0.04 vs. 8.5 +/- 0.03 (-log EC50)] and greater maximal contraction to phenylephrine (169.0 +/- 9.0 vs. 139.0 +/- 7.0% KMAX), were associated with lower immunostaining for Ang II receptor 2 and fibrinoid content of the uterine artery in ACE2 KO mice. Uterine artery flow velocities and trophoblast invasion were similar between study groups. In contrast, umbilical artery peak systolic velocities (60.2 +/- 4.5 vs. 75.1 +/- 4.5 mm/s) and the resistance index measured using VEVO 2100 ultrasound were lower in the ACE2 KO vs. WT mice. Immunostaining for pimonidazole, a marker of hypoxia, and hypoxia-inducible factor-2alpha were higher in the trophospongium and placental labyrinth of the ACE2 KO vs. WT. In summary, placental hypoxia and uterine artery dysfunction develop before major growth of the fetus occurs and may explain the fetal growth restricted phenotype.

Yang, P., et al. (2014). "Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury." Sci Rep **4**: 7027.

Since March 2013, the emergence of an avian-origin influenza A (H7N9) virus has raised concern in China. Although most infections resulted in respiratory illness, some severe cases resulted in acute respiratory distress syndrome (ARDS), which is a severe form of acute lung injury (ALI) that further contributes to morbidity. To date, no effective drugs that improve the clinical outcome of influenza A (H7N9) virus-infected patients have been identified. Angiotensin-converting enzyme (ACE) and ACE2 are involved in several pathologies such as cardiovascular functions, renal disease, and acute lung injury. In the current study, we report that ACE2 could mediate the severe acute lung injury induced by influenza A (H7N9) virus infection in an experimental mouse model. Moreover, ACE2 deficiency worsened the disease pathogenesis markedly, mainly by targeting the angiotensin II type 1 receptor (AT1). The current findings demonstrate that ACE2 plays a critical role in influenza A (H7N9) virus-induced acute lung injury, and suggest that might be a useful potential therapeutic target for future influenza A (H7N9) outbreaks.

Yang, P., et al. (2017). "[Pyr (1)]Apelin-13(1-12) Is a Biologically Active ACE2 Metabolite of the Endogenous Cardiovascular Peptide [Pyr (1)]Apelin-13." Front Neurosci **11**: 92.

Aims: Apelin is a predicted substrate for ACE2, a novel therapeutic target. Our aim was to demonstrate the endogenous presence of the putative ACE2 product [Pyr (1)]apelin-13(1-12) in human cardiovascular tissues and to confirm it retains significant biological activity for the apelin receptor in vitro and in vivo. The minimum active apelin fragment was also investigated. Methods and Results: [Pyr (1)]apelin-13 incubated with recombinant human ACE2 resulted in de novo generation of [Pyr (1)]apelin-13(1-12) identified by mass spectrometry. Endogenous [Pyr (1)]apelin-13(1-12) was detected by immunostaining in human heart and lung localized to the endothelium. Expression was undetectable in lung from patients with pulmonary arterial hypertension. In human heart [Pyr (1)]apelin-13(1-12) (pKi = 8.04 +/- 0.06) and apelin-13(F13A) (pKi = 8.07 +/- 0.24) competed with [(125)I]apelin-13 binding with nanomolar affinity, 4-fold lower than for [Pyr (1)]apelin-13 (pKi = 8.83 +/- 0.06) whereas apelin-17 exhibited highest affinity (pKi = 9.63 +/- 0.17). The rank order of potency of peptides to inhibit forskolin-stimulated cAMP was apelin-17 (pD2 = 10.31 +/- 0.28) > [Pyr (1)]apelin-13 (pD2 = 9.67 +/- 0.04) >/= apelin-13(F13A) (pD2 = 9.54 +/- 0.05) > [Pyr (1)]apelin-13(1-12) (pD2 = 9.30 +/- 0.06). The truncated peptide apelin-13(R10M) retained nanomolar potency (pD2 = 8.70 +/- 0.04) but shorter fragments exhibited low micromolar potency. In a beta-arrestin recruitment assay the rank order of potency was apelin-17 (pD2 = 10.26 +/- 0.09) >> [Pyr (1)]apelin-13 (pD2 = 8.43 +/- 0.08) > apelin-13(R10M) (pD2 = 8.26 +/- 0.17) > apelin-13(F13A) (pD2 = 7.98 +/- 0.04) >/= [Pyr (1)]apelin-13(1-12) (pD2 = 7.84 +/- 0.06) >> shorter fragments (pD2 < 6). [Pyr (1)]apelin-13(1-12) and apelin-13(F13A) contracted human saphenous vein with similar sub-nanomolar potencies and [Pyr (1)]apelin-13(1-12) was a potent inotrope in paced mouse right ventricle and human atria. [Pyr (1)]apelin-13(1-12) elicited a dose-dependent decrease in blood pressure in anesthetized rat and dose-dependent increase in forearm blood flow in human volunteers. Conclusions: We provide evidence that ACE2 cleaves [Pyr (1)]apelin-13 to [Pyr (1)]apelin-13(1-12) and this cleavage product is expressed in human cardiovascular tissues. We have demonstrated biological activity of [Pyr (1)]apelin-13(1-12) at the human and rodent apelin receptor in vitro and in vivo. Our data show that reported enhanced ACE2 activity in cardiovascular disease should not significantly compromise the beneficial effects of apelin based therapies for example in PAH.

Yang, W., et al. (2006). "Association study of ACE2 (angiotensin I-converting enzyme 2) gene polymorphisms with coronary heart disease and myocardial infarction in a Chinese Han population." Clin Sci (Lond) **111**(5): 333-340.

Results are accumulating that ACE2 (angiotensin I-converting enzyme 2) might act as a protective protein for cardiovascular diseases; however, only a few studies in human populations have been carried out. This prompted us to perform a case-control study to investigate the relationship of ACE2 polymorphisms with CHD (coronary heart disease) and MI (myocardial infarction). Three single nucleotide polymorphisms in the ACE2 gene (1075A/G, 8790A/G and 16854G/C) were genotyped by PCR-RFLP (restriction-fragment-length polymorphism) in 811 patients with CHD (of which 508 were patients with MI) and 905 normal controls in a Chinese population. The polymorphisms were in linkage disequilibrium (r (2)=0.854-0.973). Analyses were conducted by gender, because the ACE2 gene is on the X chromosome. In females, an association was detected with MI for 1075A/G (P=0.026; odds ratio=1.98) and 16854G/C (P=0.028; odds ratio=1.97) in recessive models after adjusting for covariates. In male subjects, two haplotypes (AAG and GGC) were common in frequency. In male subjects not consuming alcohol, the haplotype GGC was associated with a 1.76-fold risk of CHD [95% CI (confidence interval), 1.15-2.69; P=0.007] and a 1.77-fold risk of MI (95%

CI, 1.12-2.81; P=0.015) with environmental factors adjusted, when compared with the most common haplotype AAG. In conclusion, the results of the present study indicate that common genetic variants in the ACE2 gene might impact on MI in females, and may possibly interact with alcohol consumption to affect the risk of CHD and MI in Chinese males.

Zulli, A., et al. (2006). "Immunolocalization of ACE2 and AT2 receptors in rabbit atherosclerotic plaques." J Histochem Cytochem **54**(2): 147-150.

Evidence suggests that angiotensin type 2 receptor (AT2R) and angiotensin-converting enzyme 2 (ACE2) play a protective role in atherogenesis. These factors have not been identified in rabbit atherosclerotic plaques. Our goal was to localize ACE2 and AT2R in rabbit atherosclerotic tissues, and determine which cell types express these factors. New Zealand White rabbits were fed either a control diet or a 0.5% cholesterol diet (n=8/group) for 12 weeks. Paraffin-fixed thoracic aorta were serially sectioned and processed for immunohistochemistry using commercially available antibodies to ACE2, AT2R, RAM 11 (to identify macrophages), and alpha smooth muscle cell actin (alphaSMC) to identify smooth muscle cells and myofibroblasts. AT2R immunoreactivity, but not ACE2 immunoreactivity, was clearly present in endothelia overlying normal wall. However, both AT2R and ACE2 immunoreactivity were clearly present in endothelia overlying neo-intima formation and atherosclerotic plaques. Within plaques, both AT2R and ACE2 immunoreactivity were observed in macrophages and alphaSMC actin-positive cells. Examination of serial sections showed that the majority of cells were both ACE2- and AT2R-positive. Macrophages and alphaSMC actin-positive cells produce ACE2 and the AT2R in atherosclerotic plaques. Determining a role for these factors in the control of atherosclerosis will require additional studies.

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