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Stem Cell

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Stem Cell and ACE2 Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. 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: stem cell; ACE2; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. 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.

Chen, J., et al. (2013). "Angiotensin-converting enzyme 2 priming enhances the function of endothelial progenitor cells and their therapeutic efficacy." Hypertension **61**(3): 681-689.

Angiotensin-converting enzyme 2 (ACE2) is a lately discovered enzyme catalyzing Angiotensin II into Angiotensin 1-7. Angiotensin II has been reported to impair endothelial progenitor cell (EPC) function and is detrimental to stroke. Here, we studied the role of ACE2 in regulating EPC function in vitro and in vivo. EPCs were cultured from human renin and angiotensinogen transgenic (R+A+) mice and their controls (R-A-). In in vitro experiments, EPCs were transduced with lentivirus-ACE2 or lentivirus-green fluorescence protein. The effects of ACE2 overexpression on EPC function and endothelial NO synthase (eNOS)/nicotinamide adenine dinucleotide phosphate oxidase (Nox) expression were determined. ACE2, eNOS, and Nox inhibitors were used for pathway validation. In in vivo studies, the therapeutic efficacy of EPCs overexpressing ACE2 was determined at day 7 after ischemic stroke induced by middle cerebral artery occlusion. We found that (1) lentivirus-ACE2 transduction resulted in a 4-fold increase of ACE2 expression in EPCs. This was accompanied with an increase in eNOS expression and NO production, and a decrease in Nox2 and -4 expression and reactive oxygen species production. (2) ACE2 overexpression improved the abilities of EPC migration and tube formation, which were impaired in R+A+ mice. These effects were inhibited by ACE2 or eNOS inhibitor and further enhanced by Nox inhibitor. (3) Transfusion of lentivirus-ACE2-primed EPCs reduced cerebral infarct volume and neurological deficits, and increased cerebral microvascular density and angiogenesis. Our data demonstrate that ACE2 improves EPC function, via regulating eNOS and Nox pathways, and enhances the efficacy of EPC-based therapy for ischemic stroke.

Duan, Y., et al. (2018). "Loss of Angiotensin-Converting Enzyme 2 Exacerbates Diabetic Retinopathy by Promoting Bone Marrow Dysfunction." Stem Cells **36**(9): 1430-1440.

Angiotensin-converting enzyme 2 (ACE2) is the primary enzyme of the vasoprotective axis of the renin angiotensin system (RAS). We tested the hypothesis that loss of ACE2 would exacerbate diabetic retinopathy by promoting bone marrow dysfunction. ACE2(-/y) were crossed with Akita mice, a model of type 1 diabetes. When comparing the bone marrow of the ACE2(-/y) -Akita mice to that of Akita mice, we observed a reduction of both short-term and long-term repopulating hematopoietic stem cells, a shift of hematopoiesis toward myelopoiesis, and an impairment of lineage (-) c-kit (+) hematopoietic stem/progenitor cell (HS/PC) migration and proliferation. Migratory and proliferative dysfunction of these cells was corrected by exposure to angiotensin-1-7 (Ang-1-7), the protective peptide generated by ACE2. Over the duration of diabetes examined, ACE2 deficiency led to progressive reduction in electrical responses assessed by electroretinography and to increases in neural infarcts observed by fundus photography. Compared with Akita mice, ACE2(-/y) -Akita at 9-months of diabetes showed an increased number of acellular capillaries indicative of more severe diabetic retinopathy. In diabetic and control human subjects, CD34(+) cells, a key bone marrow HS/PC population, were assessed for changes in mRNA levels for MAS, the receptor for Ang-1-7. Levels were highest in CD34(+) cells from diabetics without retinopathy. Higher serum Ang-1-7 levels predicted protection from development of retinopathy in diabetics. Treatment with Ang-1-7 or alamandine restored the impaired migration function of CD34(+) cells from subjects with retinopathy. These data support that activation of the protective RAS within HS/PCs may represents a therapeutic strategy for prevention of diabetic retinopathy. Stem Cells 2018;36:1430-1440.

Gao, F., et al. (2014). "Mesenchymal stem cell-based angiotensin-converting enzyme 2 in treatment of acute lung injury rat induced by bleomycin." Exp Lung Res **40**(8): 392-403.

BACKGROUND: Acute lung injury (ALI) is a high incidence disease with no effective therapeutic method (mortality rate > 40%). The aim of this study was to find a new and effective therapeutic method for ALI. METHODS: After the isolation of human umbilical cord mesenchymal stem cells (HUMSCs) from cesarean fetus, we transfected the HUMSCs with Lenti-ACE2 (angiotensin-converting enzyme 2) viral particles. Then we evaluated the therapeutic effect of HUMSCs harboring ACE2 on ALI, which induced by bleomycin (BLM) in rat model. RESULTS: Our results showed that HUMSCs harboring ACE2 could attenuate ALI degree through reducing pulmonary inflammatory infiltration and degree of vascular permeability, repressing the mRNA level of pro-inflammatory cytokines, activating the mRNA level of anti-inflammatory cytokines and ACE2. Besides, results also demonstrated that HUMSCs harboring ACE2 gene had higher therapeutic effects to ALI than the single factor of HUMSCs or ACE2. CONCLUSIONS: This research provided clues for the development of effective therapeutic methods to ALI using stem cell transplantation and gene therapy.

Gurley, S. B. and T. M. Coffman (2008). "Angiotensin-converting enzyme 2 gene targeting studies in mice: mixed messages." Exp Physiol **93**(5): 538-542.

As a major regulator of blood pressure homeostasis, the renin-angiotensin system (RAS) has

been the subject of extensive scientific investigation. While the RAS was first discovered more than 100 years ago, several novel components of the system have been identified only in the last decade. One of these newer members of the RAS family is angiotensin-converting enzyme 2 (ACE2). Among the approaches used to establish a physiological role for ACE2 has been the generation of ACE2-null mouse lines using homologous recombination in embryonic stem cells. In the literature, there have been at least three lines of ACE2 knockout mice generated by gene targeting by different investigative groups. Interestingly, there are significant differences in some of the reported phenotypes of these distinct lines, especially with regard to their cardiovascular physiology. In this paper, we will review the results of published experiments using these ACE2-null mouse lines, highlighting similarities and differences in these studies and summarizing their contributions to our understanding of the physiological functions of this novel member of the RAS.

Haznedaroglu, I. C. and U. Y. Malkan (2016). "Local bone marrow renin-angiotensin system in the genesis of leukemia and other malignancies." Eur Rev Med Pharmacol Sci **20**(19): 4089-4111.

The existence of a local renin-angiotensin system (RAS) specific to the hematopoietic bone marrow (BM) microenvironment had been proposed two decades ago. Most of the RAS molecules including ACE, ACE2, AGT, AGTR1, AGTR2, AKR1C4, AKR1D1, ANPEP, ATP6AP2, CMA1, CPA3, CTSA, CTSD, CTSG, CYP11A1, CYP11B1, CYP11B2, CYP17A1, CYP21A2, DPP3, EGFR, ENPEP, GPER, HSD11B1, HSD11B2, IGF2R, KLK1, LNPEP, MAS1, MME, NR3C1, NR3C2, PREP, REN, RNPEP, and THOP1 are locally present in the BM microenvironment. Local BM RAS peptides control the hematopoietic niche, myelopoiesis, erythropoiesis, thrombopoiesis and the development of other cellular lineages. Local BM RAS is important in hematopoietic stem cell biology and microenvironment. Angiotensin II regulates the proliferation, differentiation, and engraftment of hematopoietic stem cells. Activation of Mas receptor or ACE2 promotes proliferation of CD34+ cells. BM contains a progenitor that expresses renin throughout development. Angiotensin II attenuates the migration and proliferation of CD34+ Cells and promotes the adhesion of both MNCs and CD34+ cells. Renin cells in hematopoietic organs are precursor B cells. The renin cell requires RBP-J to differentiate. Mutant renin-expressing hematopoietic precursors can cause leukemia. Deletion of RBP-J in the renin-expressing progenitors enriches the precursor B-cell gene programme. Mutant cells undergo a neoplastic transformation, and mice develop a highly penetrant B-cell leukemia with multi-organ infiltration and early death. Many biological conditions during the development and function of blood cells are mediated by RAS, such as apoptosis, cellular proliferation, intracellular signaling, mobilization, angiogenesis, and fibrosis. The aim of this paper is to review recent developments regarding the actions of local BM RAS in the genesis of leukemia and other malignancies molecules.

He, H., et al. (2015). "Mesenchymal Stem Cells Overexpressing Angiotensin-Converting Enzyme 2 Rescue Lipopolysaccharide-Induced Lung Injury." Cell Transplant **24**(9): 1699-1715.

Bone marrow-derived mesenchymal stem cells (MSCs), which have beneficial effects in acute lung injury (ALI), can serve as a vehicle for gene therapy. Angiotensin-converting enzyme 2 (ACE2), a counterregulatory enzyme of ACE that degrades angiotensin (Ang) II into Ang 1-7, has a protective role against ALI. Because ACE2 expression is severely reduced in the injured lung, a therapy targeted to improve ACE2 expression in lung might attenuate ALI. We hypothesized that MSCs overexpressing ACE2 would have further benefits in lipopolysaccharide (LPS)-induced ALI mice, when compared with MSCs alone. MSCs were transduced with ACE2 gene (MSC-ACE2) by a lentiviral vector and then infused into wild-type (WT) and ACE2 knockout (ACE2(-/y)) mice following an LPS-induced intratracheal lung injury. The results demonstrated that the lung injury of ALI mice was alleviated at 24 and 72 h after MSC-ACE2 transplantation. MSC-ACE2 improved the lung histopathology and had additional anti-inflammatory effects when compared with MSCs alone in both WT and ACE2(-/y) ALI mice. MSC-ACE2 administration also reduced pulmonary vascular permeability, improved endothelial barrier integrity, and normalized lung eNOS expression relative to the MSC group. The beneficial effects of MSC-ACE2 could be attributed to its recruitment into the injured lung and enhanced local expression of ACE2 protein without changing the serum ACE2 levels after MSC-ACE2 transplantation. The biological activity of the increased ACE2 protein decreased the Ang II amount and increased the Ang 1-7 level in the lung when compared with the ALI and MSC-only groups, thereby inhibiting the detrimental effects of accumulating Ang II. Therefore, compared to MSCs alone, the administration of MSCs overexpressing ACE2 resulted in a further improvement in the inflammatory response and pulmonary endothelial function of LPS-induced ALI mice. These additional benefits could be due to the degradation of Ang II that accompanies the targeted overexpression of ACE2 in the lung.

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.

Hilliard, L. M., et al. (2013). "The "his and hers" of the renin-angiotensin system." Curr Hypertens Rep **15**(1): 71-79.

Sex differences exist in the regulation of arterial pressure and renal function by the renin-angiotensin system (RAS). This may in part stem from a differential balance in the pressor and depressor arms of the RAS. In males, the ACE/AngII/AT (1)R pathways are enhanced, whereas, in females, the balance is shifted towards the ACE2/Ang (1-7)/MasR and AT (2)R pathways. Evidence clearly demonstrates that premenopausal women, as compared to aged-matched men, are protected from renal and cardiovascular disease, and this differential balance of the RAS between the sexes likely contributes. With aging, this cardiovascular protection in women is lost and this may be related to loss of estrogen postmenopause but the possible contribution of other sex hormones needs to be further examined. Restoration of these RAS depressor pathways in older women, or up-regulation of these in males, represents a therapeutic target that is worth pursuing.

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.

Ji, Y., et al. (2015). "Angiotensin-Converting Enzyme 2 Inhibits Apoptosis of Pulmonary Endothelial Cells During Acute Lung Injury Through Suppressing SMAD2 Phosphorylation." Cell Physiol Biochem **35**(6): 2203-2212.

BACKGROUND/AIMS: Angiotensin converting enzyme 2 (ACE2) has an established role in suppressing the severity of acute lung injury (ALI), especially when it was applied together with transplantation of human umbilical cord mesenchymal stem cells (uMSCs). Although the effects of ACE2 in ALI are believed to mainly result from its role in

hydrolyzing angiotensin II (AngII), which subsequently reduces the vascular tension and subsequent pulmonary accumulation of inflammatory cells, we and others have recently reported a possible role of ACE2 in suppressing the ALI-induced apoptosis of pulmonary endothelial cells. However, the underlying mechanisms remain undetermined. METHODS: Here, we analyzed the alteration in lung injury severity in ALI after ACE2, by histology and inflammatory cytokine levels. We analyzed apoptosis-associated proteins in lung after ALI, as well as in cultured endothelial cells treated with nitric oxide (NO). We overexpressed SMAD7 to inhibit SMAD2 signaling in cultured endothelial cells and examined its effects on NO-induced cell apoptosis. RESULTS: ACE2 alleviated severity of lung injury after ALI. ACE2 significantly decreased the ALI-induced apoptosis of pulmonary cells in vivo, and ACE2 protected endothelial cells against NO-induced apoptosis in vitro. NO induced phosphorylation of a key factor of transforming growth factor beta (TGF beta) receptor signaling, SMAD2, which could be dose-dependently inhibited by ACE2. Inhibition of SMAD2 phosphorylation through expression of its inhibitor SMAD7 significantly inhibited NO-induced cell apoptosis, without need for ACE2. CONCLUSION: Our data suggest that ACE2-mediated AngII degradation may inhibit AngII-mediated SMAD2-phophorylation, possibly through a TGFbeta-independent manner, which subsequently suppresses the ALI-induced cell death. Our results thus reveal a novel molecular pathway that controls the pathogenesis of ALI.

Joshi, S., et al. (2016). "Angiotensin converting enzyme versus angiotensin converting enzyme-2 selectivity of MLN-4760 and DX600 in human and murine bone marrow-derived cells." Eur J Pharmacol **774**: 25-33.

Angiotensin-converting enzymes, ACE and ACE2, are key members of renin angiotensin system. Activation of ACE2/Ang-(1-7) pathway enhances cardiovascular protective functions of bone marrow-derived stem/progenitor cells. The current study evaluated the selectivity of ACE2 inhibitors, MLN-4760 and DX-600, and ACE and ACE2 activities in human (hu) and murine (mu) bone marrow cells. Assays were carried out in hu and mu mononuclear cells (MNCs) and huCD34(+) cells or mu-lineage-depleted (muLin (-)) cells, human-recombinant (rh) enzymes, and mu-heart with enzyme-specific substrates. ACE or ACE2 inhibition by racemic MLN-4760, its isomers MLN-4760-A and MLN-4760-B, DX600 and captopril were characterized. MLN-4760-B is relatively less efficacious and less-selective than the racemate or MLN-4760-A at hu-rhACE2, and all three of them inhibited 43% rhACE. In huMNCs, MLN-4760-B detected 63% ACE2 with 28-fold selectivity over ACE. In huCD34(+) cells, MLN-4760-B detected 38% of ACE2 activity with 63-fold selectivity. In mu-heart and muMNCs, isomer B was 100- and 228-fold selective for ACE2, respectively. In muLin (-) cells, MLN-4760-B detected 25% ACE2 activity with a pIC50 of 6.3. The racemic mixture and MLN-4760-A showed lower efficacy and poor selectivity for ACE2 in MNCs and mu-heart. ACE activity detected by captopril was 32% and 19%, respectively, in huCD34(+) and muLin (-) cells. DX600 was less efficacious, and more selective for ACE2 compared to MLN-4760-B in all samples tested. These results suggest that MLN-4760-B is a better antagonist of ACE2 than DX600 at 10 microm concentration in human and murine bone marrow cells, and that these cells express more functional ACE2 than ACE.

Joshi, S., et al. (2019). "Hypoxic regulation of angiotensin-converting enzyme 2 and Mas receptor in human CD34(+) cells." J Cell Physiol **234**(11): 20420-20431.

CD34(+) hematopoietic stem/progenitor cells (HSPCs) are vasculogenic and hypoxia is a strong stimulus for the vasoreparative functions of these cells. Angiotensin-converting enzyme 2 (ACE2)/angiotensin-(1-7)/Mas receptor (MasR) pathway stimulates vasoprotective functions of CD34(+) cells. This study tested if ACE2 and MasR are involved in the hypoxic stimulation of CD34(+) cells. Cells were isolated from circulating mononuclear cells derived from healthy subjects (n = 46) and were exposed to normoxia (20% O2) or hypoxia (1% O2). Luciferase reporter assays were carried out in cells transduced with lentivirus carrying ACE2- or MasR- or a scramble-3'-untranslated region gene with a firefly luciferase reporter. Expressions or activities of ACE, angiotensin receptor Type 1 (AT1R), ACE2, and MasR were determined. In vitro observations were verified in HSPCs derived from mice undergoing hindlimb ischemia (HLI). In vitro exposure to hypoxia-increased proliferation and migration of CD34(+) cells in basal conditions or in response to vascular endothelial growth factor (VEGF) or stromal-derived factor 1alpha (SDF) compared with normoxia. Expression of ACE2 or MasR was increased relative to normoxia while ACE or AT1R expressions were unaltered. Luciferase activity was increased by hypoxia in cells transfected with the luciferase reporter plasmids coding for the ACE2- or MasR promoters relatively to the control. The effects of hypoxia were mimicked by VEGF or SDF under normoxia. Hypoxia-induced ADAM17-dependent shedding of functional ACE2 fragments. In mice

undergoing HLI, increased expression/activity of ACE2 and MasR were observed in the circulating HSPCs. This study provides compelling evidence for the hypoxic upregulation of ACE2 and MasR in CD34(+) cells, which likely contributes to vascular repair.

Klempin, F., et al. (2018). "Depletion of angiotensin-converting enzyme 2 reduces brain serotonin and impairs the running-induced neurogenic response." Cell Mol Life Sci **75**(19): 3625-3634.

Physical exercise induces cell proliferation in the adult hippocampus in rodents. Serotonin (5-HT) and angiotensin (Ang) II are important mediators of the pro-mitotic effect of physical activity. Here, we examine precursor cells in the adult brain of mice lacking angiotensin-converting enzyme (ACE) 2, and explore the effect of an acute running stimulus on neurogenesis. ACE2 metabolizes Ang II to Ang-(1-7) and is essential for the intestinal uptake of tryptophan (Trp), the 5-HT precursor. In ACE2-deficient mice, we observed a decrease in brain 5-HT levels and no increase in the number of BrdU-positive cells following exercise. Targeting the Ang II/AT1 axis by blocking the receptor, or experimentally increasing Trp/5-HT levels in the brain of ACE2-deficient mice, did not rescue the running-induced effect. Furthermore, mice lacking the Ang-(1-7) receptor, Mas, presented a normal neurogenic response to exercise. Our results identify ACE2 as a novel factor required for exercise-dependent modulation of adult neurogenesis and essential for 5-HT metabolism.

Lewis, S. R., et al. (2019). "Pharmacological agents for adults with acute respiratory distress syndrome." Cochrane Database Syst Rev **7**: CD004477.

BACKGROUND: Acute respiratory distress syndrome (ARDS) is a life-threatening condition caused by direct or indirect injury to the lungs. Despite improvements in clinical management (for example, lung protection strategies), mortality in this patient group is at approximately 40%. This is an update of a previous version of this review, last published in 2004. OBJECTIVES: To evaluate the effectiveness of pharmacological agents in adults with ARDS on mortality, mechanical ventilation, and fitness to return to work at 12 months. SEARCH METHODS: We searched CENTRAL, MEDLINE, Embase, and CINAHL on 10 December 2018. We searched clinical trials registers and grey literature, and handsearched reference lists of included studies and related reviews. SELECTION CRITERIA: We included randomized controlled trials (RCTs) comparing pharmacological agents with control (placebo or standard therapy) to treat adults with established ARDS. We excluded trials of nitric oxide, inhaled prostacyclins, partial liquid ventilation, neuromuscular blocking agents, fluid and nutritional interventions and medical oxygen. We excluded studies published earlier than 2000, because of changes to lung protection strategies for people with ARDS since this date. DATA COLLECTION AND ANALYSIS: Two review authors independently assessed studies for inclusion, extracted data, and assessed risks of bias. We assessed the certainty of evidence with GRADE. MAIN RESULTS: We included 48 RCTs with 6299 participants who had ARDS; two included only participants with mild ARDS (also called acute lung injury). Most studies included causes of ARDS that were both direct and indirect injuries. We noted differences between studies, for example the time of administration or the size of dose, and because of unclear reporting we were uncertain whether all studies had used equivalent lung protection strategies.We included five types of agents as the primary comparisons in the review: corticosteroids, surfactants, N-acetylcysteine, statins, and beta-agonists. We included 15 additional agents (sivelestat, mesenchymal stem cells, ulinastatin, anisodimine, angiotensin-converting enzyme (ACE) inhibitor, recombinant human ACE2 (palifermin), AP301, granulocyte-macrophage colony stimulating factor (GM-CSF), levosimendan, prostacyclins, lisofylline, ketaconazole, nitroglycerins, L-2-oxothiazolidine-4-carboxylic acid (OTZ), and penehyclidine hydrochloride).We used GRADE to downgrade outcomes for imprecision (because of few studies and few participants), for study limitations (e.g. high risks of bias) and for inconsistency (e.g. differences between study data).Corticosteroids versus placebo or standard therapyCorticosteroids may reduce all-cause mortality within three months by 86 per 1000 patients (with as many as 161 fewer to 19 more deaths); however, the 95% confidence interval (CI) includes the possibility of both increased and reduced deaths (risk ratio (RR) 0.77, 95% CI 0.57 to 1.05; 6 studies, 574 participants; low-certainty evidence). Due to the very low-certainty evidence, we are uncertain whether corticosteroids make little or no difference to late all-cause mortality (later than three months) (RR 0.99, 95% CI 0.64 to 1.52; 1 study, 180 participants), or to the duration of mechanical ventilation (mean difference (MD) -4.30, 95% CI -9.72 to 1.12; 3 studies, 277 participants). We found that ventilator-free days up to day 28 (VFD) may be improved with corticosteroids (MD 4.09, 95% CI 1.74 to 6.44; 4 studies, 494 participants; low-certainty evidence). No studies reported adverse events leading to discontinuation of study medication, or fitness to return to work at 12 months (FTR).Surfactants versus placebo or standard therapyWe are uncertain whether surfactants make little or no difference to early

mortality (RR 1.08, 95% CI 0.91 to 1.29; 9 studies, 1338 participants), or whether they reduce late all-cause mortality (RR 1.28, 95% CI 1.01 to 1.61; 1 study, 418 participants). Similarly, we are uncertain whether surfactants reduce the duration of mechanical ventilation (MD -2.50, 95% CI -4.95 to -0.05; 1 study, 16 participants), make little or no difference to VFD (MD -0.39, 95% CI -2.49 to 1.72; 2 studies, 344 participants), or to adverse events leading to discontinuation of study medication (RR 0.50, 95% CI 0.17 to 1.44; 2 studies, 88 participants). We are uncertain of these effects because we assessed them as very low-certainty. No studies reported FTR.N-aceytylcysteine versus placeboWe are uncertain whether N-acetylcysteine makes little or no difference to early mortality, because we assessed this as very low-certainty evidence (RR 0.64, 95% CI 0.32 to 1.30; 1 study, 36 participants). No studies reported late all-cause mortality, duration of mechanical ventilation, VFD, adverse events leading to study drug discontinuation, or FTR.Statins versus placeboStatins probably make little or no difference to early mortality (RR 0.99, 95% CI 0.78 to 1.26; 3 studies, 1344 participants; moderate-certainty evidence) or to VFD (MD 0.40, 95% CI -0.71 to 1.52; 3 studies, 1342 participants; moderate-certainty evidence). Statins may make little or no difference to duration of mechanical ventilation (MD 2.70, 95% CI -3.55 to 8.95; 1 study, 60 participants; low-certainty evidence). We could not include data for adverse events leading to study drug discontinuation in one study because it was unclearly reported. No studies reported late all-cause mortality or FTR.Beta-agonists versus placebo controlBeta-blockers probably slightly increase early mortality by 40 per 1000 patients (with as many as 119 more or 25 fewer deaths); however, the 95% CI includes the possibility of an increase as well as a reduction in mortality (RR 1.14, 95% CI 0.91 to 1.42; 3 studies, 646 participants; moderate-certainty evidence). Due to the very low-certainty evidence, we are uncertain whether beta-agonists increase VFD (MD -2.20, 95% CI -3.68 to -0.71; 3 studies, 646 participants), or make little or no difference to adverse events leading to study drug discontinuation (one study reported little or no difference between groups, and one study reported more events in the beta-agonist group). No studies reported late all-cause mortality, duration of mechanical ventilation, or FTR. AUTHORS' CONCLUSIONS: We found insufficient evidence to determine with certainty whether corticosteroids, surfactants, N-acetylcysteine, statins, or beta-agonists were effective at reducing mortality in people with ARDS, or duration of mechanical ventilation, or increasing ventilator-free days. Three studies awaiting classification may alter the conclusions of this review. As the potential long-term consequences of ARDS are important to survivors, future research should incorporate a longer follow-up to measure the impacts on quality of life.

Liao, X., et al. (2019). "Critical role of the endogenous renin-angiotensin system in maintaining self-renewal and regeneration potential of epidermal stem cells." Biochim Biophys Acta Mol Basis Dis **1865**(10): 2647-2656.

A tightly controlled activity of renin-angiotensin system (RAS) including renin, angiotensin-converting enzymes (ACEs), and angiotensin II (Ang II) receptors is critical not only for maintaining systemic hemodynamics and blood volume but also for controlling cell proliferation, differentiation, and tissue remodeling in target organs. ACE inhibitors or Ang II receptor type 1 (AT1R) blockers are widely used as first line drugs for the treatment of cardiovascular diseases that are caused by chronical activation of RAS. However, about 15% of patients using ACE inhibitors develop side effects in the skin and the underlying mechanisms have been poorly understood or even neglected. Herein we show an endogenous RAS in maintaining self-renewal and regeneration potential of epidermal stem cells (ESCs) thereby contributing to wound healing. Firstly, we found that ESCs may express ACE, and its members in wound edges were positively associated with wound healing in Captopril-treated rats. Secondly, we demonstrated that human ESCs had a functional RAS including ACE1, ACE2, Ang II, AT1R, and AT2R. ACE-Ang II axis maintains human ESC function via activation of both AT1R and AT2R, which are negatively regulated by each other. Ang II-induced activation of extracellular signal-regulated kinase (ERK) and signal transducers and activators of transcription (STAT)1 and STAT3 was mediated by the negative cross-talk between AT1R and AT2R in human ESCs. These results suggest that Ang II is a critical regulator of ESC function and ESC-mediated epidermal regeneration. Inappropriate interruption of Ang II-operated signaling may prejudice ESC function leading to impaired skin wound healing or even disease.

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, F., et al. (2014). "The functional study of human umbilical cord mesenchymal stem cells harbouring angiotensin-converting enzyme 2 in rat acute lung ischemia-reperfusion injury model." Cell Biochem Funct **32**(7): 580-589.

Acute lung ischemia-reperfusion injury (ALIRI) is featured as non-specific alveolar damage, lung edema and hypoxemia, often occurring within 72 h after surgery. It is the leading cause for primary graft failure and mortality after lung surgery and transplantation. Here we aimed to find a more effective therapeutic approach to treat ALIRI. We evaluated the combinational effects of human umbilical cord mesenchymal stem cells (HUMSCs) and angiotensin-converting enzyme 2 (ACE2) in the rat ALIRI model. HUMSCs were isolated for lentiviral-ACE2 transfection. Fifty rats were randomly divided into five groups: sham surgery, physiological saline (PS), ACE2, HUMSCs and HUMSCs-ACE2 group. Several physiological, biochemical and histological indicators were examined and compared among the five groups, such as blood oxygen saturation (Sat O2 %) and right ventricular systolic blood pressure (RVSBP), pulmonary morphology observations, several kinds of cell markers and the abundance of glutathione reductase (GR), glutathione peroxidase (GPX) and NAD (P)H quinone oxidoreductase (NQO1). Compared with HUMSCs and ACE2 groups, HUMSCs-ACE2 group showed lighter lung injuries, higher CD31 and vWF expression (endothelial cell surface markers), lower gamma-H2AX (DNA damage marker) and CD68 (inflammatory cell marker) and higher anti-oxidants expression (GR, GPX and NQO1). The results indicated that HUMSCs harbouring ACE2 were more effective than either HUMSCs or ACE2 alone in alleviating the ALIRI damages. The synergistic effects of HUMSCs and ACE2 provide informative clues for mechanism study and therapeutic method development of ALIRI.

Liu, Q., et al. (2020). "Mesenchymal stem cells modified with angiotensin-converting enzyme 2 are superior for amelioration of glomerular fibrosis in diabetic nephropathy." Diabetes Res Clin Pract **162**: 108093.

AIMS: This study aimed to detect the effect of angiotensin-converting enzyme (ACE) 2-modified mesenchymal stem cells (MSCs) on glomerular fibrosis in vitro and in vivo and investigate the underlying molecular mechanism. METHODS: MSCs transduced with the ACE2 gene (MSCs-ACE2) were cocultured with glomerular mesangial cells (GMCs) following Ang II stimulation. MSCs-ACE2 were transplanted into streptozotocin-induced diabetic rats. Physical, biochemical and morphological parameters were measured, and fibrotic indicators and renin-angiotensin system (RAS) components in GMCs and kidney tissues were assessed. RESULTS: The transduction efficiency of MSCs was as high as 85%. The modified MSCs secreted soluble ACE2 protein into the culture medium. After transplantation into rats with diabetes, MSCs-ACE2 targeted injured kidneys and enhanced local expression of ACE2. Compared with MSC treatment alone, MSC-ACE2 treatment was superior in reducing albuminuria and improving glomerulosclerosis. In vitro and in vivo, MSCs-ACE2 were more beneficial than MSCs alone in decreasing Ang II and increasing Ang1-7, thereby inhibiting the detrimental effects of Ang II accumulation by downregulating collagen I and fibronectin (FN) expression and inhibiting the transforming growth factor (TGF-beta)/Smad pathway. CONCLUSIONS: MSCs modified with ACE2 therapy have additional benefits to the progression of diabetic nephropathy (DN) by inhibiting renal RAS activation and reducing glomerular fibrosis.

Liu, Z., et al. (2018). "Mesenchymal stem cell-derived microvesicles alleviate pulmonary arterial hypertension by regulating renin-angiotensin system." J Am Soc Hypertens **12**(6): 470-478.

In recent years, microvesicles (MVs) derived from mesenchymal stem cells (MSCs) have been proved to be able to improve the outcome of pulmonary arterial hypertension (PAH) in many respects, but the underlying mechanisms of it still remain unclear. Because the renin-angiotensin system (RAS) has been found to be closely related to PAH, the present study was designed to investigate whether the effect of MSC-derived MVs on PAH was

correlated with RAS. MVs were isolated and purified from bone marrow MSCs. PAH rat models were established by a single intraperitoneal injection of 1% monocrotaline (MCT, 50 mg/Kg). In vivo study, after 3 weeks of MCT exposure, Nor group and PAH group were injected with 0.5 mL saline every 2 days through tail vein, whereas MVs group was injected with 0.5 mL saline containing 30mug MVs and A-779 + MVs group injected with 0.5 mL saline containing 120mug A-779 and 30mug MVs until 5 weeks of MCT exposure. Whereafter all the groups were analyzed for hemodynamic evaluation, right ventricular hypertrophy index, pulmonary vessel wall thickness index and pulmonary vessel lumen area index, the inflammation score, the collagen fiber volume fraction, the levels of Ang-(1-7) and Ang-in plasma and lung tissue, and the mRNA levels of ACE2 and ACE in the lung tissue. MVs derived from MSCs relieved the pulmonary artery pressure, right ventricular hypertrophy index, pulmonary vessel wall thickness index, pulmonary vessel lumen area index, the inflammation score, and the collagen fiber volume fraction. Moreover, in MVs group, ACE2 mRNA in the lung tissues and plasma levels of Ang-(1-7) were both upregulated compared with PAH group. On the contrary, ACE and Ang-II were decreased compared with PAH group. However, the enhanced protective effects observed in MVs group were diminished by the use of A-779, an inhibitor of Mas receptor in ACE2-Ang-(1-7)-Mas axis. MVs derived from bone marrow MSCs can exert beneficial effects against MCT-induced PAH in vivo, meanwhile shifting the balance from ACE-Ang-II-AT1R axis toward the ACE2-Ang-(1-7)-Mas axis, which might be one of the possible therapeutic mechanisms for MVs subcellular treatment.

Mallick, B., et al. (2009). "MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells." PLoS One **4**(11): e7837.

Severe acute respiratory syndrome (SARS), caused by the coronavirus SARS-CoV, is an acute infectious disease with significant mortality. A typical clinical feature associated with SARS is pulmonary fibrosis and associated lung failure. In the aftermath of the SARS epidemic, although significant progress towards understanding the underlying molecular mechanism of the infection has been made, a large gap still remains in our knowledge regarding how SARS-CoV interacts with the host cell at the onset of infection. The rapidly changing viral genome adds another variable to this equation. We have focused on a novel concept of microRNA (miRNA)-mediated host-virus interactions in bronchoalveolar stem cells (BASCs) at the onset of infection by correlating the "BASC-microRNome" with their targets within BASCs and viral genome. This work encompasses miRNA array data analysis, target prediction, and miRNA-mRNA enrichment analysis and develops a complex interaction map among disease-related factors, miRNAs, and BASCs in SARS pathway, which will provide some clues for diagnostic markers to view an overall interplay leading to disease progression. Our observation reveals the BASCs (Sca-1+ CD34+ CD45-Pecam-), a subset of Oct-4+ ACE2+ epithelial colony cells at the broncho-alveolar duct junction, to be the prime target cells of SARS-CoV infection. Upregulated BASC miRNAs-17*, -574-5p, and -214 are co-opted by SARS-CoV to suppress its own replication and evade immune elimination until successful transmission takes place. Viral Nucleocapsid and Spike protein targets seem to co-opt downregulated miR-223 and miR-98 respectively within BASCs to control the various stages of BASC differentiation, activation of inflammatory chemokines, and downregulation of ACE2. All these effectively accounts for a successful viral transmission and replication within BASCs causing continued deterioration of lung tissues and apparent loss of capacity for lung repair. Overall, this investigation reveals another mode of exploitation of cellular miRNA machinery by virus to their own advantage.

Min, F., et al. (2015). "Therapeutic effect of human umbilical cord mesenchymal stem cells modified by angiotensin-converting enzyme 2 gene on bleomycin-induced lung fibrosis injury." Mol Med Rep **11**(4): 2387-2396.

The aim of the present study was to evaluate the therapeutic effects of human umbilical cord mesenchymal stem cells (uMSCs) in the presence of angiotensinconverting enzyme 2 gene (ACE2; ACE2uMSCs) on bleomycin (BLM)induced lung injury and pulmonary fibrosis in mice. A total of 100 male C57BL/6 mice were divided at random into five groups (n=20) as follows: Control group, BLM group, ACE2 group, uMSC group and ACE2uMSC group. At 7, 14 and 28 days posttreatment, the following parameters were evaluated in lung tissue: Oxidation indexes [malondialedehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and oxidized glutathione (GSSG)]; fibrosis factors [tumor necrosis factor (TNF)alpha, interferon (IFN)gamma and transforming growth factor (TGF)beta]; inflammatory cytokines [Interleukin (IL)1, IL2, IL6 and IL10]; ACE2 gene expression; hydroxyproline and collagen type 1 messenger RNA (mRNA) concentration; as well as matrix metalloproteinase (MMPs; 2 and 9) and tissue inhibitor of metalloproteinase (TIMP)14 expression. ACE2uMSC injection following bleomycin pretreatment significantly alleviated lung injury in mice. In addition, treatment with ACE2uMSCs demonstrated a stronger therapeutic

effect than ACE2 or uMSC treatment alone, indicated by decreased expression of MDA, GSSG, TNFalpha, IFNgamma, TGFbeta, IL1, IL2, IL6, collagen type 1 mRNA, MMPs and TIMPs as well as hydroxyproline concentration, and upregulation of SOD, GSH and ACE2 and IL10. In conclusion, the results of the present study demonstrated that ACE2 and uMSCs had a synergistic therapeutic effect on bleomycininduced acute lung injury.

Qi, Y., et al. (2013). "Diminazene aceturate enhances angiotensin-converting enzyme 2 activity and attenuates ischemia-induced cardiac pathophysiology." Hypertension **62**(4): 746-752.

Angiotensin-converting enzyme 2 (ACE2) plays a critical role against myocardial infarction (MI). We hypothesized that activation of intrinsic ACE2 would be protective against ischemia-induced cardiac pathophysiology. Diminazene aceturate (DIZE), a small molecule ACE2 activator, has been used to evaluate this hypothesis. DIZE (15 mg/kg per day, s.c.) was injected 2 days before MI surgery and continued throughout the study period. MI rats showed a 62% decrease in fractional shortening (%; control, 51.1+/-3.2; DIZE alone, 52.1+/-3.2; MI, 19.1+/-3.0), a 55% decrease in contractility (dP/dtmax mm Hg/s; control, 9480+/-425.3; DIZE alone, 9585+/-597.4; MI, 4251+/-657.7), and a 27% increase in ventricular hypertrophy (mg/mm; control, 26.5+/-1.5; DIZE alone, 26.9+/-1.4; MI, 33.4+/-1.1). DIZE attenuated the MI-induced decrease in fractional shortening by 89%, improved dP/dtmax by 92%, and reversed ventricular hypertrophy by 18%. MI also significantly increased ACE and angiotensin type 1 receptor levels but decreased ACE2 activity by 40% (control, 246.2+/-25.1; DIZE alone, 254.2+/-20.6; MI, 148.9+/-29.2; RFU/min), which was reversed by DIZE treatment. Thus, DIZE treatment decreased the infarct area, attenuated LV remodeling post-MI, and restored normal balance of the cardiac renin-angiotensin system. In addition, DIZE treatment increased circulating endothelial progenitor cells, increased engraftment of cardiac progenitor cells, and decreased inflammatory cells in peri-infarct cardiac regions. All of the beneficial effects associated with DIZE treatment were abolished by C-16, an ACE2 inhibitor. Collectively, DIZE and DIZE-like small molecules may represent promising new therapeutic agents for MI.

Sato, T., et al. (2017). "ELABELA-APJ axis protects from pressure overload heart failure and angiotensin II-induced cardiac damage." Cardiovasc Res **113**(7): 760-769.

Aims: Elabela/Toddler/Apela (ELA) has been identified as a novel endogenous peptide ligand for APJ/Apelin receptor/Aplnr. ELA plays a crucial role in early cardiac development of zebrafish as well as in maintenance of self-renewal of human embryonic stem cells. Apelin was the first identified APJ ligand, and exerts positive inotropic heart effects and regulates the renin-angiotensin system. The aim of this study was to investigate the biological effects of ELA in the cardiovascular system. Methods and results: Continuous infusion of ELA peptide significantly suppressed pressure overload-induced cardiac hypertrophy, fibrosis and impaired contractility in mice. ELA treatment reduced mRNA expression levels of genes associated with heart failure and fibrosis. The cardioprotective effects of ELA were diminished in APJ knockout mice, indicating that APJ is the key receptor for ELA in the adult heart. Mechanistically, ELA downregulated angiotensin-converting enzyme (ACE) expression in the stressed hearts, whereas it showed little effects on angiotensin-converting enzyme 2 (ACE2) expression, which are distinct from the effects of Apelin. FoxM1 transcription factor, which induces ACE expression in the stressed hearts, was downregulated by ELA but not by Apelin. ELA antagonized angiotensin II-induced hypertension, cardiac hypertrophy, and fibrosis in mice. Conclusion: The ELA-APJ axis protects from pressure overload-induced heart failure possibly via suppression of ACE expression and pathogenic angiotensin II signalling. The different effects of ELA and Apelin on the expression of ACE and ACE2 implicate fine-tuned mechanisms for a ligand-induced APJ activation and downstream signalling.

Shenoy, V., et al. (2013). "Diminazene attenuates pulmonary hypertension and improves angiogenic progenitor cell functions in experimental models." Am J Respir Crit Care Med **187**(6): 648-657.

RATIONALE: Studies have demonstrated that angiotensin-converting enzyme 2 (ACE2) plays a protective role against lung diseases, including pulmonary hypertension (PH). Recently, an antitrypanosomal drug, diminazene aceturate (DIZE), was shown to exert an "off-target" effect of enhancing the enzymatic activity of ACE2 in vitro. OBJECTIVES: To evaluate the pharmacological actions of DIZE in experimental models of PH. METHODS: PH was induced in male Sprague Dawley rats by monocrotaline, hypoxia, or bleomycin challenge. Subsets of animals were simultaneously treated with DIZE. In a separate set of experiments, DIZE was administered after 3 weeks of PH induction to determine whether the drug could reverse PH. MEASUREMENTS AND MAIN RESULTS: DIZE treatment significantly prevented the development of PH in all of the animal models studied. The protective effects were associated with an increase in the

vasoprotective axis of the lung renin-angiotensin system, decreased inflammatory cytokines, improved pulmonary vasoreactivity, and enhanced cardiac function. These beneficial effects were abolished by C-16, an ACE2 inhibitor. Initiation of DIZE treatment after the induction of PH arrested disease progression. Endothelial dysfunction represents a hallmark of PH pathophysiology, and growing evidence suggests that bone marrow-derived angiogenic progenitor cells contribute to endothelial homeostasis. We observed that angiogenic progenitor cells derived from the bone marrow of monocrotaline-challenged rats were dysfunctional and were repaired by DIZE treatment. Likewise, angiogenic progenitor cells isolated from patients with PH exhibited diminished migratory capacity toward the key chemoattractant stromal-derived factor 1alpha, which was corrected by in vitro DIZE treatment. CONCLUSIONS: Our results identify a therapeutic potential of DIZE in PH therapy.

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.

Strawn, W. B., et al. (2004). "Renin-angiotensin system expression in rat bone marrow haematopoietic and stromal cells." Br J Haematol **126**(1): 120-126.

The existence of a bone marrow renin-angiotensin system (RAS) is evidenced by the association of renin, angiotensin converting enzyme (ACE), and angiotensin (Ang) II and its AT (1) and AT (2) receptors with both normal and disturbed haematopoiesis. The expression of RAS components by rat unfractionated bone marrow cells (BMC), haematopoietic-lineage BMC and cultured marrow stromal cells (MSC) was investigated to determine which specific cell types may contribute to a local bone marrow RAS. The mRNAs for angiotensinogen, renin, ACE, and AT (1a) and AT (2) receptors were present in BMC and in cultured MSC; ACE2 mRNA was detected only in BMC. Two-colour flow fluorocytometry analysis showed immunodetectable angiotensinogen, ACE, AT (1) and AT (2) receptors, and Ang II, as well as binding of Ang II to AT (1) and AT (2) receptors, in CD4(+), CD11b/c (+), CD45R (+) and CD90(+) BMC and cultured MSC; renin was found in all cell types with the exception of CD4(+) BMC. Furthermore, Ang II was detected by radioimmunoassay in MSC homogenates as well as conditioned culture medium. The presence of Ang II receptors in both haematopoietic-lineage BMC and MSC, and the de novo synthesis of Ang II by MSC suggest a potential autocrine-paracrine mechanism for local RAS-mediated regulation of haematopoiesis.

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.

Wang, L. and P. S. Leung (2013). "The role of renin-angiotensin system in cellular differentiation: implications in pancreatic islet cell development and islet transplantation." Mol Cell Endocrinol **381**(1-2): 261-271.

In addition to the well-characterized circulating renin-angiotensin system (RAS), local RAS has been identified recently in diverse tissues and organs. The presence of key components of the RAS in local tissues is important for our understanding of the patho-physiological mechanism (s) of several metabolic diseases, and may serve as a major therapeutic target for cardiometabolic syndromes. Locally generated and physiologically active RAS components have functions that are distinct from the classical vasoconstriction and fluid homeostasis actions of systemic RAS and cater specifically for local tissues. Local RAS can affect islet-cell function and structure in the adult pancreas as well as proliferation and differentiation of pancreatic stem/progenitor cells during development. Differentiation of stem/progenitor cells into insulin-expressing cells suitable for therapeutic transplantation offers a desperately needed new approach for replacement of glucose-responsive insulin producing cells in diabetic patients. Given that the generation of functional and transplantable islet cells has proven to be difficult, elucidation of RAS involvement in cellular regeneration and differentiation may propel pancreatic stem/progenitor cell development and thus beta-cell regeneration forward. This review provides a critical appraisal of current research progress on the role of the RAS, including the newly characterized ACE2/Ang-(1-7)/Mas axis in the proliferation, differentiation, and maturation of pancreatic stem/progenitor cells. It is thus plausible to propose that the AT1 stimulation could be a repair mechanism involving the AT2R as well as the ACE2/Ang-(1-7)/Mas axis in directing beta-cell development in diabetic patients using genetic and pharmaceutical manipulation of the RAS.

Xiao, F., et al. (2014). "Characterization of angiotensin-converting enzyme 2 ectodomain shedding from mouse proximal tubular cells." PLoS One **9**(1): e85958.

Angiotensin-converting enzyme 2 (ACE2) is highly expressed in the kidney proximal tubule, where it cleaves angiotensin (Ang) II to Ang-(1-7). Urinary ACE2 levels increase in diabetes, suggesting that ACE2 may be shed from tubular cells. The aim of this study was to determine if ACE2 is shed from proximal tubular cells, to characterize ACE2 fragments, and to study pathways for shedding. Studies involved primary cultures of mouse proximal tubular cells, with ACE2 activity measured using a synthetic substrate, and analysis of ACE2 fragments by immunoblots and mass spectrometry. The culture media from mouse proximal tubular cells demonstrated a time-dependent increase in ACE2 activity, suggesting constitutive ACE2 shedding. ACE2 was detected in media as two bands at approximately 90 kDa and approximately 70 kDa on immunoblots. By contrast, full-length ACE2 appeared at approximately 100 kDa in cell lysates or mouse kidney cortex. Mass spectrometry of the two deglycosylated fragments identified peptides matching mouse ACE2 at positions 18-706 and 18-577, respectively. The C-terminus of the 18-706 peptide fragment contained a non-tryptic site, suggesting that Met (706) is a candidate ACE2 cleavage site. Incubation of cells in high D-glucose (25 mM) (and to a lesser extent Ang II) for 48-72 h increased ACE2 activity in the media ($p<0.001$), an effect blocked by inhibition of a disintegrin and metalloproteinase (ADAM)17. High D-glucose increased ADAM17 activity in cell lysates ($p<0.05$). These data indicate that two glycosylated ACE2 fragments are constitutively shed from mouse proximal tubular cells. ACE2 shedding is stimulated by high D-glucose, at least partly via an ADAM17-mediated pathway. The results suggest that proximal tubular shedding of ACE2 may increase in diabetes, which could enhance degradation of Ang II in the tubular lumen, and increase levels of Ang-(1-7).

Yang, J., et al. (2016). "Pathological Ace2-to-Ace enzyme switch in the stressed heart is transcriptionally controlled by the endothelial Brg1-FoxM1 complex." Proc Natl Acad Sci U S A **113**(38): E5628-5635.

Genes encoding angiotensin-converting enzymes (Ace and Ace2) are essential for heart function regulation. Cardiac stress enhances Ace, but suppresses Ace2, expression in the heart, leading to a net production of angiotensin II that promotes cardiac hypertrophy and fibrosis. The regulatory mechanism that underlies the Ace2-to-Ace pathological switch, however, is unknown. Here we report that the Brahma-

related gene-1 (Brg1) chromatin remodeler and forkhead box M1 (FoxM1) transcription factor cooperate within cardiac (coronary) endothelial cells of pathologically stressed hearts to trigger the Ace2-to-Ace enzyme switch, angiotensin I-to-II conversion, and cardiac hypertrophy. In mice, cardiac stress activates the expression of Brg1 and FoxM1 in endothelial cells. Once activated, Brg1 and FoxM1 form a protein complex on Ace and Ace2 promoters to concurrently activate Ace and repress Ace2, tipping the balance to Ace2 expression with enhanced angiotensin II production, leading to cardiac hypertrophy and fibrosis. Disruption of endothelial Brg1 or FoxM1 or chemical inhibition of FoxM1 abolishes the stress-induced Ace2-to-Ace switch and protects the heart from pathological hypertrophy. In human hypertrophic hearts, BRG1 and FOXM1 expression is also activated in endothelial cells; their expression levels correlate strongly with the ACE/ACE2 ratio, suggesting a conserved mechanism. Our studies demonstrate a molecular interaction of Brg1 and FoxM1 and an endothelial mechanism of modulating Ace/Ace2 ratio for heart failure therapy.

Zhang, X., et al. (2015). "Combination therapy with human umbilical cord mesenchymal stem cells and angiotensin-converting enzyme 2 is superior for the treatment of acute lung ischemia-reperfusion injury in rats." Cell Biochem Funct **33**(3): 113-120.

Acute lung ischemia-reperfusion injury (ALIRI) is a serious disease that seriously affects human's life. In this study, we aimed to explore a more effective treatment method by combining human umbilical cord mesenchymal stem cells (HUMSCs) and angiotensin-converting enzyme 2 (ACE2) for ALIRI. Fifty rats were firstly divided into five groups, namely sham surgery group (sham) and four model groups (model, ACE2, HUMSCs and HUMSCs + ACE2) that were reperfused with 0.1 ml physiological saline (PS), 0.1 ml PS containing 1 x 10(6) lentiviral-ACE2/HUMSCs/ACE2 + UMSCs, respectively. Quantitative reverse transcription-PCR (qRT-PCR) and western blot assays were then conducted to detect the messenger RNA (mRNA) and protein levels of inflammatory cytokines [intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), tumour necrosis factor alpha (TNF-alpha), nuclear factor kappaB (NF-kappaB), platelet-derived growth factor (PDGF) and angiotensin II (Ang II)], antioxidant proteins [NAD (P)H quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1)], DNA damage and apoptotic indicators [BCL2-associated X (Bax), cleaved caspase-3 (C-Csp 3), cleaved-poly (ADP-ribose) polymerase (C-PARP), Y-H2AX], anti-apoptotic indicator (Bcl-2) and smooth muscle cell proliferation indicator [connexin 43 (Cx43)]. According to the qRT-PCR and western results, the mRNA and protein expression levels of ICAM-1, VCAM-1, TNF-alpha, NF-kappaB, PDGF, Bax, C-Csp 3, C-PARP and Y-H2AX were significantly higher in model group than those in sham group and they were significantly reduced by HUMSCs or ACE2 treatment ($P < 0.05$). On the contrary, Bcl-2 showed an opposite expression trend with the previous proteins. The mRNA and protein levels of NQO1 and HO-1 were sequentially increased in sham, model, ACE2, HUMSCs and HUMSCs + ACE2 groups. Besides, HUMSCs combined with ACE2 exhibited a better inhibition effect on ALIRI than HUMSCs or ACE2 alone ($P < 0.05$). In summary, HUMSCs combined with ACE2 was demonstrated to have the best therapeutic effect on ALIRI through anti-inflammation, oxidative stress and anti-apoptotic processes.

Zulli, A., et al. (2008). "Co-localization of angiotensin-converting enzyme 2-, octomer-4- and CD34-positive cells in rabbit atherosclerotic plaques." Exp Physiol **93**(5): 564-569.

Angiotensin-converting enzyme 2 (ACE2) is a novel enzyme with possible implications in the treatment of blood pressure disorders. Recent evidence suggests that an upregulation of ACE2 can be stimulated by all-trans retinoic acid (at-RA); however, at-RA also affects regulation of the stem-cell marker octomer-4 (Oct-4) and thus cellular differentiation. We have previously shown that smooth muscle cells and macrophages present within rabbit atherosclerotic plaques are positive for ACE2, Oct-4 and the haematopoietic stem-cell marker CD34. Thus, to provide evidence that possible at-RA treatment could affect both plaque cellular biology (via effects on cellular differentiation) and blood pressure (via ACE2), it is vital to show that cells with atherosclerotic plaques co-express all three markers. Thus, we sought to provide evidence that a subset of cells within atherosclerotic plaques is positive for ACE2, Oct-4 and CD34. We used New Zealand White rabbits that were fed a control diet supplemented with 0.5% cholesterol plus 1% methionine for 4 weeks and then allowed to consume a normal diet for 10 weeks. Immunohistochemistry was performed by standard techniques. We report that ACE2, Oct-4 and CD34 were all present within atherosclerotic plaques. Although macrophages were positive for all three markers, spindle-shaped cells in the media did not show all three markers. The endothelium overlying normal arterial wall showed positive Oct-4 and ACE2 immunoreactivity, but CD34 immunoreactivity was patchy, indicating that such cells might not have fully differentiated. It is concluded that cells in atherosclerotic plaques express co-express ACE2, Oct-

4 and CD34. Further studies aimed at establishing the effects of all-trans retinoic acid on blood pressure and atherosclerotic cell differentiation are warranted.

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References

1. Baidu. http://www.baidu.com. 2020.
2. Cancer Biology. http://www.cancerbio.net. 2020.
3. Chen, J., et al. (2013). "Angiotensin-converting enzyme 2 priming enhances the function of endothelial progenitor cells and their therapeutic efficacy." Hypertension 61(3): 681-689.
4. Duan, Y., et al. (2018). "Loss of Angiotensin-Converting Enzyme 2 Exacerbates Diabetic Retinopathy by Promoting Bone Marrow Dysfunction." Stem Cells 36(9): 1430-1440.
5. Gao, F., et al. (2014). "Mesenchymal stem cell-based angiotensin-converting enzyme 2 in treatment of acute lung injury rat induced by bleomycin." Exp Lung Res 40(8): 392-403.
6. Google. http://www.google.com. 2020.
7. Gurley, S. B. and T. M. Coffman (2008). "Angiotensin-converting enzyme 2 gene targeting studies in mice: mixed messages." Exp Physiol 93(5): 538-542.
8. Haznedaroglu, I. C. and U. Y. Malkan (2016). "Local bone marrow renin-angiotensin system in the genesis of leukemia and other malignancies." Eur Rev Med Pharmacol Sci 20(19): 4089-4111.
9. 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.
10. He, H., et al. (2015). "Mesenchymal Stem Cells Overexpressing Angiotensin-Converting Enzyme 2 Rescue Lipopolysaccharide-Induced Lung Injury." Cell Transplant 24(9): 1699-1715.
11. Hilliard, L. M., et al. (2013). "The "his and hers" of the renin-angiotensin system." Curr Hypertens Rep 15(1): 71-79.
12. 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.
13. Ji, Y., et al. (2015). "Angiotensin-Converting Enzyme 2 Inhibits Apoptosis of Pulmonary Endothelial Cells During Acute Lung Injury Through Suppressing SMAD2 Phosphorylation." Cell Physiol Biochem 35(6): 2203-2212.
14. Joshi, S., et al. (2016). "Angiotensin converting enzyme versus angiotensin converting enzyme-2 selectivity of MLN-4760 and DX600 in human and murine bone marrow-derived cells." Eur J Pharmacol 774: 25-33.
15. Joshi, S., et al. (2019). "Hypoxic regulation of angiotensin-converting enzyme 2 and Mas receptor in human CD34(+) cells." J Cell Physiol 234(11): 20420-20431.
16. Journal of American Science. http://www.jofamericanscience.org. 2020.
17. Klempin, F., et al. (2018). "Depletion of angiotensin-converting enzyme 2 reduces brain serotonin and impairs the running-induced neurogenic response." Cell Mol Life Sci 75(19): 3625-3634.
18. Lewis, S. R., et al. (2019). "Pharmacological agents for adults with acute respiratory distress syndrome." Cochrane Database Syst Rev 7: CD004477.
19. Liao, X., et al. (2019). "Critical role of the endogenous renin-angiotensin system in maintaining self-renewal and regeneration potential of epidermal stem cells." Biochim Biophys Acta Mol Basis Dis 1865(10): 2647-2656.
20. Life Science Journal. http://www.lifesciencesite.com. 2020.
21. 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.
22. Liu, F., et al. (2014). "The functional study of human umbilical cord mesenchymal stem cells harbouring angiotensin-converting enzyme 2 in rat acute lung ischemia-reperfusion injury model." Cell Biochem Funct 32(7): 580-589.
23. Liu, Q., et al. (2020). "Mesenchymal stem cells modified with angiotensin-converting enzyme 2 are superior for amelioration of glomerular fibrosis in diabetic nephropathy." Diabetes Res Clin Pract 162: 108093.
24. Liu, Z., et al. (2018). "Mesenchymal stem cell-derived microvesicles alleviate pulmonary arterial hypertension by regulating renin-angiotensin system." J Am Soc Hypertens 12(6): 470-478.
25. Ma H, Chen G. Stem cell. The Journal of American Science 2005;1(2):90-92. doi:10.7537/marsjas010205.14. http://www.jofamericanscience.org/journals/am-sci/0102/14-mahongbao.pdf.
26. Ma H, Cherng S. Eternal Life and Stem Cell. Nature and Science. 2007;5(1):81-96. doi:10.7537/marsnsj050107.10. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
27. Ma H, Cherng S. Nature of Life. Life Science

Journal 2005;2(1):7-15. doi:10.7537/marslsj020105.03. http://www.lifesciencesite.com/lsj/life0201/life-0201-03.pdf.
28. Ma H, Yang Y. Turritopsis nutricula. Nature and Science 2010;8(2):15-20. doi:10.7537/marsnsj080210.03. http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf.
29. Ma H. The Nature of Time and Space. Nature and science 2003;1(1):1-11. doi:10.7537/marsnsj010103.01. http://www.sciencepub.net/nature/0101/01-ma.pdf.
30. Mallick, B., et al. (2009). "MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells." PLoS One 4(11): e7837.
31. Marsland Press. http://www.sciencepub.net. 2020.
32. Marsland Press. http://www.sciencepub.org. 2020.
33. Min, F., et al. (2015). "Therapeutic effect of human umbilical cord mesenchymal stem cells modified by angiotensin-converting enzyme 2 gene on bleomycin-induced lung fibrosis injury." Mol Med Rep 11(4): 2387-2396.
34. National Center for Biotechnology Information, U.S. National Library of Medicine. http://www.ncbi.nlm.nih.gov/pubmed. 2020.
35. Nature and Science. http://www.sciencepub.net/nature. 2020.
36. Qi, Y., et al. (2013). "Diminazene aceturate enhances angiotensin-converting enzyme 2 activity and attenuates ischemia-induced cardiac pathophysiology." Hypertension 62(4): 746-752.
37. Sato, T., et al. (2017). "ELABELA-APJ axis protects from pressure overload heart failure and angiotensin II-induced cardiac damage." Cardiovasc Res 113(7): 760-769.
38. Shenoy, V., et al. (2013). "Diminazene attenuates pulmonary hypertension and improves angiogenic progenitor cell functions in experimental models." Am J Respir Crit Care Med 187(6): 648-657.
39. 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.
40. Stem Cell. http://www.sciencepub.net/stem. 2020.
41. Strawn, W. B., et al. (2004). "Renin-angiotensin system expression in rat bone marrow haematopoietic and stromal cells." Br J Haematol 126(1): 120-126.
42. 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.
43. Wang, L. and P. S. Leung (2013). "The role of renin-angiotensin system in cellular differentiation: implications in pancreatic islet cell development and islet transplantation." Mol Cell Endocrinol 381(1-2): 261-271.
44. Wikipedia. The free encyclopedia. http://en.wikipedia.org. 2020.
45. Xiao, F., et al. (2014). "Characterization of angiotensin-converting enzyme 2 ectodomain shedding from mouse proximal tubular cells." PLoS One 9(1): e85958.
46. Yang, J., et al. (2016). "Pathological Ace2-to-Ace enzyme switch in the stressed heart is transcriptionally controlled by the endothelial Brg1-FoxM1 complex." Proc Natl Acad Sci U S A 113(38): E5628-5635.
47. Zhang, X., et al. (2015). "Combination therapy with human umbilical cord mesenchymal stem cells and angiotensin-converting enzyme 2 is superior for the treatment of acute lung ischemia-reperfusion injury in rats." Cell Biochem Funct 33(3): 113-120.
48. Zulli, A., et al. (2008). "Co-localization of angiotensin-converting enzyme 2-, octomer-4- and CD34-positive cells in rabbit atherosclerotic plaques." Exp Physiol 93(5): 564-569.

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