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Vascular Endothelial Growth Factor (VEGF) and Stem Cell Research Literatures

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Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the related studies.
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Key words: cancer; life; research; literature; cell

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

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

Abarbanell, A. M., et al. (2010). "Toll-like receptor 2 mediates mesenchymal stem cell-associated myocardial recovery and VEGF production following acute ischemia-reperfusion injury." Am J Physiol Heart Circ Physiol **298**(5): H1529-1536.

Toll-like receptor 2 (TLR2), a key component of the innate immune system, is linked to inflammation and myocardial dysfunction after ischemia-reperfusion injury (I/R). Treatment of the heart with mesenchymal stem cells (MSCs) is known to improve myocardial recovery after I/R in part by paracrine factors such as VEGF. However, it is unknown whether TLR2 activation on the MSCs affects MSC-mediated myocardial recovery and VEGF production. We hypothesized that the knockout of TLR2 on the MSCs (TLR2KO MSCs) would 1) improve MSC-mediated myocardial recovery and 2) increase myocardial and MSC VEGF release. With the isolated heart perfusion system, Sprague-Dawley rat hearts were subjected to I/R and received one of three intracoronary treatments: vehicle, male wild-type MSCs (MWT MSCs), or TL2KO MSCs. All treatments were performed immediately before ischemia, and heart function was measured continuously. Postreperfusion, heart homogenates were analyzed for myocardial VEGF production. Contrary to our hypothesis, only MWT MSC treatment significantly improved the recovery of left ventricular developed pressure and the maximal positive and negative values of the first derivative of pressure. In addition, VEGF production was greatest in hearts treated with MWT MSCs. To investigate MSC production of VEGF, MSCs were activated with TNF in vitro and the supernatants collected for ELISA. In vitro basal levels of MSC VEGF production were similar. However, with TNF activation, MWT MSCs produced significantly more VEGF, whereas activated TLR2KO MSC production of VEGF was unchanged. Finally, we observed that MWT MSCs proliferated more rapidly than TLR2KO MSCs. These data indicate that TLR2 may be essential to MSC-mediated myocardial recovery and VEGF production.

Ai, W. J., et al. (2015). "R-Smad signaling-mediated VEGF expression coordinately regulates endothelial cell differentiation of rat mesenchymal stem cells." Stem Cells Dev **24**(11): 1320-1331.

A low-efficiency yield hinders the use of stem cells as a source of endothelial cells (ECs) for therapeutic vascularization, and the diversity of the transforming growth factor-beta (TGF-beta) superfamily has undermined understanding the effects of its potent vascularization-inducing. Herein, we

studied the role of the TGF-beta superfamily in EC differentiation of rat bone marrow mesenchymal stem cells (MSCs) induced by Smad2/3 and Smad1/5/8 signaling. MSCs that had been sorted by flow cytometry as CD31-negative were cultured for 14 days in medium supplemented with vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) as the control. The Smad2/3 pathway was activated by TGF-beta1 and Smad1/5/8 by bone morphogenetic proteins (BMPs). In the early phase in the Smad2/3-activated group, there were 10% CD31-positive cells, which was significantly higher than in the control group. A low Smad1/5/8 phosphorylation level after BMP4 activation doubled the number of CD31-positive cells, while a higher phosphorylation level after BMP9 activation showed no effect. A Smad2/3 inhibitor initially blocked differentiation but later promoted it, while a Smad1/5/8 inhibitor reversed the induction observed with BMPs. Moreover, the positive effects of R-Smad on differentiation were weakened by the VEGF neutralizing antibody, and a Smad3 inhibitor decreased VEGF expression and blocked differentiation in both the early and late phases. In conclusion, differentiation of ECs from MSCs via Smad2/3 signaling is stage dependent. Activation, particularly by Smad3, significantly promotes differentiation at an early phase but later is suppressive. A low Smad1/5/8 phosphorylation level has a positive effect, and R-Smad effects are partly mediated by VEGF.

An, S. S., et al. (2010). "Neuroprotective effect of combined hypoxia-induced VEGF and bone marrow-derived mesenchymal stem cell treatment." Childs Nerv Syst **26**(3): 323-331.

PURPOSES: To avoid unwanted adverse effects of higher doses of single treatment of stem cells and gene therapy and increase the therapeutic efficacies, we hypothesized the combined therapy with stem cells and gene therapy. This study assessed the neuroprotective effects of combined gene therapy and stem cell treatment under ischemic hypoxia conditions using hypoxia-inducible vascular endothelial growth factor (VEGF) and bone marrow-derived mesenchymal stem cells (BMSC). METHODS: Experimental groups included the control which was N2A cells transfected with empty vectors, the transfection only group which was N2A cells treated with pEpo-SV-VEGF alone, the BMSC only group which was N2A cells transfected with empty vectors and cocultured with BMSCs, and the combined treatment group which was N2A cells treated with pEpo-SV-VEGF and cocultured with BMSCs. Each group was transfected for 4 h and cultured at 37 degrees C and 5% CO2 for 24 h. Each group was then cultivated under hypoxic conditions (1% O2) for 12 h. Neuroprotective effects were assessed by reverse transcription polymerase chain reaction, annexin V, and cytotoxicity assay. RESULTS: Neurons exposed to hypoxic conditions exhibited neuronal apoptosis. Compared to single treatments, the combined hypoxia-inducible VEGF and BMSC treatment demonstrated a significant increase in VEGF expression and decreased neuronal apoptosis. CONCLUSIONS: These results suggest that combined pEpo-SV-VEGF and BMSC treatment is effective in protecting neurons against hypoxic ischemic injury.

Barone, A., et al. (2014). "Combined VEGF and CXCR4 antagonism targets the GBM stem cell population and synergistically improves survival in an intracranial mouse model of glioblastoma." Oncotarget **5**(20): 9811-9822.

Glioblastoma recurrence involves the persistence of a subpopulation of cells with enhanced tumor-initiating capacity (TIC) that reside within the perivascular space, or niche (PVN). Anti-angiogenic therapies may prevent the formation of new PVN but have not prevented recurrence in clinical trials, suggesting they cannot abrogate TIC activity. We hypothesized that combining anti-angiogenic therapy with blockade of PVN function would have superior anti-tumor activity. We tested this hypothesis in an established intracranial xenograft model of GBM using a monoclonal antibody specific for murine and human VEGF (mcr84) and a Protein Epitope Mimetic (PEM) CXCR4 antagonist, POL5551. When doses of POL5551 were increased to overcome an mcr84-induced improvement in vascular barrier function, combinatorial therapy significantly inhibited intracranial tumor growth and improved survival. Anti-tumor activity was associated with significant changes in tumor cell proliferation and apoptosis, and a reduction in the numbers of perivascular cells expressing the TIC marker nestin. A direct effect on TICs was demonstrated for POL5551, but not mcr84, in three primary patient-derived GBM isolates. These findings indicate that targeting the structure and function of the PVN has superior anti-tumor effect and provide a strong rationale for clinical evaluation of POL5551 and Avastin in patients with GBM.

Bianco, M., et al. (2019). "Clinical, electrophysiological and VEGF 2-year response after lenalidomide or stem cell transplantation in patients with POEMS syndrome." J Neurol Neurosurg Psychiatry **90**(3): 367-368.

Bota, D. A., et al. (2013). "Proteasome inhibition with bortezomib induces cell death in GBM stem-like cells and temozolomide-resistant glioma cell lines, but stimulates GBM stem-like cells' VEGF production and angiogenesis." J Neurosurg **119**(6): 1415-1423.

OBJECT: Recurrent malignant gliomas have inherent resistance to traditional chemotherapy. Novel therapies target specific molecular mechanisms involved in abnormal signaling and resistance to apoptosis. The proteasome is a key regulator of multiple cellular functions, and its inhibition in malignant astrocytic lines causes cell growth arrest and apoptotic cell death. The proteasome inhibitor bortezomib was reported to have very good in vitro activity against malignant glioma cell lines, with modest activity in animal models as well as in clinical trials as a single agent. In this paper, the authors describe the multiple effects of bortezomib in both in vitro and in vivo glioma models and offer a novel explanation for its seeming lack of activity. METHODS: Glioma stem-like cells (GSCs) were obtained from resected glioblastomas (GBMs) at surgery and expanded in culture. Stable glioma cell lines (U21 and D54) as well as temozolomide (TMZ)-resistant glioma cells derived from U251 and D54-MG were also cultured. GSCs from 2 different tumors, as well as D54 and U251 cells, were treated with bortezomib, and the effect of the drug was measured using an XTT cell viability assay. The activity of bortezomib was then determined in D54-MG and/or U251 cells using apoptosis analysis as well as caspase-3 activity and proteasome activity measurements. Human glioma xenograft models were created in nude mice by subcutaneous injection. Bevacizumab was administered via intraperitoneal injection at a dose of 5 mg/kg daily. Bortezomib was administered by intraperitoneal injection 1 hour after bevacizumab administration in doses of at a dose of 0.35 mg/kg on days 1, 4, 8, and 11 every 21 days. Tumors were measured twice weekly. RESULTS: Bortezomib induced caspase-3 activation and apoptotic cell death in stable glioma cell lines and in glioma stem-like cells (GSCs) derived from malignant tumor specimens Furthermore, TMZ-resistant glioma cell lines retained susceptibility to the proteasome inhibition. The bortezomib activity was directly proportional with the cells' baseline proteasome activity. The proteasome inhibition stimulated both hypoxia-inducible factor (HIF)-1alpha and vascular endothelial growth factor (VEGF) production in malignant GSCs. As such, the VEGF produced by GSCs stimulated endothelial cell growth, an effect that could be prevented by the addition of bevacizumab (VEGF antibody) to the media. Similarly, administration of bortezomib and bevacizumab to athymic mice carrying subcutaneous malignant glioma xenografts resulted in greater tumor inhibition and greater improvement in survival than administration of either drug alone. These data indicate that simultaneous proteasome inhibition and VEGF blockade offer increased benefit as a strategy for malignant glioma therapy. CONCLUSIONS: The results of this study indicate that combination therapies based on bortezomib and bevacizumab might offer an increased benefit when the two agents are used in combination. These drugs have a complementary mechanism of action and therefore can be used together to treat TMZ-resistant malignant gliomas.

Brossa, A., et al. (2015). "Sunitinib but not VEGF blockade inhibits cancer stem cell endothelial differentiation." Oncotarget **6**(13): 11295-11309.

Different mechanisms of angiogenesis and vasculogenesis are involved in the development of the tumor vasculature. Among them, cancer stem cells are known to contribute to tumor vasculogenesis through their direct endothelial differentiation. Here, we investigated the effect of anti-angiogenic therapy on vasculogenesis of cancer stem cells derived from breast and renal carcinomas. We found that all the anti-angiogenic approaches impaired proliferation and survival of cancer stem cells once differentiated into endothelial cells in vitro and reduced murine angiogenesis in vivo. At variance, only VEGF-receptor inhibition using the non-specific tyrosine kinase inhibitor Sunitinib or the anti-VEGF-receptor 2 neutralizing antibody, but not VEGF blockade using Bevacizumab, impaired the process of endothelial differentiation in vitro, suggesting a VEGF-independent mechanism. In addition, tyrosine kinase inhibition by Sunitinib but not VEGF blockade using the soluble VEGF trap sFlk1 inhibited the cancer stem cell-induced vasculogenesis in vivo. Accordingly, Sunitinib but not Bevacizumab inhibited the induction of hypoxia-inducible factor pathway occurring during endothelial differentiation under hypoxia. The present results highlight a differential effect of VEGF-receptor blockade versus VEGF inhibition in tumor vascularization. VEGFR blockade inhibits the process of tumor vasculogenesis occurring during tumor hypoxia whereas the effect of VEGF inhibition appears restricted to differentiated endothelial cells.

Chen, G., et al. (2014). "Blocking autocrine VEGF signaling by sunitinib, an anti-cancer drug, promotes embryonic stem cell self-renewal and somatic cell reprogramming." Cell Res **24**(9): 1121-1136.

Maintaining the self-renewal of embryonic stem cells (ESCs) could be achieved by activating the extrinsic signaling, i.e., the use of leukemia inhibitory factor (LIF), or blocking the intrinsic differentiation pathways, i.e., the use of GSK3 and MEK inhibitors (2i). Here we found that even in medium supplemented with LIF, mESCs still tend to differentiate toward meso-endoderm lineages after long-term culture and the culture spontaneously secretes vascular endothelial growth factors (VEGFs). Blocking VEGF signaling with sunitinib, an anti-cancer drug and a receptor

tyrosine kinase (RTK) inhibitor mainly targeting VEGF receptors (VEGFRs), is capable of maintaining the mESCs in the undifferentiated state without the need for feeder cells or LIF. Sunitinib facilitates the derivation of mESCs from blastocysts, and the mESCs maintained in sunitinib-containing medium remain pluripotent and are able to contribute to chimeric mice. Sunitinib also promotes iPSC generation from MEFs with only Oct4. Knocking down VEGFR2 or blocking it with neutralizing antibody mimicks the effect of sunitinib, indicating that blocking VEGF/VEGFR signaling is indeed beneficial to the self-renewal of mESCs. We also found that hypoxia-inducible factor alpha (HIF1alpha) and endoplasmic reticulum (ER) stress are involved in the production of VEGF in mESCs. Blocking both pathways inhibits the expression of VEGF and prevents spontaneous differentiation of mESCs. Interestingly, LIF may also exert its effect by downregulating HIF1alpha and ER stress pathways and subsequent VEGF expression. These results indicate the existence of an intrinsic differentiation pathway in mESCs by activating the autocrine VEGF signaling. Blocking VEGF signaling with sunitinib or other small molecules help to maintain the mESCs in the ground state of pluripotency.

Chen, J., et al. (2019). "The combinatory effect of sinusoidal electromagnetic field and VEGF promotes osteogenesis and angiogenesis of mesenchymal stem cell-laden PCL/HA implants in a rat subcritical cranial defect." Stem Cell Res Ther **10**(1): 379.

BACKGROUND: Restoration of massive bone defects remains a huge challenge for orthopedic surgeons. Insufficient vascularization and slow bone regeneration limited the application of tissue engineering in bone defect. The effect of electromagnetic field (EMF) on bone defect has been reported for many years. However, sinusoidal EMF (SEMF) combined with tissue engineering in bone regeneration remains poorly investigated. METHODS: In the present study, we investigated the effect of SEMF and vascular endothelial growth factor (VEGF) on osteogenic and vasculogenic differentiation of rat bone marrow-derived mesenchymal stem cells (rBMSCs). Furthermore, pretreated rBMSC- laden polycaprolactone-hydroxyapatite (PCL/HA) scaffold was constructed and implanted into the subcritical cranial defect of rats. The bone formation and vascularization were evaluated 4 and 12 weeks after implantation. RESULTS: It was shown that SEMF and VEGF could enhance the protein and mRNA expression levels of osteoblast- and endothelial cell-related markers, respectively. The combinatory effect of SEMF and VEGF slightly promoted the angiogenic differentiation of rBMSCs. The proteins of Wnt1, low-density lipoprotein receptor-related protein 6 (LRP-6), and beta-catenin increased in all inducted groups, especially in SEMF + VEGF group. The results indicated that Wnt/beta-catenin pathway might participate in the osteogenic and angiogenic differentiation of rBMSCs. Histological evaluation and reconstructed 3D graphs revealed that tissue-engineered constructs significantly promoted the new bone formation and angiogenesis compared to other groups. CONCLUSION: The combinatory effect of SEMF and VEGF raised an efficient approach to enhance the osteogenesis and vascularization of tissue-engineered constructs, which provided a useful guide for regeneration of bone defects.

Cheng, Y. S., et al. (2022). "Adipose-Derived Stem Cell-Incubated HA-Rich Sponge Matrix Implant Modulates Oxidative Stress to Enhance VEGF and TGF-beta Secretions for Extracellular Matrix Reconstruction In Vivo." Oxid Med Cell Longev **2022**: 9355692.

This study demonstrated both adipose-derived stem cells (ASCs) in vitro and in vivo combined with three-dimensional (3D) porous sponge matrices on implant wound healing. Sponge matrices were created from hyaluronic acid (HA), collagen (Col), and gelatin (Gel), constructing two types: HA-L (low content) and HA-H (high content), to be cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Fourier transform infrared spectroscopy method verified carboxyl groups of HA and amino groups of Col and Gel reacting between the raw materials and scaffolds to identify the successive cross-linking. The swelling ratios of two types of sponge matrices were analyzed by water absorption capabilities, and the results displayed both over 30-fold dry scaffold weight enhancements. In biodegradation tests, matrices were hydrolyzed over time by three cutaneous enzymes, hyaluronidase, lysozyme, and collagenase I. ASCs from rats were cultured within the HA-H scaffold, demonstrating higher antioxidative abilities and secretions on related genes and proteins compared to the other two groups. The ASC HA-H matrix promoted cell proliferation to stimulate capillary angiogenesis inducer secretions, including vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-beta). In vivo histological examinations showed ASCs from implanted HA-H implant transported into the subcutis, and rat skin cells also infiltrated into the original matrix zone to increase the extracellular matrix (ECM) reconstructions. Our experimental data revealed that the ASC HA-H sponge implant was effective in improving wound repair.

Crisostomo, P. R., et al. (2007). "Gender differences in injury induced mesenchymal stem cell apoptosis and

VEGF, TNF, IL-6 expression: role of the 55 kDa TNF receptor (TNFR1)." J Mol Cell Cardiol **42**(1): 142-149.

Concomitant pro- and anti-inflammatory properties of bone marrow stem cells (BMSC) may be an important aspect of their ability to heal injured tissue. However, very few studies have examined whether gender differences exist in BMSC function. Indeed, it remains unknown whether gender differences exist in BMSC function and ability to resist apoptosis, and if so, whether TNF receptor 1 (TNFR1) plays a role in these differences. We hypothesized that TNFR1 ablation equalizes gender differences in bone marrow mesenchymal stem cell (MSC) apoptosis, as well as expression of vascular endothelial growth factor (VEGF), TNF and interleukin (IL)-6. Mouse MSCs from male wild type (WT), female WT, male TNFR1 knockouts (TNFR1KO) and female TNFR1KO were stressed by endotoxin 200 ng/ml or 1 h hypoxia. MSC activation was determined by measuring VEGF, TNF and IL-6 production (ELISA). Differences considered significant if $p<0.05$. LPS and hypoxia resulted in significant activation in all experimental groups compared to controls. Male WT demonstrated significantly greater TNF and IL-6 and significantly less VEGF release than female WT MSCs. However, release of TNF, IL-6 and VEGF in male TNFR1 knockouts differed from male WT, but was not different from female WT MSCs. Similarly apoptosis in hypoxic male TNFRIKO differed from male WT, but it was not different from apoptosis from WT female. Female WT did not differ in TNF, IL-6 and VEGF release compared to female TNFR1KO. Gender differences exist in injury induced BMSC VEGF, TNF and IL-6 expression. TNFR1 may autoregulate VEGF, TNF and IL-6 expression in males more than females. MSCs are novel therapeutic agents for organ protection, but further study of the disparate expression of VEGF, TNF and IL-6 in males and females as well as the role of TNFR1 in these gender differences is necessary to maximize this protection.

Das, H., et al. (2009). "Stem cell therapy with overexpressed VEGF and PDGF genes improves cardiac function in a rat infarct model." PLoS One **4**(10): e7325.

BACKGROUND: Therapeutic potential was evaluated in a rat model of myocardial infarction using nanofiber-expanded human cord blood derived hematopoietic stem cells (CD133+/CD34+) genetically modified with VEGF plus PDGF genes (VIP). METHODS AND FINDINGS: Myocardial function was monitored every two weeks up to six weeks after therapy. Echocardiography revealed time dependent improvement of left ventricular function evaluated by M-mode, fractional shortening, anterior wall tissue velocity, wall motion score index, strain and strain rate in animals treated with VEGF plus PDGF overexpressed stem cells (VIP) compared to nanofiber expanded cells (Exp), freshly isolated cells (FCB) or media control (Media). Improvement observed was as follows: VIP>Exp> FCB>media. Similar trend was noticed in the exercise capacity of rats on a treadmill. These findings correlated with significantly increased neovascularization in ischemic tissue and markedly reduced infarct area in animals in the VIP group. Stem cells in addition to their usual homing sites such as lung, spleen, bone marrow and liver, also migrated to sites of myocardial ischemia. The improvement of cardiac function correlated with expression of heart tissue connexin 43, a gap junctional protein, and heart tissue angiogenesis related protein molecules like VEGF, pNOS3, NOS2 and GSK3. There was no evidence of upregulation in the molecules of oncogenic potential in genetically modified or other stem cell therapy groups. CONCLUSION: Regenerative therapy using nanofiber-expanded hematopoietic stem cells with overexpression of VEGF and PDGF has a favorable impact on the improvement of rat myocardial function accompanied by upregulation of tissue connexin 43 and pro-angiogenic molecules after infarction.

Deng, Z. G., et al. (2010). "[The effect of allogenic hematopoietic stem cell transplantation on tumor recurrence and metastasis of hepatocellular carcinoma after hepatectomy and the relationship with presence of AFP mRNA and VEGF-C mRNA in peripheral blood]." Sichuan Da Xue Xue Bao Yi Xue Ban **41**(2): 256-260.

OBJECTIVE: To evaluate the effect of alloHST on recurrence and metastasis of HCC after hepatic radical resection and investigate the relationship between AFP mRNA, VEGF-C mRNA and recurrence and metastasis of HCC after hepatic radical resection. METHODS: 22 SCID mice were randomized into 3 groups: group A-the scheduled transplantation, group, B-the single transplantation, and group C-the normal saline group as control. Human umbilical cord blood was transplanted into SCID mice by tail vein, Six weeks after AlloHST, the orthotopic tumor model in SCID mice was established by implanting histologically intact tissue under the embrane of liver. Ten days later, the mice received resection of lobe bearing tumor. The condition of recurrence and metastasis was observed 4 weeks after operation. All groups were compared by routine pathological test and the expression of AFP mRNA and MAGE-1 mRNA in peripheral blood were examined by real time quantitative reverse transcription-polymerase chain reaction (RQ-PCR). RESULTS: All of the incidence of intrahepatic recurrence rate after operation in 3 groups were 100%, but recurrent tumor

volume [(367.18 +/- 31.86) mm3, (648.26 +/- 155.22) mm3, (811.38 +/- 127.36) mm', P < 0.01)] and the incidence of lung metastasis (14.3%, 66.7%, 100%, P < 0.01) were different among groups,The inhibitory rate of group A and B was 54.7% and 20.1%. The expression of AFP mRNA in peripheral blood (1.95 +/- 0.92 vs. 5.23 +/- 1.96, 6.36 +/- 3.38, P = 0.02) and VEGF-C mRNA (2.48 +/- 2.25, 3.45 +/- 2.81, 6.60 +/- 5.81, P = 0.27) were also different that suggested the AFP mRNA and VEGF-C mRNA in peripheral blood were significantly correlated with recurrence and metastasis. CONCLUSION: AlloHST is a useful method for decreasing metastasis and recurrence in liver cancer after radical resection in early stage and appears to be quantity-effect relationship.

Diaz-Rodriguez, P., et al. (2015). "The synergistic effect of VEGF and biomorphic silicon carbides topography on in vivo angiogenesis and human bone marrow derived mesenchymal stem cell differentiation." Biomed Mater **10**(4): 045017.

Topographical features of biomaterials are able to modulate cell attachment, spreading and differentiation. The addition of growth factors to implantable biomaterials can modify these cellular responses, enhancing their therapeutic potential. The aim of this research is to establish the influence of biomorphic silicon carbide ceramics (bioSiCs) surface topography on the proliferation and osteoblastic differentiation of mesenchymal stem cells and the potential synergistic effect of the ceramic porous structure together with vascular endothelial growth factor loading (VEGF) on the surface mediated osteoblastic differentiation. Three porous bioSiCs with important differences in their microstructure were obtained from different natural precursors. Samples loaded with or without VEGF through ionic interactions were cultured with human umbilical vein endothelial cells (HUVEC) or bone marrow derived mesenchymal stem cells (hMSCs). Cell behaviour and protein activity with regard to bioSiC porous structure and surface properties were analysed. An in vivo model (Chick Chorioallantoic Membrane; CAM) was used to assess the capability of the VEGF loaded systems to promote angiogenesis. Experimental data show that loaded systems were able to control the release of VEGF for up to 15 d ensuring the activity of the protein, increasing the proliferation of HUVECs and the formation of new blood vessels in the CAM. It was found that the selection of bioSiCs with a higher pore size promoted a higher concentration of osteoblastic differentiation markers of MSCs cultured on the surface of bioSiCs. Furthermore, the addition of VEGF to the systems was able to promote a faster osteoblastic differentiation according to the qPCR results, suggesting a synergy between both the surface properties and the controlled release of the growth factor. The VEGF loaded sapelli bioSiC was found to be the most promising material for bone tissue engineering applications.

Grun, D., et al. (2016). "VEGF-A acts via neuropilin-1 to enhance epidermal cancer stem cell survival and formation of aggressive and highly vascularized tumors." Oncogene **35**(33): 4379-4387.

We identify a limited subpopulation of epidermal cancer stem cells (ECS cells), in squamous cell carcinoma, that form rapidly growing, invasive and highly vascularized tumors, as compared with non-stem cancer cells. These ECS cells grow as non-attached spheroids, and display enhanced migration and invasion. We show that ECS cell-produced vascular endothelial growth factor (VEGF)-A is required for the maintenance of this phenotype, as knockdown of VEGF-A gene expression or treatment with VEGF-A-inactivating antibody reduces these responses. In addition, treatment with bevacizumab reduces tumor vascularity and growth. Surprisingly, the classical mechanism of VEGF-A action via interaction with VEGF receptors does not mediate these events, as these cells lack VEGFR1 and VEGFR2. Instead, VEGF-A acts via the neuropilin-1 (NRP-1) co-receptor. Knockdown of NRP-1 inhibits ECS cell spheroid formation, invasion and migration, and attenuates tumor formation. These studies suggest that VEGF-A acts via interaction with NRP-1 to trigger intracellular events leading to ECS cell survival and formation of aggressive, invasive and highly vascularized tumors.

Gu, A., et al. (2009). "Role of Ceacam1 in VEGF induced vasculogenesis of murine embryonic stem cell-derived embryoid bodies in 3D culture." Exp Cell Res **315**(10): 1668-1682.

CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1), a type I transmembrane glycoprotein involved in cell-cell adhesion has been shown to act as an angiogenic factor for mouse and human endothelial cells. Based on the ability of CEACAM1 to initiate lumen formation in human mammary epithelial cells grown in 3D culture (Matrigel), we hypothesized that murine CEACAM1 may play a similar role in vasculogenesis. In order to test this hypothesis, murine embryonic stem (ES) cells stimulated with VEGF were differentiated into embryoid bodies (EB) for 8 days (-8-0 d) and transferred to Matrigel in the presence or absence of anti-CEACAM1 antibody for an additional 12 days (0-12 d). In the absence of anti-CEACAM1 antibody or in the presence of an isotype control antibody, the EB in Matrigel underwent extensive sprouting, generating lengthy vascular structures with well-defined lumina as

demonstrated by confocal microscopy, electron microscopy, and immunohistochemical analysis. Both the length and architecture of the vascular tubes were inhibited by anti-CEACAM1 mAb CC1, a mAb that blocks the cell-cell adhesion functions of CEACAM1, thus demonstrating a critical role for this cell-cell adhesion molecule in generating and maintaining vasculogenesis. QRT-PCR analysis of the VEGF treated ES cells grown under conditions that convert them to EB revealed expression of Ceacam1 as early as -5 to -3 d reaching a maximum at day 0 at which time EBs were transferred to Matrigel, thereafter levels at first declined and then increased over time. Other markers of vasculogenesis including Pecam1, VE-Cad, and Tie-1 were not detected until day 0 when EBs were transferred to Matrigel followed by a steady increase in levels, indicating later roles in vasculogenesis. In contrast, Tie-2 and Flk-1 (VEGFR2) were detected on day five of EB formation reaching a maximum at day 0 on transfer to Matrigel, similar to Ceacam1, but after which Tie-2 declined over time, while Flk-1 increased over time. QRT-PCR analysis of the anti-CEACAM1 treated ES cells revealed a significant decrease in the expression of Ceacam1, Pecam1, Tie-1, and Flk-1, while VE-Cad and Tie-2 expression were unaffected. These results suggest that the expression and signaling of CEACAM1 may affect the expression of other factors known to play critical roles in vasculogenesis. Furthermore this 3D model of vasculogenesis in an environment of extracellular matrix may be a useful model for comparison to existing models of angiogenesis.

Hajizadeh-Saffar, E., et al. (2015). "Inducible VEGF expression by human embryonic stem cell-derived mesenchymal stromal cells reduces the minimal islet mass required to reverse diabetes." Sci Rep **5**: 9322.

Islet transplantation has been hampered by loss of function due to poor revascularization. We hypothesize that co-transplantation of islets with human embryonic stem cell-derived mesenchymal stromal cells that conditionally overexpress VEGF (hESC-MSC:VEGF) may augment islet revascularization and reduce the minimal islet mass required to reverse diabetes in mice. HESC-MSCs were transduced by recombinant lentiviruses that allowed conditional (Dox-regulated) overexpression of VEGF. HESC-MSC: VEGF were characterized by tube formation assay. After co-transplantation of hESC-MSC:VEGF with murine islets in collagen-fibrin hydrogel in the omental pouch of diabetic nude mice, we measured blood glucose, body weight, glucose tolerance and serum C-peptide. As control, islets were transplanted alone or with non-transduced hESC-MSCs. Next, we compared functional parameters of 400 islets alone versus 200 islets co-transplanted with hESC-MSC:VEGF. As control, 200 islets were transplanted alone. Metabolic function of islets transplanted with hESC-MSC:VEGF significantly improved, accompanied by superior graft revascularization, compared with control groups. Transplantation of 200 islets with hESC-MSC:VEGF showed superior function over 400 islets alone. We conclude that co-transplantation of islets with VEGF-expressing hESC-MSCs allowed for at least a 50% reduction in minimal islet mass required to reverse diabetes in mice. This approach may contribute to alleviate the need for multiple donor organs per patient.

Hamerlik, P., et al. (2012). "Autocrine VEGF-VEGFR2-Neuropilin-1 signaling promotes glioma stem-like cell viability and tumor growth." J Exp Med **209**(3): 507-520.

Although vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) is traditionally regarded as an endothelial cell protein, evidence suggests that VEGFRs may be expressed by cancer cells. Glioblastoma multiforme (GBM) is a lethal cancer characterized by florid vascularization and aberrantly elevated VEGF. Antiangiogenic therapy with the humanized VEGF antibody bevacizumab reduces GBM tumor growth; however, the clinical benefits are transient and invariably followed by tumor recurrence. In this study, we show that VEGFR2 is preferentially expressed on the cell surface of the CD133(+) human glioma stem-like cells (GSCs), whose viability, self-renewal, and tumorigenicity rely, at least in part, on signaling through the VEGF-VEGFR2-Neuropilin-1 (NRP1) axis. We find that the limited impact of bevacizumab-mediated VEGF blockage may reflect ongoing autocrine signaling through VEGF-VEGFR2-NRP1, which is associated with VEGFR2-NRP1 recycling and a pool of active VEGFR2 within a cytosolic compartment of a subset of human GBM cells. Whereas bevacizumab failed to inhibit prosurvival effects of VEGFR2-mediated signaling, GSC viability under unperturbed or radiation-evoked stress conditions was attenuated by direct inhibition of VEGFR2 tyrosine kinase activity and/or shRNA-mediated knockdown of VEGFR2 or NRP1. We propose that direct inhibition of VEGFR2 kinase may block the highly dynamic VEGF-VEGFR2-NRP1 pathway and inspire a GBM treatment strategy to complement the currently prevalent ligand neutralization approach.

Han, Y., et al. (2020). "Corrigendum to "Exosomes from hypoxia-treated human adipose-derived mesenchymal stem cells enhance angiogenesis through VEGF/VEGF-R" [Int. J. Biochem. Cell Biol. 109 April (2019) 59-68]." Int J Biochem Cell Biol **126**: 105805.

He, G. H., et al. (2021). "Mesenchymal stem cell-derived exosomes inhibit the VEGF-A expression in human retinal vascular endothelial cells induced by high glucose." Int J Ophthalmol **14**(12): 1820-1827.

AIM: To determine the effect of exosomes derived from human umbilical cord blood mesenchymal stem cells (hUCMSCs) on the expression of vascular endothelial growth factor A (VEGF-A) in human retinal vascular endothelial cells (HRECs). METHODS: Exosomes were isolated from hUCMSCs using cryogenic ultracentrifugation and characterized by transmission electron microscopy, Western blotting and nanoparticle tracking analysis. HRECs were randomly divided into a normal control group (group A), a high glucose model group (group B), a high glucose group with 25 microg/mL (group C), 50 microg/mL (group D), and 100 microg/mL exosomes (group E). Twenty-four hours after coculture, the cell proliferation rate was detected using flow cytometry, and the VEGF-A level was detected using immunofluorescence. After coculture 8, 16, and 24h, the expression levels of VEGF-A in each group were detected using PCR and Western blots. RESULTS: The characteristic morphology (membrane structured vesicles) and size (diameter between 50 and 200 nm) were observed under transmission electron microscopy. The average diameter of 122.7 nm was discovered by nanoparticle tracking analysis (NTA). The exosomal markers CD9, CD63, and HSP70 were strongly detected. The proliferation rate of the cells in group B increased after 24h of coculture. Immunofluorescence analyses revealed that the upregulation of VEGF-A expression in HRECs stimulated by high glucose could be downregulated by cocultured hUCMSC-derived exosomes ($F=39.03$, $P<0.01$). The upregulation of VEGF-A protein (group C: $F=7.96$; group D: $F=17.29$; group E: $F=11.89$; 8h: $F=9.45$; 16h: $F=12.86$; 24h: $F=42.28$, $P<0.05$) and mRNA (group C: $F=4.137$; group D: $F=13.64$; group E: $F=22.19$; 8h: $F=7.253$; 16h: $F=16.98$; 24h: $F=22.62$, $P<0.05$) in HRECs stimulated by high glucose was downregulated by cocultured hUCMSC-derived exosomes ($P<0.05$). CONCLUSION: hUCMSC-derived exosomes downregulate VEGF-A expression in HRECs stimulated by high glucose in time and concentration dependent manner.

Hu, A., et al. (2019). "Involvement of stromal cell-derived factor-1alpha (SDF-1alpha), stem cell factor (SCF), fractalkine (FKN) and VEGF in TSG protection against intimal hyperplasia in rat balloon injury." Biomed Pharmacother **110**: 887-894.

BACKGROUND: Intimal hyperplasia is the major therapeutic concern after percutaneous coronary intervention. The aim of this study is to investigate effects of 2,3,4',5-tetrahydroxystilbene-2-O-beta-D glucoside (TSG) on intimal hyperplasia and the underling mechanisms through attenuating the expressions of stromal cell-derived factor-1alpha (SDF-1alpha)/CXCR4, stem cell factor (SCF)/c-kit and fractalkine (FKN)/CX3CR1, and through promoting re-endothelialization with vascular endothelial growth factor (VEGF). METHOD: Rats were operated with carotid artery balloon injury. The treatment groups were gavaged with 50 and 100 mg/kg/d of TSG. After 10 days of treatment, carotid artery pathological changes were evaluated by histology. Serum levels of SDF-1alpha, SCF, FKN and VEGF were detected by enzyme linked immunosorbent assay. The protein expressions of the receptors c-kit, CXCR4, CX3CR1, as well as CD34 and proliferating cell nuclear antigen (PCNA) were detected by immunochemistry. RESULTS: TSG dose-dependently inhibited balloon injury-induced intimal hyperplasia, as evidenced by reducing neointima area (NIA), neointima area/media area (NIA/MA), neointima area/internal elastic area (NIA/IELA), and by decreasing the protein expression of PCNA. TSG reduced serum levels of SDF-1alpha, SCF and FKN, and it also decreased the expressions of the corresponding receptors c-kit, CXCR4, CX3CR1 in neointima. Importantly, the level of VEGF in peripheral blood and the expression of CD34 in vascular walls were increased to promote re-endothelialization. CONCLUSIONS: This study clearly demonstrated that TSG was effective in inhibiting intimal hyperplasia, and this effect was mediated, at least in part, through the SCF/c-kit, SDF-1alpha/CXCR4 and FKN/CX3CR1 axes. Importantly, TSG could increase VEGF and CD34 to promote endothelial repair.

Huang, S., et al. (2015). "Pristimerin Inhibits Prostate Cancer Bone Metastasis by Targeting PC-3 Stem Cell Characteristics and VEGF-Induced Vasculogenesis of BM-EPCs." Cell Physiol Biochem **37**(1): 253-268.

BACKGROUND/AIMS: Prostate cancer (PCa) is one of the most common malignant cancers and a major leading cause of cancer deaths in men. Cancer stem-like cells are shown to be highly tumorigenic, pro-angiogenic and can significantly contribute to tumor new vessel formation and bone marrow derived-EPCs (BM-EPCs) are shown to recruit to the angiogenic switch in tumor growth and metastatic progression, suggesting the importance of targeting cancer stem cells (CSCs) and EPCs for novel tumor therapies. Pristimerin, an active component isolated from Celastraceae and Hippocrateaceae, has shown anti-tumor effects in some cell lines in previous studies. However, the effect and mechanism of Pristimerin on CSCs and EPCs in PCa bone metastasis are not well studied. METHODS: The effect of Pristimerin on PC-3 stem cell characteristics and

metastasis were detected by spheroid formation, CD133 and CD44 protein expression, matrix-gel invasive assay and colony-formation assay in vitro, VEGF and pro-inflammatory cytokines expression by ELISA assay, and tumor tumorigenicity by X-ray and MR in NOD-SCID mice model in vivo. In addition, we also detected the effect of Pristimerin on VEGF-induced vasculogenesis and protein expression of BM-EPCs. RESULTS: Pristimerin could significantly inhibit spheroid formation and protein expression of CD133 and CD44, reduce VEGF and pro-inflammation cytokines expression of PC-3 cell, and prevent the xenografted PC-3 tumor growth in the bone of nude mice. The present data also showed that Pristimerin significantly inhibited VEGF-induced vasculogenesis of BM-EPCs by suppressing the EPCs functions including proliferation, adhesion, migration, tube formation and inactivation the phosphorylation of VEGFR-2, Akt and eNOS. CONCLUSION: These data provide evidence that Pristimerin has strong potential for development as a novel agent against prostate bone metastasis by suppressing PC-3 stem cell characteristics and VEGF-induced vasculogenesis of BM-EPCs.

Huang, S., et al. (2016). "Acidic extracellular pH promotes prostate cancer bone metastasis by enhancing PC-3 stem cell characteristics, cell invasiveness and VEGF-induced vasculogenesis of BM-EPCs." Oncol Rep **36**(4): 2025-2032.

Bone metastasis is a main cause of cancer-related mortality in patients with advanced prostate cancer. Emerging evidence suggests that the acidic extracellular microenvironment plays significant roles in the growth and metastasis of tumors. However, the effects of acidity on bone metastasis of PCa remain undefined. In the present study, PC-3 cells were cultured in acidic medium (AM; pH 6.5) or neutral medium (NM; pH 7.4), aiming to investigate the effects and possible mechanisms of acidic extracellular microenvironment in bone metastasis of PCa. Our results showed that AM can promote spheroid and colony formations, cell viability and expression of stem cell characteristic-related markers in PC-3 cells. Moreover, AM stimulates MMP-9 secretion and promotes invasiveness of PC-3 cells, and these effects can be inhibited by blocking of MMP-9. Furthermore, AM stimulates VEGF secretion of PC-3 and AM conditioned medium (CMAM) promotes vasculogenesis of BM-EPCs by increasing cell viability, migration, tube formation, which involved activating the phosphorylation of VEGFR-2, Akt and P38, when pH of NM conditioned medium (CMNM) was modulated the same as AM conditioned medium (CMAM). Further studies have shown that CMNM induced vasculogenesis of BM-EPCs can be inhibited by the inhibition of VEGFR2 with DMH4. These findings suggest that acidic extracellular microenvironment may have the potential to modulate prostate cancer bone metastasis by enhancing PC-3 stem cell characteristics, cell invasiveness and VEGF-induced vasculogenesis of BM-EPCs. Improved anticancer strategies should be designed to selectively target acidic tumor microenvironment.

Hwang, J. H., et al. (2011). "Therapeutic lymphangiogenesis using stem cell and VEGF-C hydrogel." Biomaterials **32**(19): 4415-4423.

Lymphedema is a manifestation of lymphatic system insufficiency. It arises from primary lymphatic dysplasia or secondary obliteration after lymph node dissection or irradiation. Although improvement of swelling can be achieved by comprehensive non-operative therapy, treatment of this condition requires lifelong care and good compliance. Recently molecular-based treatments using VEGF-C have been investigated by several researchers. We designed the present study to determine whether the therapeutic efficacy of implanted human adipose-derived stem cells (hADSCs) could be improved by applying a gelatin hydrogel containing VEGF-C (VEGF-C hydrogel) to the site of tissue injury in a lymphedema mouse model. Four weeks after the operation, we evaluated edema and determined lymphatic vessel density at various post-operative time points. Mice treated with hADSCs and VEGF-C hydrogel showed a significantly decreased dermal edema depth compared to the groups of mice that received hADSCs only or VEGF-C hydrogel only. Immunohistochemical analysis also revealed that the hADSC/VEGF-C hydrogel group showed significantly greater lymphatic vessel regeneration than all the other groups. hADSCs were detected in the implantation sites of all mice in the hADSC/VEGF-C group, and exhibited a lymphatic endothelial differentiation phenotype as determined by co-staining PKH-labeled hADSCs for the lymphatic marker LYVE-1. Our results suggest that co-administration of hADSCs and VEGF-C hydrogel has a substantial positive effect on lymphangiogenesis.

Igarashi, Y., et al. (2016). "VEGF-C and TGF-beta reciprocally regulate mesenchymal stem cell commitment to differentiation into lymphatic endothelial or osteoblastic phenotypes." Int J Mol Med **37**(4): 1005-1013.

The direction of mesenchymal stem cell (MSC) differentiation is regulated by stimulation with various growth factors and cytokines. We recently established MSC lines, [transforming growth factor-beta (TGF-beta)-responsive SG2 cells, bone morphogenetic protein (BMP)-responsive SG3 cells, and TGF-beta/BMP-non-responsive SG5 cells],

derived from the bone marrow of green fluorescent protein-transgenic mice. In this study, to compare gene expression profiles in these MSC lines, we used DNA microarray analysis to characterize the specific gene expression profiles observed in the TGF-beta-responsive SG2 cells. Among the genes that were highly expressed in the SG2 cells, we focused on vascular endothelial growth factor (VEGF) receptor 3 (VEGFR3), the gene product of FMS-like tyrosine kinase 4 (Flt4). We found that VEGF-C, a specific ligand of VEGFR3, significantly induced the cell proliferative activity, migratory ability (as shown by Transwell migration assay), as well as the phosphorylation of extracellular signal-regulated kinase (ERK)1/2 in the SG2 cells. Additionally, VEGF-C significantly increased the expression of prospero homeobox 1 (Prox1) and lymphatic vessel endothelial hyaluronan receptor 1 (Lyve1), which are lymphatic endothelial cell markers, and decreased the expression of osteogenic differentiation marker genes in these cells. By contrast, TGF-beta significantly increased the expression of early-phase osteogenic differentiation marker genes in the SG2 cells and markedly decreased the expression of lymphatic endothelial cell markers. The findings of our study strongly suggest the following: i) that VEGF-C promotes the proliferative activity and migratory ability of MSCs; and ii) VEGF-C and TGF-beta reciprocally regulate MSC commitment to differentiation into lymphatic endothelial or osteoblastic phenotypes, respectively. Our findings provide new insight into the molecular mechanisms underlying the regenerative ability of MSCs.

Jeon, E. S., et al. (2010). "Ovarian cancer-derived lysophosphatidic acid stimulates secretion of VEGF and stromal cell-derived factor-1 alpha from human mesenchymal stem cells." Exp Mol Med **42**(4): 280-293.

Lysophosphatidic acid (LPA) stimulates growth and invasion of ovarian cancer cells and tumor angiogenesis. Cancer-derived LPA induces differentiation of human adipose tissue-derived mesenchymal stem cells (hASCs) to alpha-smooth muscle actin (alpha-SMA)-positive cancer-associated fibroblasts. Presently, we explored whether cancer-derived LPA regulates secretion of pro-angiogenic factors from hASCs. Conditioned medium (CM) from the OVCAR-3 and SKOV3 ovarian cancer cell lines stimulated secretion angiogenic factors such as stromal-derived factor-1 alpha (SDF-1 alpha) and VEGF from hASCs. Pretreatment with the LPA receptor inhibitor Ki16425 or short hairpin RNA lentiviral silencing of the LPA((1)) receptor abrogated the cancer CM-stimulated expression of alpha-SMA, SDF-1, and VEGF from hASCs. LPA induced expression of myocardin and myocardin-related transcription factor-A, transcription factors involved in smooth muscle differentiation, in hASCs. siRNA-mediated depletion of endogenous myocardin and MRTF-A abrogated the expression of alpha-SMA, but not SDF-1 and VEGF. LPA activated RhoA in hASCs and pretreatment with the Rho kinase inhibitor Y27632 completely abrogated the LPA-induced expression of alpha-SMA, SDF-1, and VEGF in hASCs. Moreover, LPA-induced alpha-SMA expression was abrogated by treatment with the ERK inhibitor U0126 or the phosphoinositide-3-kinase inhibitor LY294002, but not the PLC inhibitor U73122. LPA-induced VEGF secretion was inhibited by LY294002, whereas LPA-induced SDF-1 secretion was markedly attenuated by U0126, U73122, and LY294002. These results suggest that cancer-secreted LPA induces differentiation of hASCs to cancer-associated fibroblasts through multiple signaling pathways involving Rho kinase, ERK, PLC, and phosphoinositide-3-kinase.

Jing, J., et al. (2022). "Endothelial progenitor cells promote neural stem cell proliferation in hypoxic conditions through VEGF via the PI3K/AKT pathway." J Recept Signal Transduct Res: 1-7.

Neurons and vascular cells compose neurovascular niches in the central nervous system where endothelial cells can promote neurogenesis via direct and indirect effects. Neurocytes and vascular cells are gravely destroyed upon spinal cord injury, which severely affects spinal motor functions. Neurogenesis originates from neural stem cells (NSCs) and endothelial cells derived from endothelial progenitor cells (EPCs) in the spinal cord. To demonstrate whether EPCs promote NSC proliferation, we cultured NSCs with EPC-conditioned medium from hypoxic conditions (CM) and EPC-unconditioned medium (UCM), i.e. endothelial cell basal medium-2, as a control. The number of S-phase cells in CM were 54.73 +/- 0.67 whereas those in UCM were 26.30 +/- 0.43, and the number of cells in CM was higher than that in UCM (0.32 +/- 0.0019 vs. 0.55 +/- 0.0029). We hypothesized that the cell proliferation was promoted by vascular endothelial growth factor A (VEGFA), which is secreted by EPCs in hypoxic conditions. We then used VEGF shRNA to decrease VEGFA secretion by EPCs. NSCs were cultured in conditioned medium from shRNA transfected EPCs under hypoxia (shRNA-CM) and EPC-conditioned medium under hypoxia (CM). The number of S-phase cells in the shRNA-CM was 36.86 +/- 0.49 whereas that in CM was 53.61 +/- 0.89, and the number of cells in the shRNA-CM was lower than that in CM (0.55 +/- 0.0032 vs. 0.34 +/- 0.0029). These data indicate that EPCs could promote NSC proliferation in hypoxic condition through VEGFA secretion.

Kadar, T., et al. (2014). "Anti-VEGF therapy (bevacizumab) for sulfur mustard-induced corneal neovascularization associated with delayed limbal stem cell deficiency in rabbits." Curr Eye Res **39**(5): 439-450.

PURPOSE: To investigate the involvement of VEGF in corneal neovascularization (CNV) following sulfur mustard (SM) exposure and to test the therapeutic effects of bevacizumab (Avastin) in respect to dose, route of administration and timing. MATERIALS AND METHODS: Topical bevacizumab (6 or 25 mg/ml, x2/day) was applied to rabbit eyes, before or after appearance of NV, following SM vapor exposure, and was compared with subconjunctival injection (25 mg/ml, x2/week) and topical dexamethasone (1%, x4/day). Treatments were given for 3 weeks. VEGF levels were monitored by immunohistochemistry and ELISA assay. Clinical evaluations included slit-lamp examination, impression cytology for diagnosis of Limbal Stem Cell Deficiency (LSCD), pachymetry, measurement of NV length and histology. RESULTS: Corneal NV was developed, as early as 2 weeks after exposure, in 50-70% of the eyes, associated with increased levels of VEGF. Topical bevacizumab treatment with both doses, starting at 4 weeks, reduced vascularization. Subconjunctival injection and topical dexamethasone were more potent. A combined treatment of dexamethasone and bevacizumab improved the anti-angiogenic efficacy, yet, there was no effect on LSCD. Topical bevacizumab treatment starting at 1 week, when VEGF was elevated but before appearance of NV, had no effect. CONCLUSIONS: VEGF was involved in corneal angiogenesis in SM-induced ocular injury. Bevacizumab was beneficial in reducing CNV by both, topical or subconjunctival injection, when given as a symptomatic therapy with or without dexamethasone, however with no effect on SC deficiency. Further studies on the pathological mechanism of SM-induced ocular surface disorder may direct towards improved therapy.

Kang, T. H., et al. (2015). "Cell autonomous Vegf-C/Vegfr3 signaling in adult neural stem cells." Oncotarget **6**(37): 39387-39388.

Lanner, F., et al. (2007). "Functional arterial and venous fate is determined by graded VEGF signaling and notch status during embryonic stem cell differentiation." Arterioscler Thromb Vasc Biol **27**(3): 487-493.

OBJECTIVE: The aim of this work was to develop a mouse embryonic stem (ES) cell system addressing the early specification of the developing vasculature into functional arteries and veins. METHODS AND RESULTS: ES cells were differentiated 4 days on collagen-type IV coated dishes to obtain Flk1+ endothelial precursors. Sub-culture of these precursors for additional 4 days robustly generated, in a VEGF dose-dependent manner, mature endothelial cells. Arterial marker genes were specifically expressed in cultures differentiated with high VEGF concentration whereas the venous marker gene COUP-TFII was upregulated in endothelial cells induced through low and intermediate VEGF concentrations. This VEGF-dependent arterialization could be blocked by inhibition of Notch resulting in an arterial to venous fate switch. Functional and morphological studies, ie, measurement of sprout length, pericyte recruitment, and interleukin-I-induced leukocyte adhesion, further confirmed their arterial and venous identity. CONCLUSIONS: We conclude that endothelial cells with distinct molecular, morphological, and functional characteristics of arteries and veins can be derived through in vitro differentiation of ES cells in a VEGF dose-dependent and Notch-regulated manner.

Lee, E. Y., et al. (2009). "Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF." Wound Repair Regen **17**(4): 540-547.

Adipose-derived stem cells (ADSCs) have been shown to induce wound-healing effects. Because inflammation near the wound area induces oxygen deficiency, it is interesting to elucidate the effect of hypoxia on the function of ADSCs. In this work, we asked: (1) does hypoxia alter the wound-healing function of ADSCs? and (2) what are the major factors responsible for the alteration in the wound-healing function? Effect of hypoxia on the proliferation of ADSCs was first examined that hypoxia (2% O(2)) enhanced the proliferation of ADSCs in either the presence of serum or in the absence of serum. The conditioned medium of ADSCs harvested under hypoxia (hypoCM) significantly promoted collagen synthesis and the migration of human dermal fibroblasts, compared with that in normoxia (norCM). In the animal studies, hypoCM significantly reduced the wound area compared with norCM. Furthermore, mRNA and protein measurements showed that hypoxia up-regulated growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Inhibition of VEGF and bFGF using neutralizing antibodies reversed the migration of the wounded human dermal fibroblasts and the healing of wounds in animal experiment. Collectively, these results suggest that hypoxia increases the proliferation of ADSCs and enhances the wound-healing function of ADSCs, at least partly, by up-regulating the secretion of VEGF and bFGF.

Lee, H. L., et al. (2016). "Hypoxia-specific, VEGF-expressing neural stem cell therapy for safe and effective treatment of neuropathic pain." J Control Release **226**: 21-34.

Vascular endothelial growth factor (VEGF) is an angiogenic cytokine that stimulates the differentiation and function of vascular endothelial cells. VEGF has been implicated in improving nervous system function after injury. However, uncontrolled overexpression of VEGF increases the risk of tumor formation at the site of gene delivery. For this reason, VEGF expression needs to be strictly controlled. The goal of the present study was to understand the effects of hypoxia-induced gene expression system to control VEGF gene expression in neural stem cells (NSCs) on the regeneration of neural tissue after sciatic nerve injury. In this study, we used the erythropoietin (Epo) enhancer-SV40 promoter system (EpoSV-VEGF-NSCs) for hypoxia-specific VEGF expression. We used three types of NSCs: DsRed-NSCs as controls, SV-VEGF-NSCs as uncontrolled VEGF overexpressing NSCs, and EpoSV-VEGF-NSCs. For comparison of VEGF expression at normoxia and hypoxia, we measured the amount of VEGF secreted. VEGF expression decreased at normoxia and increased at hypoxia for EpoSV-VEGF-NSCs; thus, EpoSV-VEGF-NSCs controlled VEGF expression, dependent upon oxygenation condition. To demonstrate the therapeutic effect of EpoSV-VEGF-NSCs, we transplanted each cell line in a neuropathic pain sciatic nerve injury rat model. The transplanted EpoSV-VEGF-NSCs improved sciatic nerve functional index (SFI), mechanical allodynia, and re-myelination similar to the SV-VEGF-NSCs. Additionally, the number of blood vessels increased to a level similar to that of the SV-VEGF-NSCs. However, we did not observe tumor generation in the EpoSV-VEGF-NSC animals that were unlikely to have tumor formation in the SV-VEGF-NSCs. From our results, we determined that EpoSV-VEGF-NSCs safely regulate VEGF gene expression which is dependent upon oxygenation status. In addition, we found that they are therapeutically appropriate for treating sciatic nerve injury.

Lee, S., et al. (2015). "Enhanced therapeutic neovascularization by CD31-expressing cells and embryonic stem cell-derived endothelial cells engineered with chitosan hydrogel containing VEGF-releasing microtubes." Biomaterials **63**: 158-167.

Various stem cells and their progeny have been used therapeutically for vascular regeneration. One of the major hurdles for cell-based therapy is low cell retention in vivo, and to improve cell survival several biomaterials have been used to encapsulate cells before transplantation. Vascular regeneration involves new blood vessel formation which consists of two processes, vasculogenesis and angiogenesis. While embryonic stem cell (ESC)-derived endothelial cells (ESC-ECs) have clearer vasculogenic potency, adult cells exert their effects mainly through paracrine angiogenic activities. While these two cells have seemingly complementary advantages, there have not been any studies to date combining these two cell types for vascular regeneration. We have developed a novel chitosan-based hydrogel construct that encapsulates both CD31-expressing BM-mononuclear cells (BM-CD31(+) cells) and ESC-ECs, and is loaded with VEGF-releasing microtubes. This cell construct showed high cell survival and minimal cytotoxicity in vitro. When implanted into a mouse model of hindlimb ischemia, it induced robust cell retention, neovascularization through vasculogenesis and angiogenesis, and efficiently induced recovery of blood flow in ischemic hindlimbs. This chitosan-based hydrogel encapsulating mixed adult and embryonic cell derivatives and containing VEGF can serve as a novel platform for treating various cardiovascular diseases.

Lee, Y. B., et al. (2010). "Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture." Exp Neurol **223**(2): 645-652.

Time-released delivery of soluble growth factors (GFs) in engineered hydrogel tissue constructs promotes the migration and proliferation of embedded cells, which is an important factor for designing scaffolds that ultimately aim for neural tissue regeneration. We report a tissue engineering technique to print murine neural stem cells (C17.2), collagen hydrogel, and GF (vascular endothelial growth factor: VEGF)-releasing fibrin gel to construct an artificial neural tissue. We examined the morphological changes of the printed C17.2 cells embedded in the collagen and its migration toward the fibrin gel. The cells showed high viability (92.89+/-2.32%) after printing, which was equivalent to that of manually-plated cells. C17.2 cells printed within 1mm from the border of VEGF-releasing fibrin gel showed GF-induced changes in their morphology. The cells printed in this range also migrated toward the fibrin gel, with the total migration distance of 102.4+/-76.1microm over 3days. The cells in the control samples (fibrin without the VEGF or VEGF printed directly in collagen) neither proliferated nor migrated. The results demonstrated that bio-printing of VEGF-containing fibrin gel supported sustained release of the GF in the collagen scaffold. The presented method can be gainfully used in the development of three-dimensional (3D) artificial tissue assays and neural tissue regeneration applications.

Li, Q., et al. (2017). "VEGF treatment promotes bone marrow-derived CXCR4(+) mesenchymal stromal stem cell differentiation into vessel endothelial cells." Exp Ther Med **13**(2): 449-454.

Stem/progenitor cells serve an important role in the process of blood vessel repair. However, the mechanism of vascular repair mediated by C-X-C chemokine receptor type 4-positive (CXCR4(+)) bone marrow-derived mesenchymal stem cells (BMSCs) following myocardial infarction remains unclear. The aim of the present study was to investigate the effects of vascular endothelial growth factor (VEGF) on vessel endothelial differentiation from BMSCs. CXCR4(+) BMSCs were isolated from the femoral bone marrow of 2-month-old mice and the cells were treated with VEGF. Expression of endothelial cell markers and the functional properties were assessed by reverse transcription-quantitative polymerase chain reaction, flow cytometry and vascular formation analyses. The results indicated that the CXCR4(+) BMSCs from femoral bone marrow cells expressed putative cell surface markers of mesenchymal stem cells. Treatment with VEGF induced platelet/endothelial cell adhesion molecule-1 (PECAM-1) and von Willebrand factor (vWF) expression at the transcriptional and translational levels, compared with untreated controls. Moreover, VEGF treatment induced CXCR4(+) BMSCs to form hollow tube-like structures on Matrigel, suggesting that the differentiated endothelial cells had the functional properties of blood vessels. The results demonstrate that the CXCR4(+) BMSCs were able to differentiate into vessel endothelial cells following VEGF treatment. For cell transplantation in vascular disease, it may be concluded that CXCR4(+) BMSCs are a novel source of endothelial progenitor cells with high potential for application in vascular repair.

Lian Jin, H., et al. (2011). "Neural stem cells modified by a hypoxia-inducible VEGF gene expression system improve cell viability under hypoxic conditions and spinal cord injury." Spine (Phila Pa 1976) **36**(11): 857-864.

STUDY DESIGN: An in vitro neural hypoxia model and rat spinal cord injury (SCI) model were used to assess the regulation of therapeutic vascular endothelial growth factor (VEGF) gene expression in mouse neural stem cells (mNSCs) by the EPO (erythropoietin) enhancer or RTP801 promoter. OBJECTIVE: To increase VEGF gene expression in mNSCs under hypoxic conditions in SCI lesions but avoid unwanted overexpression of VEGF in normal sites, we developed a hypoxia-inducible gene expression system consisting of the EPO enhancer and RTP801 promoter fused to VEGF or the luciferase gene, then transfected into mNSCs. SUMMARY OF BACKGROUND DATA: On the basis of the ischemic response in the injured area, poor cell survival at the transplantation site is a consistent problem with NSC transplantation after SCI. Although VEGF directly protects neurons and enhances neurite outgrowth, uncontrolled overexpression of VEGF in uninjured tissue may cause serious adverse effects. To effectively improve NSC survival in ischemic sites after transplantation, we evaluated mNSCs modified by a hypoxia-inducible VEGF gene expression system in an SCI model. METHODS: Hypoxia-inducible luciferase or VEGF plasmids were constructed using the EPO enhancer or RTP801 promoter. The effect of these systems on targeted gene expression and cell viability was evaluated in mNSCs in both hypoxic in vitro injury and a rat SCI model in vivo. RESULTS: The gene expression system containing the EPO enhancer or RTP801 promoter significantly increased the expression of the luciferase reporter gene and therapeutic VEGF gene under hypoxic conditions. The Epo-SV-VEGF plasmid transfection group had significantly fewer apoptotic cells in vitro. This system also augmented cell viability in the in vivo SCI model. CONCLUSION: These results strongly suggest the potential utility of mNSCs modified by a hypoxia-inducible VEGF gene expression system in the development of effective stem cell transplantation protocols in SCI.

Liao, F. L., et al. (2014). "[Effect of notch signaling pathway on VEGF promoting rat mesenchymal stem cell proliferation]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **22**(4): 1068-1071.

This study was purposed to investigate the effect of Notch signaling pathway on VEGF promoting the proliferation of rat mesenchymal stem cells (MSC). Rat MSC were cultured in vitro, and the cells in logarithmic growth phase were used for experiments. The inhibitor DAPT was used to block Notch signaling pathway, and the effect of the pathway on VEGF promoting proliferation of MSC was observed. The experiment was divided into 4 groups: control, VEGF, DAPT and VEGF+DAPT. The CCK-8 was used to assay the cells proliferation of each group, while RT-PCR was used to detect the changes of related genes (Notch1, Notch2, Flk-1, Hes-1) at mRNA levels. The results indicated that the cells survival rate MSC in DAPT group and VEGF+DAPT group was low in each time point (24 h, 48 h, 72 h), the cell number decreased, and the cells became rounded. The survival rate of MSC in VEGF group was the highest; the difference of cell survival rate was statistically significant between the groups ($P < 0.01$); Compared with the control group, the mRNA expression level of Notch1, Notch2 and Flk-1 in VEGF group was raised, while the expression level of Notch1 and Notch2 in

DAPT group and VEGF+DAPT group come down, with statistically significant differences ($P < 0.05$); whereas the mRNA expression level of Hes-1 in VEGF group was down-regulated, but that in DAPT group and VEGF+DAPT group was up-regulated, and the difference was statistically significant ($P < 0.05$). Flk-1 mRNA level in DAPT group and VEGF+DAPT group was slightly lower, but the difference was not statistically significant ($P > 0.05$). It is concluded that Notch signaling pathway plays an important role in promoting the proliferation of rat MSC, treated with VEGF, however, the DAPT can weaken this effect.

Licht, T., et al. (2016). "VEGF preconditioning leads to stem cell remodeling and attenuates age-related decay of adult hippocampal neurogenesis." Proc Natl Acad Sci U S A **113**(48): E7828-E7836.

Several factors are known to enhance adult hippocampal neurogenesis but a factor capable of inducing a long-lasting neurogenic enhancement that attenuates age-related neurogenic decay has not been described. Here, we studied hippocampal neurogenesis following conditional VEGF induction in the adult brain and showed that a short episode of VEGF exposure withdrawn shortly after the generation of durable new vessels (but not under conditions where newly made vessels failed to persist) is sufficient for neurogenesis to proceed at a markedly elevated level for many months later. Continual neurogenic increase over several months was not accompanied by accelerated exhaustion of the neuronal stem cell (NSC) reserve, thereby allowing neurogenesis to proceed at a markedly elevated rate also in old mice. Neurogenic enhancement by VEGF preconditioning was, in part, attributed to rescue of age-related NSC quiescence. Remarkably, VEGF caused extensive NSC remodelling manifested in transition of the enigmatic NSC terminal arbor onto long cytoplasmic processes engaging with and spreading over even remote blood vessels, a configuration reminiscent of early postnatal "juvenile" NSCs. Together, these findings suggest that VEGF preconditioning might be harnessed for long-term neurogenic enhancement despite continued exposure to an "aged" systemic milieu.

Mantziou, S., et al. (2021). "Tinzaparin inhibits VL30 retrotransposition induced by oxidative stress and/or VEGF in HC11 mouse progenitor mammary cells: Association between inhibition of cancer stem cell proliferation and mammosphere disaggregation." Oncol Rep **46**(5).

Tinzaparin is an anticoagulant and antiangiogenic drug with inhibitory properties against tumor growth. VEGF stimulates angiogenesis, while an association between reactive oxygen species (ROS) and angiogenesis is involved in tumor progression. The present study aimed to investigate the effect of tinzaparin on VL30 retrotranspositionpositive mouse HC11 mammary stemlike epithelial cells, previously reported to be associated with induced mammosphere/cancer stem cell (CSC) generation and tumorigenesis. Under 24 h serum starvation, 15.2% nominal retrotransposition frequency was increased to 29%. Additionally, while treatment with 312 ng/ml VEGF further induced retrotransposition frequency in a dosedependent manner (up to 40.3%), preincubation with tinzaparin (2 IU/ml) for 0.54 h reduced this frequency to 18.3% in a timedependent manner, confirmed by analogous results in NIH3T3 fibroblasts. Treatment with 1040 pg/ml glucose oxidase (GO) for 24 h induced HC11 cell retrotransposition in a dosedependent manner (up to 82.5%), while a 3 h preincubation with tinzaparin (1 or 2 IU/ml) elicited a 13.5 or 25.5% reduction in retrotransposition, respectively. Regarding tumorigenic VL30 retrotranspositionpositive HC11 cells, treatment with 2 IU/ml tinzaparin for 5 days reduced proliferation rate in a timedependent manner (up to ~55%), and after 3 weeks, disaggregated soft agarformed foci, as well as lowadherent mammospheres, producing single mesenchymallike cells with a ~50% reduced retrotransposition. With respect to the VL30 retrotransposition mechanism: While 12 ng/ml VEGF increased the level of VL30 and endogenous reverse transcriptase (enRT) transcripts ~1.41 and ~1.16fold, respectively, subsequent tinzaparin treatment reduced both endogenous/ROS and VEGFinduced levels 1.15 and 0.40fold (VL30) and 0.60 and 0.52fold (enRT), respectively. To the best of our knowledge, these data demonstrate for the first time, the novel inhibition activity of tinzaparin against ROS and VEGFinduced VL30 retrotransposition, and the proliferation and/or aggregation of mouse HC11 mammosphere/tumorinitiating CSCs, thus contributing to the inhibition of VL30 retrotranspositioninduced primary tumor growth.

Markel, T. A., et al. (2008). "VEGF is critical for stem cell-mediated cardioprotection and a crucial paracrine factor for defining the age threshold in adult and neonatal stem cell function." Am J Physiol Heart Circ Physiol **295**(6): H2308-2314.

Bone marrow mesenchymal stem cells (MSCs) may be a novel treatment modality for organ ischemia, possibly through the release of beneficial paracrine factors. However, an age threshold likely exists as to when MSCs gain their beneficial protective properties. We hypothesized that 1) VEGF would be a crucial stem cell paracrine mediator in providing postischemic myocardial protection and 2) small-interfering (si)RNA ablation of VEGF in adult MSCs (aMSCs) would equalize the differences observed

between aMSC- and neonatal stem cell (nMSC)-mediated cardioprotection. Female adult Sprague-Dawley rat hearts were subjected to ischemia-reperfusion injury via Langendorff-isolated heart preparation (15 min equilibration, 25 min ischemia, and 60 min reperfusion). MSCs were harvested from adult and 2.5-wk-old neonatal mice and cultured under normal conditions. VEGF was knocked down in both cell lines by VEGF siRNA. Immediately before ischemia, one million aMSCs or nMSCs with or without VEGF knockdown were infused into the coronary circulation. The cardiac functional parameters were recorded. VEGF in cell supernatants was measured via ELISA. aMSCs produced significantly more VEGF than nMSCs and were noted to increase postischemic myocardial recovery compared with nMSCs. The knockdown of VEGF significantly decreased VEGF production in both cell lines, and the pretreatment of these cells impaired stem cell-mediated myocardial function. The knockdown of VEGF in adult stem cells equalized the myocardial functional differences observed between adult and neonatal stem cells. Therefore, VEGF is a critical paracrine mediator in facilitating postischemic myocardial recovery and likely plays a role in mediating the observed age threshold during stem cell therapy.

Mesnieres, M., et al. (2021). "Fetal hematopoietic stem cell homing is controlled by VEGF regulating the integrity and oxidative status of the stromal-vascular bone marrow niches." Cell Rep **36**(8): 109618.

Hematopoietic stem and progenitor cell (HSPC) engraftment after transplantation during anticancer treatment depends on support from the recipient bone marrow (BM) microenvironment. Here, by studying physiological homing of fetal HSPCs, we show the critical requirement of balanced local crosstalk within the skeletal niche for successful HSPC settlement in BM. Transgene-induced overproduction of vascular endothelial growth factor (VEGF) by osteoprogenitor cells elicits stromal and endothelial hyperactivation, profoundly impacting the stromal-vessel interface and vascular architecture. Concomitantly, HSPC homing and survival are drastically impaired. Transcriptome profiling, flow cytometry, and high-resolution imaging indicate alterations in perivascular and endothelial cell characteristics, vascular function and cellular metabolism, associated with increased oxidative stress within the VEGF-enriched BM environment. Thus, developmental HSPC homing to bone is controlled by local stromal-vascular integrity and the oxidative-metabolic status of the recipient milieu. Interestingly, irradiation of adult mice also induces stromal VEGF expression and similar osteo-angiogenic niche changes, underscoring that our findings may contribute targets for improving stem cell therapies.

Ng, Y. S., et al. (2004). "Identification of genes involved in VEGF-mediated vascular morphogenesis using embryonic stem cell-derived cystic embryoid bodies." Lab Invest **84**(9): 1209-1218.

The vasculature forms during development via two processes, vasculogenesis and angiogenesis, in which vessels form de novo from angioblast precursors or as sprouts from pre-existing vessels, respectively. A common and critical aspect of both processes is vascular morphogenesis, which includes branching of endothelial cell cords and lumen formation. Although ample evidence support the central role of vascular endothelial growth factor (VEGF) in both vasculogenesis and angiogenesis, the role of VEGF in vascular morphogenesis is unclear and little is known about the regulation of vascular morphogenesis, in general. We have used the in vitro vessel differentiation system of embryonic stem (ES) cell-derived cystic embryonic bodies (CEB) as a model for studying VEGF-mediated vessel formation. Whereas CEB formed from wild-type ES cells make well-formed vessel-like structures, CEB derived from VEGF-null ES cells contain PECAM-1-positive endothelial cells, but these cells do not participate in vascular morphogenesis. Using gene expression microarray analysis to compare gene expression in these two systems, we have been able to identify many genes and novel ESTs that are downstream of VEGF function, and which may be involved in VEGF-mediated vascular morphogenesis including caveolin-1 and HEY-1. These results support using the CEB model, in combination with gene knockout ES cells, for studying vascular morphogenesis.

Ni, X., et al. (2017). "Lentiviral vector-mediated co-overexpression of VEGF and Bcl-2 improves mesenchymal stem cell survival and enhances paracrine effects in vitro." Int J Mol Med **40**(2): 418-426.

Mesenchymal stem cell (MSC) transplantation has emerged as a promising therapy for ischemic heart disease; however, the low survival rate of transplanted cells limits their therapeutic efficacy. The aim of this study was to investigate whether the dual genetic modification of vascular endothelial growth factor (VEGF) and Bcell lymphoma2 (Bcl2) confers a higher expression level of the target genes, better survival and a stronger paracrine effect in MSCs in an adverse environment than the modification of the individual genes. For this purpse, a lentiviral vector was constructed by using a selfcleaving T2A peptide sequence to link and achieve the cooverexpression of VEGF and Bcl2. Rat MSCs were transfected to obtain

cell lines that exhibited a stable overexpression. An in vitro model of oxygen glucose deprivation (OGD) was applied to mimic the ischemic microenvironment, and cell apoptosis, autophagy and the paracrine effects were then determined. Compared with the MSCs in which individual genes were modified and the control MSCs, the MSCs which were subjected to dual genetic modification had a higher expression level of the target genes, a more rapid proliferation, reduced apoptosis, decreased autophagy and an enhanced paracrine effect. Furthermore, the suppression of autophagy was found to contribute to the inhibition of apoptosis in this in vitro OGD model. On the whole, these data indicate that the cooverexpression of VEGF and Bcl2 protects MSCs in an ischemic environment by inhibiting apoptosis, suppressing autophagy and enhancing the paracrine effects.

Nowicki, M., et al. (2017). "VEGF, ANGPT1, ANGPT2, and MMP-9 expression in the autologous hematopoietic stem cell transplantation and its impact on the time to engraftment." Ann Hematol **96**(12): 2103-2112.

As a site of complicated interactions among cytokines, bone marrow niche has been the subject of many scientific studies, mainly in the context of the proteins influencing damage or recovery of endothelium after allogeneic hematopoietic stem cell transplantation (HSCT). In this study, we aimed at exploring mutual correlations of bone marrow niche cytokines involved in the homing and mobilization of hematopoietic stem cells, as well as in angiogenesis. The aim of our study was to evaluate levels of cytokines: VEGF, angiopoietin-1 (ANGPT1), angiopoietin-2 (ANGPT2), and matrix metalloproteinase 9 (MMP-9) during autologous HSCT and to examine their influence on hematological recovery. Forty-three patients with hematological malignancies (33 multiple myeloma, 10 lymphoma) were enrolled in the study. Plasma samples were taken at five time points: before conditioning treatment (BC), on transplantation day (0) and 7 (+7), 14 (+14), and 21 (+21) days after HSCT. The cytokine levels were evaluated by ELISA method. Our study revealed decreased levels of VEGF, ANGPT1, and MMP-9 in the early post-transplant period as compared to the baseline (BC). ANGPT2 was decreased after conditioning treatment, but tended to increase from day +7. On day +7, positive correlations between ANGPT1 level as well as MMP-9 and the time to engraftment were observed. As opposite to ANGPT1, negative correlation between ANGPT2 level on day +7 after HSCT and the time to hematological recovery was noticed. Our study suggests that investigated cytokines are an important part of bone marrow environment and significantly influence the time to engraftment after HSCT.

Pakravan, K., et al. (2017). "MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses in vitro angiogenesis through modulating the mTOR/HIF-1alpha/VEGF signaling axis in breast cancer cells." Cell Oncol (Dordr) **40**(5): 457-470.

BACKGROUND: Human mesenchymal stem cells (MSCs) have been shown to be involved in the formation and modulation of tumor stroma and in interacting with tumor cells, partly through their secretome. Exosomes are nano-sized intraluminal multi-vesicular bodies secreted by most types of cells and have been found to mediate intercellular communication through the transfer of genetic information via coding and non-coding RNAs to recipient cells. Since exosomes are considered as protective and enriched sources of shuttle microRNAs (miRNAs), we hypothesized that exosomal transfer of miRNAs from MSCs may affect tumor cell behavior, particularly angiogenesis. METHODS: Exosomes derived from MSCs were isolated and characterized by scanning electron microscopy analyses, dynamic light scattering measurements, and Western blotting. Fold changes in miR-100 expression levels were calculated in exosomes and their corresponding donor cells by qRT-PCR. The effects of exosomal transfer of miR-100 from MSCs were assessed by qRT-PCR and Western blotting of the mTOR/HIF-1alpha/VEGF signaling axis in breast cancer cells. The quantification of secreted VEGF protein was determined by enzyme-linked immunosorbent assay. The putative paracrine effects of MSC-derived exosomes on tumor angiogenesis were explored by in vitro angiogenesis assays including endothelial cell proliferation, migration and tube formation assays. RESULTS: We found that MSC-derived exosomes induce a significant and dose-dependent decrease in the expression and secretion of vascular endothelial growth factor (VEGF) through modulating the mTOR/HIF-1alpha signaling axis in breast cancer-derived cells. We also found that miR-100 is enriched in MSC-derived exosomes and that its transfer to breast cancer-derived cells is associated with the down-regulation of VEGF in a time-dependent manner. The putative role of exosomal miR-100 transfer in regulating VEGF expression was substantiated by the ability of anti-miR-100 to rescue the inhibitory effects of MSC-derived exosomes on the expression of VEGF in breast cancer-derived cells. In addition, we found that down-regulation of VEGF mediated by MSC-derived exosomes can affect the vascular behavior of endothelial cells in vitro. CONCLUSIONS: Overall, our findings suggest that exosomal transfer of miR-100 may be a novel mechanism underlying the paracrine effects of MSC-

derived exosomes and may provide a means by which these vesicles can modulate vascular responses within the microenvironment of breast cancer cells.

Park, S. H., et al. (2021). "Endogenous Stem Cell-Based In Situ Tissue Regeneration Using Electrostatically Interactive Hydrogel with a Newly Discovered Substance P Analog and VEGF-Mimicking Peptide." Small **17**(40): e2103244.

The use of chemoattractants to promote endogenous stem cell-based in situ tissue regeneration has recently garnered much attention. This study is the first to assess the endogenous stem cell migration using a newly discovered substance P (SP) analog (SP1) by molecular dynamics simulations as an efficient chemoattractant. Further, a novel strategy based on electrostatic interaction using cationic chitosan (Ch) and anionic hyaluronic acid (HA) to prepare an SP1-loaded injectable C/H formulation without SP1 loss is developed. The formulation quickly forms an SP1-loaded C/H hydrogel in situ through in vivo injection. The newly discovered SP1 is found to possess human mesenchymal stromal cells (hMSCs) migration-inducing ability that is approximately two to three times higher than that of the existing SP. The designed VEGF-mimicking peptide (VP) chemically reacts with the hydrogel (C/H-VP) to sustain the release of VP, thus inducing vasculogenic differentiation of the hMSCs that migrate toward the C/H-VP hydrogel. Similarly, in animal experiments, SP1 attracts a large number of hMSCs toward the C/H-VP hydrogel, after which VP induces vasculogenic differentiation. Collectively, these findings indicate that SP1-loaded C/H-VP hydrogels are a promising strategy to facilitate endogenous stem cell-based in situ tissue regeneration.

Pelegri, N. G., et al. (2019). "Rat Hippocampal Neural Stem Cell Modulation Using PDGF, VEGF, PDGF/VEGF, and BDNF." Stem Cells Int **2019**: 4978917.

Neural stem cells have become the focus of many studies as they have the potential to differentiate into all three neural lineages. This may be utilised to develop new and novel ways to treat neurological conditions such as spinal cord and brain injuries, especially if the stem cells can be modulated in vivo without additional invasive surgical procedures. This research is aimed at investigating the effects of the growth factors vascular endothelial growth factor, platelet-derived growth factor, brain-derived neurotrophic factor, and vascular endothelial growth factor/platelet-derived growth factor on hippocampal-derived neural stem cells. Cell growth and differentiation were assessed using immunohistochemistry and glutaminase enzyme assay. Cells were cultured for 14 days and treated with different growth factors at two different concentrations 20 ng/mL and 100 ng/mL. At 2 weeks, cells were fixed, and immunohistochemistry was conducted to determine cellular differentiation using antibodies against GFAP, nestin, OSP, and NF200. The cell medium supernatant was also collected during treatment to determine glutaminase levels secreted by the cells as an indicator of neural differentiation. VEGF/PDGF at 100 ng/mL had the greatest influence on cellular proliferation of HNSC, which also stained positively for nestin, OSP, and NF200. In comparison, HNSC in other treatments had poorer cell health and adhesion. HNSC in all treatment groups displayed some differentiation markers and morphology, but this is most significant in the 100 ng/ml VEGF/PDGF treatment. VEGF/PDGF combination produced the optimal effect on the HNSCs inducing the differentiation pathway exhibiting oligodendrocytic and neuronal markers. This is a promising finding that should be further investigated in the brain and spinal cord injury.

Penna, C., et al. (2013). "Pharmacologically active microcarriers influence VEGF-A effects on mesenchymal stem cell survival." J Cell Mol Med **17**(1): 192-204.

Resistance of transplanted mesenchymal stem cells (MSCs) in post-ischemic heart is limited by their poor vitality. Vascular-endothelial-growth-factor-A (VEGF-A) as such or slowly released by fibronectin-coated pharmacologically-active-microcarriers (FN-PAM-VEGF) could differently affect survival kinases and anti-apoptotic mediator (e.g. Bcl-2). Therefore VEGF-A or FN-PAM-VEGF could differently enhance cell proliferation, and/or resistance to hypoxia/reoxygenation (H/R) of MSCs. To test these hypotheses MSCs were incubated for 6-days with VEGF-A alone or with FN-PAM-VEGF. In addition, MSCs pre-treated for 24-hrs with VEGF-A or FN-PAM-VEGF were subsequently exposed to H/R (72-hrs 3% O(2) and 3-hrs of reoxygenation). Cell-proliferation and post-hypoxic vitality were determined. Kinases were studied at 30-min., 1- and 3-days of treatment. Cell-proliferation increased about twofold ($P < 0.01$) 6-days after VEGF-A treatment, but by a lesser extent (55% increase) with FN-PAM-VEGF ($P < 0.05$). While MSC pre-treatment with VEGF-A confirmed cell-proliferation, pre-treatment with FN-PAM-VEGF protected MSCs against H/R. In the early phase of treatments, VEGF-A increased phospho-Akt, phospho-ERK-1/2 and phospho-PKCepsilon compared to the untreated cells or FN-PAM-VEGF. Afterword, kinase phosphorylations were higher with VGEF, except for ERK-1/2, which was similarly increased by both treatments at 3 days. Only FN-PAM-VEGF significantly increased Bcl-2 levels. After H/R, lactate

dehydrogenase release and cleaved Caspase-3 levels were mainly reduced by FN-PAM-VEGF. While VEGF-A enhances MSC proliferation in normoxia, FN-PAM-VEGF mainly hampers post-hypoxic MSC death. These different effects underscore the necessity of approaches suited to the various conditions. The use of FN-PAM-VEGF could be considered as a novel approach for enhancing MSC survival and regeneration in hostile environment of post-ischemic tissues.

Philipp, D., et al. (2021). "VEGF Contributes to Mesenchymal Stem Cell-Mediated Reversion of Nor1-Dependent Hypertrophy in iPS Cell-Derived Cardiomyocytes." Stem Cells Int **2021**: 8888575.

Myocardial hypertrophy is present in many heart diseases, representing a strong predictor of adverse cardiovascular outcomes. Regarding therapeutic intervention, mesenchymal stem cells (MSCs) have been suggested to significantly reduce cardiac hypertrophy and progression to heart failure. Preconditioning of MSCs was previously demonstrated to highly improve their paracrine activity resulting in modulation of immune responses and the progression of diseases. Here, we studied the effects of bone marrow-derived preconditioned MSCs on hypertrophied induced pluripotent stem cell-derived cardiomyocytes (iPS-CM) and also sought to identify MSC-derived antihypertrophic molecules. Phenylephrine (PE) was used to induce hypertrophy in murine iPS-CM, and markers of hypertrophy were identified by microarray analysis. Murine MSCs were treated with IFN-gamma and IL-1beta to enhance their paracrine activity, and transcriptional profiling was performed by microarray analysis. Hypertrophied iPS-CM were subsequently cocultured with preconditioned MSCs or MSC-conditioned medium (CM), respectively. Effects on hypertrophied iPS-CM were studied by cell area quantification, real-time PCR, and western blot. In some experiments, cells were incubated with fractions of MSC-CM obtained by ultrafiltration or by MSC-CM supplemented with inhibitory antibodies. Intracellular and extracellular levels of vascular endothelial growth factor (VEGF) were evaluated by western blot and ELISA. PE-induced hypertrophy in iPS-CM was associated with an upregulation of neuron-derived orphan receptor (Nor1) expression, activation of Akt, and inhibition of both strongly prevented hypertrophy induction in iPS-CM. VEGF secreted by preconditioned MSCs provoked hypertrophy regression in iPS-CM, and a negative correlation between Nor1 expression and hypertrophic growth could be evidenced. Our results demonstrate that Nor1 expression strongly supports hypertrophy in iPS-CM. Moreover, the secretome of preconditioned MSCs triggered regression of hypertrophy in iPS-CM in a VEGF-dependent manner. We suggest that the delivery of the MSC-derived secretome may represent a therapeutic strategy to limit cardiac hypertrophy. However, additional in vivo studies are needed to prove this hypothesis.

Piao, Y., et al. (2012). "Glioblastoma resistance to anti-VEGF therapy is associated with myeloid cell infiltration, stem cell accumulation, and a mesenchymal phenotype." Neuro Oncol **14**(11): 1379-1392.

Vascular endothelial growth factor (VEGF) is a critical regulator of angiogenesis. Inhibiting the VEGF-VEGF receptor (R) signal transduction pathway in glioblastoma has recently been shown to delay progression, but the relative benefit and mechanisms of response and failure of anti-VEGF therapy and VEGFR inhibitors are not well understood. The purpose of our study was to evaluate the relative effectiveness of VEGF sequestration and/or VEGFR inhibition on orthotopic tumor growth and the mechanism(s) of treatment resistance. We evaluated, not only, the effects of anti-VEGF therapy (bevacizumab), anti-VEGFR therapy (sunitinib), and the combination on the survival of mice bearing orthotopic gliomas, but also the differential effects of the treatments on tumor vascularity, cellular proliferation, mesenchymal and stem cell markers, and myeloid cell infiltration using flow cytometry and immunohistochemistry. Bevacizumab significantly prolonged survival compared with the control or sunitinib alone. Both antiangiogenic agents initially reduced infiltration of macrophages and tumor vascularity. However, multitargeted VEGFR inhibition, but not VEGF sequestration, rapidly created a vascular gradient and more rapidly induced tumor hypoxia. Re-infiltration of macrophages was associated with the induction of hypoxia. Combination treatment with bevacizumab and sunitinib improved animal survival compared with bevacizumab therapy alone. However, at the time of tumor progression, a significant increase in CD11b(+)/Gr1(+) granulocyte infiltration was observed, and tumors developed aggressive mesenchymal features and increased stem cell marker expression. Collectively, our results demonstrate a more prolonged decrease in tumor vascularity with bevacizumab than with sunitinib, associated with a delay in the development of hypoxia and sustained reduction of infiltrated myeloid cells.

Ping, S., et al. (2019). "Stem cell factor and granulocyte colony-stimulating factor promote brain repair and improve cognitive function through VEGF-A in a mouse model of CADASIL." Neurobiol Dis **132**: 104561.

Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy

(CADASIL) is a cerebral small vascular disease caused by NOTCH3 gene mutation in vascular smooth muscle cells (VSMCs), leading to ischemic stroke and vascular dementia. To date, the pathogenesis of CADASIL remains poorly understood, and there is no treatment that can slow the progression of CADASIL. Using a transgenic mouse model of CADASIL (TgNotch3R90C), this study reveals novel findings for understanding CADASIL pathogenesis that decreased cerebral vascular endothelial growth factor (VEGF/VEGF-A) is linked to reduced cerebral blood vessel density. Reduced endothelial cell (EC) proliferation and angiogenesis are seen in TgNotch3R90C mouse brain-isolated ECs. Decreased dendrites, axons, and synapses in the somatosensory and motor cortex layer 2/3 and in the hippocampal CA1, and reduced neurogenesis in both the subventricular zone and subgranular zone occur in 15-month-old TgNotch3R90C mice. These reductions in neuron structures, synapses, and neurogenesis are significantly correlated to decreased cerebral vasculature in the corresponding areas. Impaired spatial learning and memory in TgNotch3R90C mice are significantly correlated with the reduced cerebral vasculature, neuron structures, and synapses. Repeated treatment of stem cell factor and granulocyte colony-stimulating factor (SCF+G-CSF) at 9 and 10months of age improves cognitive function, increases cerebral VEGF/VEGF-A, restores cerebral vasculature, and enhances regeneration of neuronal structures, synaptogenesis and neurogenesis in TgNotch3R90C mice. Pretreatment with Avastin, an angiogenesis inhibitor by neutralizing VEGF-A, completely eliminates the SCF+G-CSF-enhanced cognitive function, vascular and neuronal structure regeneration, synaptogenesis and neurogenesis in TgNotch3R90C mice. SCF+G-CSF-enhanced EC proliferation and angiogenesis in TgNotch3R90C mouse brain-isolated ECs are also blocked by Avastin pretreatment. These data suggest that SCF+G-CSF treatment may repair Notch3R90C mutation-damaged brain through the VEGF-A-mediated angiogenesis. This study provides novel insight into the involvement of VEGF/VEGF-A in the pathogenesis of CADASIL and sheds light on the mechanism underlying the SCF+G-CSF-enhanced brain repair in CADASIL.

Ping, Y. F., et al. (2011). "The chemokine CXCL12 and its receptor CXCR4 promote glioma stem cell-mediated VEGF production and tumour angiogenesis via PI3K/AKT signalling." J Pathol **224**(3): 344-354.

Chemokines and their receptors are actively involved in inflammation, immune responses, and cancer development. Here we report the detection of CD133(+) glioma stem-like cells (GSCs) co-expressing a chemokine receptor CXCR4 in human primary glioma tissues. These GSCs were located in areas adjacent to tumour vascular capillaries, suggesting an association between GSCs and tumour angiogenesis. To test this hypothesis, we isolated CD133(+) GSCs from surgical specimens of human primary gliomas and glioma cell lines. As compared to CD133(-) cells, CD133(+) GSCs expressed significantly higher levels of CXCR4 mRNA and protein, and migrated more efficiently in response to the CXCR4 ligand CXCL12. In addition, CXCL12 induced vascular endothelial growth factor (VEGF) production by CD133(+) GSCs via activation of the PI3K/AKT signalling pathway. Furthermore, knocking down of CXCR4 using RNA interference or inhibition of CXCR4 function by an antagonist AMD3100 not only reduced VEGF production by CD133(+) GSCs in vitro, but also attenuated the growth and angiogenesis of tumour xenografts in vivo formed by CD133(+) GSCs in SCID mice. These results indicate that CXCL12 and its receptor CXCR4 promote GSC-initiated glioma growth and angiogenesis by stimulating VEGF production.

Purpura, K. A., et al. (2008). "Analysis of the temporal and concentration-dependent effects of BMP-4, VEGF, and TPO on development of embryonic stem cell-derived mesoderm and blood progenitors in a defined, serum-free media." Exp Hematol **36**(9): 1186-1198.

OBJECTIVE: To develop a robust serum-free (SF) system for generation of hemogenic mesoderm and blood progenitors from pluripotent cells. MATERIALS AND METHODS: Embryonic stem cells (ESCs) maintained in N2B27 supplemented with leukemia inhibitory factor (LIF) and bone morphogenetic protein (BMP)-4 were induced to differentiate into Brachyury/T-expressing cells (measured using a green fluorescent protein reporter) and myeloid-erythroid colony-forming cells (ME-CFCs), by removing LIF, changing the base media formulation, and via the time- and concentration-dependent addition of other factors. RESULTS: Presence of 10 ng/mL BMP-4 permitted the emergence of cells expressing T and the vascular endothelial growth factor receptor (VEGFR)-2, however, <5% of the cells were double-positive on day 4. Adjusting the SF media formulation allowed only 5 ng/mL BMP-4 to yield 24% +/- 4% Brachyury-green fluorescent protein VEGFR-2(+) cells by day 4. These cells could develop into ME-CFC, producing 4.4 +/- 0.8 CFC per 1000 cells at day 8. We also examined the timing and concentration sensitivity of BMP-4, VEGF, and thrombopoietin (TPO) during differentiation. BMP-4 with 50 ng/mL TPO generated 232 +/- 48 CFC per 5 x 10(4) cells, similar to the serum-control, and this response could be enhanced to 292 +/- 42 CFC per 5 x 10(4) cells by early (between day 0-5), but not late (after day 5) VEGF treatment. CONCLUSION:

Moving to SF systems facilitates directed differentiation by eliminating confounding signals. This article describes modifications to the N2B27 media that amplify mesoderm induction and extends earlier work defining blood progenitor cell induction from ESC with BMP-4, VEGF, and TPO.

Quittet, M. S., et al. (2015). "Effects of mesenchymal stem cell therapy, in association with pharmacologically active microcarriers releasing VEGF, in an ischaemic stroke model in the rat." Acta Biomater **15**: 77-88.

Few effective therapeutic interventions are available to limit brain damage and functional deficits after ischaemic stroke. Within this context, mesenchymal stem cell (MSC) therapy carries minimal risks while remaining efficacious through the secretion of trophic, protective, neurogenic and angiogenic factors. The limited survival rate of MSCs restricts their beneficial effects. The usefulness of a three-dimensional support, such as a pharmacologically active microcarrier (PAM), on the survival of MSCs during hypoxia has been shown in vitro, especially when the PAMs were loaded with vascular endothelial growth factor (VEGF). In the present study, the effect of MSCs attached to laminin-PAMs (LM-PAMs), releasing VEGF or not, was evaluated in vivo in a model of transient stroke. The parameters assessed were infarct volume, functional recovery and endogenous cellular reactions. LM-PAMs induced the expression of neuronal markers by MSCs both in vitro and in vivo. Moreover, the prolonged release of VEGF increased angiogenesis around the site of implantation of the LM-PAMs and facilitated the migration of immature neurons towards the ischaemic tissue. Nonetheless, MSCs/LM-PAMs-VEGF failed to improve sensorimotor functions. The use of LM-PAMs to convey MSCs and to deliver growth factors could be an effective strategy to repair the brain damage caused by a stroke.

Randolph, L. N., et al. (2019). "Sex-dependent VEGF expression underlies variations in human pluripotent stem cell to endothelial progenitor differentiation." Sci Rep **9**(1): 16696.

Human pluripotent stem cells (hPSCs) offer tremendous promise in tissue engineering and cell-based therapies because of their unique combination of two properties: pluripotency and a high proliferative capacity. To realize this potential, development of efficient hPSC differentiation protocols is required. In this work, sex-based differences are identified in a GSK3 inhibitor based endothelial progenitor differentiation protocol. While male hPSCs efficiently differentiate into CD34 (+)CD31(+) endothelial progenitors upon GSK3 inhibition, female hPSCs showed limited differentiation capacity using this protocol. Using VE-cadherin-GFP knockin reporter cells, female cells showed significantly increased differentiation efficiency when treated with VEGF during the second stage of endothelial progenitor differentiation. Interestingly, male cells showed no significant change in differentiation efficiency with VEGF treatment, but did show augmented early activation of VE-cadherin expression. A sex-based difference in endogenous expression of VEGF was identified that is likely the underlying cause of discrepancies in sex-dependent differentiation efficiency. These findings highlight the importance of sex differences in progenitor biology and the development of new stem cell differentiation protocols.

Ray, R., et al. (2008). "Deleterious effects of endogenous and exogenous testosterone on mesenchymal stem cell VEGF production." Am J Physiol Regul Integr Comp Physiol **294**(5): R1498-1503.

Modulating the paracrine effects of bone marrow mesenchymal stem cells (BMSCs) may be important for the treatment of ischemic myocardial tissue. In this regard, endogenous estrogen may enhance BMSC vascular endothelial growth factor (VEGF) production. However, little information exists regarding the effect of testosterone on stem cell function. We hypothesized that 1) endogenous or exogenous estrogen will enhance stem cell production of VEGF and 2) endogenous or exogenous testosterone will inhibit BMSC VEGF production. BMSCs were collected from adult male, female, castrated male, and ovariectomized female rats. One hundred thousand cells were incubated with testosterone (1, 10, or 100 nM) or estrogen (0.15, 1.5, or 15 nM) for 48 h. Cell supernatants were collected, and VEGF was measured by ELISA. BMSCs harvested from castrated males, normal females, and ovariectomized females produced more VEGF compared with normal males. Castration was associated with the highest level (1,018 +/- 98.26 pg/ml) of VEGF production by BMSCs, which was significantly more than that produced by BMSCs harvested from normal male and normal female animals. Exogenous testosterone significantly reduced VEGF production in BMSCs harvested from ovariectomized females in a dose-dependent manner. Exogenous estrogen did not alter BMSC VEGF production. These findings suggest that testosterone may work on BMSCs to decrease protective growth factor production and that effective removal of testosterone's deleterious effects via castration may prove to be beneficial in terms of protective factor production. By manipulating the mechanisms that BMSCs use to produce growth factors, we may be able

to engineer stem cells to produce maximum growth factors during therapeutic use.

Rizvanov, A. A., et al. (2008). "Human umbilical cord blood cells transfected with VEGF and L(1)CAM do not differentiate into neurons but transform into vascular endothelial cells and secrete neuro-trophic factors to support neuro-genesis-a novel approach in stem cell therapy." Neurochem Int **53**(6-8): 389-394.

Genetically modified mono-nuclear cell fraction from human umbilical cord blood (HUCB) expressing human vascular endothelial growth factor (VEGF) and mouse neural L(1) cell adhesion molecule (L(1)CAM) were used for gene-stem cell therapy of transgenic (G)93(A) mice adopted as an animal amyotrophic lateral sclerosis (ALS) model. We generated non-viral plasmid constructs, expressing human VEGF(165) (pcDNA-VEGF) and mouse neural L(1) cell adhesion molecule (pcDNA-mL(1)CAM). Mono-nuclear fraction of HUCB cells were transiently transfected by electro-poration with a mixture of expression plasmids (pcDNA-VEGF+pcDNA-mL(1)CAM). Sixteen transgenic female and male mice were randomly assigned to three groups: (1) transplantation of genetically modified HUCB cells expressing L(1) and VEGF (n=6), (2) transplantation of un-transfected HUCB cells (n=5), and (3) control group (n=5). In first two experimental groups 1x10(6) cells were injected retro-orbitally in pre-symptomatic 22-25-week-old (G)93(A) mice. Our results demonstrate that HUCB cells successfully grafted into nervous tissue of ALS mice and survived for over 3 months. Therefore, genetically modified HUCB cells migrate in the spinal cord parenchyma, proliferate, but instead of transforming into nerve cells, they differentiate into endothelial cells forming new blood vessels. We propose that: (A) expression of mouse neural L(1)CAM is responsible for increased homing and subsequent proliferation of transplanted cells at the site of neuro-degeneration, (B) expression of human VEGF directs HUCB cell differentiation into endothelial cells, and (C) neuro-protective effect may stem from the delivery of various neuro-trophic factors from newly formed blood vessels.

Roitbak, T., et al. (2008). "Neural stem/progenitor cells promote endothelial cell morphogenesis and protect endothelial cells against ischemia via HIF-1alpha-regulated VEGF signaling." J Cereb Blood Flow Metab **28**(9): 1530-1542.

Vascular cells provide a neural stem/progenitor cell (NSPC) niche that regulates expansion and differentiation of NSPCs within the germinal zones of the embryonic and adult brain under both physiologic and pathologic conditions. Here, we examined the NSPC-endothelial cell (NSPC/EC) interaction under conditions of ischemia, both in vitro and after intracerebral transplantation. In culture, embryonic mouse NSPCs supported capillary morphogenesis and protected ECs from cell death induced by serum starvation or by transient oxygen and glucose deprivation (OGD). Neural stem/progenitor cells constitutively expressed hypoxia-inducible factor 1alpha (HIF-1alpha) transcription factor and vascular endothelial growth factor (VEGF), both of which were increased approximately twofold after the exposure of NSPCs to OGD. The protective effects of NSPCs on ECs under conditions of serum starvation and hypoxia were blocked by pharmacological inhibitors of VEGF signaling, SU1498 and Flt-1-Fc. After intracerebral transplantation, NSPCs continued to express HIF-1alpha and VEGF, and promoted microvascular density after focal ischemia. These studies support a role for NSPCs in stabilization of vasculature during ischemia, mediated via HIF-1alpha-VEGF signaling pathways, and suggest therapeutic application of NSPCs to promote revascularization and repair after brain injury.

Sedrakyan, S., et al. (2017). "Amniotic fluid stem cell-derived vesicles protect from VEGF-induced endothelial damage." Sci Rep **7**(1): 16875.

Injection of amniotic fluid stem cells (AFSC) delays the course of progression of renal fibrosis in animals with Alport Syndrome, enhancing kidney function and improving survival. The mechanisms responsible for these protective outcomes are still largely unknown. Here, we showed that vascular endothelial growth factor (VEGF) signaling within the glomeruli of Alport mice is strongly elevated early on in the disease, causing glomerular endothelial cell damage. Intraventricular injected AFSC that homed within the glomeruli showed strong modulation of the VEGF activity, particularly in glomerular endothelial cells. To investigate this phenomenon we hypothesized that extracellular vesicles (EVs) produced by the AFSC could be responsible for the observed renoprotection. AFSC derived EVs presented exosomal and stem cell markers on their surface membrane, including VEGFR1 and VEGFR2. EVs were able to modulate VEGF in glomerular endothelial cells by effectively trapping the excess VEGF through VEGFR1-binding preventing cellular damage. In contrast, VEGFR1/sVEGFR1 knockout EVs failed to show similar protection, thus indicating that VEGF trapping is a potentially viable mechanism for AFSC-EV mediated renoprotection. Taken together, our findings establish that EVs secreted by AFSC could target a specific signaling pathway within the glomerulus, thus representing a new potential glomerulus-specific targeted intervention.

Shetty, A. K., et al. (2005). "Stem/progenitor cell proliferation factors FGF-2, IGF-1, and VEGF exhibit early decline during the course of aging in the hippocampus: role of astrocytes." Glia **51**(3): 173-186.

Dentate neurogenesis, important for learning and memory, declines dramatically by middle age. Although studies have shown that this age-related decrease can be reversed to some extent by exogenous applications of mitogenic factors, it is unclear whether one or more of these factors exhibits decline by middle age. We hypothesize that multiple stem/progenitor cell proliferation factors exhibit early decline during the course of aging in the hippocampus, and some of these declines are linked to age-related alterations in hippocampal astrocytes. We measured the concentrations of fibroblast growth factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) in the hippocampus of young, middle-aged, and aged F344 rats, using enzyme-linked immunosorbent assay (ELISA). In addition, we quantified the total number of FGF-2 immunopositive (FGF-2+) and glial fibrillary acidic protein immunopositive (GFAP+) cells in the dentate gyrus and the entire hippocampus. Our results provide new evidence that the concentrations of FGF-2, IGF-1, and VEGF decline considerably by middle age but remain steady between middle age and old age. Further, decreased concentrations of FGF-2 during aging are associated with decreased numbers of FGF-2+ astrocytes. Quantification of GFAP+ cells, and GFAP and FGF-2 dual immunostaining analyses, reveal that aging does not decrease the total number of astrocytes but fractions of astrocytes that express FGF-2 decline considerably by middle age. Thus, dramatically decreased dentate neurogenesis by middle age is likely linked to reduced concentrations of FGF-2, IGF-1, and VEGF in the hippocampus, as each of these factors can individually influence the proliferation of stem/progenitor cells in the dentate gyrus. Additionally, the results demonstrate that decreased FGF-2 concentration during aging is a consequence of age-related impairment in FGF-2 synthesis by astrocytes.

Smadja, D. M., et al. (2015). "Treprostinil indirectly regulates endothelial colony forming cell angiogenic properties by increasing VEGF-A produced by mesenchymal stem cells." Thromb Haemost **114**(4): 735-747.

Pulmonary vasodilators and prostacyclin therapy in particular, have markedly improved the outcome of patients with pulmonary hypertension (PH). Endothelial dysfunction is a key feature of PH, and we previously reported that treprostinil therapy increases number and proliferative potential of endothelial colony forming cells (ECFC) isolated from PH patients' blood. In the present study, the objective was to determine how treprostinil contributes to the proangiogenic functions of ECFC. We examined the effect of treprostinil on ECFC obtained from cord blood in terms of colony numbers, proliferative and clonogenic properties in vitro, as well as in vivo vasculogenic properties. Surprisingly, treprostinil inhibited viability of cultured ECFC but did not modify their clonogenic properties or the endothelial differentiation potential from cord blood stem cells. Treprostinil treatment significantly increased the vessel-forming ability of ECFC combined with mesenchymal stem cells (MSC) in Matrigel implanted in nude mice. In vitro, ECFC proliferation was stimulated by conditioned media from treprostinil-pretreated MSC, and this effect was inhibited either by the use of VEGF-A blocking antibodies or siRNA VEGF-A in MSC. Silencing VEGF-A gene in MSC also blocked the pro-angiogenic effect of treprostinil in vivo. In conclusion, increased VEGF-A produced by MSC can account for the increased vessel formation observed during treprostinil treatment. The clinical relevance of these data was confirmed by the high level of VEGF-A detected in plasma from patients with paediatric PH who had been treated with treprostinil. Moreover, our results suggest that VEGF-A level in patients could be a surrogate biomarker of treprostinil efficacy.

Song, S. Y., et al. (2010). "The pivotal role of VEGF in adipose-derived-stem-cell-mediated regeneration." Expert Opin Biol Ther **10**(11): 1529-1537.

IMPORTANCE OF THE FIELD: Several lines of evidence suggest that VEGF is a key regulator of the paracrine effects of adipose-derived stem cells (ASCs), but the mechanism of action remains to be identified. AREAS COVERED IN THIS REVIEW: This brief review discusses the following research questions: i) Does VEGF increase the proliferation/migration and differentiation of ASCs?; ii) Does VEGF mediate the paracrine effects of ASCs?; and iii) How is VEGF synthesized, and which factors regulate VEGF secretion? WHAT THE READER WILL GAIN: External stimuli such as hypoxia may activate receptor tyrosine kinases in the membrane of ASCs, which, in turn, phosphorylate extracellular signal regulated kinase (ERK) and members of the Akt signaling pathway, stabilizing hypoxia inducible factor 1alpha (HIF-1alpha) that are primary regulators of VEGF expression. Secreted VEGF directly stimulates ASCs via VEGF receptors in an autocrine manner and regenerates damaged neighboring cells in a paracrine manner. TAKE HOME MESSAGE: Most studies of stem cell regeneration have focused on differentiation of ASCs and their building block function; however,

the paracrine effects of ASCs should also be the focus of attention.

Stratman, A. N., et al. (2011). "VEGF and FGF prime vascular tube morphogenesis and sprouting directed by hematopoietic stem cell cytokines." Blood **117**(14): 3709-3719.

Here, we demonstrate a novel, direct-acting, and synergistic role for 3 hematopoietic stem cell cytokines: stem cell factor, interleukin-3, and stromal derived factor-1alpha, in controlling human endothelial cell (EC) tube morphogenesis, sprouting, and pericyte-induced tube maturation under defined serum-free conditions in 3-dimensional matrices. Angiogenic cytokines such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) alone or VEGF/FGF combinations do not support these responses. In contrast, VEGF and FGF prime EC responses to hematopoietic cytokines via up-regulation of c-Kit, IL-3Ralpha, and C-X-C chemokine receptor type 4 from either human ECs or embryonic quail vessel explants. In support of these findings, EC Runx1 is demonstrated to be critical in coordinating vascular morphogenic responses by controlling hematopoietic cytokine receptor expression. Combined blockade of hematopoietic cytokines or their receptors in vivo leads to blockade of developmental vascularization in quail embryos manifested by vascular hemorrhage and disrupted vascular remodeling events in multiple tissue beds. This work demonstrates a unique role for hematopoietic stem cell cytokines in vascular tube morphogenesis and sprouting and further demonstrates a novel upstream priming role for VEGF and FGF to facilitate the action of promorphogenic hematopoietic cytokines.

Sun, J., et al. (2010). "VEGF-mediated angiogenesis stimulates neural stem cell proliferation and differentiation in the premature brain." Biochem Biophys Res Commun **394**(1): 146-152.

This study investigated the effects of angiogenesis on the proliferation and differentiation of neural stem cells in the premature brain. We observed the changes in neurogenesis that followed the stimulation and inhibition of angiogenesis by altering vascular endothelial growth factor (VEGF) expression in a 3-day-old rat model. VEGF expression was overexpressed by adenovirus transfection and down-regulated by siRNA interference. Using immunofluorescence assays, Western blot analysis, and real-time PCR methods, we observed angiogenesis and the proliferation and differentiation of neural stem cells. Immunofluorescence assays showed that the number of vWF-positive areas peaked at day 7, and they were highest in the VEGF up-regulation group and lowest in the VEGF down-regulation group at every time point. The number of neural stem cells, neurons, astrocytes, and oligodendrocytes in the subventricular zone gradually increased over time in the VEGF up-regulation group. Among the three groups, the number of these cells was highest in the VEGF up-regulation group and lowest in the VEGF down-regulation group at the same time point. Western blot analysis and real-time PCR confirmed these results. These data suggest that angiogenesis may stimulate the proliferation of neural stem cells and differentiation into neurons, astrocytes, and oligodendrocytes in the premature brain.

Sun, J., et al. (2010). "Endothelial cells promote neural stem cell proliferation and differentiation associated with VEGF activated Notch and Pten signaling." Dev Dyn **239**(9): 2345-2353.

To investigate whether and how endothelial cells affect neurogenesis, we established a system to co-culture endothelial cells and brain slices of neonatal rat and observed how subventricular zone cells differentiate in the presence of endothelial cells. In the presence of endothelial cells, neural stem cells increased in number, as did differentiated neurons and glia. The augmentation of neurogenesis was reversed by diminishing vascular endothelial growth factor (VEGF) expression in endothelial cells with RNA interference (RNAi). Microarray analysis indicated that expression levels of 112 genes were significantly altered by co-culture and that expression of 81 of the 112 genes recovered to normal levels following RNAi of VEGF in endothelial cells. Pathway mapping showed an enrichment of genes in the Notch and Pten pathways. These data indicate that endothelial cells promote neural stem cell proliferation and differentiation associated with VEGF, possibly by activating the Notch and Pten pathways.

Tang, J. M., et al. (2015). "VEGF-A promotes cardiac stem cell engraftment and myocardial repair in the infarcted heart." Int J Cardiol **183**: 221-231.

BACKGROUND: The objective of this study was to determine whether vascular endothelial growth factor (VEGF)-A subtypes improve cardiac stem cell (CSC) engraftment and promote CSC-mediated myocardial repair in the infarcted heart. METHODS: CSCs were treated with VEGF receptor (VEGFR) inhibitors, VCAM-1 antibody (VCAM-1-Ab), or PKC-alpha inhibitor followed by the treatment with VEGF-A. CSC adhesion assays were performed in vitro. In vivo, the PKH26-labeled and VCAM-1-Ab or PKC-alpha inhibitor pre-treated CSCs were treated with VEGF-A followed by implantation into infarcted rat hearts. The hearts were then collected for measuring CSC engraftment and evaluating cardiac fibrosis and function 3 or 28days after the CSC transplantation.

RESULTS: All three VEGF-A subtypes promoted CSC adhesion to extracellular matrix and endothelial cells. VEGF-A-mediated CSC adhesion required VEGFR and PKCalpha signaling. Importantly, VEGF-A induced VCAM-1, but not ICAM-1 expression in CSCs through PKCalpha signaling. In vivo, VEGF-A promoted the engraftment of CSCs in infarcted hearts, which was attenuated by PKCalpha inhibitor or VCAM-1-Ab. Moreover, VEGF-A-mediated CSC engraftment resulted in a reduction in infarct size and fibrosis. Functional studies showed that the transplantation of the VEGF-A-treated CSCs stimulated extensive angiomyogenesis in infarcted hearts as indicated by the expression of cardiac troponin T and von Willebrand factor, leading to an improved performance of left ventricle. Blockade of PKCalpha signaling or VCAM-1 significantly diminished the beneficial effects of CSCs treated with VEGF-A. CONCLUSION: VEGF-A promotes myocardial repair through, at least in part, enhancing the engraftment of CSCs mediated by PKCalpha/VCAM-1 pathway.

Tang, J. M., et al. (2011). "VEGF/SDF-1 promotes cardiac stem cell mobilization and myocardial repair in the infarcted heart." Cardiovasc Res **91**(3): 402-411.

AIMS: The objective of this study was to investigate whether vascular endothelial growth factor (VEGF) secreted by mesenchymal stem cells (MSC) improves myocardial survival and the engraftment of implanted MSC in infarcted hearts and promotes recruitment of stem cells through paracrine release of myocardial stromal cell-derived factor-1alpha (SDF-1alpha). METHODS AND RESULTS: VEGF-expressing MSC ((VEGF)MSC)-conditioned medium enhanced SDF-1alpha expression in heart slices and H9C2 cardiomyoblast cells via VEGF and the vascular endothelial growth factor receptor (VEGFR). The (VEGF)MSC-conditioned medium markedly promoted cardiac stem cell (CSC) migration at least in part via the SDF-1alpha/CXCR4 pathway and involved binding to VEGFR-1 and VEGFR-3. In vivo, (VEGF)MSC-stimulated SDF-1alpha expression in infarcted hearts resulted in massive mobilization and homing of bone marrow stem cells and CSC. Moreover, VEGF-induced SDF-1alpha guided the exogenously introduced CSC in the atrioventricular groove to migrate to the infarcted area, leading to a reduction in infarct size. Functional studies showed that (VEGF)MSC transplantation stimulated extensive angiomyogenesis in infarcted hearts as indicated by the expression of cardiac troponin T, CD31, and von Willebrand factor and improved the left ventricular performance, whereas blockade of SDF-1alpha or its receptor by RNAi or antagonist significantly diminished the beneficial effects of (VEGF)MSC. CONCLUSION: Exogenously expressed VEGF promotes myocardial repair at least in part through SDF-1alpha/CXCR4-mediated recruitment of CSC.

Tang, Y. L., et al. (2004). "Autologous mesenchymal stem cell transplantation induce VEGF and neovascularization in ischemic myocardium." Regul Pept **117**(1): 3-10.

Neovascularization induced by vascular endothelial growth factor (VEGF) represents an appealing approach for treating ischemic heart disease. However, VEGF therapy has been associated with transient therapeutic effects and potential risk for hemangioma growth. Adult mesenchymal stem cells (MSCs) derived from bone marrow are a promising source for tissue regeneration and repair. In order to achieve a safe and persistent angiogenic effect, we have explored the potential of autologous MSCs transplantation to enhance angiogenesis and cardiac function of ischemic hearts. One week after myocardial infarction induced by occlusion of left anterior descending artery, autologous MSCs expanded in vitro was administrated intramyocardially into the infarct area of the same donor rats. By 2 months, MSCs implantation significantly elevated VEGF expression levels, accompanied by increased vascular density and regional blood flow in the infarct zone. The neovascularization resulted in a decreased apoptosis of hypertrophied myocytes and markedly improved the left ventricular contractility (ejection fraction: 79.9+/-7.6% vs. 37.2+/-6.9% in control animals). Therefore, mechanisms underlying MSCs improvement of cardiac functions may involve neovascularization induced by differentiation of MSCs to endothelial cells and para-secretion of growth factors, in addition to the apoptosis reduction and previously reported cardiomyocytes regeneration. Two months after cell transplantation, there are significant improvement of left ventricular function. Hence, autologous MSCs transplantation may represent a promising therapeutic strategy free of ethical concerns and immune rejection, for neovascularization in ischemic heart diseases.

Wang, J. H., et al. (2008). "[Effects of adipose-derived stem cell transplantation on the angiogenesis and the expression of bFGF and VEGF in the brain post focal cerebral ischemia in rats]." Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi **24**(10): 958-961.

AIM: To investigate the effects of adipose-derived stem cell (ADSC) transplantation on the angiogenesis in the brain post focal cerebral ischemia in rats. METHODS: 72 male adult Sprague-Dawley rats were randomly divided into 4 groups: sham-operated group, middle cerebral artery occlusion (MCAO) group, vehicle group and MCAO+ADSC-treated group (n=18). A permenant focal cerebral

ischemia model was established with the modified Longa's method. ADSC were labeled by DAPI before transplantation. One day after right MCAO, 30 muL of cell suspension containing 1x10(6) cells were injected into the lateral ventricle of MCAO+ADSC-treated group and the same dose of PBS was given to the vehicle group. On D4, D7 and D14 after MCAO, the rats were killed to detect the regeneration of microvessel and the expression of bFGF and VEGF in ischemic region by immunohistochemistry and RT-PCR. RESULTS: A lot of microvessel proliferate in the injured cortex reached peak in 2 weeks. The microvessel density in the brain tissues of rats treated with ADSC was higher than that in MCAO group and vehicle group (P<0.01). The expression of bFGF and VEGF in the brain tissues of MCAO+ADSC-treated group was higher than that in MCAO group and vehicle group on D4, D7 and D14 post MCAO. CONCLUSION: The transplantation of ADSC can promote the revascularization of cerebral ischemia in rats partly by enhancing bFGF and VEGF synthesis in brain.

Wang, M., et al. (2007). "STAT3 mediates bone marrow mesenchymal stem cell VEGF production." J Mol Cell Cardiol **42**(6): 1009-1015.

The mechanisms by which mesenchymal stem cells (MSCs) may protect native tissue are incompletely understood. Understanding the mechanisms by which these cells release factors such as vascular endothelial growth factor (VEGF), may lead to enhanced protection. We hypothesized that stress, in the form of hypoxia or TNF, activates MSCs to release VEGF by STAT3 and p38 MAPK dependent mechanisms. Mouse MSCs from wild type (WT) and STAT3 knockout mice (STAT3KO) were harvested and purified by a single-step method using adhesion. The release of VEGF was analyzed by using MSC conditioned media under hypoxia or TNF stimulation with or without p38 MAPK inhibition. Activation of STAT3 and p38 MAPK was determined by analysis of cell lysates. MSCs released VEGF under normoxia, which was associated with constitutive STAT3 activity. STAT3 deficiency resulted in decreased MSC production of VEGF. In response to hypoxia or TNF, MSCs produced more VEGF, which was correlated with hypoxia or TNF activated p38 MAPK and STAT3. The p38 MAPK inhibitor significantly decreased hypoxia-induced or TNF-stimulated VEGF production in WT. Additionally, STAT3 ablation neutralized hypoxia-induced MSC release of VEGF. No effect of p38 MAPK inhibitor alone was observed on MSC release of VEGF in WT. However, inhibition of p38 MAPK blocked release of VEGF in STAT3KO MSCs. MSCs are a potent source of VEGF, the production of which is mediated by STAT3 under normoxia partly; however, following hypoxia or TNF exposure, MSC release of VEGF is mediated by both STAT3 and p38 MAPK.

Wang, X., et al. (2006). "Bioenergetic and functional consequences of stem cell-based VEGF delivery in pressure-overloaded swine hearts." Am J Physiol Heart Circ Physiol **290**(4): H1393-1405.

In an established swine model of severe left ventricular (LV) hypertrophy (LVH), the bioenergetic and functional consequences of transplanting autologous mesenchymal stem cells (MSCs) overexpressing vascular endothelial growth factor (VEGF-MSCs) into the LV were evaluated; transplantation was accomplished by infusion of VEGF-MSCs into the interventricular cardiac vein. Specifically, the hypertrophic response to aortic banding was compared in seven pigs treated with 30 million VEGF-MSCs, eight pigs treated with 30 million MSCs without VEGF modification, and 19 untreated LVH pigs. Eight pigs without banding or cell transplantation (normal) were also studied. Four weeks postbanding, LV wall thickening (MRI), myocardial blood flow (MBF), high-energy phosphate levels ((31)P magnetic resonance spectroscopy), and hemodynamic measurements were obtained under basal conditions and during a catecholamine-induced high cardiac workstate (HCW). Although 9 of 19 untreated banded pigs developed clinical evidence of biventricular failure, no MSCs-treated animal developed heart failure. MSCs engraftment was present in both cell transplant groups, and both baseline and HCW MBF values were significantly increased in hearts receiving VEGF-MSCs compared with other groups ($P < 0.05$). During HCW, cardiac inotropic reserve (defined as the percent increase of rate pressure product at HCW relative to baseline) was normal in the VEGF-MSCs group and significantly decreased in all other banded groups. Additionally, during HCW, the myocardial energetic state [reflected by the phosphocreatine-to-ATP ratio (PCr/ATP)] of VEGF-MSCs-treated hearts remained stable, whereas in all other groups, PCr/ATP decreased significantly from baseline values ($P < 0.05$, each group). Myocardial von Willebrand factor and VEGF mRNA expressions and myocardial capillary density were significantly increased in VEGF-MSCs-treated hearts ($P < 0.05$). Hence, in the pressure-overloaded LV, transplantation of VEGF-MSCs prevents LV decompensation, induces neovascularization, attenuates hypertrophy, and improves MBF, myocardial bioenergetic characteristics, and contractile performance.

Wang, Y., et al. (2008). "TGF-alpha increases human mesenchymal stem cell-secreted VEGF by MEK- and PI3-K- but not JNK- or ERK-dependent mechanisms."

Am J Physiol Regul Integr Comp Physiol **295**(4): R1115-1123.

Transforming growth factor-alpha (TGF-alpha) may be an important mediator of wound healing and the injury response. Human bone marrow mesenchymal stem cells (MSCs) release VEGF as a potentially beneficial paracrine response; however, it remains unknown whether TGF-alpha stimulates the production of VEGF from MSCs and, if so, by which mechanisms. We hypothesized that TGF-alpha would increase human MSC VEGF production by MAP kinase kinase (MAPKK/MEK), phosphatidylinositol 3-kinase (PI3-K)-, ERK, and JNK-dependent mechanisms. To study this, MSCs were cultured and divided into the following groups: 1) with vehicle; 2) with various stimulants alone: TGF-alpha, TNF-alpha, or TGF-alpha+TNF-alpha; 3) with individual kinase inhibitors alone (two different inhibitors for each of the following kinases: MEK, PI3-K, ERK, or JNK); and 4) with the above stimulants and each of the eight inhibitors. After 24-h incubation, a TGF-alpha dose-response curve demonstrated that low-dose TGF-alpha (500 pg/ml) suppressed MSC production of VEGF compared with vehicle (502 +/- 16 pg/10(5) cells/ml to 332 +/- 9 pg/10(5) cells/ml), while high-dose TGF-alpha (250 ng/ml) significantly increased MSC VEGF production (603 +/- 24 pg/10(5) cells/ml). High-dose TGF-alpha also increased TNF-alpha-stimulated release of VEGF from MSCs. MSCs exposed to TGF-alpha and/or TNF-alpha also demonstrated increased activation of PI3-K, JNK, and ERK. The TGF-alpha-stimulated production of VEGF by MSCs and the additive effect of TNF-alpha and TGF-alpha on VEGF production were abolished by MEK and PI3-K inhibition, but not ERK or JNK inhibition. Our data suggest that TGF-alpha increases VEGF production in MSCs via MEK- and PI3-K- but not ERK- or JNK-dependent mechanisms.

Wang, Z., et al. (2011). "Clinicopathologic correlation of cancer stem cell markers CD44, CD24, VEGF and HIF-1alpha in ductal carcinoma in situ and invasive ductal carcinoma of breast: an immunohistochemistry-based pilot study." Pathol Res Pract **207**(8): 505-513.

CD24(-/low)CD44(+) cells have been identified as putative cancer stem cells (CSCs) in breast cancer. However, the expression of these markers, as well as their association with clinical parameters or tumor microenvironment of breast cancer, remains largely unknown. In the present study, we examined the expression of CD44, CD24, VEGF, and HIF-1alpha in human breast tumor tissues and assessed their clinicopathological correlations. We investigated tissue samples, including 117 cases of invasive ductal carcinoma (IDCa), 14 cases of ductal carcinoma in situ (DCIS), and 15 cases of intraductal hyperplasia (IDH) from breast tissues. The expression of CD44, CD24, HIF-1alpha, and VEGF was evaluated using immunohistochemical staining. CD24, CD44, HIF-1alpha, and VEGF were expressed in 49 (41.9%), 51 (43.6%), 32 (27.4%), and 97 cases (82.9%), respectively, in IDCa. CD24(-/low)CD44(+) cells were noted in 48 (41.3%) cases. The levels of CD24 and VEGF expression correlated positively with tumor malignancy ($P<0.05$). Meanwhile, the expression of CD24, CD44, and VEGF correlated significantly positively with increasing tumor grade ($P<0.05$). In addition, associations between CD44 and VEGF, CD24 and VEGF, HIF-1alpha and VEGF, CD24(-/low)CD44(+) and VEGF, CD24(-/low)CD44(+) and HIF-1alpha were also observed ($P<0.05$). The HIF-1alpha expression level was relatively higher in early stage breast cancer patients with CD24(-/low)CD44(+) cells. Taken together, our results suggest that CD24 and VEGF may play important roles in breast tumorigenesis and progression, while HIF-1alpha may play a role in the early stage of breast carcinogenesis.

Wen, N., et al. (2019). "Bromodomain inhibitor jq1 induces cell cycle arrest and apoptosis of glioma stem cells through the VEGF/PI3K/AKT signaling pathway." Int J Oncol **55**(4): 879-895.

Bromodomain and extraterminal domain proteins, especially bromodomaincontaining protein 4 (Brd4), have recently emerged as therapeutic targets for several cancers, although the role and mechanism of Brd4 in glioblastoma multiforme (GBM) are unclear. In this study, we aimed to explore the underlying mechanisms of the antitumor effects of Brd4 and the bromodomain inhibitor JQ1 on glioma stem cells (GSCs). In vitro, JQ1 and small interfering RNAs targeting Brd4 (siBrd4) inhibited the proliferation and selfrenewal of GSCs. In vivo, JQ1 significantly inhibited the growth of xenograft GSCs tumors. The RNAseq analysis revealed that the PI3KAKT pathway played an important role in GBM. Vascular endothelial growth factor (VEGF) and VEGF receptor 2 phosphorylation was downregulated by exposure to JQ1 in GSCs, thereby reducing PI3K and AKT activity. In addition, treatment with JQ1 inhibited MMP expression, thereby inhibiting degradation of the extracellular matrix by MMP and angiogenesis in GBM tumors. Suppression of AKT phosphorylation inhibited the expression of the retinoblastoma/E2F1 complex, resulting in cell cycle arrest. In addition, treatment with siBrd4 or JQ1 induced apoptosis by activating AKT downstream target genes involved in apoptosis. In conclusion, these results suggest that Brd4 has great potential as a therapeutic target, and JQ1 has notable antitumor effects against GBM which may be mediated via the VEGF/PI3K/AKT signaling pathway.

Wen, Z., et al. (2014). "MicroRNA-377 regulates mesenchymal stem cell-induced angiogenesis in ischemic hearts by targeting VEGF." PLoS One **9**(9): e104666.

MicroRNAs have been appreciated in various cellular functions, including the regulation of angiogenesis. Mesenchymal-stem-cells (MSCs) transplanted to the MI heart improve cardiac function through paracrine-mediated angiogenesis. However, whether microRNAs regulate MSC induced angiogenesis remains to be clarified. Using microRNA microarray analysis, we identified a microRNA expression profile in hypoxia-treated MSCs and observed that among all dysregulated microRNAs, microRNA-377 was decreased the most significantly. We also validated that vascular endothelial growth factor (VEGF) is a target of microRNA-377 using dual-luciferase reporter assay and Western-blotting. Knockdown of endogenous microRNA-377 promoted tube formation in human umbilical vein endothelial cells. We then engineered rat MSCs with lentiviral vectors to either overexpress microRNA-377 (MSC miR-377) or knockdown microRNA-377 (MSC Anti-377) to investigate whether microRNA-377 regulated MSC-induced myocardial angiogenesis, using MSCs infected with lentiviral empty vector to serve as controls (MSC Null). Four weeks after implantation of the microRNA-engineered MSCs into the infarcted rat hearts, the vessel density was significantly increased in MSC Anti-377-hearts, and this was accompanied by reduced fibrosis and improved myocardial function as compared to controls. Adverse effects were observed in MSC miR-377-treated hearts, including reduced vessel density, impaired myocardial function, and increased fibrosis in comparison with MSC Null-group. These findings indicate that hypoxia-responsive microRNA-377 directly targets VEGF in MSCs, and knockdown of endogenous microRNA-377 promotes MSC-induced angiogenesis in the infarcted myocardium. Thus, microRNA-377 may serve as a novel therapeutic target for stem cell-based treatment of ischemic heart disease.

Xiao, Z., et al. (2007). "Upregulation of Flk-1 by bFGF via the ERK pathway is essential for VEGF-mediated promotion of neural stem cell proliferation." Cell Res **17**(1): 73-79.

Neural stem cells (NSCs) constitute the cellular basis for embryonic brain development and neurogenesis. The process is regulated by NSC niche including neighbor cells such as vascular and glial cells. Since both vascular and glial cells secrete vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), we assessed the effect of VEGF and bFGF on NSC proliferation using nearly homogeneous NSCs that were differentiated from mouse embryonic stem cells. VEGF alone did not have any significant effect. When bFGF was added, however, VEGF stimulated NSC proliferation in a dose-dependent manner, and this stimulation was inhibited by ZM323881, a VEGF receptor (Flk-1)-specific inhibitor. Interestingly, ZM323881 also inhibited cell proliferation in the absence of exogenous VEGF, suggesting that VEGF autocrine plays a role in the proliferation of NSCs. The stimulatory effect of VEGF on NSC proliferation depends on bFGF, which is likely due to the fact that expression of Flk-1 was upregulated by bFGF via phosphorylation of ERK1/2. Collectively, this study may provide insight into the mechanisms by which microenvironmental niche signals regulate NSCs.

Xie, X., et al. (2007). "Genetic modification of embryonic stem cells with VEGF enhances cell survival and improves cardiac function." Cloning Stem Cells **9**(4): 549-563.

Cardiac stem cell therapy remains hampered by acute donor cell death posttransplantation and the lack of reliable methods for tracking cell survival in vivo. We hypothesize that cells transfected with inducible vascular endothelial growth factor 165 (VEGF(165)) can improve their survival as monitored by novel molecular imaging techniques. Mouse embryonic stem (ES) cells were transfected with an inducible, bidirectional tetracycline (Bi-Tet) promoter driving VEGF(165) and renilla luciferase (Rluc). Addition of doxycycline induced Bi-Tet expression of VEGF(165) and Rluc significantly compared to baseline ($p<0.05$). Expression of VEGF(165) enhanced ES cell proliferation and inhibited apoptosis as determined by Annexin-V staining. For noninvasive imaging, ES cells were transduced with a double fusion (DF) reporter gene consisting of firefly luciferase and enhanced green fluorescence protein (Fluc-eGFP). There was a robust correlation between cell number and Fluc activity ($R(2)=0.99$). Analysis by immunostaining, histology, and RT-PCR confirmed that expression of Bi-Tet and DF systems did not affect ES cell self-renewal or pluripotency. ES cells were differentiated into beating embryoid bodies expressing cardiac markers such as troponin, Nkx2.5, and beta-MHC. Afterward, 5 x 10(5) cells obtained from these beating embryoid bodies or saline were injected into the myocardium of SV129 mice (n=36) following ligation of the left anterior descending (LAD) artery. Bioluminescence imaging (BLI) and echocardiography showed that VEGF(165) induction led to significant improvements in both transplanted cell survival and cardiac function ($p<0.05$). This is the first study to demonstrate imaging of embryonic stem cell-mediated gene therapy targeting cardiovascular disease. With further validation, this platform may have broad

applications for current basic research and further clinical studies.

Xu, J., et al. (2008). "MAPK/ERK signalling mediates VEGF-induced bone marrow stem cell differentiation into endothelial cell." J Cell Mol Med **12**(6A): 2395-2406.

Multi-potent adult progenitor cells (MAPCs) differentiate into endothelial cells (ECs) in the presence of vascular endothelial growth factor (VEGF). The mechanism(s) of VEGF-induced differentiation of MAPCs to ECs are not yet known. We, therefore, examined the role of mitogen-activated protein kinase/extracellular signal-regulated kinase (p42/44-MAPK/ERK1/2) signalling in endothelial differentiation from bone marrow stem cells. We observed that VEGF stimulation of MAPCs for 14 days results in a significant expression of endothelial-specific gene and/or proteins including von Willebrand factor (vWF), vascular endothelial-cadherin (VE-cadherin), VEGF receptor-2 (VEGFR2), and CD31. Up-regulation of EC-specific markers was accompanied by a cobblestone morphology, expression of endothelial nitric oxide synthase (eNOS), and Dil-Ac-LDL uptake, typical for EC morphology and function. VEGF induced a sustained activation of p42 MAPK/ERK, but not that of p44 MAPK/ERK during the course of MAPCs differentiation in a time-dependent manner up to 14 days. VEGF-induced activation of p42 MAPK/ERK also led to the nuclear translocation of MAPK/ERK1/2. Incubation of MAPCs with MAPK/ERK1/2 phosphorylation inhibitor PD98059 blocked the sustained VEGF-induced MAPK/ERK1/2 phosphorylation as well as its nuclear translocation in the differentiating MAPCs. Inhibition of MAPK/ERK1/2 phosphorylation by PD98059 also blocked the expression of EC-specific genes in these cells and their differentiation to ECs. These data suggest that VEGF induces MAPC differentiation into EC via a. MAPK/ERK1/2 signalling pathway-mediated mechanism in vitro.

Yang, J., et al. (2015). "Buyang Huanwu Decoction (BYHWD) Enhances Angiogenic Effect of Mesenchymal Stem Cell by Upregulating VEGF Expression After Focal Cerebral Ischemia." J Mol Neurosci **56**(4): 898-906.

Buyang Huanwu decoction (BYHWD) has been used for centuries to treat paralysis and stroke. Previously, we have demonstrated that BYHWD combined with mesenchymal stem cell (MSC) transplantation attenuates ischemic injury partly by upregulating angiogenesis. However, the mechanisms of this drug for stroke treatment are not completely understood. Here, we aimed to clarify the mechanism of BYHWD on angiogenesis mediated by MSCs. Firstly, we verified microvessels with a size of 50-100 nm produced by either MSCs or MSCs treated by 500 mug/ml BYHWD. These exosomes were purified and found to be able to activate vascular endothelial growth factor (VEGF) expression in endothelial cells (ECs). Moreover, exosomes from MSCs and MSCs treated by BYHWD induced elevated microRNA (miRNA)-126 expression and reduced miR-221 and miR-222 expression. In MSCs, disruption of dicer, an enzyme responsible for miRNA maturation, by dicer small interfering RNA (siRNA), or RNase pretreatment abolished this ability of the exosomes. Additionally, exosomes from MSCs treated by BYHWD promoted VEGF and Ki-67 expression and augmented vascular density in rat brain after bilateral carotid artery ligation. In conclusion, our study revealed that BYHWD exposure augmented angiogenetic miRNA and VEGF expression in exosomes secreted by MSCs and elevated angiogenesis in rat brain.

Ye, L., et al. (2018). "MiR-126 enhances VEGF expression in induced pluripotent stem cell-derived retinal neural stem cells by targeting spred-1." Int J Clin Exp Pathol **11**(2): 1023-1030.

Pathological retinal neovascularization (RNV) is a leading cause of vision loss in several ocular diseases; however, the underlying molecular mechanisms involved in the development of RNV remain unclear. It has been shown that microRNAs contribute to the process of angiogenesis, which has received greater attention by investigators who study the progression of RNV. In the present study, we investigated the function of miR-126 expression in retinal neural stem cells derived from induced pluripotent stem cell (IPSs) obtained from patients with RNV. During the induction process, the levels of both miR126 and vascular endothelial growth factor C (VEGF-C) gradually decreased, while the levels of spred-1 significantly increased. The existence of conserved miR-126-binding sites in spred-1 mRNA was predicted by computational algorithms, and verified by the luciferase reporter assay. The use of miR-126 mimics revealed dramatically reduced levels of spred-1, and increased levels of VEGF. When using shRNA to target spred-1, the resultant decreased levels of spred-1 were associated with significantly enhanced levels of VEGF expression. Our results demonstrate that miR-126 promotes VEGF expression in IPS cells by suppressing spred-1 expression, which contributes to angiogenesis during the progression of RNV. These findings suggest that miR-126 and spred-1 might serve as novel molecular targets for treating RNV-related ocular diseases.

Yeh, T. S., et al. (2014). "Baculovirus-transduced, VEGF-expressing adipose-derived stem cell sheet for

the treatment of myocardium infarction." Biomaterials **35**(1): 174-184.

Cell sheet technology has been widely employed for the treatment of myocardial infarction (MI), but cell sheet fabrication generally requires the use of thermo-responsive dishes. Here we developed a method for the preparation of adipose-derived stem cell (ASC) sheet that obviated the need of thermo-responsive dishes. This method only required the seeding of rabbit ASC onto 6-well plates at an appropriate cell density and culture in appropriate medium, and the cells were able to develop into ASC sheet in 2 days. The ASC sheet allowed for transduction with the hybrid baculovirus at efficiencies >97%, conferring robust and prolonged (>35 days) overexpression of vascular endothelial growth factor (VEGF). The ASC sheet was easily detached by brief (10 s) trypsinization and saline wash, while retaining the extracellular matrix and desired physical properties. The ASC sheet formation and VEGF expression promoted cell survival under hypoxia in vitro. Epicardial implantation of the VEGF-expressing ASC sheet to rabbit MI models reduced the infarct size and improved cardiac functions to non-diseased levels, as judged from the left ventrical ejection fraction/myocardial perfusion. The VEGF-expressing ASC sheet also effectively prevented myocardial wall thinning, suppressed myocardium fibrosis and enhanced blood vessel formation. These data implicated the potential of this method for the preparation of genetically engineered ASC sheet and future MI treatment.

Yuan, L., et al. (2011). "VEGF-modified human embryonic mesenchymal stem cell implantation enhances protection against cisplatin-induced acute kidney injury." Am J Physiol Renal Physiol **300**(1): F207-218.

The implantation of mesenchymal stem cells (MSC) has been reported as a new technique to restore renal tubular structure and improve renal function in acute kidney injury (AKI). Vascular endothelial growth factor (VEGF) plays an important role in the renoprotective function of MSC. Whether upregulation of VEGF by a combination of MSC and VEGF gene transfer could enhance the protective effect of MSC in AKI is not clear. We investigated the effects of VEGF-modified human embryonic MSC (VEGF-hMSC) in healing cisplatin-injured renal tubular epithelial cells (TCMK-1) with a coculture system. We found that TCMK-1 viability declined 3 days after cisplatin pretreatment and that coculture with VEGF-hMSC enhanced cell protection via mitogenic and antiapoptotic actions. In addition, administration of VEGF-hMSC in a nude mouse model of cisplatin-induced kidney injury offered better protective effects on renal function, tubular structure, and survival as represented by increased cell proliferation, decreased cellular apoptosis, and improved peritubular capillary density. These data suggest that VEGF-modified hMSC implantation could provide advanced benefits in the protection against AKI by increasing antiapoptosis effects and improving microcirculation and cell proliferation.

Yun, S. P., et al. (2009). "Role of HIF-1alpha and VEGF in human mesenchymal stem cell proliferation by 17beta-estradiol: involvement of PKC, PI3K/Akt, and MAPKs." Am J Physiol Cell Physiol **296**(2): C317-326.

17beta-Estradiol (E(2)) is a steroid hormone well known for its roles in the regulation of various cell functions. However, the precise role that E(2) plays in the proliferation of human mesenchymal stem cells (hMSCs) has not been completely elucidated. In the present study, we examined the effects of E(2) on cell proliferation and the related signaling pathways using hMSCs. We showed that E(2), at > or =10(-9) M, significantly increased [3H]thymidine incorporation after 24 h of incubation, and E(2) also increased [3H]thymidine incorporation at >6 h. Also, E(2) significantly increased the percentage of the cell population in the S phase based on FACS analysis. Moreover, E(2) increased estrogen receptor (ER), PKC, phosphatidylinositol 3-kinase (PI3K)/Akt, and MAPK phosphorylation. Subsequently, these signaling molecules were involved in an E(2)-induced increase of [3H]thymidine incorporation. E(2) also increased hypoxia-inducible factor (HIF)-1alpha and VEGF protein levels. These levels of protein expression were inhibited by ICI-182,780 (10(-6) M, an ER antagonist), staurosporine and bisindolylmaleimide I (10(-6) M, a PKC inhibitor), LY-294002 (10(-6) M, a PI3K inhibitor), Akt inhibitor (10(-5) M), SP-600125 (10(-6) M, a SAPK/JNK inhibitor), and PD-98059 (10(-5) M, a p44/42 MAPKs inhibitor). In addition, HIF-1alpha small interfering (si)RNA and ICI-182,780 inhibited E(2)-induced VEGF expression and cell proliferation. VEGF siRNA also significantly inhibited E(2)-induced cell proliferation. In conclusion, E(2) partially stimulated hMSC proliferation via HIF-1alpha activation and VEGF expression through PKC, PI3K/Akt, and MAPK pathways.

Zeng, L., et al. (2006). "HDAC3 is crucial in shear- and VEGF-induced stem cell differentiation toward endothelial cells." J Cell Biol **174**(7): 1059-1069.

Reendothelialization involves endothelial progenitor cell (EPC) homing, proliferation, and differentiation, which may be influenced by fluid shear stress and local flow pattern. This study aims to elucidate the role of laminar flow on embryonic stem

(ES) cell differentiation and the underlying mechanism. We demonstrated that laminar flow enhanced ES cell-derived progenitor cell proliferation and differentiation into endothelial cells (ECs). Laminar flow stabilized and activated histone deacetylase 3 (HDAC3) through the Flk-1-PI3K-Akt pathway, which in turn deacetylated p53, leading to p21 activation. A similar signal pathway was detected in vascular endothelial growth factor-induced EC differentiation. HDAC3 and p21 were detected in blood vessels during embryogenesis. Local transfer of ES cell-derived EPC incorporated into injured femoral artery and reduced neointima formation in a mouse model. These data suggest that shear stress is a key regulator for stem cell differentiation into EC, especially in EPC differentiation, which can be used for vascular repair, and that the Flk-1-PI3K-Akt-HDAC3-p53-p21 pathway is crucial in such a process.

Zhang, W., et al. (2014). "VEGF and BMP-2 promote bone regeneration by facilitating bone marrow stem cell homing and differentiation." Eur Cell Mater **27**: 1-11; discussion 11-12.

Vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2) have been widely used in the fields of tissue engineering and regenerative medicine to stimulate angiogenesis and bone formation. The goal of this study was to determine whether VEGF and BMP-2 are involved in the homing of bone marrow stem cells (BMSCs) for bone regeneration and to provide insights into their mechanism of action. The chemoattraction of BMSCs to VEGF and BMP-2 was analysed in vitro using a checkerboard assay. VEGF and BMP-2 stimulated the chemotaxis of BMSCs but not chemokinesis. In vivo, both VEGF and BMP-2 also have been confirmed to induce the homing of tail vein injected BMSCs to the site of silk scaffold subcutaneous implantation in nude mice. When the scaffolds were implanted in the rabbit skull defects, more SSEA+ mesenchymal stem cells were mobilised and homed to silk scaffolds containing VEGF and/or BMP-2. More importantly, autogenic BMSCs were reinjected via the ear vein after labelling with lenti-GFP, and the cells were detected to home to the defects and differentiate into endothelial cells and osteogenic cells induced by VEGF and BMP-2. Finally, perfusion with Microfil showed that initial angiogenesis was enhanced in tissue-engineered complexes containing VEGF. Observations based on microCT assay and histological study revealed that bone formation was accelerated on BMP-2-containing scaffolds. These findings support our hypothesis that the localised release of VEGF and BMP-2 promote bone regeneration, in part by facilitating the mobilisation of endogenous stem cells and directing the differentiation of these cells into endothelial and osteogenic lineages.

Zhang, Y. K., et al. (2010). "Effects of buyang huanwu tang combined with bone marrow mesenchymal stem cell transplantation on the expression of VEGF and Ki-67 in the brain tissue of the cerebral ischemia-reperfusion model rat." J Tradit Chin Med **30**(4): 278-282.

OBJECTIVE: To explore the mechanism of Buyang Huanwu Tang (Decoction Invigorating Yang for Recuperation) combined with bone marrow mesenchymal stem cells (MSCs) transplantation in protecting nerves of cerebral ischemic injury. METHODS: Local cerebral ischemia-reperfusion rat model was established with modified Zea-Longa thread-occlusion method, and MSCs were injected into the caudal vein, and Buyang Huanwu Tang was administrated. Vascular endothelial growth factor (VEGF) and Ki-67 expression in the ischemic side of the brain in the cerebral ischemic-reperfusion rat were detected with immuno-histochemical staining method. RESULTS: VEGF and Ki-67 expressions were significantly up-regulated in the MSCs group and the combination group, with significant differences as compared with the model group and the sham operation group ($P < 0.05$), and with the most strongest effect in the combination group. CONCLUSION: Buyang Huanwu Tang combined with MSCs transplantation repairs the injured blood vessels and lesion tissues possibly by up-regulation of VEGF and Ki-67 expression.

Zhao, H., et al. (2020). "VEGF Promotes Endothelial Cell Differentiation from Human Embryonic Stem Cells Mainly Through PKC-varepsilon/eta Pathway." Stem Cells Dev **29**(2): 90-99.

Human embryonic stem cells (hESCs) have unlimited proliferation capacity and can differentiate into most types of somatic cells. We previously described that the overexpression of FLI1 as well as the activation of protein kinase C (FLI1-PKC) could rapidly and efficiently differentiate hESCs into endothelial cells (ECs). However, the relationship between vascular endothelial growth factor (VEGF) and PKC in hESC-EC differentiation is debated, and the roles of different PKC isoforms in hESC-EC differentiation remain unknown. In this study, after 2 days of induction, we found that the overexpression of FLI1 and the addition of VEGF-A to hESCs (FLI1-VEGF) could generate 19.6% +/- 5.4% ECs. The induction efficiency of ECs was reduced from 72.3% +/- 7.6% to 37.9% +/- 4.9% following the addition VEGF-A siRNA to the FLI1-PKC system. However, the induction of ECs was nearly completely abrogated following the addition of the pan-PKC inhibitor to the

FLI1-VEGF, FLI1-PKC, or other systems. The above results suggested that VEGF can partially replace PKC, but PKC plays a more critical role downstream of VEGF during hESC-EC induction. To further investigate which PKC isoform was mainly involved in converting hESCs to ECs, siRNAs were used to knock down nine PKC isoforms in the FLI1-PKC system. Only the knockdown of PKC-varepsilon or PKC-eta significantly decreased the induction efficiency of ECs to 51.1% +/- 5.8% or 50.3% +/- 5.1%, respectively. PKC-varepsilon/eta siRNA could suppress EC induction in other differentiation systems. Moreover, RNA-seq and quantitative polymerase chain reaction analysis also showed that only the expression of PKC-varepsilon and PKC-eta was robustly upregulated during hESC-EC induction. In summary, our results suggested that VEGF promoted the differentiation of ECs derived from hESCs, which mainly depended on PKC, specifically the PKC-varepsilon and PKC-eta pathways.

Zhao, T., et al. (2022). "microRNA-15b-5p shuttled by mesenchymal stem cell-derived extracellular vesicles protects podocytes from diabetic nephropathy via downregulation of VEGF/PDK4 axis." J Bioenerg Biomembr **54**(1): 17-30.

Diabetic nephropathy (DN) is a severe complication of diabetes lethal for end-stage renal disease, with less treatment methodologies and uncertain pathogenesis. In the current study, we determined the role of mesenchymal stem cells (MSCs)-derived extracellular vesicles (EVs) containing microRNA (miR)-15b-5p in DN. After extraction and identification of MSC-derived EVs, mouse podocyte line MPC5 was selected to establish an in vitro high-glucose (HG) cell model, where expression of miR-15b-5p, pyruvate dehydrogenase kinase 4 (PDK4) and VEGFA expression in tissues and cells were determined. The loss- and gain- function assays were conducted to determine the roles of miR-15b-5p, PDK4 and VEGFA. MPC5 cells were then co-cultured with MSC-derived EVs and their biological behaviors were detected by Western blot, CCK-8 assay, and flow cytometry. The binding relationship between miR-15b-5p and PDK43 by dual luciferase reporter gene assay. The expression of miR-15b-5p was downregulated in podocytes under HG environment, but highly expressed in mouse MSCs-derived EVs. EVs-derived miR-15b-5p could protect MPC5 cell apoptosis and inflammation. miR-15b-5p inhibited the expression of PDK4 by directly bound to the 3'UTR region of PDK4 gene. miR-15b-5p inhibits VEGF expression by binding to PDK4. Inhibition of PDK4 decreased VEGFA expression and reduced apoptosis and inflammation. Collectively, miR-15b-5p shuttled by MSC-derived EV can play protective roles in HG-induced mouse podocyte injury, possibly by targeting PDK4 and decreasing the VEGFA expression.

Zhou, S., et al. (2017). "Injectable Mussel-Inspired Immobilization of Platelet-Rich Plasma on Microspheres Bridging Adipose Micro-Tissues to Improve Autologous Fat Transplantation by Controlling Release of PDGF and VEGF, Angiogenesis, Stem Cell Migration." Adv Healthc Mater **6**(22).

Platelets-rich plasma (PRP) can produce growth factors (GFs) to improve angiogenesis. However, direct injection of PRP does not lead to highly localized GFs. The current study employs a mussel-inspired polydopamine to immobilize PRP on gelatin microspheres (GMs) with the purpose of bridging adipose micro-tissues to help implanted fat survive (GM-pDA-PRP). Enhanced PRP adhesion leads to a prolonged and localized production of GFs, which is verified by platelet counting and by ELISA of vascular endothelial growth factors (VEGFs) and of platelet derived growth factors (PDGFs). The GM-pDA-PRP "hatches" a microenvironment for the proliferation of adipose-derived stem cells. After the adipose micro-tissue has bridged with GM-pDA-PRP after 16 weeks, triple-fluorescence staining reveals that the mature adipocytes, blood vessels, and capillaries are arranged like in normal adipose tissue. The survival fat increases significantly compared to that in control, PRP, and GM-PRP groups (84.8 +/- 11.4% versus 47.8 +/- 8.9%, 56.9 +/- 9.7%, and 60.2 +/- 10.5%, respectively). Both histological assessments and CD31 immunofluorescence indicate that the improvement of angiogenesis in GM-pDA-PRP is higher than in the fat graft group (6.4-fold in quantitative CD31 positive cells). The CD34 positive cells in the GM-pDA-PRP group are around 3.5-fold the amount in the fat graft group, which suggests that more stem cells migrate to the implant area. Cell proliferation staining shows that the number of Ki67 positive cells is around five times as high as that in the fat graft group.

Zhu, X. J., et al. (2014). "[Exogenous VEGF promotes hematopoietic stem cell mobilization]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **22**(1): 154-159.

This study was aimed to investigate the effect of exogenous VEGF on hematopoietic stem cell mobilization and immune system. The C57BL/6J mice were randomly divided into the normal control group, VEGF short-term group (5 d) and VEGF long-term group (27 d). Mice in the experimental group were injected ip with VEGF (100 ng/d); mice in control group were injected ip with PBS. The white blood cell (WBC) count and the ratio of lymphocyte in the peripheral blood at different time point were assayed by hemacytometer. The percentage of hematopoietic

stem cell (HSC), lymphocyte subgroup, regulatory T cell (Treg), myeloid-derived suppressor cells (MDSC) in the peripheral blood and spleen of different groups were detected by flow cytometry. The morphological changes of spleen and spleen index of mice in the control and long-term group were observed by microscopy. The results showed that the absolute number of WBC in the peripheral blood of mice significantly increased after injection of VEGF, and the peak value was at day 3. The percentage of Lin(-)Sca-1(+)CD117(+) cells in the peripheral blood and spleen of the long-term group were significantly higher than that in the normal control group ($P < 0.05$). The spleen of the mice in VEGF long-term group was larger than that of the control group, the spleen index also increased ($P < 0.05$), and remarkable extramedullary hematopoietic signs were found in the HE stained sections. There was no significant change in the total ratio of lymphocytes in the peripheral blood after injection, but the percentage of CD3(+) cells and the CD3(+)/B220(+) ratio in the long-term group deceased; the percentages of Treg and Gr-1(+)CD11b(+) MDSC in the experimental groups increased ($P < 0.05$), which more significantly increased in the long-term group than that in the short-term group ($P < 0.05$). It is concluded that the exogenous VEGF promotes hematopoietic stem cell mobilization, and at same time up-regulates the many kinds of suppressive immune cell levels which leads to changes of immuno-function.

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