Genetically Enhanced Stem Cell Research Literatures

Mark Herbert, PhD

39-06 Main Street, Flushing, NY 11354, USA, ma8080@gmail.com

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

[Mark Herbert, PhD. **Genetically Enhanced Stem Cell Research Literatures.** *Academ Arena* 2018;10(10):28-64]. ISSN 1553-992X (print); ISSN 2158-771X (online). http://www.sciencepub.net/academia. 5. doi:10.7537/marsaaj101018.05.

Key words: stem cell; life; research; literature; genetically enhanced

Introduction

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

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

Abraham, N. G., et al. (2008). "Bone marrow stem cell transplant into intra-bone cavity prevents type 2 diabetes: role of heme oxygenase-adiponectin." J Autoimmun **30**(3): 128-135.

Increase in endothelial cell sloughing and diminished function of endothelial stem cell progenitors in diabetic subjects are well known phenomena. We hypothesized that transplantation of bone marrow stem cells (BMSCs) including mesenchymal stem cells but not limited to CD34(+) stem cells into type 2 diabetic ob mice would restore insulin sensitivity and glucose tolerance. This approach, when combined with induction of HO-1 (a cytoprotective antioxidant system) in the recipient, would further improve bone marrow function. Sublethally irradiated ob mice received BMSC or CD34(+) stem cells from B129SF2/J mice (genetically related) via i.v. or intra bone marrow-bone marrow transplantation (IBM-BMT) at a dose of 5 x 10(6) cells. CD34(+) i.v. administration to ob mice modestly improved glucose tolerance, whereas BMSC administered by the IBM-BMT significantly increased BMSC function, serum adiponectin and glucose tolerance. Induction of HO-1 in the recipients greatly enhanced the ability of BMSC to prevent diabetes. These findings suggest that transplantation of BMSC-mesenchymal stem cells via IBM-BMT in conjunction with induction of HO-1 can eradicate type 2 diabetes. The beneficial effect of HO-1 induction further suggests that the abnormality in endothelial progenitor cells is due to mesenchymal stem cell-stromal cell disorder exacerbated by oxidative stress and decreases in adiponectin. Thus, transplantation of BMSC using the IBM-BMT strategy in conjunction with HO-1 induction offers a novel approach for the treatment of type 2 diabetes.

Ambrosone, A., et al. (2012). "Hymyc1 downregulation promotes stem cell proliferation in Hydra vulgaris." PLoS One **7**(1): e30660.

Hydra is a unique model for studying the mechanisms underlying stem cell biology. The activity of the three stem cell lineages structuring its body constantly replenishes mature cells lost due to normal tissue turnover. By a poorly understood mechanism, stem cells are maintained through self-renewal while concomitantly producing differentiated progeny. In vertebrates, one of many genes that participate in regulating stem cell homeostasis is the protooncogene c-myc, which has been recently identified also in Hydra, and found expressed in the interstitial stem cell lineage. In the present paper, by developing a novel strategy of RNA interference-mediated gene silencing (RNAi) based on an enhanced uptake of small interfering RNAi (siRNA), we provide molecular and

biological evidence for an unexpected function of the Hydra myc gene (Hymyc1) in the homeostasis of the interstitial stem cell lineage. We found that Hymyc1 inhibition impairs the balance between stem cell self renewal/differentiation, as shown by the accumulation of stem cell intermediate and terminal differentiation products in genetically interfered animals. The identical phenotype induced by the 10058-F4 inhibitor, a disruptor of c-Myc/Max dimerization, demonstrates the specificity of the RNAi approach. We show the kinetic and the reversible feature of Hymyc1 RNAi, together with the effects displayed on regenerating animals. Our results show the involvement of Hymyc1 in the control of interstitial stem cell dynamics, provide new clues to decipher the molecular control of the cell and tissue plasticity in Hydra, and also provide further insights into the complex myc network in higher organisms. The ability of Hydra cells to uptake double stranded RNA and to trigger a RNAi response lays the foundations of a comprehensive analysis of the RNAi response in Hydra allowing us to track back in the evolution and the origin of this process.

Bahrambeigi, V., et al. (2015). "Genetically modified murine adipose-derived mesenchymal stem cells producing interleukin-2 favor B16F10 melanoma cell proliferation." Immunol Invest **44**(3): 216-236.

Adipose-derived mesenchymal stem cells (ADSCs) are attractive tools for cancer gene therapy due to their intrinsic tropism to the tumor environment. Interleukin-2 (IL2) is recognized as a key regulatory molecule, which enhances the activity and growth of the immune effector cell function. High-Dose IL2 Therapy is an option for treatment of malignant melanoma but has frequent, often serious and sometimes life-threatening side effects. Here we investigated the effect of genetically modified ADSCs (GM-ADSCs) expressing IL2 in immunocompetent mouse models of subcutaneous and lung metastatic melanoma. Prior to in vivo studies, we demonstrated that IL2 produced by GM-ADSCs may act as a growth factor for melanoma cells due to the increased viability and reduced apoptosis of melanoma cells after in vitro treatment. Subcutaneous co-injection of IL2-expressing ADSCs with melanoma cells significantly enhanced the melanoma tumor growth. Furthermore, histological analysis of subcutaneous tumors for IL2 and Melan-A (a melanocytic differentiation marker) confirmed that most of cells in melanoma/IL2-ADSC co-injected tumors are melanoma cells, not IL2-ADSCs. In pulmonary metastases model, melanoma cells were injected intravenously and 10 days later mice were treated by systematical injection of GM-ADSCs. Intravenously injected IL2-ADSCs engrafted into melanoma lung tumors but were unable to reduce melanoma lung metastases. Besides, administered IL2-ADSCs significantly reduced systemic CD4+ cells and did not impact the total survival of lung metastases melanoma bearing mice. In conclusion, this study showed that IL2-producing ADSCs can favor B16F10 melanoma cell proliferation. Therefore, therapies utilizing IL2 have to be taken into careful consideration.

Balyasnikova, I. V., et al. (2010). "Genetic modification of mesenchymal stem cells to express a single-chain antibody against EGFRvIII on the cell surface." J Tissue Eng Regen Med **4**(4): 247-258.

Human adult mesenchymal stem cells (hMSCs) are under active investigation as cellular carriers for gene therapy. hMSCs possess natural tropism toward tumours; however, the targeting of hMSCs to specific cell populations within tumours is unexplored. In the case of glioblastoma multiforme (GBM), at least half of the tumours express EGFRvIII on the cell surface, an ideal target for antibody-mediated gene/drug delivery. In this study, we investigated the feasibility of genetically modifying hMSCs to express a single-chain antibody (scFv) to EGFRvIII on their surfaces. Nucleofection was used to transfect hMSCs with cDNA encoding scFv EGFRvIII fused with PDGFR or human B7-1 transmembrane domains. The expression of scFv EGFRvIII on the cell surface was assessed by FACS. A stable population of scFv EGFRvIII-expressing hMSCs was selected, based on antibiotic resistance, and enriched using FACS. We found that nucleofection allows the efficient expression of scFv EGFRvIII on the cell surface of hMSCs. hMSCs transfected with the construct encoding scFv EGFRvIII as a fusion with PDGFRtm showed scFv EGFRvIII expression in up to 86% of cells. Most importantly, human MSCs expressing scFv against EGFRvIII demonstrated enhanced binding to U87-EGFRvIII cells in vitro and significantly increased retention in human U87-EGFRvIII-expressing tumours in vivo. In summary, we provide the first conclusive evidence of genetic modification of hMSCs with a single-chain antibody against an antigen expressed on the surface of tumour cells, thereby opening up a new venue for enhanced delivery of gene therapy applications in the context of malignant brain cancer.

Bobis-Wozowicz, S., et al. (2015). "Human Induced Pluripotent Stem Cell-Derived Microvesicles Transmit RNAs and Proteins to Recipient Mature Heart Cells Modulating Cell Fate and Behavior." Stem Cells **33**(9): 2748-2761.

Microvesicles (MVs) are membrane-enclosed cytoplasmic fragments released by normal and activated cells that have been described as important mediators of cell-to-cell communication. Although the ability of human induced pluripotent stem cells

(hiPSCs) to participate in tissue repair is being increasingly recognized, the use of hiPSC-derived MVs (hiPSC-MVs) in this regard remains unknown. Accordingly, we investigated the ability of hiPSC-MVs to transfer bioactive molecules including mRNA, microRNA (miRNA), and proteins to mature target cells such as cardiac mesenchymal stromal cells (cMSCs), and we next analyzed effects of hiPSC-MVs on fate and behavior of such target cells. The results show that hiPSC-MVs derived from integration-free hiPSCs cultured under serum-free and feeder-free conditions are rich in mRNA, miRNA, and proteins originated from parent cells; however, the levels of expression vary between donor cells and MVs. Importantly, we found that transfer of hiPSC components by hiPSC-MVs impacted on transcriptome and proteomic profiles of target cells as well as exerted proliferative and protective effects on cMSCs, and enhanced their cardiac and endothelial differentiation potential. hiPSC-MVs also transferred exogenous transcripts from genetically modified hiPSCs that opens new perspectives for future strategies to enhance MV content. We conclude that hiPSC-MVs are effective vehicles for transferring iPSC attributes to adult somatic cells, and hiPSC-MV-mediated horizontal transfer of RNAs and proteins to injured tissues may be used for therapeutic tissue repair. In this study, for the first time, we propose a new concept of use of hiPSCs as a source of safe acellular bioactive derivatives for tissue regeneration.

Brunelli, S., et al. (2007). "Nitric oxide release combined with nonsteroidal antiinflammatory activity prevents muscular dystrophy pathology and enhances stem cell therapy." Proc Natl Acad Sci U S A **104**(1): 264-269.

Duchenne muscular dystrophy is a relatively common disease that affects skeletal muscle, leading to progressive paralysis and death. There is currently no resolutive therapy. We have developed a treatment in which we combined the effects of nitric oxide with nonsteroidal antiinflammatory activity by using HCT 1026, a nitric oxide-releasing derivative of flurbiprofen. Here, we report the results of long-term (1-year) oral treatment with HCT 1026 of two murine models for limb girdle and Duchenne muscular dystrophies (alpha-sarcoglycan-null and mdx mice). In both models, HCT 1026 significantly ameliorated the morphological, biochemical, and functional phenotype in the absence of secondary effects, efficiently slowing down disease progression. HCT 1026 acted by reducing inflammation, preventing muscle damage, and preserving the number and function of satellite cells. HCT 1026 was significantly more effective than the corticosteroid prednisolone, which was analyzed in parallel. As an additional beneficial effect, HCT 1026 enhanced the therapeutic efficacy of arterially delivered donor stem cells, by increasing 4-fold their ability to migrate and reconstitute muscle fibers. The therapeutic strategy we propose is not selective for a subset of mutations; it provides ground for immediate clinical experimentation with HCT 1026 alone, which is approved for use in humans; and it sets the stage for combined therapies with donor or autologous, genetically corrected stem cells.

Burke, D., et al. (2013). "The role of oxygen as a regulator of stem cell fate during fracture repair in TSP2-null mice." J Orthop Res **31**(10): 1585-1596.

It is often difficult to decouple the relative importance of different factors in regulating MSC differentiation. Genetically modified mice provide model systems whereby some variables can be manipulated while others are kept constant. Fracture repair in thrombospondin-2 (TSP2)-null mice is characterized by reduced endochondral ossification and enhanced intramembranous bone formation. The proposed mechanism for this shift in MSC fate is that increased vascular density and hence oxygen availability in TSP2-null mice regulates differentiation. However, TSP2 is multifunctional and regulates other aspects of the regenerative cascade, such as MSC proliferation. The objective of this study is to use a previously developed computational model of tissue differentiation, in which substrate stiffness and oxygen tension regulate stem cell differentiation, to simulate potential mechanisms which may drive alterations in MSC fate in TSP2-null mice. Four models (increased cell proliferation, increased numbers of MSCs in the marrow decreased cellular oxygen consumption, and an initially stiffer callus) were not predictive of experimental observations in TSP2-null mice. In contrast, increasing the rate of angiogenic progression led to a prediction of greater intramembranous ossification, diminished endochondral ossification, and a reduced region of hypoxia in the fracture callus similar to that quantified experimentally by the immunohistochemical detection of pimonidazole adducts that develop with hypoxia. This study therefore provides further support for the hypothesis that oxygen availability during early fracture healing is a key regulator of MSC bipotential differentiation, and furthermore, it highlights the advantages of integrating computational models with genetically modified mouse studies for further elucidating mechanisms regulating stem cell fate.

Castellanos, D. A., et al. (2002). "TrkC overexpression enhances survival and migration of neural stem cell transplants in the rat spinal cord." Cell Transplant **11**(3): 297-307.

Although CNS axons have the capacity to regenerate after spinal cord injury when provided with a permissive substrate, the lack of appropriate synaptic target sites for regenerating fibers may limit restoration of spinal circuitry. Studies in our laboratory are focused on utilizing neural stem cells to provide new synaptic target sites for regenerating spinal axons following injury. As an initial step, rat neural precursor cells genetically engineered to overexpress the tyrosine kinase C (trkC) neurotrophin receptor were transplanted into the intact rat spinal cord to evaluate their survival and differentiation. Cells were either pretreated in vitro prior to transplantation with trkC ligand neurotrophin-3 (NT-3) to initiate differentiation or exposed to NT-3 in vivo following transplantation via gelfoam or Oxycel. Both treatments enhanced survival of trkC-overexpressing stem cells to nearly 100%, in comparison with approximately 30-50% when either NT-3 or trkC was omitted. In addition, increased migration of trkC-overexpressing cells throughout the spinal gray matter was noted, particularly following in vivo NT-3 exposure. The combined trkC expression and NT-3 treatment appeared to reduce astrocytic differentiation of transplanted neural precursors. Decreased cavitation and increased beta-tubulin fibers were noted in the vicinity of transplanted cells, although the majority of transplanted cells appeared to remain in an undifferentiated state. These findings suggest that genetically engineered neural stem cells in combination with neurotrophin treatment may be a useful addition to strategies for repair of spinal neurocircuitry following injury.

Chao, J., et al. (2014). "Kallikrein-kinin in stem cell therapy." World J Stem Cells **6**(4): 448-457.

The tissue kallikrein-kinin system exerts a wide spectrum of biological activities in the cardiovascular, renal and central nervous systems. Tissue kallikrein-kinin modulates the proliferation, viability, mobility and functional activity of certain stem cell populations, namely mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), mononuclear cell subsets and neural stem cells. Stimulation of these stem cells by tissue kallikrein-kinin may lead to protection against renal, cardiovascular and neural damage by inhibiting apoptosis, inflammation, fibrosis and oxidative stress and promoting neovascularization. Moreover, MSCs and EPCs genetically modified with tissue kallikrein are resistant to hypoxia- and oxidative stress-induced apoptosis, and offer enhanced protective actions in animal models of heart and kidney injury and hindlimb ischemia. In addition, activation of the plasma kallikrein-kinin system promotes EPC recruitment to the inflamed synovium of arthritic rats. Conversely, cleaved high molecular weight kininogen, a product of plasma kallikrein, reduces the viability and vasculogenic activity of EPCs. Therefore, kallikrein-kinin provides a new approach in enhancing the efficacy of stem cell therapy for human diseases.

Chen, Y. B., et al. (2015). "Mesenchymal stem cell-based HSP70 promoter-driven VEGFA induction by resveratrol promotes angiogenesis in a mouse model." Cell Stress Chaperones **20**(4): 643-652.

Several studies of stem cell-based gene therapy have indicated that long-lasting regeneration following vessel ischemia may be stimulated through VEGFA gene therapy and/or MSC transplantation for reduction of ischemic injury in limb ischemia and heart failure. The therapeutic potential of MSC transplantation can be further improved by genetically modifying MSCs with genes which enhance angiogenesis following ischemic injury. In the present study, we aimed to develop an approach in MSC-based therapy for repair and mitigation of ischemic injury and regeneration of damaged tissues in ischemic disease. HSP70 promoter-driven VEGFA expression was induced by resveratrol (RSV) in MSCs, and in combination with known RSV biological functions, the protective effects of our approach were investigated by using ex vivo aortic ring coculture system and a 3D scaffolds in vivo model. Results of this investigation demonstrated that HSP promoter-driven VEGFA expression in MSC increased approximately 2-fold over the background VEGFA levels upon HSP70 promoter induction by RSV. Exposure of HUVEC cells to medium containing MSC in which VEGFA had been induced by cis-RSV enhanced tube formation in the treated HUVEC cells. RSV-treated MSC cells differentiated into endothelial-like phenotypes, exhibiting markedly elevated expression of endothelial cell markers. These MSCs also induced aortic ring sprouting, characteristic of neovascular formation from pre-existing vessels, and additionally promoted neovascularization at the MSC transplantation site in a mouse model. These observations support a hypothesis that VEGFA expression induced by cis-RSV acting on the HSP70 promoter in transplanted MSC augments the angiogenic effects of stem cell gene therapy. The use of an inducible system also vastly reduces possible clinical risks associated with constitutive VEGFA expression.

Chuang, C. K., et al. (2007). "Baculovirus as a new gene delivery vector for stem cell engineering and bone tissue engineering." Gene Ther **14**(19): 1417-1424.

Baculovirus has emerged as a novel vector for in vitro and in vivo gene delivery due to its low cytotoxicity and non-replication nature in mammalian cells, but the applications of baculovirus in the genetic

modification of human mesenchymal stem cells (hMSCs) and tissue engineering are yet to be reported. In this study, we genetically engineered hMSCs with a baculovirus (Bac-CB) expressing bone morphogenetic protein-2 (BMP-2). Bac-CB transduction of hMSCs at a multiplicity of infection of 40 triggered effective differentiation of hMSCs into osteoblasts. Supertransduction at day 6 after initial transduction enhanced the BMP-2 expression and further accelerated the in vitro osteogenesis, as confirmed by alkaline phosphatase assay, Alizarin red staining and reverse transcription-polymerase chain reaction analysis of osteoblastic genes. Implantation of the supertransduced cells at ectopic sites in the nude mice resulted in efficient cell differentiation into osteoblasts at week 2 and induced progressive mineralization and partial bone formation at week 6, as confirmed by hematoxylin and eosin, immunohistochemical and Alizarin red staining. These data collectively demonstrated, for the first time, the potential of baculovirus in hMSCs engineering and implicated its use in bone tissue engineering.

Clarke, M., et al. (2017). "Transcriptional regulation of SPROUTY2 by MYB influences myeloid cell proliferation and stem cell properties by enhancing responsiveness to IL-3." Leukemia **31**(4): 957-966.

Myeloproliferative neoplasms (MPN), which overproduce blood cells in the bone marrow, have recently been linked with a genetically determined decrease in expression of the MYB transcription factor. Here, we use a mouse MYB knockdown model with an MPN-like phenotype to show how lower levels of MYB lead to stem cell characteristics in myeloid progenitors. The altered progenitor properties feature elevated cytokine responsiveness, especially to interleukin-3, which results from increased receptor expression and increased MAPK activity leading to enhanced phosphorylation of a key regulator of protein synthesis, ribosomal protein S6. MYB acts on MAPK signaling by directly regulating transcription of the gene encoding the negative modulator SPRY2. This mechanistic insight points to pathways that might be targeted therapeutically in MPN.

Cunha, C., et al. (2011). "Genetically-determined hyperfunction of the S100B/RAGE axis is a risk factor for aspergillosis in stem cell transplant recipients." PLoS One **6**(11): e27962.

Invasive aspergillosis (IA) is a major threat to the successful outcome of hematopoietic stem cell transplantation (HSCT), although individual risk varies considerably. Recent evidence has established a pivotal role for a danger sensing mechanism implicating the S100B/receptor for advanced glycation end products (RAGE) axis in antifungal immunity. The association of selected genetic variants in the S100B/RAGE axis with susceptibility to IA was investigated in 223 consecutive patients undergoing HSCT. Furthermore, studies addressing the functional consequences of these variants were performed. Susceptibility to IA was significantly associated with two distinct polymorphisms in RAGE (-374T/A) and S100B (+427C/T) genes, the relative contribution of each depended on their presence in both transplantation counterparts [patient SNP (RAGE), adjusted hazard ratio (HR), 1.97; P = 0.042 and donor SNP (RAGE), HR, 2.03; P = 0.047] or in donors (SNP (S100B), HR, 3.15; P = 7.8e-(4)) only, respectively. Functional assays demonstrated a gain-of-function phenotype of both variants, as shown by the enhanced expression of inflammatory cytokines in RAGE polymorphic cells and increased S100B secretion in vitro and in vivo in the presence of the S100B polymorphism. These findings point to a relevant role of the danger sensing signaling in human antifungal immunity and highlight a possible contribution of a genetically-determined hyperfunction of the S100B/RAGE axis to susceptibility to IA in the HSCT setting.

Daadi, M. M., et al. (2009). "Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain." Mol Ther **17**(7): 1282-1291.

Real-time imaging of transplanted stem cells is essential for understanding their interactions in vivo with host environments, for tracking cell fate and function and for successful delivery and safety monitoring in the clinical setting. In this study, we used bioluminescence (BLI) and magnetic resonance imaging (MRI) to visualize the fate of grafted human embryonic stem cell (hESC)-derived human neural stem cells (hNSCs) in stroke-damaged rat brain. The hNSCs were genetically engineered with a lentiviral vector carrying a double fusion (DF) reporter gene that stably expressed enhanced green fluorescence protein (eGFP) and firefly luciferase (fLuc) reporter genes. The hNSCs were self-renewable, multipotent, and expressed markers for neural stem cells. Cell survival was tracked noninvasively by MRI and BLI for 2 months after transplantation and confirmed histologically. Electrophysiological recording from grafted GFP (+) cells and immuno-electronmicroscopy demonstrated connectivity. Grafted hNSCs differentiated into neurons, into oligodendrocytes in stroke regions undergoing remyelination and into astrocytes extending processes toward stroke-damaged vasculatures. Our data suggest that the combination of BLI and MRI modalities provides reliable real-time monitoring of cell fate.

De Vocht, N., et al. (2012). "Multimodal imaging of stem cell implantation in the central nervous system of mice." J Vis Exp (64): e3906.

During the past decade, stem cell transplantation has gained increasing interest as primary or secondary therapeutic modality for a variety of diseases, both in preclinical and clinical studies. However, to date results regarding functional outcome and/or tissue regeneration following stem cell transplantation are quite diverse. Generally, a clinical benefit is observed without profound understanding of the underlying mechanism (s). Therefore, multiple efforts have led to the development of different molecular imaging modalities to monitor stem cell grafting with the ultimate aim to accurately evaluate survival, fate and physiology of grafted stem cells and/or their micro-environment. Changes observed in one or more parameters determined by molecular imaging might be related to the observed clinical effect. In this context, our studies focus on the combined use of bioluminescence imaging (BLI), magnetic resonance imaging (MRI) and histological analysis to evaluate stem cell grafting. BLI is commonly used to non-invasively perform cell tracking and monitor cell survival in time following transplantation, based on a biochemical reaction where cells expressing the Luciferase-reporter gene are able to emit light following interaction with its substrate (e.g. D-luciferin). MRI on the other hand is a non-invasive technique which is clinically applicable and can be used to precisely locate cellular grafts with very high resolution, although its sensitivity highly depends on the contrast generated after cell labeling with an MRI contrast agent. Finally, post-mortem histological analysis is the method of choice to validate research results obtained with non-invasive techniques with highest resolution and sensitivity. Moreover end-point histological analysis allows us to perform detailed phenotypic analysis of grafted cells and/or the surrounding tissue, based on the use of fluorescent reporter proteins and/or direct cell labeling with specific antibodies. In summary, we here visually demonstrate the complementarities of BLI, MRI and histology to unravel different stem cell- and/or environment-associated characteristics following stem cell grafting in the CNS of mice. As an example, bone marrow-derived stromal cells, genetically engineered to express the enhanced Green Fluorescent Protein (eGFP) and firefly Luciferase (fLuc), and labeled with blue fluorescent micron-sized iron oxide particles (MPIOs), will be grafted in the CNS of immune-competent mice and outcome will be monitored by BLI, MRI and histology (Figure 1).

Deveza, L., et al. (2013). "Paracrine release from nonviral engineered adipose-derived stem cells promotes endothelial cell survival and migration in vitro." Stem Cells Dev **22**(3): 483-491.

Stem cells hold great potential for therapeutic angiogenesis due to their ability to directly contribute to new vessel formation or secrete paracrine signals. Adipose-derived stem cells (ADSCs) are a particularly attractive autologous cell source for therapeutic angiogenesis due to their ease of isolation and relative abundance. Gene therapy may be used to further enhance the therapeutic efficacy of ADSCs by overexpressing desired therapeutic factors. Here, we developed vascular endothelial growth factor (VEGF)-overexpressing ADSCs utilizing poly (beta-amino esters) (PBAEs), a hydrolytically biodegradable polymer, and examined the effects of paracrine release from nonviral modified ADSCs on the angiogenic potential of human umbilical vein endothelial cells (HUVECs) in vitro. PBAE polymeric vectors delivered DNA into ADSCs with high efficiency and low cytotoxicity, leading to an over 3-fold increase in VEGF production by ADSCs compared with Lipofectamine 2000. Paracrine release from PBAE/VEGF-transfected ADSCs enhanced HUVEC viability and decreased HUVEC apoptosis under hypoxia. Further, paracrine release from PBAE/VEGF-transfected ADSCs significantly enhanced HUVEC migration and tube formation, two critical cellular processes for effective angiogenesis. Our results demonstrate that genetically engineered ADSCs using biodegradable polymeric nanoparticles may provide a promising autologous cell source for therapeutic angiogenesis in treating cardiovascular diseases.

Doering, C. B., et al. (2018). "Preclinical Development of a Hematopoietic Stem and Progenitor Cell Bioengineered Factor VIII Lentiviral Vector Gene Therapy for Hemophilia A." Hum Gene Ther **29**(10): 1183-1201.

Genetically modified, autologous hematopoietic stem and progenitor cells (HSPCs) represent a new class of genetic medicine. Following this therapeutic paradigm, we are developing a product candidate, designated CD68-ET3-LV CD34(+), for the treatment of the severe bleeding disorder, hemophilia A. The product consists of autologous CD34(+) cells transduced with a human immunodeficiency virus 1-based, monocyte lineage-restricted, self-inactivating lentiviral vector (LV), termed CD68-ET3-LV, encoding a bioengineered coagulation factor VIII (fVIII) transgene, termed ET3, designed for enhanced expression. This vector was shown capable of high-titer manufacture under clinical scale and Good Manufacturing Practice. Biochemical and

immunogenicity testing of recombinant ET3, as well as safety and efficacy testing of CD68-ET3-LV HSPCs, were utilized to demonstrate overall safety and efficacy in murine models. In the first model, administration of CD68-ET3-LV-transduced stem-cell antigen-1(+) cells to hemophilia A mice resulted in sustained plasma fVIII production and hemostatic correction without signs of toxicity. Patient-derived, autologous mobilized peripheral blood (mPB) CD34(+) cells are the clinical target cells for ex vivo transduction using CD68-ET3-LV, and the resulting genetically modified cells represent the investigational drug candidate. In the second model, CD68-ET3-LV gene transfer into mPB CD34(+) cells isolated from normal human donors was utilized to obtain in vitro and in vivo pharmacology, pharmacokinetic, and toxicology assessment. CD68-ET3-LV demonstrated reproducible and efficient gene transfer into mPB CD34(+) cells, with vector copy numbers in the range of 1 copy per diploid genome equivalent without affecting clonogenic potential. Differentiation of human CD34(+) cells into monocytes was associated with increased fVIII production, supporting the designed function of the CD68 promoter. To assess in vivo pharmacodynamics, CD68-ET3-LV CD34(+) cell product was administered to immunodeficient mice. Treated mice displayed sustained plasma fVIII levels and no signs of product related toxicity. Collectively, the findings of the current study support the preclinical safety and efficacy of CD68-ET3-LV CD34(+).

Dufourcq, P., et al. (2008). "Secreted frizzled-related protein-1 enhances mesenchymal stem cell function in angiogenesis and contributes to neovessel maturation." Stem Cells **26**(11): 2991-3001.

Mesenchymal stem cell (MSC) transplantation offers a great angiogenic opportunity in vascular regenerative medicine. The canonical Wnt/beta-catenin signaling pathway has been demonstrated to play an essential role in stem cell fate. Recently, genetic studies have implicated the Wnt/Frizzled (Fz) molecular pathway, namely Wnt7B and Fz4, in blood growth regulation. Here, we investigated whether MSC could be required in shaping a functional vasculature and whether secreted Frizzled-related protein-1 (sFRP1), a modulator of the Wnt/Fz pathway, could modify MSC capacities, endowing MSC to increase vessel maturation. In the engraftment model, we show that murine bone marrow-derived MSC induced a beneficial vascular effect through a direct cellular contribution to vascular cells. MSC quickly organized into primitive immature vessel tubes connected to host circulation; this organization preceded host endothelial cell (EC) and smooth muscle cell (SMC) recruitment to later form mature neovessel. MSC sustained neovessel organization and maturation. We report here that sFRP1 forced expression enhanced MSC surrounding neovessel, which was correlated with an increase in vessel maturation and functionality. In vitro, sFRP1 strongly increased platelet-derived growth factor-BB (PDGF-BB) expression in MSC and enhanced beta-catenin-dependent cell-cell contacts between MSC themselves and EC or SMC. In vivo, sFRP1 increased their functional integration around neovessels and vessel maturation through a glycogen synthase kinase 3 beta (GSK3beta)-dependent pathway. sFRP1-overexpressing MSC compared with control MSC were well elongated and in a closer contact with the vascular wall, conditions required to achieve an organized mature vessel wall. We propose that genetically modifying MSC to overexpress sFRP1 may be potentially effective in promoting therapeutic angiogenesis/arteriogenesis processes. Disclosure of potential conflicts of interest is found at the end of this article.

Duncan, G., et al. (2017). "Drug-Mediated Shortening of Action Potentials in LQTS2 Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes." Stem Cells Dev **26**(23): 1695-1705.

Cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSCs) are now a well-established modality for modeling genetic disorders of the heart. This is especially so for long QT syndrome (LQTS), which is caused by perturbation of ion channel function, and can lead to fainting, malignant arrhythmias and sudden cardiac death. LQTS2 is caused by mutations in KCNH2, a gene whose protein product contributes to IKr (also known as HERG), which is the predominant repolarizing potassium current in CMs. beta-blockers are the mainstay treatment for patients with LQTS, functioning by reducing heart rate and arrhythmogenesis. However, they are not effective in around a quarter of LQTS2 patients, in part, because they do not correct the defining feature of the condition, which is excessively prolonged QT interval. Since new therapeutics are needed, in this report, we biopsied skin fibroblasts from a patient who was both genetically and clinically diagnosed with LQTS2. By producing LQTS-hiPSC-CMs, we assessed the impact of different drugs on action potential duration (APD), which is used as an in vitro surrogate for QT interval. Not surprisingly, the patient's own beta-blocker medication, propranolol, had a marginal effect on APD in the LQTS-hiPSC-CMs. However, APD could be significantly reduced by up to 19% with compounds that enhanced the IKr current by direct channel binding or by indirect mediation through the PPARdelta/protein 14-3-3 epsilon/HERG pathway. Drug-induced enhancement of an alternative potassium current, IKATP, also reduced APD by up to

21%. This study demonstrates the utility of LQTS-hiPSC-CMs in evaluating whether drugs can shorten APD and, importantly, shows that PPARdelta agonists may form a new class of therapeutics for this condition.

Duong, T. T., et al. (2018). "Use of induced pluripotent stem cell models to probe the pathogenesis of Choroideremia and to develop a potential treatment." Stem Cell Res **27**: 140-150.

Choroideremia (CHM) is a rare monogenic, X-linked recessive inherited retinal degeneration resulting from mutations in the Rab Escort Protein-1 (REP1) encoding CHM gene. The primary retinal cell type leading to CHM is unknown. In this study, we explored the utility of induced pluripotent stem cell-derived models of retinal pigmented epithelium (iPSC-RPE) to study disease pathogenesis and a potential gene-based intervention in four different genetically distinct forms of CHM. A number of abnormal cell biologic, biochemical, and physiologic functions were identified in the CHM mutant cells. We then identified a recombinant adeno-associated virus (AAV) serotype, AAV7m8, that is optimal for both delivering transgenes to iPSC-RPEs as well as to appropriate target cells (RPE cells and rod photoreceptors) in the primate retina. To establish the proof of concept of AAV7m8 mediated CHM gene therapy, we developed AAV7m8.hCHM, which delivers the human CHM cDNA under control of CMV-enhanced chicken beta-actin promoter (CssA). Delivery of AAV7m8.hCHM to CHM iPSC-RPEs restored protein prenylation, trafficking and phagocytosis. The results confirm that AAV-mediated delivery of the REP1-encoding gene can rescue defects in CHM iPSC-RPE regardless of the type of disease-causing mutation. The results also extend our understanding of mechanisms involved in the pathophysiology of choroideremia.

Erben, U., et al. (1999). "Differential effects of a stem cell factor-immunoglobulin fusion protein on malignant and normal hematopoietic cells." Cancer Res **59**(12): 2924-2930.

We genetically connected the extracellular domain of human stem cell factor to the Fc-portion of human IgG1. The chimeric recombinant stem cell factor IgG1 fusion protein (rSCF-IgG1) had an apparent approximately Mr 190,000 and consisted of three identical covalently linked subunits. It specifically bound to c-kit and the high affinity Fc gamma receptor, respectively. Liquid phase rSCF-IgG1 was, on a molar basis, about eight times more potent than native human rSCF in stimulating the proliferation of c-kit-positive leukemic cell lines and of nonmalignant CD34-positive hematopoietic progenitor cells. Although the effective dose conferring half maximum of [methyl-3H]thymidine uptake by liquid phase and solid phase-bound rSCF-IgG1 were comparable, the plateau level of [methyl-3H]thymidine uptake by malignant cells was decreased by the latter, whereas proliferation of nonmalignant progenitor cells was supported. Liquid phase rSCF-IgG1 had a 2-fold increased potential to maintain primitive nonmalignant progenitor cells in stroma-free long-term culture compared with rSCF. Liquid phase rSCF-IgG1 caused enhanced and prolonged receptor phosphorylation and a more rapid down modulation of c-kit. Our data support the concept that solid phase-attachment of rSCF-IgG1 is sufficient for alteration of biological function and that rSCF-IgG1 partially blocks SCF-stimulated malignant cell growth while supporting normal progenitor cells.

Fu, K. Y., et al. (2011). "Sciatic nerve regeneration by microporous nerve conduits seeded with glial cell line-derived neurotrophic factor or brain-derived neurotrophic factor gene transfected neural stem cells." Artif Organs **35**(4): 363-372.

Neurotrophic factors such as the glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) promote nerve cell survival and regeneration, but their efficacy in repairing a longer gap defect of rat sciatic nerve (15 mm) has not been established. In this study, two recombinant mammalian vectors containing either rat GDNF gene or BDNF gene were constructed and each was transfected into neural stem cells (NSCs). It was found that the transfection of GDNF or BDNF gene into NSCs led to significantly enhanced expression of GDNF or BDNF mRNA. The amount of GDNF or BDNF protein secreted from the transfected NSCs showed a 3.3-fold or 2.5-fold increase than that from nontransfected NSCs, respectively. The regeneration capacity of rat sciatic nerve in a poly (D,L-lactide) conduit seeded with GDNF or BDNF-transfected NSCs was evaluated by the histology, functional gait, and electrophysiology after 8 weeks of implantation. It was observed that the degree of myelination and the size of regenerated tissue in the conduits seeded with GDNF- and BDNF-transfected NSCs were higher than those seeded with the nontransfected NSCs. Conduits seeded with GDNF-transfected NSCs had the greatest number of blood vessels. The functional recovery assessed by the functional gait and electrophysiology was significantly improved for conduits seeded with GDNF or BDNF-transfected NSCs. It was concluded that the genetically modified NSCs may have potential applications in promoting nerve regeneration and functional recovery.

Gerli, M. F., et al. (2014). "Transplantation of induced pluripotent stem cell-derived mesoangioblast-

like myogenic progenitors in mouse models of muscle regeneration." J Vis Exp (83): e50532.

Patient-derived iPSCs could be an invaluable source of cells for future autologous cell therapy protocols. iPSC-derived myogenic stem/progenitor cells similar to pericyte-derived mesoangioblasts (iPSC-derived mesoangioblast-like stem/progenitor cells: IDEMs) can be established from iPSCs generated from patients affected by different forms of muscular dystrophy. Patient-specific IDEMs can be genetically corrected with different strategies (e.g. lentiviral vectors, human artificial chromosomes) and enhanced in their myogenic differentiation potential upon overexpression of the myogenesis regulator MyoD. This myogenic potential is then assessed in vitro with specific differentiation assays and analyzed by immunofluorescence. The regenerative potential of IDEMs is further evaluated in vivo, upon intramuscular and intra-arterial transplantation in two representative mouse models displaying acute and chronic muscle regeneration. The contribution of IDEMs to the host skeletal muscle is then confirmed by different functional tests in transplanted mice. In particular, the amelioration of the motor capacity of the animals is studied with treadmill tests. Cell engraftment and differentiation are then assessed by a number of histological and immunofluorescence assays on transplanted muscles. Overall, this paper describes the assays and tools currently utilized to evaluate the differentiation capacity of IDEMs, focusing on the transplantation methods and subsequent outcome measures to analyze the efficacy of cell transplantation.

Goncalves, M. A., et al. (2006). "Human mesenchymal stem cells ectopically expressing full-length dystrophin can complement Duchenne muscular dystrophy myotubes by cell fusion." Hum Mol Genet **15**(2): 213-221.

Duchenne muscular dystrophy (DMD) is the most prevalent inheritable muscle disease. It is caused by mutations in the approximately 2.5-megabase dystrophin (Dys) encoding gene. Therapeutic attempts at DMD have relied on injection of allogeneic Dys-positive myoblasts. The immune rejection of these cells and their limited availability have prompted the search for alternative therapies and sources of myogenic cells. Stem cell-based gene therapy aims to restore tissue function by the transplantation of gene-corrected autologous cells. It depends on (i) the capacity of stem cells to participate in tissue regeneration and (ii) the efficient genetic correction of defective autologous stem cells. We explored the potential of bone marrow-derived human mesenchymal stem cells (hMSCs) genetically modified with the full-length Dys-coding sequence to engage in myogenesis. By tagging hMSCs with enhanced green fluorescent protein (EGFP) or the membrane dye PKH26, we demonstrated that they could participate in myotube formation when cultured together with differentiating human myoblasts. Experiments performed with EGFP-marked hMSCs and DsRed-labeled DMD myoblasts revealed that the EGFP-positive DMD myotubes were also DsRed-positive indicating that hMSCs participate in human myogenesis through cellular fusion. Finally, we showed that hMSCs transduced with a tropism-modified high-capacity hybrid viral vector encoding full-length Dys could complement the genetic defect of DMD myotubes.

Hacke, K., et al. (2009). "Suppression of HLA expression by lentivirus-mediated gene transfer of siRNA cassettes and in vivo chemoselection to enhance hematopoietic stem cell transplantation." Immunol Res **44**(1-3): 112-126.

Current approaches for hematopoietic stem cell (HSC) and organ transplantation are limited by donor and host-mediated immune responses to allo-antigens. Application of these therapies is limited by the toxicity of preparative and post-transplant immunosuppressive regimens and a shortage of appropriate HLA-matched donors. We have been exploring two complementary approaches for genetically modifying donor cells that achieve long-term suppression of cellular proteins that elicit host immune responses to mismatched donor antigens, and provide a selective advantage to genetically engineered donor cells after transplantation. The first approach is based on recent advances that make feasible targeted down-regulation of HLA expression. Suppression of HLA expression could help to overcome limitations imposed by extensive HLA polymorphisms that restrict the availability of suitable donors. Accordingly, we have recently investigated whether knockdown of HLA by RNA interference (RNAi) enables allogeneic cells to evade immune recognition. For efficient and stable delivery of short hairpin-type RNAi constructs (shRNA), we employed lentivirus-based gene transfer vectors that integrate into genomic DNA, thereby permanently modifying transduced donor cells. Lentivirus-mediated delivery of shRNA targeting pan-Class I and allele-specific HLA achieved efficient and dose-dependent reduction in surface expression of HLA in human cells, and enhanced resistance to allo-reactive T lymphocyte-mediated cytotoxicity, while avoiding non-MHC restricted killing. Complementary strategies for genetic engineering of HSC that would provide a selective advantage for transplanted donor cells and enable successful engraftment with less toxic preparative and immunosuppressive regimens would increase the numbers of individuals to whom HLA

suppression therapy could be offered. Our second strategy is to provide a mechanism for in vivo selection of genetically modified HSC and other donor cells. We have uniquely combined transplantation during the neonatal period, when tolerance may be more readily achieved, with a positive selection strategy for in vivo amplification of drug-resistant donor HSC. This model system enables the evaluation of mechanisms of tolerance induction to neo-antigens, and allogeneic stem cells during immune ontogeny. HSC are transduced ex vivo by lentivirus-mediated gene transfer of P140K-O (6)-methylguanine-methyltransferase (MGMT (P140K)). The MGMT (P140K) DNA repair enzyme confers resistance to benzylguanine, an inhibitor of endogenous MGMT, and to chloroethylating agents such as BCNU. In vivo chemoselection enables enrichment of donor cells at the stem cell level. Using complementary approaches of in vivo chemoselection and RNAi-induced silencing of HLA expression may enable the generation of histocompatibility-enhanced, and eventually, perhaps "universally" compatible cellular grafts.

Halum, S. L., et al. (2008). "Optimization of autologous muscle stem cell survival in the denervated hemilarynx." Laryngoscope **118**(7): 1308-1312.

OBJECTIVE: Current treatments for vocal fold paralysis are suboptimal in that they fail to restore dynamic function. Autologous muscle stem cell (MSC) therapy is a promising potential therapy for vocal fold paralysis in that it can attenuate denervation-induced muscle atrophy and provide a vehicle for delivery of neurotrophic factors, thereby potentially selectively guiding reinnervation. The goal of this project was to characterize optimal conditions for injected autologous MSC survival in the thyroarytenoid (TA) muscle following recurrent laryngeal nerve (RLN) injury by local administration of adjuvant factors. STUDY DESIGN: Animal experiment. METHODS: Unilateral RLN transection and sternocleidomastoid muscle (approximately 1 g) biopsies were performed in 20 male Wistar rats. One month later, 10 autologous MSCs labeled via retroviral-enhanced green fluorescent protein (EGFP) transduction were injected into the denervated hemilarynx of each animal with one of four adjuvant therapies: cardiotoxin [(CTX) 10 M], insulin-like growth factor-1 [(IGF- 1) 100 microg/mL], ciliary neurotrophic factor [(CNTF) 50 microg/mL], or saline. Animals were euthanized 1 month later and larynges harvested, sectioned, and analyzed for MSC survival. RESULTS: All specimens demonstrate extensive MSC survival, with fusion of the MSCs with the denervated myofibers. Based on mean fluorescent intensity of the laryngeal specimens, IGF-1 and CNTF had the greatest positive influence on MSC survival. Myofiber diameters demonstrated myofiber atrophy to be inversely related to MSC survival, with the least atrophy in the groups having the greatest MSC survival. CONCLUSIONS: Autologous MSC therapy may be a future treatment for vocal fold paralysis. These findings support a model whereby MSCs genetically engineered to secrete CNTF and/or IGF-1 may not only promote neural regeneration, but also enhance MSC survival in an autocrine fashion.

Harrison, D. E., et al. (1994). "Splenic primitive hematopoietic stem cell (PHSC) activity is enhanced by steel factor because of PHSC proliferation." Blood **83**(11): 3146-3151.

To test whether primitive hematopoietic stem cells (PHSCs) are stimulated by Steel (SI) factor (c-kit ligand) in vivo, donor mice were studied after three or seven daily injections of SI factor. PHSC activity was measured as long-term erythroid and lymphoid competitive repopulating ability. Cells to be tested (usually marrow or spleen cells from treated donors) were mixed with untreated competitor marrow that produces erythrocytes and lymphocytes that are genetically distinguishable from the donors by differences in hemoglobin (Hb) and glucosephosphate isomerase (GPI) markers. These cell mixtures were injected into lethally irradiated hosts, and after 111 to 293 days, functional abilities of donor PHSC populations were assessed and expressed as percentages of donor-type Hb and GPI in the host's circulating erythrocytes and lymphocytes, respectively. A striking increase in splenic PHSC activity occurred after seven daily injections of SI factor, with a much smaller increase after three daily injections. Both three and seven daily injections of SI factor slightly reduced marrow PHSC activity. Rapid cycling greatly increases PHSC vulnerability to 5-fluorouracil (5FU). To test whether SI factor stimulates PHSCs into rapid cycling, donor mice were given a dose of 5FU in addition to SI factor. The increase in splenic PHSCs after 7 days of treatment with SI factor occurred to a similar degree whether donors were or were not treated with 5FU on day 8. However, a dose of 5FU on day 4 of the SI factor treatments almost totally prevented the increase in splenic PHSC activity. Apparently this increased activity requires PHSC cycling throughout the period of SI factor treatment.

Harrison, F., et al. (2013). "Hematopoietic stem cell gene therapy for the multisystemic lysosomal storage disorder cystinosis." Mol Ther **21**(2): 433-444.

Cystinosis is an autosomal recessive metabolic disease that belongs to the family of lysosomal storage disorders (LSDs). The defective gene is CTNS encoding the lysosomal cystine transporter, cystinosin. Cystine accumulates in all tissues and leads to organ

damage including end-stage renal disease. Using the Ctns (-/-) murine model for cystinosis, we tested the use of hematopoietic stem and progenitor cells (HSPC) genetically modified to express a functional CTNS transgene using a self-inactivating-lentiviral vector (SIN-LV). We showed that transduced cells were capable of decreasing cystine content in all tissues and improved kidney function. Transduced HSPC retained their differentiative capabilities, populating all tissue compartments examined and allowing long-term expression of the transgene. Direct correlation between the levels of lentiviral DNA present in the peripheral blood and the levels present in tissues were demonstrated, which could be useful to follow future patients. Using a new model of cystinosis, the DsRed Ctns (-/-) mice, and a LV driving the expression of the fusion protein cystinosin-enhanced green fluorescent protein (eGFP), we showed that cystinosin was transferred from CTNS-expressing cells to Ctns-deficient adjacent cells in vitro and in vivo. This transfer led to cystine decreases in Ctns-deficient cells in vitro. These data suggest that the mechanism of cross-correction is possible in cystinosis.

He, J. Q., et al. (2007). "Human embryonic stem cell-derived cardiomyocytes: drug discovery and safety pharmacology." Expert Opin Drug Discov **2**(5): 739-753.

Human embryonic stem cells (hESCs) can provide potentially unlimited quantities of a wide range of human cell types that can be used in drug discovery and development, basic research and regenerative medicine. In this review, the authors describe the differentiation of hESCs into cardiomyocytes and outline the properties of hESC-derived cardiomyocytes (hESC-CMs), including their cardiac-type action potentials and contractile characteristics. In vitro cellular assays using hESC-CMs, which can be genetically engineered to create target-specific reporters as well as human disease models, will have applications at multiple stages of the drug discovery process. Furthermore, cardiac safety pharmacology assays evaluating the risk of proarrhythmic side effects associated with QT prolongation may be enhanced in their predictive value with the use of hESC-CMs.

Hedlund, E., et al. (2007). "Selection of embryonic stem cell-derived enhanced green fluorescent protein-positive dopamine neurons using the tyrosine hydroxylase promoter is confounded by reporter gene expression in immature cell populations." Stem Cells **25**(5): 1126-1135.

Transplantation of mouse embryonic stem (mES) cells can restore function in Parkinson disease models, but can generate teratomas. Purification of dopamine neurons derived from embryonic stem cells by fluorescence-activated cell sorting (FACS) could provide a functional cell population for transplantation while eliminating the risk of teratoma formation. Here we used the tyrosine hydroxylase (TH) promoter to drive enhanced green fluorescent protein (eGFP) expression in mES cells. First, we evaluated 2.5-kilobase (kb) and 9-kb TH promoter fragments and showed that clones generated using the 9-kb fragment produced significantly more eGFP+/TH+ neurons. We selected the 9-kb TH clone with the highest eGFP/TH overlap for further differentiation, FACS, and transplantation experiments. Grafts contained large numbers of eGFP+ dopamine neurons of an appropriate phenotype. However, there were also numerous eGFP+ cells that did not express TH and did not have a neuronal morphology. In addition, we found cells in the grafts representing all three germ layers. Based on these findings, we examined the expression of stem cell markers in our eGFP+ population. We found that a majority of eGFP+ cells were stage-specific embryonic antigen-positive (SSEA-1+) and that the genetically engineered clones contained more SSEA-1+ cells after differentiation than the original D3 mES cells. By negative selection of SSEA-1, we could isolate a neuronal eGFP+ population of high purity. These results illustrate the complexity of using genetic selection to purify mES cell-derived dopamine neurons and provide a comprehensive analysis of cell selection strategies based on tyrosine hydroxylase expression. Disclosure of potential conflicts of interest is found at the end of this article.

Henig, I., et al. (2017). "[Hematopoietic Stem Cell Transplantation in the Perspective of Time and up to the 2000 Transplant at the Rambam Health Care Campus]." Harefuah **156**(9): 589-594.

INTRODUCTION: Stem cell transplantation is indicated in hematological malignancies as well as some solid tumors and congenital abnormalities. Autologous transplantation allows the administration of high dose chemotherapy without prolonged bone marrow aplasia. Allogeneic transplantation allows us to give the patient a new immune system that can locate and destroy remaining tumor cells. First attempts in patients began in 1939. Improved outcomes occurred after discovering the human leukocyte antigen system which allowed for matching the donor to the patient. Immunosuppression therapy to prevent graft versus host disease also improved the outcomes. Since the 1970's, more and more centers in North America and Europe opened stem cell transplantation programs. Today it is performed worldwide and on December 2012, the one million milestone transplant worldwide was achieved. The

bone marrow transplantation program started at Rambam Health Care campus on September 1995. Since then 2000 transplantations were performed at Rambam. A third of these procedures were allogeneic and two thirds were autologous. In the last decade patient survival has improved significantly due to better supportive care and the use of reduced intensity conditioning relying on the graft versus tumor effect (GVT). New ways to reduce graft versus host disease (GVHD), while improving GVT effect are based on manipulating T cells in the graft and on genetically engineered T cell with enhanced antitumor cytotoxicity. In the future, allogeneic transplantation will become more complex, more individualized and more efficient.

Hsiao, F. S., et al. (2011). "Toward an ideal animal model to trace donor cell fates after stem cell therapy: production of stably labeled multipotent mesenchymal stem cells from bone marrow of transgenic pigs harboring enhanced green fluorescence protein gene." J Anim Sci **89**(11): 3460-3472.

The discovery of postnatal mesenchymal stem cells (MSC) with their general multipotentiality has fueled much interest in the development of cell-based therapies. Proper identification of transplanted MSC is crucial for evaluating donor cell distribution, differentiation, and migration. Lack of an efficient marker of transplanted MSC has precluded our understanding of MSC-related regenerative studies, especially in large animal models such as pigs. In the present study, we produced transgenic pigs harboring an enhanced green fluorescent protein (EGFP) gene. The pigs provide a reliable and reproducible source for obtaining stable EGFP-labeled MSC, which is very useful for donor cell tracking after transplantation. The undifferentiated EGFP-tagged MSC expressed a greater quantity of EGFP while maintaining MSC multipotentiality. These cells exhibited homogeneous surface epitopes and possessed classic trilineage differentiation potential into osteogenic, adipogenic, and chondrogenic lineages, with robust EGFP expression maintained in all differentiated progeny. Injection of donor MSC can dramatically increase the thickness of infarcted myocardium and improve cardiac function in mice. Moreover, the MSC, with their strong EGFP expression, can be easily distinguished from the background autofluorescence in myocardial infarcts. We demonstrated an efficient, effective, and easy way to identify MSC after long-term culture and transplantation. With the transgenic model, we were able to obtain stem or progenitor cells in earlier passages compared with the transfection of traceable markers into established MSC. Because the integration site of the transgene was the same for all cells, we lessened the potential for positional effects and the heterogeneity of the stem cells. The EGFP-transgenic pigs may serve as useful biomedical and agricultural models of somatic stem cell biology.

Jungling, K., et al. (2006). "N-cadherin transsynaptically regulates short-term plasticity at glutamatergic synapses in embryonic stem cell-derived neurons." J Neurosci **26**(26): 6968-6978.

The cell adhesion molecule N-cadherin has been proposed to regulate synapse formation in mammalian central neurons. This is based on its synaptic localization enabling alignment of presynaptic and postsynaptic specializations by an adhesion mechanism. However, a potential role of N-cadherin in regulating synaptic transmission has remained elusive. In this paper, a functional analysis of N-cadherin knock-out synapses was enabled by in vitro neuronal differentiation of mouse embryonic stem cells circumventing the early embryonic lethality of mice genetically null for N-cadherin. In our in vitro system, initial synapse formation was not altered in the absence of N-cadherin, which might be attributable to compensatory mechanisms. Here, we demonstrate that N-cadherin is required for regulating presynaptic function at glutamatergic synapses. An impairment in the availability of vesicles for exocytosis became apparent selectively during high activity. Short-term plasticity was strongly altered with synaptic depression enhanced in the absence of N-cadherin. Most intriguingly, facilitation was converted to depression under specific stimulation conditions. This indicates an important role of N-cadherin in the control of short-term plasticity. To analyze, whether N-cadherin regulates presynaptic function by a transsynaptic mechanism, we studied chimeric cultures consisting of wild-type neocortical neurons and ES cell-derived neurons. With N-cadherin absent only postsynaptically, we observed a similar increase in short-term synaptic depression as found in its complete absence. This indicates a retrograde control of short-term plasticity by N-cadherin. In summary, our results revealed an unexpected involvement of a synaptic adhesion molecule in the regulation of short-term plasticity at glutamatergic synapses.

Kara, R. J., et al. (2012). "A mouse model for fetal maternal stem cell transfer during ischemic cardiac injury." Clin Transl Sci **5**(4): 321-328.

Fetal cells enter the maternal circulation during pregnancies and can persist in blood and tissues for decades, creating a state of physiologic microchimerism. Microchimerism refers to acquisition of cells from another individual and can be due to bidirectional cell traffic between mother and fetus during pregnancy. Peripartum cardiomyopathy, a rare cardiac disorder associated with high mortality rates

has the highest recovery rate amongst all etiologies of heart failure although the reason is unknown. Collectively, these observations led us to hypothesize that fetal cells enter the maternal circulation and may be recruited to the sites of myocardial disease or injury. The ability to genetically modify mice makes them an ideal system for studying the phenomenon of microchimerism in cardiac disease. Described here is a mouse model for ischemic cardiac injury during pregnancy designed to study microchimerism. Wild-type virgin female mice mated with eGFP male mice underwent ligation of the left anterior descending artery to induce a myocardial infarction at gestation day 12. We demonstrate the selective homing of eGFP cells to the site of cardiac injury without such homing to noninjured tissues suggesting the presence of precise signals sensed by fetal cells enabling them to target diseased myocardium specifically.

Kensah, G., et al. (2013). "Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro." Eur Heart J **34**(15): 1134-1146.

AIMS: We explored the use of highly purified murine and human pluripotent stem cell (PSC)-derived cardiomyocytes (CMs) to generate functional bioartificial cardiac tissue (BCT) and investigated the role of fibroblasts, ascorbic acid (AA), and mechanical stimuli on tissue formation, maturation, and functionality. METHODS AND RESULTS: Murine and human embryonic/induced PSC-derived CMs were genetically enriched to generate three-dimensional CM aggregates, termed cardiac bodies (CBs). Addressing the critical limitation of major CM loss after single-cell dissociation, non-dissociated CBs were used for BCT generation, which resulted in a structurally and functionally homogenous syncytium. Continuous in situ characterization of BCTs, for 21 days, revealed that three critical factors cooperatively improve BCT formation and function: both (i) addition of fibroblasts and (ii) ascorbic acid supplementation support extracellular matrix remodelling and CB fusion, and (iii) increasing static stretch supports sarcomere alignment and CM coupling. All factors together considerably enhanced the contractility of murine and human BCTs, leading to a so far unparalleled active tension of 4.4 mN/mm (2) in human BCTs using optimized conditions. Finally, advanced protocols were implemented for the generation of human PSC-derived cardiac tissue using a defined animal-free matrix composition. CONCLUSION: BCT with contractile forces comparable with native myocardium can be generated from enriched, PSC-derived CMs, based on a novel concept of tissue formation from non-dissociated cardiac cell aggregates. In combination with the successful generation of tissue using a defined animal-free matrix, this represents a major step towards clinical applicability of stem cell-based heart tissue for myocardial repair.

Kermani, A. J., et al. (2008). "Characterization and genetic manipulation of human umbilical cord vein mesenchymal stem cells: potential application in cell-based gene therapy." Rejuvenation Res **11**(2): 379-386.

Stem cells are defined by two main characteristics: self-renewal capacity and commitment to multi-lineage differentiation. The cells have a great therapeutic potential in repopulating damaged tissues as well as being genetically manipulated and used in cell-based gene therapy. Umbilical cord vein is a readily available and inexpensive source of stem cells that are capable of generating various cell types. Despite the recent isolation of human umbilical cord vein mesenchymal stem cells (UVMSC), the self-renewal capacity and the potential clinical application of the cells are not well known. In the present study, we have successfully isolated and cultured human UVMSCs. Our data further revealed that the isolated cells express the self-renewal genes Oct-4, Nanog, ZFX, Bmi-1, and Nucleostemin; but not Zic-3, Hoxb-4, TCL-1, Tbx-3 and Esrrb. In addition, our immunocytochemistry results revealed the expression of SSEA-4, but not SSEA-3, TRA-1-60, and TRA-1-81 embryonic stem cell surface markers in the cells. Also, we were able to transfect the cells with a reporter, enhanced green fluorescent protein (EGFP), and a therapeutic human brain-derived neurotrophic factor (hBDNF) gene by means of electroporation and obtained a stable cell line, which could constantly express both transgenes. The latter data provide further evidence on the usefulness of umbilical cord vein mesenchymal stem cells as a readily available source of stem cells, which could be genetically manipulated and used in cell-based gene therapy applications.

Kilty, I. C., et al. (1999). "Isolation of a potential neural stem cell line from the internal capsule of an adult transgenic rat brain." J Neurochem **73**(5): 1859-1870.

A thermosensitive mutation of simian virus 40 large T antigen (LTA) gene, the tsA58 gene, was cloned downstream of the 6-kbp neurofilament light chain promoter in pPOLYIII and injected into the pronucleus of fertilised oocytes of Sprague-Dawley rats to develop a strain harbouring six copies of the transgene. Immunocytochemical staining of hemizygous adult tissues with antibodies to the C-terminus of LTA showed that the inactive form of LTA was expressed only in the fibres of the internal capsule and in the choroid plexus of the brain.

Culturing the former region at 33 degrees C, the permissive temperature for LTA, yielded a cell line, NF2C, which produced active LTA and grew at 33 degrees C but which produced only inactive LTA and eventually died at the non-permissive temperature of 39 degrees C. This clonal cell line was heterogeneous at 33 degrees C, producing the precursor neuronal cell marker nestin and the glial-specific markers glial fibrillary acidic protein, vimentin and S100A1, as well as weakly producing the neuronal cell markers 68-kDa neurofilament protein (NF68) and microtubule-associated protein 2 (MAP2) in different subpopulations of cells. However, at 39 degrees C, the cells produced dendritic, neuronal-like processes and elevated levels of NF68 and MAP2, as well as the neuronal markers synaptophysin, neurone-specific enolase, and low levels of tau, all determined by western blotting and immunofluorescent staining. Basic fibroblast growth factor enhanced the growth of the cells at 33 degrees C but also enhanced the formation of dendritic neuronal-like processes at 39 degrees C. It is suggested that NF2C represents a potential stem cell line from adult brain that expresses precursor and glial cell markers at 33 degrees C but undergoes partial differentiation to a neuronal cell phenotype at 39 degrees C.

Kim, E., et al. (2011). "Magnetically enhanced adeno-associated viral vector delivery for human neural stem cell infection." Biomaterials **32**(33): 8654-8662.

Gene therapy technology is a powerful tool to elucidate the molecular cues that precisely regulate stem cell fates, but developing safe vehicles or mechanisms that are capable of delivering genes to stem cells with high efficiency remains a challenge. In this study, we developed a magnetically guided adeno-associated virus (AAV) delivery system for gene delivery to human neural stem cells (hNSCs). Magnetically guided AAV delivery resulted in rapid accumulation of vectors on target cells followed by forced penetration of the vectors across the plasma membrane, ultimately leading to fast and efficient cellular transduction. To combine AAV vectors with the magnetically guided delivery, AAV was genetically modified to display hexa-histidine (6xHis) on the physically exposed loop of the AAV2 capsid (6xHis AAV), which interacted with nickel ions chelated on NTA-biotin conjugated to streptavidin-coated superparamagnetic iron oxide nanoparticles (NiStNPs). NiStNP-mediated 6xHis AAV delivery under magnetic fields led to significantly enhanced cellular transduction in a non-permissive cell type (i.e., hNSCs). In addition, this delivery method reduced the viral exposure times required to induce a high level of transduction by as much as to 2-10 min of hNSC infection, thus demonstrating the great potential of magnetically guided AAV delivery for numerous gene therapy and stem cell applications.

Kimelman, N., et al. (2007). "Review: gene- and stem cell-based therapeutics for bone regeneration and repair." Tissue Eng **13**(6): 1135-1150.

Many clinical conditions require regeneration or implantation of bone. This is one focus shared by neurosurgery and orthopedics. Current therapeutic options (bone grafting and protein-based therapy) do not provide satisfying solutions to the problem of massive bone defects. In the past few years, gene- and stem cell-based therapy has been extensively studied to achieve a viable alternative to current solutions offered by modern medicine for bone-loss repair. The use of adult stem cells for bone regeneration has gained much focus. This unique population of multipotential cells has been isolated from various sources, including bone marrow, adipose, and muscle tissues. Genetic engineering of adult stem cells with potent osteogenic genes has led to fracture repair and rapid bone formation in vivo. It is hypothesized that these genetically modified cells exert both an autocrine and a paracrine effects on host stem cells, leading to an enhanced osteogenic effect. The use of direct gene delivery has also shown much promise for in vivo bone repair. Several viral and nonviral methods have been used to achieve substantial bone tissue formation in various sites in animal models. To advance these platforms to the clinical setting, it will be mandatory to overcome specific hurdles, such as control over transgene expression, viral vector toxicity, and prolonged culture periods of therapeutic stem cells. This review covers a prospect of cell and gene therapy for bone repair as well as some very recent advancements in stem cell isolation, genetic engineering, and exogenous control of transgene expression.

Klingener, M., et al. (2014). "N-cadherin promotes recruitment and migration of neural progenitor cells from the SVZ neural stem cell niche into demyelinated lesions." J Neurosci **34**(29): 9590-9606.

Discrete cellular microenvironments regulate stem cell pools and their development, as well as function in maintaining tissue homeostasis. Although the signaling elements modulating neural progenitor cells (NPCs) of the adult subventricular zone (SVZ) niche are fairly well understood, the pathways activated following injury and the resulting outcomes, are less clear. In the present study, we used mouse models of demyelination and proteomics analysis to identify molecular cues present in the adult SVZ niche during injury, and analyzed their role on NPCs in the

context of promoting myelin repair. Proteomic analysis of SVZ tissue from mice with experimental demyelination identified several proteins that are known to play roles in NPC proliferation, adhesion, and migration. Among the proteins found to be upregulated were members of the N-cadherin signaling pathway. During the onset of demyelination in the subcortical white matter (SCWM), activation of epidermal growth factor receptor (EGFR) signaling in SVZ NPCs stimulates the interaction between N-cadherin and ADAM10. Upon cleavage and activation of N-cadherin signaling by ADAM10, NPCs undergo cytoskeletal rearrangement and polarization, leading to enhanced migration out of the SVZ into demyelinated lesions of the SCWM. Genetically disrupting either EGFR signaling or ADAM10 inhibits this pathway, preventing N-cadherin regulated NPC polarization and migration. Additionally, in vivo experiments using N-cadherin gain- and loss-of-function approaches demonstrated that N-cadherin enhances the recruitment of SVZ NPCs into demyelinated lesions. Our data revealed that EGFR-dependent N-cadherin signaling physically initiated by ADAM10 cleavage is the response of the SVZ niche to promote repair of the injured brain.

Knorr, D. A. and D. S. Kaufman (2010). "Pluripotent stem cell-derived natural killer cells for cancer therapy." Transl Res **156**(3): 147-154.

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) provide an accessible, genetically tractable, and homogenous starting cell population to efficiently study human blood cell development. These cell populations provide platforms to develop new cell-based therapies to treat both malignant and nonmalignant hematological diseases. Our group previously demonstrated the ability of hESC-derived hematopoietic precursors to produce functional natural killer (NK) cells as well as an explanation of the underlying mechanism responsible for the inefficient development of T and B cells from hESCs. hESCs and iPSCs, which can be engineered reliably in vitro, provide an important new model system to study human lymphocyte development and produce enhanced cell-based therapies with the potential to serve as a "universal" source of antitumor lymphocytes. This review will focus on the application of hESC-derived NK cells with currently used and novel therapeutics for clinical trials, barriers to translation, and future applications through genetic engineering approaches.

Koide, Y., et al. (2007). "Two distinct stem cell lineages in murine bone marrow." Stem Cells **25**(5): 1213-1221.

Mesenchymal stem cells (MSC), a distinct type of adult stem cell, are easy to isolate, culture, and manipulate in ex vivo culture. These cells have great plasticity and potential for therapeutic application, but their properties are poorly understood because of their low frequency and the lack of knowledge on cell surface markers and their location of origin. The present study was designed to address the undefined lineage relationship of hematopoietic and mesenchymal stem cells. Genetically marked, highly purified hematopoietic stem cells (HSCs) were transplanted into wild-type animals and, after bone marrow repopulation, the progeny were rigorously investigated for differentiation potential into mesenchymal tissues by analyzing in vitro differentiation into mesenchymal tissues. None/very little of the hematopoietic cells contributed to colony-forming units fibroblast activity and mesenchymal cell differentiation; however, unfractionated bone marrow cells resulted in extensive replacement of not only hematopoietic cells but also mesenchymal cells, including MSCs. As a result, we concluded that purified HSCs have no significant potency to differentiate into mesenchymal lineage. The data strongly suggest that hematopoietic cells and mesenchymal lineage cells are derived from individual lineage-specific stem cells. In addition, we succeeded in visualizing mesenchymal lineage cells using in vivo microimaging and immunohistochemistry. Flow cytometric analysis revealed CD140b (PDGFRbeta) could be a specific marker for mesenchymal lineage cells. The results may reinforce the urgent need for a more comprehensive view of the mesenchymal stem cell identity and characteristics. Disclosure of potential conflicts of interest is found at the end of this article.

Kugler, J., et al. (2015). "A Bmp Reporter Transgene Mouse Embryonic Stem Cell Model as a Tool to Identify and Characterize Chemical Teratogens." Toxicol Sci **146**(2): 374-385.

Embryonic stem cells (ESCs) were first isolated from mouse embryos more than 30 years ago. They have proven invaluable not only in generating genetically modified mice that allow for analysis of gene function in tissue development and homeostasis but also as models for genetic disease. In addition, ESCs in vitro are finding inroads in pharmaceutical and toxicological testing, including the identification of teratogenic compounds. Here, we describe the use of a bone morphogenetic protein (Bmp)-reporter ESC line, isolated from a well-characterized transgenic mouse line, as a new tool for the identification of chemical teratogens. The Bmp-mediated expression of the green fluorescent protein enabled the quantification of dose- and time-dependent effects of valproic acid as well as retinoic acid. Significant

effects were detectable at concentrations that were comparable to the ones observed in the classical embryonic stem cell test, despite the fact that the reporter gene is expressed in distinct cell types, including endothelial and endodermal cells. Thus these cells provide a valuable new tool for the identification and characterization of relevant mechanisms of embryonic toxicity.

Lan, Y., et al. (2012). "[Establishment of an albumin and cytokeratin 19 genetically-modified embryonic stem cell line and evaluation of its hepatoblast differentiation capacities]." Zhonghua Gan Zang Bing Za Zhi **20**(1): 50-54.

OBJECTIVE: To establish a gene-modified embryonic stem (ES; E14.1-2) cell line with hepatoblast differentiation reporter genes, albumin (ALB) and cytokeratin 19 (CK19), labeled to facilitate study of their potential applicability as differentiated hepatoblasts. METHODS: Two expression vectors were constructed, one with the ALB promotor driving the enhanced green fluorescent protein (EGFP) and anti-neomycin genes (pAlb-EGFP), and the other with the CK19 promotor driving the red fluorescence protein and anti-hygromycin genes (pCK19-hCD25-IRES-tdTOMATO). The linearized vectors were electroporated into the E14.1 line, and double reporter genes-modified ES cells (E14.1-2) were selected by neomycin and hygromycin. E14.1-2 hepatoblast differentiation was induced by exposure to growth factors (BMP4 and bFGF) and evidenced by embryoid body formation. Fluorescence-activated cell sorting (FACS) and reverse transcription-polymerase chain reaction (RT-PCR) were used to confirm whether differentiated cells were hepatoblast-like and to quantify the differentiation efficiency. RESULTS: The pAlb-EGFP and pCK19-hCD25-IRES-tdTOMATO vectors were shown to specifically activate ALB and CK19 expression. The E14.1-2 cell line with labeled ALB and CK19 was established, and shown to have pluripotency by RT-PCR detection of pluripotent markers' expression, namely Oct4 and SSEA-1. After 22 days of induction, 21.27% of the differentiated hepatoblasts were detected by FACS as positive for ALB and CK19 expression. CONCLUSIONS: A gene-modified ES cell line was generated with hepatocyte differentiation reporter genes ALB and CK19 labeled. The differentiation of the resultant E14.1-2 line was technically simple to qualify and quantify, and will likely aid future studies of hepatoblast characteristics.

Lee, M. S., et al. (2017). "Enhanced Cell Growth of Adipocyte-Derived Mesenchymal Stem Cells Using Chemically-Defined Serum-Free Media." Int J Mol Sci **18**(8).

The multipotency and anti-inflammatory effects of mesenchymal stem cells (MSCs) make them attractive for cell therapy in regenerative medicine. A large number of MSCs is required for efficient therapy owing to the low homing efficiency of MSCs to target sites. Furthermore, owing to limitations in obtaining sufficient amounts of MSCs, in vitro expansion of MSCs that preserves their differentiation and proliferative potential is essential. The animal factor included in culture media also limits clinical application. In this study, adipose-derived MSCs showed a significantly higher proliferation rate in STK2, a chemically-defined medium, than in DMEM/FBS. The expression of MSC surface markers was increased in the culture using STK2 compared to that using DMEM/FBS. Tri-lineage differentiation analyses showed that MSCs cultured in STK2 were superior to those cultured in DMEM/FBS. In addition, MSCs cultured in STK2 showed a reduced senescence rate, small and homogenous cell size, and were more genetically stable compared to those cultured in DMEM/FBS. Furthermore, secretome analysis showed that the expression of factors related to proliferation/migration, anti-inflammation, and differentiation were increased in STK2 culture medium compared to DMEM/FBS. Taken together, these results suggest that culture using STK2 medium offers many advantages through which it is possible to obtain safer, superior, and larger numbers of MSCs.

Lepperhof, V., et al. (2014). "Bioluminescent imaging of genetically selected induced pluripotent stem cell-derived cardiomyocytes after transplantation into infarcted heart of syngeneic recipients." PLoS One **9**(9): e107363.

Cell loss after transplantation is a major limitation for cell replacement approaches in regenerative medicine. To assess the survival kinetics of induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CM) we generated transgenic murine iPSC lines which, in addition to CM-specific expression of puromycin N-acetyl-transferase and enhanced green fluorescent protein (EGFP), also constitutively express firefly luciferase (FLuc) for bioluminescence (BL) in vivo imaging. While undifferentiated iPSC lines generated by random integration of the transgene into the genome retained stable FLuc activity over many passages, the BL signal intensity was strongly decreased in purified iPS-CM compared to undifferentiated iPSC. Targeted integration of FLuc-expression cassette into the ROSA26 genomic locus using zinc finger nuclease (ZFN) technology strongly reduced transgene silencing in iPS-CM, leading to a several-fold higher BL compared to iPS-CM expressing FLuc from random genomic loci. To investigate the survival

kinetics of iPS-CM in vivo, purified CM obtained from iPSC lines expressing FLuc from a random or the ROSA26 locus were transplanted into cryoinfarcted hearts of syngeneic mice. Engraftment of viable cells was monitored by BL imaging over 4 weeks. Transplanted iPS-CM were poorly retained in the myocardium independently of the cell line used. However, up to 8% of cells survived for 28 days at the site of injection, which was confirmed by immunohistological detection of EGFP-positive iPS-CM in the host tissue. Transplantation of iPS-CM did not affect the scar formation or capillary density in the periinfarct region of host myocardium. This report is the first to determine the survival kinetics of drug-selected iPS-CM in the infarcted heart using BL imaging and demonstrates that transgene silencing in the course of iPSC differentiation can be greatly reduced by employing genome editing technology. FLuc-expressing iPS-CM generated in this study will enable further studies to reduce their loss, increase long-term survival and functional integration upon transplantation.

Li, Z., et al. (2013). "Bone marrow mesenchymal stem cells are an attractive donor cell type for production of cloned pigs as well as genetically modified cloned pigs by somatic cell nuclear transfer." Cell Reprogram **15**(5): 459-470.

The somatic cell nuclear transfer (SCNT) technique has been widely applied to clone pigs or to produce genetically modified pigs. Currently, this technique relies mainly on using terminally differentiated fibroblasts as donor cells. To improve cloning efficiency, only partially differentiated multipotent mesenchymal stem cells (MSCs), thought to be more easily reprogrammed to a pluripotent state, have been used as nuclear donors in pig SCNT. Although in vitro-cultured embryos cloned from porcine MSCs (MSCs-embryos) were shown to have higher preimplantation developmental ability than cloned embryos reconstructed from fibroblasts (Fs-embryos), the difference in in vivo full-term developmental rate between porcine MSCs-embryos and Fs-embryos has not been investigated so far. In this study, we demonstrated that blastocyst total cell number and full-term survival abilities of MSCs-embryos were significantly higher than those of Fs-embryos cloned from the same donor pig. The enhanced developmental potential of MSCs-embryos may be associated with their nuclear donors' DNA methylation profile, because we found that the methylation level of imprinting genes and repeat sequences differed between MSCs and fibroblasts. In addition, we showed that use of transgenic porcine MSCs generated from transgene plasmid transfection as donor cells for SCNT can produce live transgenic cloned pigs. These results strongly suggest that porcine bone marrow MSCs are a desirable donor cell type for production of cloned pigs and genetically modified cloned pigs via SCNT.

Lin, S., et al. (2017). "Synergistic effects on mesenchymal stem cell-based cartilage regeneration by chondrogenic preconditioning and mechanical stimulation." Stem Cell Res Ther **8**(1): 221.

BACKGROUND: Mesenchymal stem cells (MSCs) hold promising translational potential in cartilage regeneration. However, the efficacy of MSC-based tissue engineering is not satisfactory in the treatment of cartilage defect because of the inevitable cellular functional changes during ex vivo cell expansion. How to maintain the chondrogenic capacity of MSCs to improve their therapeutic outcomes remains an outstanding question. METHODS: Bone marrow-derived MSCs were firstly primed in chondrogenic induction medium which was then replaced with normal growth medium to attain the manipulated cells (M-MSCs). Methacrylated hyaluronic acid (MeHA) was synthesized as a scaffold to encapsulate the cells. The MSC- or M-MSC-laden constructs were treated with dynamic compressive loading (DL) in a bioreactor or with free loading (FL) for 14 days. Afterwards, the constructs were implanted in nude mice or rat models of osteochondral defects to test their efficiency in cartilage regeneration or repair. RESULTS: Data showed that the resulting M-MSCs exhibited superior chondrogenic differentiation potential and survivability compared with untreated MSCs. More importantly, we found that DL significantly promoted neocartilage formation in the MeHA hydrogel encapsulated with M-MSCs after 30 days of implantation in nude mice. Furthermore, the constructs laden with M-MSCs after DL for 14 days significantly enhanced cartilage healing in a rat model of osteochondral defect. CONCLUSIONS: Findings from this study highlight the importance of maintaining chondrogenic potential of MSCs by in-vitro chondrogenic preconditioning and a synergistic effect of mechanical stimulation in cartilage engineering, which may shed light on the stem cell-based tissue engineering for cartilage repair.

Marei, H. E., et al. (2013). "Over-expression of hNGF in adult human olfactory bulb neural stem cells promotes cell growth and oligodendrocytic differentiation." PLoS One **8**(12): e82206.

The adult human olfactory bulb neural stem/progenitor cells (OBNC/PC) are promising candidate for cell-based therapy for traumatic and neurodegenerative insults. Exogenous application of NGF was suggested as a promising therapeutic strategy for traumatic and neurodegenerative diseases,

however effective delivery of NGF into the CNS parenchyma is still challenging due mainly to its limited ability to cross the blood-brain barrier, and intolerable side effects if administered into the brain ventricular system. An effective method to ensure delivery of NGF into the parenchyma of CNS is the genetic modification of NSC to overexpress NGF gene. Overexpression of NGF in adult human OBNSC is expected to alter their proliferation and differentiation nature, and thus might enhance their therapeutic potential. In this study, we genetically modified adult human OBNS/PC to overexpress human NGF (hNGF) and green fluorescent protein (GFP) genes to provide insight about the effects of hNGF and GFP genes overexpression in adult human OBNS/PC on their in vitro multipotentiality using DNA microarray, immunophenotyping, and Western blot (WB) protocols. Our analysis revealed that OBNS/PC-GFP and OBNS/PC-GFP-hNGF differentiation is a multifaceted process involving changes in major biological processes as reflected in alteration of the gene expression levels of crucial markers such as cell cycle and survival markers, stemness markers, and differentiation markers. The differentiation of both cell classes was also associated with modulations of key signaling pathways such MAPK signaling pathway, ErbB signaling pathway, and neuroactive ligand-receptor interaction pathway for OBNS/PC-GFP, and axon guidance, calcium channel, voltage-dependent, gamma subunit 7 for OBNS/PC-GFP-hNGF as revealed by GO and KEGG. Differentiated OBNS/PC-GFP-hNGF displayed extensively branched cytoplasmic processes, a significant faster growth rate and up modulated the expression of oligodendroglia precursor cells markers (PDGFRalpha, NG2 and CNPase) respect to OBNS/PC-GFP counterparts. These findings suggest an enhanced proliferation and oligodendrocytic differentiation potential for OBNS/PC-GFP-hNGF as compared to OBNS/PC-GFP.

Men, H., et al. (2012). "Germline transmission of a novel rat embryonic stem cell line derived from transgenic rats." Stem Cells Dev **21**(14): 2606-2612.

Germline-competent rat embryonic stem (ES) cell lines are important resources for the creation of mutant rat models using ES-cell-based gene targeting technology. The ability to isolate germline-competent ES cell lines from any rat strain, including genetically modified strains, would allow for more sophisticated genetic manipulations without extensive breeding. Sprague Dawley (SD) males carrying an enhanced green fluorescent protein (EGFP) transgene were used as the founder animals for the derivation of ES cell lines. A number of ES cell lines were established and subjected to rigorous quality control testing that included assessment of pluripotency factor expression, karyotype analysis, and pathogen/sterility testing. Two male ES cell lines, SD-Tg.EC1/Rrrc and SD-Tg.EC8/Rrrc, were injected into blastocysts recovered from a cross of Dark Agouti (DA) males with SD females. Resulting chimeric animals were bred with wild-type SD mates to verify the germline transmissibility of the ES cell lines by identifying pups carrying the ES cell line-derived EGFP transgene. While both ES cell lines gave rise to chimeric animals, only SD-Tg.EC1 was germline competent. This confirms the feasibility of deriving germline-competent ES cell lines from transgenic rat strains and provides a novel ES cell line with a stable green fluorescent protein (GFP) reporter for future genetic manipulations to create new rat models.

Men, H. and E. C. Bryda (2013). "Derivation of a germline competent transgenic Fischer 344 embryonic stem cell line." PLoS One **8**(2): e56518.

Embryonic stem (ES) cell-based gene manipulation is an effective method for the generation of mutant animal models in mice and rats. Availability of germline-competent ES cell lines from inbred rat strains would allow for creation of new genetically modified models in the desired genetic background. Fischer344 (F344) males carrying an enhanced green fluorescence protein (EGFP) transgene were used as the founder animals for the derivation of ES cell lines. After establishment of ES cell lines, rigorous quality control testing that included assessment of pluripotency factor expression, karyotype analysis, and pathogen/sterility testing was conducted in selected ES cell lines. One male ES cell line, F344-Tg.EC4011, was further evaluated for germline competence by injection into Dark Agouti (DA) X Sprague Dawley (SD) blastocysts. Resulting chimeric animals were bred with wild-type SD mates and germline transmissibility of the ES cell line was confirmed by identification of pups carrying the ES cell line-derived EGFP transgene. This is the first report of a germline competent F344 ES cell line. The availability of a new germline competent ES cell line with a stable fluorescence reporter from an inbred transgenic rat strain provides an important new resource for genetic manipulations to create new rat models.

Mohseni Nodehi, S., et al. (2012). "Enhanced ADCC activity of affinity maturated and Fc-engineered mini-antibodies directed against the AML stem cell antigen CD96." PLoS One **7**(8): e42426.

CD96, a cell surface antigen recently described to be preferentially expressed on acute myeloid leukemia (AML) leukemic stem cells (LSC) may represent an interesting target structure for the development of antibody-based therapeutic approaches. The v-regions from the CD96-specific hybridoma TH-111 were

isolated and used to generate a CD96-specific single chain fragment of the variable regions (scFv). An affinity maturated variant resulting in 4-fold enhanced CD96-binding was generated by random mutagenesis and stringent selection using phage display. The affinity maturated scFv CD96-S32F was used to generate bivalent mini-antibodies by genetically fusing an IgG1 wild type Fc region or a variant with enhanced CD16a binding. Antibody dependent cell-mediated cytotoxicity (ADCC) experiments revealed that Fc engineering was essential to trigger significant effector cell-mediated lysis when the wild type scFv was used. The mini-antibody variant generated by fusing the affinity-maturated scFv with the optimized Fc variant demonstrated the highest ADCC activity (2.3-fold enhancement in efficacy). In conclusion, our data provide proof of concept that CD96 could serve as a target structure for effector cell-mediated lysis and demonstrate that both enhancing affinity for CD96 and for CD16a resulted in mini-antibodies with the highest cytolytic potential.

Moreno, R., et al. (2012). "Fetal liver-derived mesenchymal stem cell engraftment after allogeneic in utero transplantation into rabbits." Stem Cells Dev **21**(2): 284-295.

Prenatal transplantation of genetically engineered mesenchymal stem cells (MSCs) might benefit prevention or treatment of early-onset genetic disorders due to the cells' intrinsic regenerative potential plus the acquired advantage from therapeutic transgene expression. However, a thorough assessment of the safety, accessibility, and behavior of these MSCs in the fetal environment using appropriate animal models is required before we can advance toward a clinical application. We have recently shown that fetal rabbit liver MSCs (fl-MSCs) have superior growth rate, clonogenic capability, and in vitro adherence and differentiation abilities compared with adult rabbit bone marrow MSCs. In this follow-up study, we report safe and widespread distribution of recombinant pSF-EGFP retrovirus-transduced fl-MSCs (EGFP (+)-fl-MSCs) in neonatal rabbit tissues at 10 days after fetal allogeneic transplantation through both intrahepatic and intra-amniotic administration. Conversely, a more restricted biodistribution pattern according to the route of administration was apparent in the young rabbits intervened at 16 weeks after fetal EGFP (+)-fl-MSC transplantation. Furthermore, the presence of these cells in the recipients' tissues, tracked with the reporter provirus, was inversely related to the developmental stage of the fetuses at the time of intervention. Long-term engraftment was confirmed both by fluorescence in situ hybridization analysis on touch tissue imprints using a chromosome Y-specific BAC probe, and by immunohistochemical localization of EGFP expression. Finally, there was no evidence of immune responses against the transplanted EGFP (+)-fl-MSCs or the EGFP transgenic product in the treated young rabbits. Thus, cell transplantation approaches using genetically engineered fetal MSCs may prove particularly valuable to frontier medical treatments for congenital birth defects in perinatology.

Myers, T. J., et al. (2010). "Mesenchymal stem cells at the intersection of cell and gene therapy." Expert Opin Biol Ther **10**(12): 1663-1679.

IMPORTANCE OF THE FIELD: Mesenchymal stem cells have the ability to differentiate into osteoblasts, chondrocytes and adipocytes. Along with differentiation, MSCs can modulate inflammation, home to damaged tissues and secrete bioactive molecules. These properties can be enhanced through genetic-modification that would combine the best of both cell and gene therapy fields to treat monogenic and multigenic diseases. AREAS COVERED IN THIS REVIEW: Findings demonstrating the immunomodulation, homing and paracrine activities of MSCs followed by a summary of the current research utilizing MSCs as a vector for gene therapy, focusing on skeletal disorders, but also cardiovascular disease, ischemic damage and cancer. WHAT THE READER WILL GAIN: MSCs are a possible therapy for many diseases, especially those related to the musculoskeletal system, as a standalone treatment, or in combination with factors that enhance the abilities of these cells to migrate, survive or promote healing through anti-inflammatory and immunomodulatory effects, differentiation, angiogenesis or delivery of cytolytic or anabolic agents. TAKE HOME MESSAGE: Genetically-modified MSCs are a promising area of research that would be improved by focusing on the biology of MSCs that could lead to identification of the natural and engrafting MSC-niche and a consensus on how to isolate and expand MSCs for therapeutic purposes.

Nakamura, Y., et al. (2013). "Enhanced wound healing by topical administration of mesenchymal stem cells transfected with stromal cell-derived factor-1." Biomaterials **34**(37): 9393-9400.

The objective of this study was to investigate the ability of mesenchymal stem cells (MSC) genetically engineered with stromal cell-derived factor-1 (SDF-1) to heal skin wounds. When transfected with SDF-1 plasmid DNA, MSC which were isolated from the bone marrow of rats, secreted SDF-1 for 7 days. In vitro cell migration assay revealed that the SDF-1-engineered MSC (SDF-MSC) enhanced the migration of MSC and dermal fibroblasts to a significantly greater extent than MSC. The SDF-MSC secreted vascular endothelial growth factor, hepatocyte growth

factor, and interleukin 6 at a significantly high level. A skin defect model of rats was prepared and MSC and SDF-MSC were applied to the wound to evaluate wound healing in terms of wound size and histological examinations. The wound size decreased significantly faster with SDF-MSC treatment than with MSC and PBS treatments. The length of the neoepithelium and the number of blood vessels newly formed were significantly larger. A cell-tracing experiment with fluorescently labeled cells demonstrated that the percent survival of SDF-MSC in the tissue treated was significantly high compared with that of MSC. It was concluded that SDF-1 genetic engineering is a promising way to promote the wound healing activity of MSC for a skin defect.

Neri, M., et al. (2011). "Neural stem cell gene therapy ameliorates pathology and function in a mouse model of globoid cell leukodystrophy." Stem Cells **29**(10): 1559-1571.

Murine neural stem cells (mNSCs), either naive or genetically modified to express supranormal levels of beta-galactocerebrosidase (GALC), were transplanted into the brain of Twitcher mice, a murine model of globoid cell leukodystrophy, a severe sphingolipidosis. Cells engrafted long-term into the host cytoarchitecture, producing functional GALC. Levels of enzyme activity in brain and spinal cord tissues were enhanced when GALC-overexpressing NSC were used. Enzymatic correction correlated with reduced tissue storage, decreased activation of astroglia and microglia, delayed onset of symptoms, and longer lifespan. Mechanisms underlying the therapeutic effect of mNSC included widespread enzyme distribution, cross-correction of host cells, anti-inflammatory activity, and neuroprotection. Similar cell engraftment and metabolic correction were reproduced using human NSC. Thus, NSC gene therapy rapidly reconstitutes sustained and long-lasting enzyme activity in central nervous system tissues. Combining this approach with treatments targeting the systemic disease associated with leukodystrophies may provide significant therapeutic benefit.

Nicholas, C. R., et al. (2007). "A method for single-cell sorting and expansion of genetically modified human embryonic stem cells." Stem Cells Dev **16**(1): 109-117.

Genetic modification of human embryonic stem (hES) cells is essential for studies of gene function and differentiation. The expression of transgenes may direct tissue-specific differentiation and aid in the identification of various differentiated cell types. Stable genomic integration of transgenes is optimal because hES cell differentiation can span several days to weeks and include numerous cell divisions, and establishing homogeneous modified cell lines will facilitate research studies. Herein we provide a method for producing and expanding hES cell lines from single cells that have been isolated by fluorescence-activated cell sorting (FACS) following genetic modification by lentivirus vectors. Using this method, we have established enhanced green fluorescent protein (eGFP)-expressing hES cell lines that are pluripotent, contain a diploid chromosomal content, and stably express eGFP following more than 2 months of routine culture and in vivo differentiation.

Nimchuk, Z. L., et al. (2015). "Plant stem cell maintenance by transcriptional cross-regulation of related receptor kinases." Development **142**(6): 1043-1049.

The CLAVATA3 (CLV3)-CLAVATA1 (CLV1) ligand-receptor kinase pair negatively regulates shoot stem cell proliferation in plants. clv1 null mutants are weaker in phenotype than clv3 mutants, but the clv1 null phenotype is enhanced by mutations in the related receptor kinases BARELY ANY MERISTEM 1, 2 and 3 (BAM1, 2 and 3). The basis of this genetic redundancy is unknown. Here, we demonstrate that the apparent redundancy in the CLV1 clade is in fact due to the transcriptional repression of BAM genes by CLV1 signaling. CLV1 signaling in the rib meristem (RM) of the shoot apical meristem is necessary and sufficient for stem cell regulation. CLV3-CLV1 signaling in the RM represses BAM expression in wild-type Arabidopsis plants. In clv1 mutants, ectopic BAM expression in the RM partially complements the loss of CLV1. BAM regulation by CLV1 is distinct from CLV1 regulation of WUSCHEL, a proposed CLV1 target gene. In addition, quadruple receptor mutants are stronger in phenotype than clv3, pointing to the existence of additional CLV1/BAM ligands. These data provide an explanation for the genetic redundancy seen in the CLV1 clade and reveal a novel feedback operating in the control of plant stem cells.

Novosadova, E. V., et al. (2005). "Different effects of enhanced and reduced expression of pub gene on the formation of embryoid bodies by cultured embryonic mouse stem cell." Bull Exp Biol Med **140**(1): 153-158.

The effects of pub gene on proliferation and initial stages of differentiation of embryonic mouse stem cells were studied in vitro. To this end we used enhanced expression of human pub gene (hpub) and suppression of expression of mouse endogenous pub gene with RNA-interference in embryonic stem cells. Proliferative activity of genetically modified polyclonal lines of the embryonic stem cells transfected with plasmids carrying expressing hpub gene or plasmids generating small interference RNA to

this gene did not differ from that of the control cells. Inhibition of expression of endogenous pub gene in embryonic stem cells using small interference RNA 2-fold decreased the formation of embryoid bodies, at the same time additional expression of exogenous hpub gene almost 2-fold increased their number in comparison with the control. It was hypothesized that pub gene participates in early stages of differentiation of embryonic stem cells leading to the formation of embryoid bodies.

Olivier, S., et al. (2010). "EB66 cell line, a duck embryonic stem cell-derived substrate for the industrial production of therapeutic monoclonal antibodies with enhanced ADCC activity." MAbs **2**(4): 405-415.

Monoclonal antibodies (mAbs) represent the fastest growing class of therapeutic proteins. The increasing demand for mAb manufacturing and the associated high production costs call for the pharmaceutical industry to improve its current production processes or develop more efficient alternative production platforms. The experimental control of IgG fucosylation to enhance antibody dependent cell cytotoxicity (ADCC) activity constitutes one of the promising strategies to improve the efficacy of monoclonal antibodies and to potentially reduce the therapeutic cost. We report here that the EB66 cell line derived from duck embryonic stem cells can be efficiently genetically engineered to produce mAbs at yields beyond a 1 g/L, as suspension cells grown in serum-free culture media. EB66 cells display additional attractive grown characteristics such as a very short population doubling time of 12 to 14 hours, a capacity to reach very high cell density (> 30 million cells/mL) and a unique metabolic profile resulting in low ammonium and lactate accumulation and low glutamine consumption, even at high cell densities. Furthermore, mAbs produced on EB66 cells display a naturally reduced fucose content resulting in strongly enhanced ADCC activity. The EB66 cells have therefore the potential to evolve as a novel cellular platform for the production of high potency therapeutic antibodies.

Papayannopoulou, T., et al. (2003). "The role of G-protein signaling in hematopoietic stem/progenitor cell mobilization." Blood **101**(12): 4739-4747.

The directed migration of mature leukocytes to inflammatory sites and the lymphocyte trafficking in vivo are dependent on G protein-coupled receptors and delivered through pertussis toxin (Ptx)-sensitive Gi-protein signaling. In the present study, we explored the in vivo role of G-protein signaling on the redistribution or mobilization of hematopoietic stem/progenitor cells (HPCs). A single injection of Ptx in mice elicits a long-lasting leukocytosis and a progressive increase in circulating colony-forming unit-culture (CFU-C) and colony-forming unit spleen (CFU-S). We found that the prolonged effect is sustained by a continuous slow release of Ptx bound to red blood cells or other cells and is potentially enhanced by an indirect influence on cell proliferation. Plasma levels of certain cytokines (interleukin 6 [IL-6], granulocyte colony-stimulating factor [G-CSF]) increase days after Ptx treatment, but these are unlikely initiators of mobilization. In addition to normal mice, mice genetically deficient in monocyte chemotactic protein 1 (MCP-1), matrix metalloproteinase 9 (MMP-9), G-CSF receptor, beta2 integrins, or selectins responded to Ptx treatment, suggesting independence of Ptx-response from the expression of these molecules. Combined treatments of Ptx with anti-very late activation antigen (anti-VLA-4), uncovered potentially important insight in the interplay of chemokines/integrins, and the synergy of Ptx with G-CSF appeared to be dependent on MMP-9. As Ptx-mobilized kit+ cells display virtually no response to stromal-derived factor 1 (SDF-1) in vitro, our data suggest that disruption of CXCR4/SDF-1 signaling may be the underlying mechanism of Ptx-induced mobilization and indirectly reinforce the notion that active signaling through this pathway is required for continuous retention of cells within the bone marrow. Collectively, our data unveil a novel example of mobilization through pharmacologic modulation of signaling.

Paul, A., et al. (2012). "PAMAM dendrimer-baculovirus nanocomplex for microencapsulated adipose stem cell-gene therapy: in vitro and in vivo functional assessment." Mol Pharm **9**(9): 2479-2488.

The present study aims to develop a new stem cell based gene delivery system consisting of human adipose tissue derived stem cells (hASCs) genetically modified with self-assembled nanocomplex of recombinant baculovirus and PAMAM dendrimer (Bac-PAMAM) to overexpress the vascular endothelial growth factor (VEGF). Cells were enveloped into branched PEG surface functionalized polymeric microcapsules for efficient transplantation. In vitro analysis confirmed efficient transduction of hASCs expressing 7.65 +/- 0.86 ng functionally active VEGF per 10(6) microencapsulated hASCs (ASC-VEGF). To determine the potential of the developed system, chronically infarcted rat hearts were treated with either empty microcapsules (MC), microencapsulated hASCs expressing MGFP reporter protein (MC+ASC-MGFP), or MC+ASC-VEGF, and analyzed for 10 weeks. Post-transplantation data confirmed higher myocardial VEGF expressions with significantly enhanced neovasculature in the

MC+ASC-VEGF group. In addition, the cardiac performance, as measured by percentage ejection fraction, also improved significantly in the MC+ASC-VEGF group (48.6 +/- 6.1%) compared to that in MC+ASC-MGFP (38.8 +/- 5.3%) and MC groups (31.5 +/- 3.3%). Collectively, these data demonstrate the feasibility of this system for improved stem cell therapy applications.

Pickard, M. R., et al. (2015). "Using magnetic nanoparticles for gene transfer to neural stem cells: stem cell propagation method influences outcomes." J Funct Biomater **6**(2): 259-276.

Genetically engineered neural stem cell (NSC) transplants offer a key strategy to augment neural repair by releasing therapeutic biomolecules into injury sites. Genetic modification of NSCs is heavily reliant on viral vectors but cytotoxic effects have prompted development of non-viral alternatives, such as magnetic nanoparticle (MNPs). NSCs are propagated in laboratories as either 3-D suspension "neurospheres" or 2-D adherent "monolayers". MNPs deployed with oscillating magnetic fields ("magnetofection technology") mediate effective gene transfer to neurospheres but the efficacy of this approach for monolayers is unknown. It is important to address this issue as oscillating magnetic fields dramatically enhance MNP-based transfection in transplant cells (e.g., astrocytes and oligodendrocyte precursors) propagated as monolayers. We report for the first time that oscillating magnetic fields enhanced MNP-based transfection with reporter and functional (basic fibroblast growth factor; FGF2) genes in monolayer cultures yielding high transfection versus neurospheres. Transfected NSCs showed high viability and could re-form neurospheres, which is important as neurospheres yield higher post-transplantation viability versus monolayer cells. Our results demonstrate that the combination of oscillating magnetic fields and a monolayer format yields the highest efficacy for MNP-mediated gene transfer to NSCs, offering a viable non-viral alternative for genetic modification of this important neural cell transplant population.

Povey, J., et al. (1998). "Enhanced retroviral transduction of 5-fluorouracil-resistant human bone marrow (stem) cells using a genetically modified packaging cell line." Blood **92**(11): 4080-4089.

Pluripotent hematopoietic stem cells (PHSC) are rare cells capable of multilineage differentiation, long-term reconstituting activity and extensive self-renewal. Such cells are the logical targets for many forms of corrective gene therapy, but are poor targets for retroviral mediated gene transfer owing to their quiescence, as retroviral transduction requires that the target cells be cycling. To try and surmount this problem we have constructed a retroviral producer line that expresses the membrane-bound form of human stem cell factor (SCF) on its cell surface. These cells are capable, therefore, of delivering a growth signal concomitant with recombinant retroviral vector particles. In this report we describe the use of this cell line to transduce a highly quiescent population of cells isolated from adult human bone marrow using the 5-fluorouracil (FU) resistance technique of Berardi et al. Quiescent cells selected using this technique were transduced by cocultivation with retroviral producers expressing surface bound SCF or with the parent cell line that does not. Following coculture, the cells were plated in long-term bone marrow culture for a further 5 weeks, before plating the nonadherent cells in semisolid media. Colonies forming in the semisolid media over the next 14 days were analyzed by polymerase chain reaction for the presence of the retroviral vector genome. Over six experiments, the transduction frequency of the quiescent 5-FU resistant cells using the SCF-expressing producer line averaged about 20%, whereas those transduced using the parent producer line showed evidence of reduced levels or no transduction.

Pruitt, S. C., et al. (2007). "Reduced Mcm2 expression results in severe stem/progenitor cell deficiency and cancer." Stem Cells **25**(12): 3121-3132.

Mcm2 is a component of the DNA replication licensing complex that marks DNA replication origins during G1 of the cell cycle for use in the subsequent S-phase. It is expressed in stem/progenitor cells in a variety of regenerative tissues in mammals. Here, we have used the Mcm2 gene to develop a transgenic mouse in which somatic stem/progenitor cells can be genetically modified in the adult. In these mice, a tamoxifen-inducible form of Cre recombinase is integrated 3' to the Mcm2 coding sequence and expressed via an internal ribosome entry site (IRES). Heterozygous Mcm2(IRES-CreERT2/wild-type (wt)) mice are phenotypically indistinguishable from wild-type at least through 1 year of age. In bigenic Mcm2(IRES-CreERT2/wt); Z/EG reporter mice, tamoxifen-dependent enhanced green fluorescence protein expression is inducible in a wide variety of somatic stem cells and their progeny. However, in Mcm2(IRES-CreERT2/IRES-CreERT2) homozygous embryos or mouse embryonic fibroblasts, Mcm2 is reduced to approximately one-third of wild-type levels. Despite the fact that these mice develop normally and are asymptomatic as young adults, life span is greatly reduced, with most surviving to only approximately 10-12 weeks of age. They demonstrate severe deficiencies in the proliferative cell compartments of a variety of tissues, including the subventricular zone of

the brain, muscle, and intestinal crypts. However, the immediate cause of death in most of these animals is cancer, where the majority develop lymphomas. These studies directly demonstrate that deficiencies in the function of the core DNA replication machinery that are compatible with development and survival nonetheless result in a chronic phenotype leading to stem cell deficiency in multiple tissues and cancer. Disclosure of potential conflicts of interest is found at the end of this article.

Psatha, N., et al. (2014). "Superior long-term repopulating capacity of G-CSF+plerixafor-mobilized blood: implications for stem cell gene therapy by studies in the Hbb (th-3) mouse model." Hum Gene Ther Methods **25**(6): 317-327.

High numbers of genetically modified hematopoietic stem cells (HSCs) equipped with enhanced engrafting potential are required for successful stem cell gene therapy. By using thalassemia as a model, we investigated the functional properties of hematopoietic stem and progenitor cells (HSPCs) from Hbb (th3)/45.2(+) mice after mobilization with G-CSF, plerixafor, or G-CSF+plerixafor and the engraftment kinetics of primed cells after competitive primary and noncompetitive secondary transplantation. G-CSF+plerixafor yielded the highest numbers of HSPCs, while G-CSF+plerixafor-mobilized Hbb (th3)/45.2(+) cells, either unmanipulated or transduced with a reporter vector, achieved faster hematologic reconstitution and higher levels of donor chimerism over all other types of mobilized cells, after competitive transplantation to B6.BoyJ/45.1(+) recipients. The engraftment benefit observed in the G-CSF+plerixafor group was attributed to the more primitive stem cell phenotype of G-CSF+plerixafor-LSK cells, characterized by higher CD150(+)/CD48 expression. Moreover, secondary G-CSF+plerixafor recipients displayed stable or even higher chimerism levels as compared with primary engrafted mice, thus maintaining or further improving engraftment levels over G-CSF- or plerixafor-secondary recipients. Plerixafor-primed cells displayed the lowest competiveness over all other mobilized cells after primary or secondary transplantation, probably because of the higher frequency of more actively proliferating LK cells. Overall, the higher HSC yields, the faster hematological recovery, and the superiority in long-term engraftment indicate G-CSF+plerixafor-mobilized blood as an optimal graft source, not only for thalassemia gene therapy, but also for stem cell gene therapy applications in general.

Qiu, X. C., et al. (2015). "Donor mesenchymal stem cell-derived neural-like cells transdifferentiate into myelin-forming cells and promote axon regeneration in rat spinal cord transection." Stem Cell Res Ther **6**: 105.

INTRODUCTION: Severe spinal cord injury often causes temporary or permanent damages in strength, sensation, or autonomic functions below the site of the injury. So far, there is still no effective treatment for spinal cord injury. Mesenchymal stem cells (MSCs) have been used to repair injured spinal cord as an effective strategy. However, the low neural differentiation frequency of MSCs has limited its application. The present study attempted to explore whether the grafted MSC-derived neural-like cells in a gelatin sponge (GS) scaffold could maintain neural features or transdifferentiate into myelin-forming cells in the transected spinal cord. METHODS: We constructed an engineered tissue by co-seeding of MSCs with genetically enhanced expression of neurotrophin-3 (NT-3) and its high-affinity receptor tropomyosin receptor kinase C (TrkC) separately into a three-dimensional GS scaffold to promote the MSCs differentiating into neural-like cells and transplanted it into the gap of a completely transected rat spinal cord. The rats received extensive post-operation care, including cyclosporin A administrated once daily for 2 months. RESULTS: MSCs modified genetically could differentiate into neural-like cells in the MN + MT (NT-3-MSCs + TrKC-MSCs) group 14 days after culture in the GS scaffold. However, after the MSC-derived neural-like cells were transplanted into the injury site of spinal cord, some of them appeared to lose the neural phenotypes and instead transdifferentiated into myelin-forming cells at 8 weeks. In the latter, the MSC-derived myelin-forming cells established myelin sheaths associated with the host regenerating axons. And the injured host neurons were rescued, and axon regeneration was induced by grafted MSCs modified genetically. In addition, the cortical motor evoked potential and hindlimb locomotion were significantly ameliorated in the rat spinal cord transected in the MN + MT group compared with the GS and MSC groups. CONCLUSION: Grafted MSC-derived neural-like cells in the GS scaffold can transdifferentiate into myelin-forming cells in the completely transected rat spinal cord.

Quarta, M., et al. (2017). "Bioengineered constructs combined with exercise enhance stem cell-mediated treatment of volumetric muscle loss." Nat Commun **8**: 15613.

Volumetric muscle loss (VML) is associated with loss of skeletal muscle function, and current treatments show limited efficacy. Here we show that bioconstructs suffused with genetically-labelled muscle stem cells (MuSCs) and other muscle resident cells (MRCs) are effective to treat VML injuries in

mice. Imaging of bioconstructs implanted in damaged muscles indicates MuSCs survival and growth, and ex vivo analyses show force restoration of treated muscles. Histological analysis highlights myofibre formation, neovascularisation, but insufficient innervation. Both innervation and in vivo force production are enhanced when implantation of bioconstructs is followed by an exercise regimen. Significant improvements are also observed when bioconstructs are used to treat chronic VML injury models. Finally, we demonstrate that bioconstructs made with human MuSCs and MRCs can generate functional muscle tissue in our VML model. These data suggest that stem cell-based therapies aimed to engineer tissue in vivo may be effective to treat acute and chronic VML.

Rodriguez, R. T., et al. (2007). "Manipulation of OCT4 levels in human embryonic stem cells results in induction of differential cell types." Exp Biol Med (Maywood) **232**(10): 1368-1380.

To fully understand self-renewal and pluripotency and their regulation in human embryonic stem cells (hESCs), it is necessary to generate genetically modified cells and analyze the consequences of elevated and reduced expression of genes. Genes expressed in hESCs using plasmid vectors, however, are subject to silencing. Moreover, hESCs have a low plating efficiency when dissociated to single cells, making creation of subcloned lines inefficient. In addition to overexpression experiments, it is important to perform loss-of-function studies, which can be achieved rapidly using RNA interference (RNAi). We report stable long-term expression of enhanced green fluorescent protein (eGFP) in hESCs using a lentiviral vector, and establishment of an eGFP-expressing subline (RG6) using manual dissection. To demonstrate the efficacy of RNAi in hESCs, an RNAi expression vector was used to achieve reduced expression of eGFP in hESCs. To evaluate the role of OCT4 in the regulation of hESC self-renewal and differentiation, a vector expressing a hairpin RNA targeting endogenous expression of OCT4 was constructed. In a novel experiment in hESCs, the OCT4 cDNA sequence was cloned into an expression vector to allow for the transient upregulation of OCT4 in hESCs. The ability to manipulate levels of OCT4 above and below enodogenous levels allows the determination of OCT4 function in hESCs. Specifically, reduced expression of OCT4 in hESCs promoted upregulation of markers indicative of mesoderm and endoderm differentiation, and elevated levels of OCT4 in hESCs promoted upregulation of markers indicative of endoderm derivatives. Thus, both upregulation and downregulation of Oct4 in hESCs results in differentiation, but with patterns distinct from parallel experiments in mice.

Roudkenar, M. H., et al. (2018). "Lipocalin 2 enhances mesenchymal stem cell-based cell therapy in acute kidney injury rat model." Cytotechnology **70**(1): 103-117.

Acute kidney injury (AKI) is one of the most common health-threatening diseases in the world. There is still no effective medical treatment for AKI. Recently, Mesenchymal stem cell (MSC)-based therapy has been proposed for treatment of AKI. However, the microenvironment of damaged kidney tissue is not favorable for survival of MSCs which would be used for therapeutic intervention. In this study, we genetically manipulated MSCs to up-regulate lipocalin-2 (Lcn2) and investigated whether the engineered MSCs (MSC-Lcn2) could improve cisplatin-induced AKI in a rat model. Our results revealed that up-regulation of Lcn2 in MSCs efficiently enhanced renal function. MSC Lcn2 up-regulates expression of HGF, IGF, FGF and VEGF growth factors. In addition, they reduced molecular biomarkers of kidney injury such as KIM-1 and Cystatin C, while increased the markers of proximal tubular epithelium such as AQP-1 and CK18 following cisplatin-induced AKI. Overall, here we over-expressed Lcn2, a well-known cytoprotective factor against acute ischemic renal injury, in MSCs. This not only potentiated beneficial roles of MSCs for cell therapy purposes but also suggested a new modality for treatment of AKI.

Sabatino, M., et al. (2016). "Generation of clinical-grade CD19-specific CAR-modified CD8+ memory stem cells for the treatment of human B-cell malignancies." Blood **128**(4): 519-528.

Long-lived, self-renewing, multipotent T memory stem cells (TSCM) can trigger profound and sustained tumor regression but their rareness poses a major hurdle to their clinical application. Presently, clinically compliant procedures to generate relevant numbers of this T-cell population are undefined. Here, we provide a strategy for deriving large numbers of clinical-grade tumor-redirected TSCM starting from naive precursors. CD8(+)CD62L (+)CD45RA (+) naive T cells enriched by streptamer-based serial-positive selection were activated by CD3/CD28 engagement in the presence of interleukin-7 (IL-7), IL-21, and the glycogen synthase-3beta inhibitor TWS119, and genetically engineered to express a CD19-specific chimeric antigen receptor (CD19-CAR). These conditions enabled the generation of CD19-CAR-modified CD8(+) TSCM that were phenotypically, functionally, and transcriptomically equivalent to their naturally occurring counterpart. Compared with

CD8(+) T cells generated with clinical protocols currently under investigation, CD19-CAR-modified CD8(+) TSCM exhibited enhanced metabolic fitness and mediated robust, long-lasting antitumor responses against systemic acute lymphoblastic leukemia xenografts. This clinical-grade platform provides the basis for a phase 1 trial evaluating the activity of CD19-CAR-modified CD8(+) TSCM in patients with B-cell malignancies refractory to prior allogeneic hematopoietic stem cell transplantation.

Shujia, J., et al. (2008). "Stable therapeutic effects of mesenchymal stem cell-based multiple gene delivery for cardiac repair." Cardiovasc Res **77**(3): 525-533.

AIMS: We have previously shown that transplantation of mesenchymal stem cells (MSCs) co-overexpressing angiopoietin-1 (Ang-1) and Akt prevented cell apoptosis, enhanced angiogenesis, and improved left ventricular heart function. The present study was designed to determine the persistence of therapeutic benefits on longer term basis. METHODS AND RESULTS: Acute myocardial infarction model was developed in 30 young female Fischer-344 rats by permanent ligation of the left anterior descending coronary artery. The animals were grouped (n = 10) to receive 70 microL Dulbecco's modified Eagle's medium (DMEM) without cells (DMEM group 1) or containing 3 x 10(6) non-transduced male MSCs (MSC group 2) or transduced MSCs co-overexpressing Ang-1 and Akt (MAA group 3). The injections were carried out intramyocardially in the free wall of left ventricle at multiple sites. Three months after cell transplantation, real-time polymerase chain reaction for the rat sry gene, confocal imaging, and immunohistochemical studies revealed the extensive survival and myogenic differentiation of the PKH67-labelled cell graft. Blood vessel density was significantly higher in the MAA group ($P < 0.05$) at 3 months compared with the other groups. Blood vessel maturation index as determined by double-fluorescent immunostaining for vWFactor VIII and smooth muscle actin showed that most of the newly formed vessels matured to develop a smooth muscle covering in MAA group. Sonographic assessment of heart function showed that heart function deteriorated in the DMEM group, whereas the functional benefits were stable over a period of 3 months following transplantation of transfected cells. CONCLUSION: Engraftment of genetically modified MSCs co-overexpressing Ang-1 and Akt produced long-term histological and functional benefits in an infarcted heart.

Sifuentes, C. J., et al. (2016). "Rapid, Dynamic Activation of Muller Glial Stem Cell Responses in Zebrafish." Invest Ophthalmol Vis Sci **57**(13): 5148-5160.

Purpose: Zebrafish neurons regenerate from Muller glia following retinal lesions. Genes and signaling pathways important for retinal regeneration in zebrafish have been described, but our understanding of how Muller glial stem cell properties are regulated is incomplete. Mammalian Muller glia possess a latent neurogenic capacity that might be enhanced in regenerative therapies to treat degenerative retinal diseases. Methods: To identify transcriptional changes associated with stem cell properties in zebrafish Muller glia, we performed a comparative transcriptome analysis from isolated cells at 8 and 16 hours following an acute photic lesion, prior to the asymmetric division that produces retinal progenitors. Results: We report a rapid, dynamic response of zebrafish Muller glia, characterized by activation of pathways related to stress, nuclear factor-kappaB (NF-kappaB) signaling, cytokine signaling, immunity, prostaglandin metabolism, circadian rhythm, and pluripotency, and an initial repression of Wnt signaling. When we compared publicly available transcriptomes of isolated mouse Muller glia from two retinal degeneration models, we found that mouse Muller glia showed evidence of oxidative stress, variable responses associated with immune regulation, and repression of pathways associated with pluripotency, development, and proliferation. Conclusions: Categories of biological processes/pathways activated following photoreceptor loss in regeneration-competent zebrafish Muller glia, which distinguished them from mouse Muller glia in retinal degeneration models, included cytokine signaling (notably NF-kappaB), prostaglandin E2 synthesis, expression of core clock genes, and pathways/metabolic states associated with pluripotency. These regulatory mechanisms are relatively unexplored as potential mediators of stem cell properties likely to be important in Muller glial cells for successful retinal regeneration.

Singh, G., et al. (2012). "Successful derivation of EGFP-transgenic embryonic stem cell line from a genetically non-permissive FVB/N mouse." Am J Stem Cells **1**(2): 163-173.

Derivation of embryonic stem (ES)-cell lines from genetically non-permissive mouse strains, such as FVB/N, has been difficult, despite this strain offering advantages for mouse transgenesis for developmental studies. We earlier generated beta-actin promoter-driven enhanced green fluorescent protein (EGFP)-transgenic FVB/N mice, expressing EGFP in all cells. Here, by optimizing culture system and using RESGROTM ES-cell culture medium, we successfully derived EGFP-transgenic ES-cell line, 'GS-2' line,

from F1 hybrid blastocysts, from wild-type 129/SvJ female X EGFP-transgenic homozygous FVB/N male. The GS-2 ES-cell line exhibited all defining criteria of a typical ES-cell line, including normal colony morphology and karyotype (40,XY), high replication-expansion efficiency (passages: >100), expression of pluripotent markers (Oct-4, Nanog, Sox-2, SSEA-1 and others) and, embryoid body (EB) development and EB differentiation to ecto-/meso-/endo-dermal cell types, expressing nestin, BMP-4 and alpha-fetoprotein, respectively. GS-2 ES-cells formed (i) teratoma containing three germ lineage-derived cell types, (ii) chimeric blastocysts and fetuses, following their aggregation with wild-type 8-cell embryos, (iii) functional cardiac clusters and (iv) predominantly neural cell types when EBs were developed in KOSR-supplemented medium. Taken together, we derived a robust EGFP-transgenic GS-2 ES-cell line, from a non-permissive transgenic (FVB/N) mouse by a single cross to 129/SvJ wild-type mouse. The GS-2 ES-cell line exhibited full differentiation potential, in vitro/in vivo, providing enormous opportunity for stem cell research, including experimental cell transplantation studies.

Sivasankaran, A., et al. (2018). "Machine Learning Approach to Predicting Stem Cell Donor Availability." Biol Blood Marrow Transplant.

The success of unrelated donor stem cell transplants depends on not only finding genetically matched donors, but also donor availability. On average 50% of potential donors in the National Marrow Donor Program database are unavailable for a variety of reasons, after initially matching a patient, with significant variations in availability among subgroups (eg, by race or age). Several studies have established univariate donor characteristics associated with availability. Individual consideration of each applicable characteristic is laborious. Extrapolating group averages to the individual-donor level tends to be highly inaccurate. In the current environment with enhanced donor data collection, we can make better estimates of individual donor availability. We propose a machine learning based approach to predict availability of every registered donor, and evaluate the predictive power on a test cohort of 44,544 requests to be.77 based on the area under the receiver-operating characteristic curve. We propose that this predictor should be used during donor selection to reduce the time to transplant.

Smith, N. J., et al. (2010). "Haematopoietic stem cell transplantation does not retard disease progression in the psycho-cognitive variant of late-onset metachromatic leukodystrophy." J Inherit Metab Dis **33 Suppl 3**: S471-475.

Haematopoietic stem cell transplantation has an unproven role in the management of late-onset metachromatic leukodystrophy: theoretically justified through the engraftment of enzyme-replete haematopoietic progenitors and restoration of capacity for sulphatide catabolism in neural tissue through enzyme recapture, the long-term outcome is unknown. The rarity of the psycho-cognitive variant and slow progression of late-onset disease impairs evaluation of treatment. We report detailed clinical and neuropsychological assessments after haematopoietic stem-cell transplantation in a patient with a late-onset psycho-cognitive form of metachromatic leukodystrophy. Cognitive decline, indistinguishable from the natural course of the disease, was serially documented over 11 years despite complete donor chimaerism and correction of leukocyte arylsulphatase A to wild type values; subtle motor deterioration was similarly noted and progressive cerebral volume loss was evident upon magnetic resonance imaging. Sensory nerve conduction deteriorated 17 months post-transplantation with apparent stabilisation at 11-year review. Haematopoietic stem-cell transplantation was ineffective for this rare attenuated variant of metachromatic leukodystrophy. In the few patients identified pre-symptomatically or with early-phase disease, clear recommendations are lacking; when transplantation is considered, umbilical cord blood grafts from enzyme-replete donors with adjunctive mesenchymal stem cell infusions from the same source may be preferable. Improved outcomes will depend on enhanced awareness and early diagnosis of the disease, so that promising interventions such as genetically modified, autologous stem cell transplantation have the best opportunity of success.

Song, M., et al. (2012). "Induced pluripotent stem cell research: a revolutionary approach to face the challenges in drug screening." Arch Pharm Res **35**(2): 245-260.

Discovery of induced pluripotent stem (iPS) cells in 2006 provided a new path for cell transplantation and drug screening. The iPS cells are stem cells derived from somatic cells that have been genetically reprogrammed into a pluripotent state. Similar to embryonic stem (ES) cells, iPS cells are capable of differentiating into three germ layers, eliminating some of the hurdles in ES cell technology. Further progress and advances in iPS cell technology, from viral to non-viral systems and from integrating to non-integrating approaches of foreign genes into the host genome, have enhanced the existing technology, making it more feasible for clinical applications. In particular, advances in iPS cell technology should enable autologous transplantation and more efficient drug discovery. Cell transplantation may lead to

improved treatments for various diseases, including neurological, endocrine, and hepatic diseases. In studies on drug discovery, iPS cells generated from patient-derived somatic cells could be differentiated into specific cells expressing specific phenotypes, which could then be used as disease models. Thus, iPS cells can be helpful in understanding the mechanisms of disease progression and in cell-based efficient drug screening. Here, we summarize the history and progress of iPS cell technology, provide support for the growing interest in iPS cell applications with emphasis on practical uses in cell-based drug screening, and discuss some challenges faced in the use of this technology.

Sui, B., et al. (2016). "Allogeneic Mesenchymal Stem Cell Therapy Promotes Osteoblastogenesis and Prevents Glucocorticoid-Induced Osteoporosis." Stem Cells Transl Med **5**(9): 1238-1246.

UNLABELLED:: Gene-modified mesenchymal stem cell (MSC)-like cells with enhanced bone marrow homing and osteogenesis have been used in treating glucocorticoid-induced murine osteoporosis (GIOP). Recent preclinical studies have further demonstrated the immunomodulatory and anticatabolic potential of allogeneic MSCs in treating osteoporosis under inflammatory and autoimmune conditions. In this study, we investigated whether systemic infusion of allogeneic MSCs without genetic manipulation could prevent GIOP, whether anabolic and anticatabolic effects existed, and whether homing or immunomodulation underlay the putative therapeutic effects. Allogeneic bone marrow-derived MSCs (BMMSCs) were isolated, identified, and systemically infused into mice treated with excessive dexamethasone. We revealed that allogeneic MSC transplantation prevented the reduction of bone mass and strength in GIOP. Bone histomorphometric analyses of bone remodeling demonstrated the maintenance of bone formation and osteoblast survival after MSC therapy. Using green fluorescent protein (GFP)-labeled BMMSCs, we showed that donor BMMSCs (GFP) homed and inhabited recipient bone marrow for at least 4 weeks and prevented recipient bone marrow cell apoptosis, as shown by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling. Furthermore, donor BMMSCs (GFP) committed to Osterix (Osx) (+) osteoblast progenitors and induced recipient osteoblastogenesis, as exhibited by GFP-Osx double-labeling immunofluorescence analysis. No anticatabolic effects or systemic immunomodulatory effects of infused BMMSCs were detected. These findings demonstrated that allogeneic MSC therapy prevented GIOP by inhabiting and functioning in recipient bone marrow, which promoted osteoblastogenesis, which in turn maintained bone formation. Our findings provide important information regarding cell-based anabolic therapy for GIOP and uncover MSC behaviors following the homing event. SIGNIFICANCE: This study revealed the therapeutic potential of systemically infused, genetically unmodified allogeneic MSCs in glucocorticoid-induced osteoporosis. The donor MSCs inhabited recipient bone marrow and promoted osteoblastogenesis. The therapeutic effects were based on maintenance of bone formation. These results provide important information regarding cell-based anabolic therapy for glucocorticoid-induced osteoporosis and uncover previously unrecognized mesenchymal stem cell behaviors following a homing event. The current study also indicates that minimizing the time of cell culture confers an advantage for increasing transplanted mesenchymal stem cells to the targeted organ to promote therapeutic effects.

Turgeman, G., et al. (2001). "Engineered human mesenchymal stem cells: a novel platform for skeletal cell mediated gene therapy." J Gene Med **3**(3): 240-251.

BACKGROUND: Human mesenchymal stem cells (hMSCs) are pluripotent cells that can differentiate to various mesenchymal cell types. Recently, a method to isolate hMSCs from bone marrow and expand them in culture was described. Here we report on the use of hMSCs as a platform for gene therapy aimed at bone lesions. METHODS: Bone marrow derived hMSCs were expanded in culture and infected with recombinant adenoviral vector encoding the osteogenic factor, human BMP-2. The osteogenic potential of genetically engineered hMSCs was assessed in vitro and in vivo. RESULTS: Genetically engineered hMSCs displayed enhanced proliferation and osteogenic differentiation in culture. In vivo, transplanted genetically engineered hMSCs were able to engraft and form bone and cartilage in ectopic sites, and regenerate bone defects (non-union fractures) in mice radius bone. Importantly, the same results were obtained with hMSCs isolated from a patient suffering from osteoporosis. CONCLUSIONS: hMSCs represent a novel platform for skeletal gene therapy and the present results suggest that they can be genetically engineered to express desired therapeutic proteins inducing specific differentiation pathways. Moreover, hMSCs obtained from osteoporotic patients can restore their osteogenic activity following human BMP-2 gene transduction, an important finding in the future planning of gene therapy treatment for osteoporosis.

Wagner, K. U. and G. H. Smith (2005). "Pregnancy and stem cell behavior." J Mammary Gland Biol Neoplasia **10**(1): 25-36.

The identification of cancer-initiating epithelial subtypes (i.e. cancer stem cells) is important for gaining a more comprehensive understanding of the process of neoplastic transformation and tumorigenesis. Since reproductive history has a major impact on breast tumorigenesis, it is reasonable to assume that pregnancy and lactation have enduring effects on the cancer susceptibility of multipotent progenitors. Using the Cre-lox technology as a tool to genetically label pregnancy-hormone-responsive cells, we identified a mammary epithelial subtype that is abundant in parous females. These pregnancy-induced mammary epithelial cells (PI-MECs) originate from differentiating cells during the first pregnancy and lactation cycle. They do not undergo apoptosis during postlactational remodeling, and they persist throughout the remainder of a female's life. In this review, we discuss the biological relevance of PI-MECs in multiparous females and their important stem cell-like features, such as self renewal, as well as their ability to produce progeny with diverse cellular fates. Using appropriate animal models, we further demonstrate that PI-MECs are cellular targets for pregnancy-enhanced mammary tumorigenesis.

Wang, Y., et al. (2014). "Suicide gene-mediated sequencing ablation revealed the potential therapeutic mechanism of induced pluripotent stem cell-derived cardiovascular cell patch post-myocardial infarction." Antioxid Redox Signal **21**(16): 2177-2191.

AIMS: This study is designed to assess the protective cardiac effects after myocardial infarction (MI) of (i) cardiovascular progenitor cells (PC) differentiated directly into cardiomyocytes (CM) and endothelial cells (ECs) at the injury site, as separable from the effects of (ii) paracrine factors released from PC. RESULTS: In vivo: bi-cell patch containing induced pluripotent stem cell (iPSC)-derived CM and EC (BIC) was transplanted onto the infarcted heart. BIC were transduced with herpes simplex virus thymidine kinase "suicide" gene driven by cardiac NCX1 or endothelial vascular endothelium-cadherin promoter. IGF-1alpha and VEGF levels released from ischemic tissues were significantly enhanced in the BIC patch treatment group. Heart function, infarction size, and vessel density were significantly improved after BIC patch treatment. These effects were completely abolished in the group given ganciclovir (GCV) at week 1 as a suicide gene activator, and partially abolished in the group given GCV at week 3 as compared with the untreated cell patch group. INNOVATION: This study was designed to distinguish between cell-based and noncell-based therapeutic effects of PC lineages after MI. PCs derived from iPSC were genetically modified to express "suicide" gene. iPSC-derived CM and EC were then ablated in situ at week 1 and 3 by intraperitoneal administration of GCV. This enabled direct assessment of the effects of iPSC transplantation on myocardial function and tissue regeneration potential. CONCLUSIONS: Data support a mechanism in which iPSC-derived cardiovascular lineages contribute directly to improved cardiac performance and attenuated remodeling. Paracrine factors provide additional support to the restoration of heart function.

Wang, Z., et al. (2008). "Neuregulin-1 promotes cardiomyocyte differentiation of genetically engineered embryonic stem cell clones." BMB Rep **41**(10): 699-704.

Embryonic stem (ES) cell-derived cardiomyocytes (ESCMs) must be specifically purified in order to prevent teratoma formation, and this confusing issue has hampered their clinical application. We therefore investigated a technique to generate pure labeled ESCMs for possible use in cardiac repair. We generated transgenic ES cell lines expressing enhanced green fluorescent protein (EGFP) under the transcriptional control of the alpha-cardiac myosin heavy chain (alpha-MHC) promoter. Differentiated EGFP-positive ES cells displayed characteristics of CMs. Furthermore, neuregulin-1 (NRG-1) upregulated the expression of the cardiac-restricted transcription factors Nkx2.5 and GATA-4, as well as differentiated CM factors (alpha-MHC, beta-MHC). Immunohistochemistry demonstrated that NRG-1 increased expression of cardiac-specific troponin T in the beating foci of the embryoid bodies. This work revealed a potential method for specifically labeling and enriching ESCMs by combining genetically-engineered ES cell clones and exogenous growth factor treatment.

Wei, Y., et al. (2014). "Ncor2 is required for hematopoietic stem cell emergence by inhibiting Fos signaling in zebrafish." Blood **124**(10): 1578-1585.

Nuclear receptor corepressors (Ncors) are important for developmental and homeostatic processes in vertebrates, which exert transcriptional repression by coordinating with histone deacetylases. However, little is known about their roles in definitive hematopoiesis. In this study, we show that in zebrafish, ncor2 is required for hematopoietic stem cell (HSC) development by repressing fos-vegfd signaling. ncor2 is specifically expressed in the aorta-gonad-mesonephros (AGM) region in zebrafish embryos. ncor2 deficiency reduced the population of HSCs in both the AGM region and T cells in the thymus. Mechanistically, ncor2 knockdown upregulated fos transcription by modulating the acetylation level in the fos promoter region, which then enhanced Vegfd

signaling. Consequently, the augmented Vegfd signaling induced Notch signaling to promote the arterial endothelial fate, therefore, possibly repressing the hemogenic endothelial specification, which is a prerequisite for HSC emergence. Thus, our findings identify a novel regulatory mechanism for Ncor2 through Fos-Vegfd-Notch signaling cascade during HSC development in zebrafish embryos.

Xia, Z., et al. (2004). "Macrophagic response to human mesenchymal stem cell and poly (epsilon-caprolactone) implantation in nonobese diabetic/severe combined immunodeficient mice." J Biomed Mater Res A **71**(3): 538-548.

Nonobese diabetic, severe combined immunodeficient (NOD/SCID) mice are extensively used to assess in vivo potentials for human cellular differentiation, development, and neophysiology. They are not only deficient in T and B cells, but also exhibit macrophage dysfunction and an absence of circulating complement. However, the survival of engrafted human mesenchymal stem cells (hMSCs) is limited and minimal mature bone tissue develops from implanted hMSCs in this model. The aim of the present study was to investigate the response to such implants in NOD/SCID mice. To this end, hMSCs genetically marked with enhanced green fluorescent protein, a biodegradable polymer, poly (epsilon-caprolactone) (PCL), and a bioconstruct incorporating the enhanced green fluorescent protein-labeled hMSCs with PCL after culture together for 3 weeks in vitro, were implanted into NOD/SCID mice and followed for up to 10 weeks. Monocytes/macrophages appeared to be the major invading cell type in all the implants and remained in the materials regardless of whether or not hMSCs were present over the time periods studied. When the hMSCs were implanted without the PCL scaffold, host macrophage invasion was also observed with the majority of hMSCs being eliminated within 2 weeks. Multinuclear giant cells or foreign body giant cells were seen in the cases of PCL implantation. These cells slowly infiltrated into the central core of the materials over a 10-week period of implantation with neutrophils and mast cells also being observed. In conclusion, in NOD/SCID mice, monocytes/macrophages still effectively respond to the implantation of xenografts and biopolymers with functional migration, phagocytosis, adhesion, foreign body recognition and formation of multinuclear giant cells, or foreign body giant cells. Thus, these animals still retain a level of innate immune responsiveness to these implantations and in addition may provoke a physiological environment that is unsuitable for extensive intramembranous ossification by engrafted hMSCs.

Xiong, Q., et al. (2011). "A fibrin patch-based enhanced delivery of human embryonic stem cell-derived vascular cell transplantation in a porcine model of postinfarction left ventricular remodeling." Stem Cells **29**(2): 367-375.

It is unknown how to use human embryonic stem cell (hESC) to effectively treat hearts with postinfarction left ventricular (LV) remodeling. Using a porcine model of postinfarction LV remodeling, this study examined the functional improvement of enhanced delivery of combined transplantation of hESC-derived endothelial cells (ECs) and hESC-derived smooth muscle cells (SMCs) with a fibrin three-dimensional (3D) porous scaffold biomatrix. To facilitate tracking the transplanted cells, the hESCs were genetically modified to stably express green fluorescent protein and luciferase (GFP/Luc). Myocardial infarction (MI) was created by ligating the first diagonal coronary artery for 60 minutes followed by reperfusion. Two million each of GFP/Luc hESC-derived ECs and SMCs were seeded in the 3D porous biomatrix patch and applied to the region of ischemia/reperfusion for cell group (MI+P+C, n = 6), whereas biomatrix without cell (MI+P, n = 5), or saline only (MI, n = 5) were applied to control group hearts with same coronary artery ligation. Functional outcome (1 and 4 weeks follow-up) of stem cell transplantation was assessed by cardiac magnetic resonance imaging. The transplantation of hESC-derived vascular cells resulted in significant LV functional improvement. Significant engraftment of hESC-derived cells was confirmed by both in vivo and ex vivo bioluminescent imaging. The mechanism underlying the functional beneficial effects of cardiac progenitor transplantation is attributed to the increased neovascularization. These findings demonstrate a promising therapeutic potential of using these hESC-derived vascular cell types and the mode of patch delivery.

Yan, B., et al. (2013). "Regulation of PTEN/Akt pathway enhances cardiomyogenesis and attenuates adverse left ventricular remodeling following thymosin beta4 Overexpressing embryonic stem cell transplantation in the infarcted heart." PLoS One **8**(9): e75580.

Thymosin beta4 (Tbeta4), a small G-actin sequestering peptide, mediates cell proliferation, migration, and angiogenesis. Whether embryonic stem (ES) cells, overexpressing Tbeta4, readily differentiate into cardiac myocytes in vitro and in vivo and enhance cardioprotection following transplantation post myocardial infarction (MI) remains unknown. Accordingly, we established stable mouse ES cell lines, RFP-ESCs and Tbeta4-ESCs, expressing RFP and an RFP-Tbeta4 fusion protein, respectively. In vitro, the

number of spontaneously beating embryoid bodies (EBs) was significantly increased in Tbeta4-ESCs at day 9, 12 and 15, compared with RFP-ESCs. Enhanced expression of cardiac transcriptional factors GATA-4, Mef2c and Txb6 in Tbeta4-EBs, as confirmed with real time-PCR analysis, was accompanied by the increased number of EB areas stained positive for sarcomeric alpha-actin in Tbeta4-EBs, compared with the RFP control, suggesting a significant increase in functional cardiac myocytes. Furthermore, we transplanted Tbeta4-ESCs into the infarcted mouse heart and performed morphological and functional analysis 2 weeks after MI. There was a significant increase in newly formed cardiac myocytes associated with the Notch pathway, a decrease in apoptotic nuclei mediated by an increase in Akt and a decrease in levels of PTEN. Cardiac fibrosis was significantly reduced, and left ventricular function was significantly augmented in the Tbeta4-ESC transplanted group, compared with controls. It is concluded that genetically modified Tbeta4-ESCs, potentiates their ability to turn into cardiac myocytes in vitro as well as in vivo. Moreover, we also demonstrate that there was a significant decrease in both cardiac apoptosis and fibrosis, thus improving cardiac function in the infarcted heart.

Yanagihara, K., et al. (2018). "Treatment of Bone Defects by Transplantation of Genetically Modified Mesenchymal Stem Cell Spheroids." Mol Ther Methods Clin Dev **9**: 358-366.

Cell transplantation is promising for regenerative medicine. A combination of a three-dimensional spheroid culture system with gene transfection was developed to enhance the therapeutic effects of mesenchymal stem cell (MSC) transplantation. The spheroid cell culture system is based on micropatterned substrates composed of a regular array of 100-mum-diameter cell-adhesion areas coated with a temperature-responsive polymer, poly (N-isopropylacrylamide-co-methacrylic acid), which allows for spheroid detachment by simply cooling the plates. In this study, MSC spheroids were transfected with plasmid DNA encoding runt-related transcription factor 2 (Runx2) and tested for their ability to enhance bone regeneration. In vitro analyses revealed that osteogenic differentiation of the MSCs was enhanced by forming spheroids and was further promoted by Runx2 expression. The enhanced osteogenic differentiation was maintained under pathological conditions, such as hypoxia and inflammation. Transplanting Runx2-transfected MSC spheroids into bone defects on rat femurs induced significantly faster bone regeneration compared with nontransfected MSC spheroids or genetically modified MSCs from conventional monolayer culture. MSC migration into the bone defect area was enhanced by upregulation of cell-migration-related genes. In conclusion, genetically modified MSC spheroids are effective for enhancing bone regeneration, providing a promising option for cell transplantation therapy in the fields of regenerative medicine.

Yang, Q., et al. (2016). "RNA polymerase II pausing modulates hematopoietic stem cell emergence in zebrafish." Blood **128**(13): 1701-1710.

The promoter-proximal pausing of RNA polymerase II (Pol II) plays a critical role in regulating metazoan gene transcription. Despite the prevalence of Pol II pausing across the metazoan genomes, little is known about the in vivo effect of Pol II pausing on vertebrate development. We use the emergence of hematopoietic stem cells (HSCs) in zebrafish embryos as a model to investigate the role of Pol II pausing in vertebrate organogenesis. Disrupting Pol II pausing machinery causes a severe reduction of HSC specification, a defect that can be effectively rescued by inhibiting Pol II elongation. In pausing-deficient embryos, the transforming growth factor beta (TGFbeta) signaling is elevated due to enhanced transcription elongation of key pathway genes, leading to HSC inhibition; in contrast, the interferon-gamma (IFN-gamma) signaling and its downstream effector Jak2/Stat3, which are required for HSC formation, are markedly attenuated owing to reduced chromatin accessibility on IFN-gamma receptor genes. These findings reveal a novel transcription mechanism instructing HSC fate by pausing-mediated differential regulation of key signaling pathways.

Yao, J., et al. (2012). "Tissue inhibitor of matrix metalloproteinase-3 or vascular endothelial growth factor transfection of aged human mesenchymal stem cells enhances cell therapy after myocardial infarction." Rejuvenation Res **15**(5): 495-506.

Mesenchymal stem cell (MSC) transplantation has been proposed as a potential therapeutic approach for ischemic heart disease, but the regenerative capacity of these cells decreases with age. In this study, we genetically engineered old human MSCs (O-hMSCs) with tissue inhibitor of matrix metalloproteinase-3 (TIMP3) and vascular endothelial growth factor (VEGF) and evaluated the effects on the efficacy of cell-based gene therapy in a rat myocardial infarction (MI) model. Cultured O-hMSCs were transfected with TIMP3 (O-TIMP3) or VEGF (O-VEGF) and compared with young hMSCs (Y-hMSCs) and non-transfected O-hMSCs for growth, clonogenic capacity, and differentiation potential. In vivo, rats were subjected to left coronary artery ligation with subsequent injection of Y-hMSCs, O-hMSCs, O-TIMP3, O-VEGF, or medium. Echocardiography was

performed prior to and at 1, 2, and 4 weeks after MI. Myocardial levels of matrix metalloproteinase-2 (MMP2), MMP9, TIMP3, and VEGF were assessed at 1 week. Hemodynamics, morphology, and histology were measured at 4 weeks. In vitro, genetically modified O-hMSCs showed no changes in growth, colony formation, or multi-differentiation capacity. In vivo, transplantation with O-TIMP3, O-VEGF, or Y-hMSCs increased capillary density, preserved cardiac function, and reduced infarct size compared to O-hMSCs and medium control. O-TIMP3 and O-VEGF transplantation enhanced TIMP3 and VEGF expression, respectively, in the treated animals. O-hMSCs genetically modified with TIMP3 or VEGF can increase angiogenesis, prevent adverse matrix remodeling, and restore cardiac function to a degree similar to Y-hMSCs. This gene-modified cell therapy strategy may be a promising clinical treatment to rejuvenate stem cells in elderly patients.

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.

Zan, X., et al. (2012). "Polyvalent display of RGD motifs on turnip yellow mosaic virus for enhanced stem cell adhesion and spreading." Acta Biomater **8**(8): 2978-2985.

Turnip yellow mosaic virus (TYMV) is a stable 28 nm icosahedral plant virus that can be isolated in gram quantities. In order to study the polyvalent effect of Arg-Gly-Asp (RGD) clustering on the response of bone marrow stem cells (BMSCs), an RGD motif was genetically displayed on the coat protein of the TYMV capsid. Composite films composed of either wild-type TYMV or TYMV-RGD44, in combination with poly (allylamine hydrochloride) (PAH), were fabricated by a layer-by-layer adsorption of virus and PAH. The deposition process was studied by quartz crystal microbalance, UV-visible spectroscopy and atomic force microscopy. BMSC adhesion assays showed enhanced cell adhesion and spreading on TYMV-RGD44 coated substrates compared to native TYMV. These results demonstrate the potential of TYMV as a viable scaffold for bioactive peptide display and cell culturing studies.

Zeng, W., et al. (2013). "Viral transduction of male germline stem cells results in transgene transmission after germ cell transplantation in pigs." Biol Reprod **88**(1): 27.

Genetic modification of germline stem cells (GSCs) is an alternative approach to generate large transgenic animals where transgenic GSCs are transplanted into a recipient testis to generate donor-derived transgenic sperm. The objective of the present study was to explore the application of viral vectors in delivering an enhanced green fluorescent protein (EGFP) transgene into GSCs for production of transgenic gametes through germ cell transplantation. Both adeno-associated virus (AAV)- and lentivirus (LV)-based vectors were effective in transducing pig GSCs, resulting in the production of transgenic sperm in recipient boars. Twenty-one boars treated with busulfan to deplete endogenous GSCs and nine nontreated boars received germ cell transplantation at 12 wk of age. Semen was collected from recipient boars from 5 to 7 mo posttransplantation when boars became sexually mature, and semen collection continued for as long as 5 yr for some boars. The percentage of ejaculates that were positive for the EGFP transgene ranged from 0% to 54.8% for recipients of AAV vector-transduced germ cells (n = 17) and from 0% to 25% for recipients of LV vector-transduced germ cells (n = 5). When semen from two AAV recipients was used for in vitro fertilization (IVF), 9.09% and 64.3% of embryos were transgenic. Semen collected from two LV-vector recipients produced 7.7% and 26.3% transgenic IVF embryos. Here, we not only demonstrated AAV-mediated GSC

transduction in another large animal model (pigs) but also showed, to our knowledge for the first time, that LV-mediated GSC transduction resulted in transgene transmission in pigs.

Zhang, P., et al. (2005). "[Plasmid transfection of rat bone marrow mesenchymal stem cells by cationic lipid for gene-modified cell transplantation therapy]." Zhongguo Yi Xue Ke Xue Yuan Xue Bao **27**(4): 504-508.

OBJECTIVE: To investigate the feasibility of gene transfection of bone marrow mesenchymal stem cell (BMSC) by cationic lipid. METHODS: The relative optimal transfection condition was determined by scale transfection experiment in vitro and the transient transfection efficiency of enhanced green fluorescence protein (EGFP) gene for rat BMSC was determined with Lipofectamine2000 (LP2000). The relationship between cell cycle status and the expression of the gene was analyzed. The intensity and the ratio of EGFP gene expression versus time was determined by flow cytometry. In the in vivo study, the transgenic rat BMSC was injected into the myocardium of inbred rats, and the in vivo transcription of EGFP gene and the EGFP-expressing BMSC were traced in the myocardium after transplantation using reverse transcription-polymerase chain reaction and fluorescent microscopy, respectively. RESULTS: EGFP gene transfection efficiency in BMSC was different under different transfection condition (DNA concentration and DNA: LP2000). Cationic lipid-mediated transfection demonstrated marked toxicity to BMSC. Cell cycle status restricted the expression efficiency of the gene introduced by cationic lipid. The EGFP expressing-BMSC and in vivo transcription of the EGFP gene could be detected in rat myocardium post implantation. CONCLUSION: Cationic lipid is an effective carrier for gene-modified cell transplantation therapy.

Zhang, Z., et al. (2016). "Mammary-Stem-Cell-Based Somatic Mouse Models Reveal Breast Cancer Drivers Causing Cell Fate Dysregulation." Cell Rep **16**(12): 3146-3156.

Cancer genomics has provided an unprecedented opportunity for understanding genetic causes of human cancer. However, distinguishing which mutations are functionally relevant to cancer pathogenesis remains a major challenge. We describe here a mammary stem cell (MaSC) organoid-based approach for rapid generation of somatic genetically engineered mouse models (GEMMs). By using RNAi and CRISPR-mediated genome engineering in MaSC-GEMMs, we have discovered that inactivation of Ptpn22 or Mll3, two genes mutated in human breast cancer, greatly accelerated PI3K-driven mammary tumorigenesis. Using these tumor models, we have also identified genetic alterations promoting tumor metastasis and causing resistance to PI3K-targeted therapy. Both Ptpn22 and Mll3 inactivation resulted in disruption of mammary gland differentiation and an increase in stem cell activity. Mechanistically, Mll3 deletion enhanced stem cell activity through activation of the HIF pathway. Thus, our study has established a robust in vivo platform for functional cancer genomics and has discovered functional breast cancer mutations.

Zhao, Y., et al. (2014). "Pim-1 kinase cooperates with serum signals supporting mesenchymal stem cell propagation." Cells Tissues Organs **199**(2-3): 140-149.

Mesenchymal stem cells (MSCs) are currently undergoing testing in several clinical settings. The propagation of MSCs from multiple species in culture is an important step in furthering our understanding of these progenitor cells. Pim-1, a proto-oncogenic serine/threonine kinase, regulates cell proliferation, survival, and differentiation. Although it has been shown that Pim-1 participates in signal transduction mediating mitogenic action in MSCs, its roles in the modulation of MSC propagation remain to be defined. Understanding of ovine MSCs transduced with Pim-1 may provide improved ovine models for cellular therapy development. Using genetically modified ovine MSCs that constitutively overexpressed Pim-1 (MSC expressing PIM-1 and ZsGreen protein), we evaluated the impact of elevated Pim-1 activity on the proliferation, survival, and differentiation of MSCs in culture. Our results showed that Pim-1 enhanced the intrinsic molecular signals of growth and survival implicated in the mediation of serum signaling under normal culture conditions (10% serum). We found that Pim-1 promoted MSC proliferation irrespectively of the serum concentration, but with a decreased proliferation rate compared to increased serum concentrations, relative to the control vector-transduced MSC expressing ZsGreen protein. Further, Pim-1 prevented MSC apoptosis induced by hypoxia or serum deprivation as evidenced by enhanced mitochondria integrity and reduced annexin V binding. Interestingly, the phenotype and multilineage differentiation potential of the cells were not influenced by Pim-1. Taken together, these observations demonstrate that Pim-1 kinase cooperates with exogenous serum signals supporting MSC propagation in the ovine model.

Zhu, K., et al. (2014). "Nanovector-based prolyl hydroxylase domain 2 silencing system enhances the efficiency of stem cell transplantation for infarcted myocardium repair." Int J Nanomedicine **9**: 5203-5215.

Mesenchymal stem cell (MSC) transplantation has attracted much attention in myocardial infarction

therapy. One of the limitations is the poor survival of grafted cells in the ischemic microenvironment. Small interfering RNA-mediated prolyl hydroxylase domain protein 2 (PHD2) silencing in MSCs holds tremendous potential to enhance their survival and paracrine effect after transplantation. However, an efficient and biocompatible PHD2 silencing system for clinical application is lacking. Herein, we developed a novel PHD2 silencing system based on arginine-terminated generation 4 poly (amidoamine) (Arg-G4) nanoparticles. The system exhibited effective and biocompatible small interfering RNA delivery and PHD2 silencing in MSCs in vitro. After genetically modified MSC transplantation in myocardial infarction models, MSC survival and paracrine function of IGF-1 were enhanced significantly in vivo. As a result, we observed decreased cardiomyocyte apoptosis, scar size, and interstitial fibrosis, and increased angiogenesis in the diseased myocardium, which ultimately attenuated ventricular remodeling and improved heart function. This work demonstrated that an Arg-G4 nanovector-based PHD2 silencing system could enhance the efficiency of MSC transplantation for infarcted myocardium repair.

Zhu, K., et al. (2016). "Nanoparticles-Assisted Stem Cell Therapy for Ischemic Heart Disease." Stem Cells Int **2016**: 1384658.

Stem cell therapy has attracted increasing attention as a promising treatment strategy for cardiac repair in ischemic heart disease. Nanoparticles (NPs), with their superior physical and chemical properties, have been widely utilized to assist stem cell therapy. With the help of NPs, stem cells can be genetically engineered for enhanced paracrine profile. To further understand the fate and behaviors of stem cells in ischemic myocardium, imaging NPs can label stem cells and be tracked in vivo under multiple modalities. Besides that, NPs can also be used to enhance stem cell retention in myocardium. These facts have raised efforts on the development of more intelligent and multifunctional NPs for cellular application. Herein, an overview of the applications of NPs-assisted stem cell therapy is given. Key issues and future prospects are also critically addressed.

Ziegler, A. N., et al. (2014). "Insulin-like growth factor-II (IGF-II) and IGF-II analogs with enhanced insulin receptor-a binding affinity promote neural stem cell expansion." J Biol Chem **289**(8): 4626-4633.

The objective of this study was to employ genetically engineered IGF-II analogs to establish which receptor (s) mediate the stemness promoting actions of IGF-II on mouse subventricular zone neural precursors. Neural precursors from the subventricular zone were propagated in vitro in culture medium supplemented with IGF-II analogs. Cell growth and identity were analyzed using sphere generation and further analyzed by flow cytometry. F19A, an analog of IGF-II that does not bind the IGF-2R, stimulated an increase in the proportion of neural stem cells (NSCs) while decreasing the proportion of the later stage progenitors at a lower concentration than IGF-II. V43M, which binds to the IGF-2R with high affinity but which has low binding affinity to the IGF-1R and to the A isoform of the insulin receptor (IR-A) failed to promote NSC growth. The positive effects of F19A on NSC growth were unaltered by the addition of a functional blocking antibody to the IGF-1R. Altogether, these data lead to the conclusion that IGF-II promotes stemness of NSCs via the IR-A and not through activation of either the IGF-1R or the IGF-2R.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

References

1. Abraham, N. G., et al. (2008). "Bone marrow stem cell transplant into intra-bone cavity prevents type 2 diabetes: role of heme oxygenase-adiponectin." J Autoimmun 30(3): 128-135.
2. Ambrosone, A., et al. (2012). "Hymyc1 downregulation promotes stem cell proliferation in Hydra vulgaris." PLoS One 7(1): e30660.
3. Bahrambeigi, V., et al. (2015). "Genetically modified murine adipose-derived mesenchymal stem cells producing interleukin-2 favor B16F10 melanoma cell proliferation." Immunol Invest 44(3): 216-236.
4. Baidu. http://www.baidu.com. 2018.
5. Balyasnikova, I. V., et al. (2010). "Genetic modification of mesenchymal stem cells to express a single-chain antibody against EGFRvIII on the cell surface." J Tissue Eng Regen Med 4(4): 247-258.
6. Bobis-Wozowicz, S., et al. (2015). "Human Induced Pluripotent Stem Cell-Derived Microvesicles Transmit RNAs and Proteins to Recipient Mature Heart Cells Modulating Cell Fate and Behavior." Stem Cells 33(9): 2748-2761.
7. Brunelli, S., et al. (2007). "Nitric oxide release combined with nonsteroidal antiinflammatory activity prevents muscular dystrophy pathology and enhances stem cell therapy." Proc Natl Acad Sci U S A 104(1): 264-269.
8. Burke, D., et al. (2013). "The role of oxygen as a regulator of stem cell fate during fracture repair in TSP2-null mice." J Orthop Res 31(10): 1585-1596.

9. Castellanos, D. A., et al. (2002). "TrkC overexpression enhances survival and migration of neural stem cell transplants in the rat spinal cord." Cell Transplant 11(3): 297-307.
10. Chao, J., et al. (2014). "Kallikrein-kinin in stem cell therapy." World J Stem Cells 6(4): 448-457.
11. Chen, Y. B., et al. (2015). "Mesenchymal stem cell-based HSP70 promoter-driven VEGFA induction by resveratrol promotes angiogenesis in a mouse model." Cell Stress Chaperones 20(4): 643-652.
12. Chuang, C. K., et al. (2007). "Baculovirus as a new gene delivery vector for stem cell engineering and bone tissue engineering." Gene Ther 14(19): 1417-1424.
13. Clarke, M., et al. (2017). "Transcriptional regulation of SPROUTY2 by MYB influences myeloid cell proliferation and stem cell properties by enhancing responsiveness to IL-3." Leukemia 31(4): 957-966.
14. Cunha, C., et al. (2011). "Genetically-determined hyperfunction of the S100B/RAGE axis is a risk factor for aspergillosis in stem cell transplant recipients." PLoS One 6(11): e27962.
15. Daadi, M. M., et al. (2009). "Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain." Mol Ther 17(7): 1282-1291.
16. De Vocht, N., et al. (2012). "Multimodal imaging of stem cell implantation in the central nervous system of mice." J Vis Exp (64): e3906.
17. Deveza, L., et al. (2013). "Paracrine release from nonviral engineered adipose-derived stem cells promotes endothelial cell survival and migration in vitro." Stem Cells Dev 22(3): 483-491.
18. Doering, C. B., et al. (2018). "Preclinical Development of a Hematopoietic Stem and Progenitor Cell Bioengineered Factor VIII Lentiviral Vector Gene Therapy for Hemophilia A." Hum Gene Ther 29(10): 1183-1201.
19. Dufourcq, P., et al. (2008). "Secreted frizzled-related protein-1 enhances mesenchymal stem cell function in angiogenesis and contributes to neovessel maturation." Stem Cells 26(11): 2991-3001.
20. Duncan, G., et al. (2017). "Drug-Mediated Shortening of Action Potentials in LQTS2 Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes." Stem Cells Dev 26(23): 1695-1705.
21. Duong, T. T., et al. (2018). "Use of induced pluripotent stem cell models to probe the pathogenesis of Choroideremia and to develop a potential treatment." Stem Cell Res 27: 140-150.
22. Erben, U., et al. (1999). "Differential effects of a stem cell factor-immunoglobulin fusion protein on malignant and normal hematopoietic cells." Cancer Res 59(12): 2924-2930.
23. Fu, K. Y., et al. (2011). "Sciatic nerve regeneration by microporous nerve conduits seeded with glial cell line-derived neurotrophic factor or brain-derived neurotrophic factor gene transfected neural stem cells." Artif Organs 35(4): 363-372.
24. Gerli, M. F., et al. (2014). "Transplantation of induced pluripotent stem cell-derived mesoangioblast-like myogenic progenitors in mouse models of muscle regeneration." J Vis Exp (83): e50532.
25. Goncalves, M. A., et al. (2006). "Human mesenchymal stem cells ectopically expressing full-length dystrophin can complement Duchenne muscular dystrophy myotubes by cell fusion." Hum Mol Genet 15(2): 213-221.
26. Google. http://www.google.com. 2018.
27. Hacke, K., et al. (2009). "Suppression of HLA expression by lentivirus-mediated gene transfer of siRNA cassettes and in vivo chemoselection to enhance hematopoietic stem cell transplantation." Immunol Res 44(1-3): 112-126.
28. Halum, S. L., et al. (2008). "Optimization of autologous muscle stem cell survival in the denervated hemilarynx." Laryngoscope 118(7): 1308-1312.
29. Harrison, D. E., et al. (1994). "Splenic primitive hematopoietic stem cell (PHSC) activity is enhanced by steel factor because of PHSC proliferation." Blood 83(11): 3146-3151.
30. Harrison, F., et al. (2013). "Hematopoietic stem cell gene therapy for the multisystemic lysosomal storage disorder cystinosis." Mol Ther 21(2): 433-444.
31. He, J. Q., et al. (2007). "Human embryonic stem cell-derived cardiomyocytes: drug discovery and safety pharmacology." Expert Opin Drug Discov 2(5): 739-753.
32. Hedlund, E., et al. (2007). "Selection of embryonic stem cell-derived enhanced green fluorescent protein-positive dopamine neurons using the tyrosine hydroxylase promoter is confounded by reporter gene expression in immature cell populations." Stem Cells 25(5): 1126-1135.
33. Henig, I., et al. (2017). "[Hematopoietic Stem Cell Transplantation in the Perspective of Time and up to the 2000 Transplant at the Rambam Health Care Campus]." Harefuah 156(9): 589-594.
34. Hsiao, F. S., et al. (2011). "Toward an ideal animal model to trace donor cell fates after stem

cell therapy: production of stably labeled multipotent mesenchymal stem cells from bone marrow of transgenic pigs harboring enhanced green fluorescence protein gene." J Anim Sci 89(11): 3460-3472.
35. Jungling, K., et al. (2006). "N-cadherin transsynaptically regulates short-term plasticity at glutamatergic synapses in embryonic stem cell-derived neurons." J Neurosci 26(26): 6968-6978.
36. Kara, R. J., et al. (2012). "A mouse model for fetal maternal stem cell transfer during ischemic cardiac injury." Clin Transl Sci 5(4): 321-328.
37. Kensah, G., et al. (2013). "Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro." Eur Heart J 34(15): 1134-1146.
38. Kermani, A. J., et al. (2008). "Characterization and genetic manipulation of human umbilical cord vein mesenchymal stem cells: potential application in cell-based gene therapy." Rejuvenation Res 11(2): 379-386.
39. Kilty, I. C., et al. (1999). "Isolation of a potential neural stem cell line from the internal capsule of an adult transgenic rat brain." J Neurochem 73(5): 1859-1870.
40. Kim, E., et al. (2011). "Magnetically enhanced adeno-associated viral vector delivery for human neural stem cell infection." Biomaterials 32(33): 8654-8662.
41. Kimelman, N., et al. (2007). "Review: gene- and stem cell-based therapeutics for bone regeneration and repair." Tissue Eng 13(6): 1135-1150.
42. Klingener, M., et al. (2014). "N-cadherin promotes recruitment and migration of neural progenitor cells from the SVZ neural stem cell niche into demyelinated lesions." J Neurosci 34(29): 9590-9606.
43. Knorr, D. A. and D. S. Kaufman (2010). "Pluripotent stem cell-derived natural killer cells for cancer therapy." Transl Res 156(3): 147-154.
44. Koide, Y., et al. (2007). "Two distinct stem cell lineages in murine bone marrow." Stem Cells 25(5): 1213-1221.
45. Kugler, J., et al. (2015). "A Bmp Reporter Transgene Mouse Embryonic Stem Cell Model as a Tool to Identify and Characterize Chemical Teratogens." Toxicol Sci 146(2): 374-385.
46. Lan, Y., et al. (2012). "[Establishment of an albumin and cytokeratin 19 genetically-modified embryonic stem cell line and evaluation of its hepatoblast differentiation capacities]." Zhonghua Gan Zang Bing Za Zhi 20(1): 50-54.
47. Lee, M. S., et al. (2017). "Enhanced Cell Growth of Adipocyte-Derived Mesenchymal Stem Cells Using Chemically-Defined Serum-Free Media." Int J Mol Sci 18(8).
48. Lepperhof, V., et al. (2014). "Bioluminescent imaging of genetically selected induced pluripotent stem cell-derived cardiomyocytes after transplantation into infarcted heart of syngeneic recipients." PLoS One 9(9): e107363.
49. Li, Z., et al. (2013). "Bone marrow mesenchymal stem cells are an attractive donor cell type for production of cloned pigs as well as genetically modified cloned pigs by somatic cell nuclear transfer." Cell Reprogram 15(5): 459-470.
50. Lin, S., et al. (2017). "Synergistic effects on mesenchymal stem cell-based cartilage regeneration by chondrogenic preconditioning and mechanical stimulation." Stem Cell Res Ther 8(1): 221.
51. Ma H, Chen G. Stem cell. The Journal of American Science 2005;1(2):90-92.
52. Ma H, Cherng S. Eternal Life and Stem Cell. Nature and Science. 2007;5(1):81-96.
53. Ma H, Cherng S. Nature of Life. Life Science Journal 2005;2(1):7-15.
54. Ma H, Yang Y. Turritopsis nutricula. Nature and Science 2010;8(2):15-20. http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf.
55. Ma H. The Nature of Time and Space. Nature and science 2003;1(1):1-11. Nature and science 2007;5(1):81-96.
56. Marei, H. E., et al. (2013). "Over-expression of hNGF in adult human olfactory bulb neural stem cells promotes cell growth and oligodendrocytic differentiation." PLoS One 8(12): e82206.
57. Marsland Press. http://www.sciencepub.net. 2018.
58. Men, H. and E. C. Bryda (2013). "Derivation of a germline competent transgenic Fischer 344 embryonic stem cell line." PLoS One 8(2): e56518.
59. Men, H., et al. (2012). "Germline transmission of a novel rat embryonic stem cell line derived from transgenic rats." Stem Cells Dev 21(14): 2606-2612.
60. Mohseni Nodehi, S., et al. (2012). "Enhanced ADCC activity of affinity maturated and Fc-engineered mini-antibodies directed against the AML stem cell antigen CD96." PLoS One 7(8): e42426.
61. Moreno, R., et al. (2012). "Fetal liver-derived mesenchymal stem cell engraftment after allogeneic in utero transplantation into rabbits." Stem Cells Dev 21(2): 284-295.
62. Myers, T. J., et al. (2010). "Mesenchymal stem cells at the intersection of cell and gene therapy." Expert Opin Biol Ther 10(12): 1663-1679.

63. Nakamura, Y., et al. (2013). "Enhanced wound healing by topical administration of mesenchymal stem cells transfected with stromal cell-derived factor-1." Biomaterials 34(37): 9393-9400.
64. National Center for Biotechnology Information, U.S. National Library of Medicine. http://www.ncbi.nlm.nih.gov/pubmed. 2018.
65. Neri, M., et al. (2011). "Neural stem cell gene therapy ameliorates pathology and function in a mouse model of globoid cell leukodystrophy." Stem Cells 29(10): 1559-1571.
66. Nicholas, C. R., et al. (2007). "A method for single-cell sorting and expansion of genetically modified human embryonic stem cells." Stem Cells Dev 16(1): 109-117.
67. Nimchuk, Z. L., et al. (2015). "Plant stem cell maintenance by transcriptional cross-regulation of related receptor kinases." Development 142(6): 1043-1049.
68. Novosadova, E. V., et al. (2005). "Different effects of enhanced and reduced expression of pub gene on the formation of embryoid bodies by cultured embryonic mouse stem cell." Bull Exp Biol Med 140(1): 153-158.
69. Olivier, S., et al. (2010). "EB66 cell line, a duck embryonic stem cell-derived substrate for the industrial production of therapeutic monoclonal antibodies with enhanced ADCC activity." MAbs 2(4): 405-415.
70. Papayannopoulou, T., et al. (2003). "The role of G-protein signaling in hematopoietic stem/progenitor cell mobilization." Blood 101(12): 4739-4747.
71. Paul, A., et al. (2012). "PAMAM dendrimer-baculovirus nanocomplex for microencapsulated adipose stem cell-gene therapy: in vitro and in vivo functional assessment." Mol Pharm 9(9): 2479-2488.
72. Pickard, M. R., et al. (2015). "Using magnetic nanoparticles for gene transfer to neural stem cells: stem cell propagation method influences outcomes." J Funct Biomater 6(2): 259-276.
73. Povey, J., et al. (1998). "Enhanced retroviral transduction of 5-fluorouracil-resistant human bone marrow (stem) cells using a genetically modified packaging cell line." Blood 92(11): 4080-4089.
74. Pruitt, S. C., et al. (2007). "Reduced Mcm2 expression results in severe stem/progenitor cell deficiency and cancer." Stem Cells 25(12): 3121-3132.
75. Psatha, N., et al. (2014). "Superior long-term repopulating capacity of G-CSF+plerixafor-mobilized blood: implications for stem cell gene therapy by studies in the Hbb (th-3) mouse model." Hum Gene Ther Methods 25(6): 317-327.
76. Qiu, X. C., et al. (2015). "Donor mesenchymal stem cell-derived neural-like cells transdifferentiate into myelin-forming cells and promote axon regeneration in rat spinal cord transection." Stem Cell Res Ther 6: 105.
77. Quarta, M., et al. (2017). "Bioengineered constructs combined with exercise enhance stem cell-mediated treatment of volumetric muscle loss." Nat Commun 8: 15613.
78. Rodriguez, R. T., et al. (2007). "Manipulation of OCT4 levels in human embryonic stem cells results in induction of differential cell types." Exp Biol Med (Maywood) 232(10): 1368-1380.
79. Roudkenar, M. H., et al. (2018). "Lipocalin 2 enhances mesenchymal stem cell-based cell therapy in acute kidney injury rat model." Cytotechnology 70(1): 103-117.
80. Sabatino, M., et al. (2016). "Generation of clinical-grade CD19-specific CAR-modified CD8+ memory stem cells for the treatment of human B-cell malignancies." Blood 128(4): 519-528.
81. Shujia, J., et al. (2008). "Stable therapeutic effects of mesenchymal stem cell-based multiple gene delivery for cardiac repair." Cardiovasc Res 77(3): 525-533.
82. Sifuentes, C. J., et al. (2016). "Rapid, Dynamic Activation of Muller Glial Stem Cell Responses in Zebrafish." Invest Ophthalmol Vis Sci 57(13): 5148-5160.
83. Singh, G., et al. (2012). "Successful derivation of EGFP-transgenic embryonic stem cell line from a genetically non-permissive FVB/N mouse." Am J Stem Cells 1(2): 163-173.
84. Sivasankaran, A., et al. (2018). "Machine Learning Approach to Predicting Stem Cell Donor Availability." Biol Blood Marrow Transplant.
85. Smith, N. J., et al. (2010). "Haematopoietic stem cell transplantation does not retard disease progression in the psycho-cognitive variant of late-onset metachromatic leukodystrophy." J Inherit Metab Dis 33 Suppl 3: S471-475.
86. Song, M., et al. (2012). "Induced pluripotent stem cell research: a revolutionary approach to face the challenges in drug screening." Arch Pharm Res 35(2): 245-260.
87. Sui, B., et al. (2016). "Allogeneic Mesenchymal Stem Cell Therapy Promotes Osteoblastogenesis and Prevents Glucocorticoid-Induced Osteoporosis." Stem Cells Transl Med 5(9): 1238-1246.
88. Turgeman, G., et al. (2001). "Engineered human mesenchymal stem cells: a novel platform for

skeletal cell mediated gene therapy." J Gene Med 3(3): 240-251.
89. Wagner, K. U. and G. H. Smith (2005). "Pregnancy and stem cell behavior." J Mammary Gland Biol Neoplasia 10(1): 25-36.
90. Wang, Y., et al. (2014). "Suicide gene-mediated sequencing ablation revealed the potential therapeutic mechanism of induced pluripotent stem cell-derived cardiovascular cell patch post-myocardial infarction." Antioxid Redox Signal 21(16): 2177-2191.
91. Wang, Z., et al. (2008). "Neuregulin-1 promotes cardiomyocyte differentiation of genetically engineered embryonic stem cell clones." BMB Rep 41(10): 699-704.
92. Wei, Y., et al. (2014). "Ncor2 is required for hematopoietic stem cell emergence by inhibiting Fos signaling in zebrafish." Blood 124(10): 1578-1585.
93. Wikipedia. The free encyclopedia. http://en.wikipedia.org. 2018.
94. Wikipedia. The free encyclopedia. Stem cell. https://en.wikipedia.org/wiki/Stem_cell. 2018.
95. Xia, Z., et al. (2004). "Macrophagic response to human mesenchymal stem cell and poly (epsilon-caprolactone) implantation in nonobese diabetic/severe combined immunodeficient mice." J Biomed Mater Res A 71(3): 538-548.
96. Xiong, Q., et al. (2011). "A fibrin patch-based enhanced delivery of human embryonic stem cell-derived vascular cell transplantation in a porcine model of postinfarction left ventricular remodeling." Stem Cells 29(2): 367-375.
97. Yan, B., et al. (2013). "Regulation of PTEN/Akt pathway enhances cardiomyogenesis and attenuates adverse left ventricular remodeling following thymosin beta4 Overexpressing embryonic stem cell transplantation in the infarcted heart." PLoS One 8(9): e75580.
98. Yanagihara, K., et al. (2018). "Treatment of Bone Defects by Transplantation of Genetically Modified Mesenchymal Stem Cell Spheroids." Mol Ther Methods Clin Dev 9: 358-366.
99. Yang, Q., et al. (2016). "RNA polymerase II pausing modulates hematopoietic stem cell emergence in zebrafish." Blood 128(13): 1701-1710.
100. Yao, J., et al. (2012). "Tissue inhibitor of matrix metalloproteinase-3 or vascular endothelial growth factor transfection of aged human mesenchymal stem cells enhances cell therapy after myocardial infarction." Rejuvenation Res 15(5): 495-506.
101. 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.
102. Zan, X., et al. (2012). "Polyvalent display of RGD motifs on turnip yellow mosaic virus for enhanced stem cell adhesion and spreading." Acta Biomater 8(8): 2978-2985.
103. Zeng, W., et al. (2013). "Viral transduction of male germline stem cells results in transgene transmission after germ cell transplantation in pigs." Biol Reprod 88(1): 27.
104. Zhang, P., et al. (2005). "[Plasmid transfection of rat bone marrow mesenchymal stem cells by cationic lipid for gene-modified cell transplantation therapy]." Zhongguo Yi Xue Ke Xue Yuan Xue Bao 27(4): 504-508.
105. Zhang, Z., et al. (2016). "Mammary-Stem-Cell-Based Somatic Mouse Models Reveal Breast Cancer Drivers Causing Cell Fate Dysregulation." Cell Rep 16(12): 3146-3156.
106. Zhao, Y., et al. (2014). "Pim-1 kinase cooperates with serum signals supporting mesenchymal stem cell propagation." Cells Tissues Organs 199(2-3): 140-149.
107. Zhu, K., et al. (2014). "Nanovector-based prolyl hydroxylase domain 2 silencing system enhances the efficiency of stem cell transplantation for infarcted myocardium repair." Int J Nanomedicine 9: 5203-5215.
108. Zhu, K., et al. (2016). "Nanoparticles-Assisted Stem Cell Therapy for Ischemic Heart Disease." Stem Cells Int 2016: 1384658.
109. Ziegler, A. N., et al. (2014). "Insulin-like growth factor-II (IGF-II) and IGF-II analogs with enhanced insulin receptor-a binding affinity promote neural stem cell expansion." J Biol Chem 289(8): 4626-4633.