

Marrow Stem Cell Literatures

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Abstract: The definition of stem cell is “an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell”. Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on marrow stem cell.

[Smith MH. **Marrow Stem Cell Literatures.** *Stem Cell* 2012;3(4):6-56] (ISSN 1545-4570).
<http://www.sciencepub.net/stem>. 2

Key words: stem cell; life; gene; DNA; protein; marrow

Literatures

Agarwal, R., S. Doren, et al. (1995). "Long-term culture of chronic myelogenous leukemia marrow cells on stem cell factor-deficient stroma favors benign progenitors." *Blood* **85**(5): 1306-12.

Long-term culture of marrow from patients with chronic myelogenous leukemia (CML) has been reported to favor the outgrowth of bcr/abl- progenitor cells in some patients. We examined the effect of the presence of soluble or transmembrane forms of stem cell factor (SCF) in long-term cultures of CML marrow. CD34-enriched cells from CML patients in advanced chronic phase or accelerated phase were plated on immortalized fetal liver stromal cells from homozygous SCF-deficient SI/SI mice (SI/SI4) with or without the addition of soluble human SCF, SI/SI4 cells expressing high levels of the transmembrane form of human SCF (SI/SIh220), or primary human allogeneic stroma. Cells were removed from cultures and plated weekly in colony assays. The clonogenic cell output from cultures completely lacking SCF was lower over the first 2 to 3 weeks, but by 5 weeks was similar to the clonogenic cell output from the other culture conditions. Analysis of bcr/abl transcripts from individual colonies showed a lower percentage of malignant progenitors present in long-term cultures completely deficient in SCF than under the other culture conditions, particularly compared with primary human stroma-containing long-term cultures. SCF may specifically favor malignant versus benign progenitor cells present in the marrow of CML patients, and an abnormal proliferative response to SCF in very primitive cells may be an underlying defect in the pathophysiology of this disease.

Alderuccio, F., J. Chan, et al. (2009). "Gene therapy and bone marrow stem-cell transfer to treat autoimmune disease." *Trends Mol Med* **15**(8): 344-51.

Current treatment of human autoimmune disease by autologous bone marrow stem-cell transfer is hampered by frequent disease relapses. This is most probably owing to re-emergent self-reactive lymphocytes. Gene therapy combined with bone marrow stem cells has successfully introduced genes lacking in immunodeficiencies. Because the bone marrow compartment has a key role in establishing immune tolerance, this combination strategy should offer a rational approach to prevent re-emergent self-reactive lymphocytes by establishing solid, life-long immune tolerance to causative self-antigen. Indeed, we have recently demonstrated the success of this combination approach to prevent and cure an experimental autoimmune disease. We suggest that this combination strategy has the potential for translation to treat human autoimmune diseases in which causative self-antigens are known.

Ara, T., K. Tokoyoda, et al. (2003). "Long-term hematopoietic stem cells require stromal cell-derived factor-1 for colonizing bone marrow during ontogeny." *Immunity* **19**(2): 257-67.

The physiological role of SDF-1 on hematopoietic stem cells (HSCs) remains elusive. We show that colonization of bone marrow by HSCs in addition to myeloid cells is severely impaired in SDF-1(-/-) embryos by a long-term repopulation assay. Colonization of spleen by HSCs was also affected, but to a lesser extent. Enforced expression of SDF-1 under the control of vascular-specific Tie-2 regulatory sequences could completely rescue the reduction of HSCs but not myeloid cells in SDF-1(-/-) bone marrow. SDF-1 was detected in the vicinity of the

vascular endothelial cells in fetal bone marrow. SDF-1 plays a critical role in colonization of bone marrow by HSCs and myeloid cells during ontogeny, and the mechanisms by which SDF-1 functions are distinct between HSCs and myeloid cells.

Aye, M. T., S. Hashemi, et al. (1992). "Expression of stem cell factor and c-kit mRNA in cultured endothelial cells, monocytes and cloned human bone marrow stromal cells (CFU-RF)." *Exp Hematol* **20**(4): 523-7.

Previously, we have shown that conditioned medium from a subpopulation of human marrow stromal cells (CFU-RF) contain an activity able to stimulate the growth of macroscopic epo-dependent erythroid colonies. The ligand for the product of the c-kit proto-oncogene (also known as stem cell factor or SCF), among other activities, has been reported to have similar effects on erythroid colony growth. We have also presented data showing that SCF together with phytohemagglutinin-stimulated leukocyte conditioned medium can stimulate erythroid colony growth in the presence of antibodies to erythropoietin. Using the human SCF cDNA probe (K. Zsebo, Amgen Inc.) we now show that cells derived from CFU-RF colonies express SCF but not c-kit. Human umbilical vein endothelial cells were also found to express SCF and this expression was increased by addition of monocyte supernatant, IL-1 beta or thrombin. Cells of the human erythroleukemia cell line HEL were found to express c-kit but not SCF. Neither c-kit nor SCF mRNA were detected in phytohemagglutinin-stimulated lymphocytes. Together, these data support the view that the behaviour of proliferating erythroid stem cells in the marrow, which may express c-kit, could be regulated by membrane-bound SCF present on surrounding stromal cells.

Bakondi, B., I. S. Shimada, et al. (2009). "CD133 identifies a human bone marrow stem/progenitor cell sub-population with a repertoire of secreted factors that protect against stroke." *Mol Ther* **17**(11): 1938-47.

The reparative properties of bone marrow stromal cells (BMSCs) have been attributed in part to the paracrine action of secreted factors. We isolated typical human BMSCs by plastic adherence and compared them with BMSC sub-populations isolated by magnetic-activated cell sorting against CD133 (CD133-derived BMSCs, CD133BMSCs) or CD271 [p75 low-affinity nerve growth factor receptor (p75LNGFR), p75BMSCs]. Microarray assays of expressed genes, and enzyme-linked immunosorbent assays (ELISAs) of selected growth factors and cytokines secreted under normoxic and hypoxic conditions demonstrated that the three transit-

amplifying progenitor cell populations were distinct from one another. CD133BMSC-conditioned medium (CdM) was superior to p75BMSC CdM in protecting neural progenitor cells against cell death during growth factor/nutrient withdrawal. Intracardiac (arterial) administration of concentrated CD133BMSC CdM provided neuroprotection and significantly reduced cortical infarct volumes in mice following cerebral ischemia. In support of the paracrine hypothesis for BMSC action, intra-arterial infusion of CD133BMSC CdM provided significantly greater protection against stroke compared with the effects of CD133BMSC (cell) administration. CdM from CD133BMSCs also provided superior protection against stroke compared with that conferred by CdM from p75BMSCs or typically isolated BMSCs. CD133 identifies a sub-population of nonhematopoietic stem/progenitor cells from adult human bone marrow, and CD133BMSC CdM may provide neuroprotection for patients with stroke.

Ball, S. G., A. C. Shuttleworth, et al. (2004). "Direct cell contact influences bone marrow mesenchymal stem cell fate." *Int J Biochem Cell Biol* **36**(4): 714-27.

Adult bone marrow-derived mesenchymal stem cells (MSC) can differentiate into various cell types of mesenchymal origin, but mechanisms regulating such cellular changes are unclear. We have conducted co-culture experiments to examine whether mesenchymal stem cell differentiation is influenced by indirect or direct contact with differentiated cells. Cultured adult mesenchymal stem cells showed some characteristics of synthetic state vascular smooth muscle cells (SMC). When co-cultured with vascular endothelial cells (EC) without cell contact, they exhibited abundant well-organised smooth muscle alpha-actin (alpha-actin) filaments. Direct co-culture with endothelial cells resulted in increased smooth muscle alpha-actin mRNA and protein, yet also comprehensive disruption of smooth muscle alpha-actin filament organisation. In order to assess whether these cell contact effects on mesenchymal stem cells were cell type specific, we also analysed direct co-cultures of mesenchymal stem cells with dermal fibroblasts. However, these experiments were characterised by the appearance of abundant spindle-shaped myofibroblast-like cells containing organised smooth muscle alpha-actin filaments. Thus, direct contact with distinct differentiated cells may be a critical determinant of mesenchymal stem cell fate in blood vessels and other connective tissues.

Ballas, C. B., S. P. Zielske, et al. (2002). "Adult bone marrow stem cells for cell and gene therapies: implications for greater use." *J Cell Biochem Suppl* **38**: 20-8.

There is excitement generated almost daily about the possible uses of stem cells to treat human disease. Much of the interest of late is generated by embryonic stem cells (ESCs). As exciting as ESCs may be, they are quite controversial for moral reasons, given their source. They are also scientifically controversial since they are much less well understood than the original, long-standing, and clinically successful hematopoietic stem cell (HSC). HSCs have the distinct advantage of being reasonably well characterized and have been proven in the clinic. They can be isolated by simple procedures directly from the bone marrow or from peripheral blood after being stimulated (mobilized). They can then be manipulated and delivered to a patient, often producing a cure. Their biology provides the paradigm by which all other stem cells are judged, and they have little in the way of moral controversy surrounding them given they are isolated from adults who have consented to the procedure. Another putative stem cell has gained momentum in the last few years; the mesenchymal stem cell (MSC). MSCs appear to have much in common with HSCs. They were originally characterized from bone marrow, are capable of differentiating along multiple lineages and, at least in vitro, have significant expansion capability. Unlike HSCs, they have not yet been definitively shown to function as stem cells, despite their ability to differentiate into various mesenchymal cell types under the right culture conditions. Still, there is mounting evidence these cells may be useful, if not as true stem cells then at least as vehicles for emerging cell and gene therapies, especially in the field of tissue engineering. While this is an important endpoint, it is more important to thoroughly understand stem cell biology. That understanding can then be applied toward the ultimate goal of using these cells not just for various forms of therapy, but rather as a tool to discover the mechanisms and means to bring about directed repair and regeneration of damaged or diseased tissues and organs. The excitement of HSCs and MSCs has been muted somewhat by the excitement surrounding ESCs, primarily due to the fact HSCs and MSCs are viewed as limited to specific cell types while ESCs could potentially be applied to any cell type. Recent information indicates HSCs, MSCs, and other cells in general may have more universal differentiation abilities than previously thought.

Barbash, I. M., P. Chouraqui, et al. (2003). "Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution." *Circulation* **108**(7): 863-8.

BACKGROUND: Systemic delivery of bone marrow-derived mesenchymal stem cells (BM-MSCs) is an attractive approach for myocardial repair. We aimed to test this strategy in a rat model after myocardial infarction (MI). **METHODS AND RESULTS:** BM-MSCs were obtained from rat bone marrow, expanded in vitro to a purity of >50%, and labeled with ^{99m}Tc exametazine, fluorescent dye, LacZ marker gene, or bromodeoxyuridine. Rats were subjected to MI by transient coronary artery occlusion or to sham MI. ^{99m}Tc-labeled cells (4x10⁶) were transfused into the left ventricular cavity of MI rats either at 2 or 10 to 14 days after MI and were compared with sham-MI rats or MI rats treated with intravenous infusion. Gamma camera imaging and isolated organ counting 4 hours after intravenous infusion revealed uptake of the ^{99m}Tc-labeled cells mainly in the lungs, with significantly smaller amounts in the liver, heart, and spleen. Delivery by left ventricular cavity infusion resulted in drastically lower lung uptake, better uptake in the heart, and specifically higher uptake in infarcted compared with sham-MI hearts. Histological examination at 1 week after infusion identified labeled cells either in the infarcted or border zone but not in remote viable myocardium or sham-MI hearts. Labeled cells were also identified in the lung, liver, spleen, and bone marrow. **CONCLUSIONS:** Systemic intravenous delivery of BM-MSCs to rats after MI, although feasible, is limited by entrapment of the donor cells in the lungs. Direct left ventricular cavity infusion enhances migration and colonization of the cells preferentially to the ischemic myocardium.

Barese, C., N. Pech, et al. (2007). "Granulocyte colony-stimulating factor prior to nonmyeloablative irradiation decreases murine host hematopoietic stem cell function and increases engraftment of donor marrow cells." *Stem Cells* **25**(6): 1578-85.

The use of nonmyeloablative conditioning prior to bone marrow transplantation is an important component of transplantation-based therapies for nonmalignant blood diseases. In this study, treatment of recipient mice with granulocyte colony-stimulating factor (G-CSF) prior to low-dose total body irradiation (LD-TBI) enhanced long-term engraftment of freshly isolated congenic marrow 1.5- to 2-fold more than treatment with LD-TBI alone. This combined regimen was also evaluated in a mouse model of X-linked chronic granulomatous disease (X-CGD), where neutrophils have a defective NADPH oxidase due to genetic deletion of the gp91(phox) subunit. Long-term engraftment of male X-CGD bone marrow cells cultured ex vivo for retroviral transduction of gp91(phox) was enhanced by approximately 40% when female X-CGD recipients were pretreated with

G-CSF prior to 300 cGy. These data confirm that sequential treatment with G-CSF and LD-TBI prior to transplantation increases long-term engraftment of donor marrow, and they extend this approach to transplantation of murine donor marrow cultured *ex vivo* for gene transfer. Additional studies showed that the administration of G-CSF prior to LD-TBI did not alter early homing of donor marrow cells. However, the combined regimen significantly decreased the content of long-term repopulating cells in recipient marrow compared with LD-TBI alone, as assessed in competitive assays, which may contribute to the enhanced engraftment of donor marrow cells. Disclosure of potential conflicts of interest is found at the end of this article.

Becker, P. S. (2002). "Hematopoietic stem cell gene therapy for inherited bone marrow disorders: past accomplishments and continued challenges." *J Cell Biochem Suppl* **38**: 55-64.

From the time that the genes encoding the defective proteins were cloned for a number of inherited diseases, it became a goal to correct those conditions by restoring the normal gene and thereby, its product. For the inherited disorders affecting the blood and its progenitor cells, the hematopoietic stem cells were the ideal target cells for gene transfer, because the normal gene would then be transferred to all of the progeny cells, theoretically for the lifetime of the recipient. However, the tasks of isolating the hematopoietic stem cells, introducing the new genes in such a manner as to preserve engraftment of the manipulated cells, and achieving long-term gene expression, have not been straightforward in the clinical trial setting, although there has been moderate success for cells *in vitro*, and in murine studies. With the report of clinical efficacy of gene transfer in children with X-linked severe combined immunodeficiency disease, the dream of clinical gene transfer to hematopoietic cells has become a reality. But there are still significant impediments remaining for a number of diseases. The innovations of introduction of synthetic receptors that confer growth advantage, the use of lentiviral vectors with increased stem cell transduction efficiency, and the addition of modified promoter/enhancer sequences to augment and preserve gene expression may bring wider success to gene therapy clinical trials for bone marrow disorders in the near future.

Billadeau, D., F. Prosper, et al. (1997). "Sequential analysis of bone marrow and peripheral blood after stem cell transplant for myeloma shows disparate tumor involvement." *Leukemia* **11**(9): 1565-70.

Treatment with combination chemotherapy has not resulted in long-term remissions in multiple

myeloma (MM) despite advances in drug discovery and protocol improvement over the last 25 years. Increasingly, peripheral blood (PB) stem cell transplants (PBSCT) are being used along with chemotherapy and total body irradiation as treatment for multiple myeloma. Although the majority of tumor cells are found within the bone marrow (BM), tumor cells circulate in the PB in patients with MM. Therefore, one potential problem with PBSCT is contamination of the stem cell harvests with tumor cells. Although substantial reduction in BM tumor load is achieved after chemotherapy and autologous transplantation, most patients still relapse. In an attempt to identify and quantitate the residual tumor within sequential BM and PB samples of patients with MM following autologous PB stem cell transplants we have used a tumor-specific detection assay, allele-specific oligonucleotide-PCR (ASO-PCR). We found that while the BM tumor burden may fluctuate in some patients by as much as 4-logs after transplant, the PB tumor remains quite stable, and does not reflect the tumor burden in the BM. Moreover, analysis of PB involvement over time was not predictive of marrow involvement or of potential relapse. These results suggest that the PB is frequently involved in MM and further indicate that it represents a compartment that is only minimally altered by intensive therapy.

Bodine, D. M., N. E. Seidel, et al. (1996). "Bone marrow collected 14 days after *in vivo* administration of granulocyte colony-stimulating factor and stem cell factor to mice has 10-fold more repopulating ability than untreated bone marrow." *Blood* **88**(1): 89-97.

We have examined the repopulating ability of bone marrow and peripheral blood cells collected immediately and at intervals after treatment of donor mice with the combination of granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF). Using a competitive repopulation assay we showed that the repopulating ability of peripheral blood cells was highest immediately after cytokine treatment and declined to normal levels within 6 weeks of the termination of treatment with G-CSF and SCF. In contrast the repopulating ability of bone marrow cells was low immediately after cytokine treatment and increased to levels that were 10-fold or more greater than marrow from untreated mice by 14 days after termination of treatment with G-CSF and SCF. This high level of repopulating activity declined to normal levels by 6 weeks after termination of treatment with G-CSF and SCF. The high level of repopulating ability was confirmed by injecting cells from G-CSF- and SCF-treated donors into unconditioned recipients. Peripheral blood cells collected immediately after treatment with G-CSF and SCF engrafted into unconditioned mice sevenfold better than an

equivalent number of bone marrow cells from untreated mice. Likewise, bone marrow cells collected 14 days after treatment of the donor animal with G-CSF and SCF engrafted at 10-fold higher levels than an equivalent number of bone marrow cells from untreated mice. We conclude that the treatment of donor mice with G-CSF and SCF causes a transient increase in the repopulating ability of peripheral blood and later of bone marrow. These observations may have applications to clinical hematopoietic stem cell transplantation.

Bosnakovski, D., M. Mizuno, et al. (2006). "Gene expression profile of bovine bone marrow mesenchymal stem cell during spontaneous chondrogenic differentiation in pellet culture system." *Jpn J Vet Res* **53**(3-4): 127-39.

Bovine bone marrow mesenchymal stem cells (MSCs) cultured in condensate culture, spontaneous and independent for any external biostimulants, undergo chondrogenic differentiation. In the present study, the bovine MSC chondrogenesis pathway was studied by analyzing stage-specific gene expression using quantitative "Real Time" reverse transcriptase polymerase chain reaction (qRT-PCR). Results showed that bovine MSCs underwent complete chondrogenesis; the initial stage was characterized by expression of sox9 messenger ribonucleic acid (mRNA), followed by high transcription of chondrocyte specific genes, collagen type II and IX, biglycan and cartilage oligomeric matrix protein, and the final prehypertrophic and/or hypertrophic stage was distinguished by increased expression of collagen type X. From day 7 to day 14 of differentiation increased mRNA expression of the transforming growth factors beta1 and beta2, basic fibroblast growth factor (FGF 2), bone morphogenic protein 6 (BMP 6), insulin-like growth factors 1, parathyroid hormone related peptide and indian hedgehog (Ihh) were detected. These results suggest that these well known chondrogenic growth factors may play a role in bovine chondrogenesis in autocrine and/or paracrine manner. On day 21 of the culture, FGF 2, BMP 6 and Ihh were highly expressed, compared to cells cultured in monolayer manner, which suggests a possible function in maintaining the terminal stage of differentiation. This data extends our knowledge about the unusual species-specific bovine MSC chondrogenesis, allowing us to define the phenotype of the differentiated cells. Furthermore, this study contributes to our understanding of known chondrogenic-growth factors in autocrine and/or paracrine manner playing a role in the spontaneous differentiation.

Bunting, K. D., J. Galipeau, et al. (1998). "Transduction of murine bone marrow cells with an MDR1 vector enables ex vivo stem cell expansion, but these expanded grafts cause a myeloproliferative syndrome in transplanted mice." *Blood* **92**(7): 2269-79.

Attempts to expand repopulating hematopoietic cells ex vivo have yielded only modest amplification in stem cell numbers. We now report that expression of an exogenous human multi-drug resistance 1 (MDR1) gene enables dramatic ex vivo stem cell expansion in the presence of early acting hematopoietic cytokines. Bone marrow cells were transduced with retroviral vectors expressing either the MDR1 gene or a variant of human dihydrofolate reductase (DHFR), and then expanded for 12 days in the presence of interleukin-3 (IL-3), IL-6, and stem cell factor. When these cells were injected into nonirradiated mice, high levels of long-term engraftment were only seen with MDR1-transduced grafts. To verify that expansion of MDR1-transduced repopulating cells had occurred, competitive repopulation assays were performed using MDR1 expanded grafts. These experiments showed progressive expansion of MDR1-transduced repopulating cells over the expansion period, with a 13-fold overall increase in stem cells after 12 days. In all of the experiments, mice transplanted with expanded MDR1-transduced stem cells developed a myeloproliferative disorder characterized by high peripheral white blood cell counts and splenomegaly. These results show that MDR1-transduced stem cells can be expanded in vitro using hematopoietic cytokines without any drug selection, but enforced stem cell self-renewal divisions can have adverse consequences.

Cancelas, J. A., M. Jansen, et al. (2006). "The role of chemokine activation of Rac GTPases in hematopoietic stem cell marrow homing, retention, and peripheral mobilization." *Exp Hematol* **34**(8): 976-85.

Signaling downstream from the chemokine receptor CXCR4, the tyrosine kinase receptor c-kit and beta1-integrins has been shown to be crucial in the regulation of migration, homing, and engraftment of hematopoietic stem cells and progenitors. Each of these receptors signal through Rac-type Rho guanosine triphosphatases (GTPases). Rac GTPases play a major role in the organization of the actin cytoskeleton and also in the control of gene expression and the activation of proliferation and survival pathways. Here we review the specific roles of the members of the Rac subfamily of the Rho GTPase family in regulating the intracellular signaling of hematopoietic cells responsible for regulation of

homing, marrow retention, and peripheral mobilization.

Cavazzana-Calvo, M., C. Bagnis, et al. (1999). "Peripheral stem cells in bone marrow transplantation. Peripheral blood stem cell and gene therapy." *Baillieres Best Pract Res Clin Haematol* **12**(1-2): 129-38.

Mobilized peripheral blood stem cells characterized by sustained re-populating ability could be optimal target cells for ex-vivo gene transfer. In spite of very attractive preliminary results obtained in the murine studies, therapeutically efficient gene transfer and expression in human targeted cells must be proven. In recent years, effort has been spent on the identification of factors limiting gene transfer efficiency of haematopoietic stem cells. Increasing knowledge concerning haematopoiesis and gene transfer has helped in identifying a number of limiting factors. These factors as well as the strategies that showed increased retroviral infection of haematopoietic stem cells will be discussed. Finally, the results of the clinical trials will be reported.

Chen, C. H., M. L. Ho, et al. (2005). "Green tea catechin enhances osteogenesis in a bone marrow mesenchymal stem cell line." *Osteoporos Int* **16**(12): 2039-45.

Green tea has been reported to possess antioxidant, antitumorogenic, and antibacterial qualities that regulate the endocrine system. Previous epidemiological studies found that the bone mineral density (BMD) of postmenopausal women with a habit of tea drinking was higher than that of women without habitual tea consumption. However, the effects of green tea catechins on osteogenic function have rarely been investigated. In this study, we tested (-)-epigallocatechin-3-gallate (EGCG), one of the green tea catechins, on cell proliferation, the mRNA expressions of relevant osteogenic markers, alkaline phosphatase (ALP) activity and mineralization. In a murine bone marrow mesenchymal stem cell line, D1, the mRNA expressions of core binding factors $\alpha 1$ (Cbfa1/Runx2), osterix, osteocalcin, ALP increased after 48 h of EGCG treatment. ALP activity was also significantly augmented upon EGCG treatment for 4 days, 7 days and 14 days. Furthermore, mineralizations assayed by Alizarin Red S and von Kossa stain were enhanced after EGCG treatment for 2-4 weeks in D1 cell cultures. However, a 24-h treatment of EGCG inhibited thymidine incorporation of D1 cells. These results demonstrated that long-term treatment of EGCG increases the expressions of osteogenic genes, elevates ALP activity and eventually stimulates mineralization, in spite of its inhibitory effect on proliferation. This finding

suggests that the stimulatory effects of EGCG on osteogenesis of mesenchymal stem cells may be one of the mechanisms that allow tea drinkers to possess higher BMD.

Chung, N., B. K. Jee, et al. (2009). "HOX gene analysis of endothelial cell differentiation in human bone marrow-derived mesenchymal stem cells." *Mol Biol Rep* **36**(2): 227-35.

Human bone marrow-derived mesenchymal stem cells (hMSCs) have been shown to possess multilineage differentiation potential. HOX genes function in transcriptional regulators, and are involved in stem cell differentiation. The aim of the present study was to demonstrate HOX genes that are related to angiogenesis. To identify the expression patterns of 37 HOX genes in the endothelial cell differentiation of hMSCs, we analyzed HOX genes through profiling with multiplex RT-PCR. The results showed that the expression patterns of four HOX genes, HOXA7, HOXB3, HOXA3, and HOXB13, significantly changed during angiogenesis. The expression levels of HOXA7 and HOXB3 were dramatically increased, whereas those of HOXA3 and HOXB13 were decreased during endothelial cell differentiation. When further analysis of the expressions of these HOX genes was performed with real-time PCR and an immunoblot assay, the expression patterns were also found to be well-matched with the results of multiplex RT-PCR. Here, we report that HOXA7, HOXB3, HOXA3, and HOXB13 might be involved in the angiogenesis of hMSCs.

Chung, N. G., D. C. Jeong, et al. (2004). "Cotransplantation of marrow stromal cells may prevent lethal graft-versus-host disease in major histocompatibility complex mismatched murine hematopoietic stem cell transplantation." *Int J Hematol* **80**(4): 370-6.

Marrow stromal cells (MSC) produce a microenvironment supporting hematopoiesis and may contribute immune tolerance because of low immunogenicity and the suppressive effect of alloreactivity. We investigated whether cotransplantation of MSC could prevent lethal graft-versus-host disease (GVHD) in major histocompatibility complex mismatched allogeneic murine hematopoietic stem cell transplantation (HSCT) using female BALB/c (H-2d, recipient) and C3H/He (H-2k, donor) mice. MSC were obtained from C3H/He bone marrow cells (BMC). MSC and irradiated BALB/c splenocytes (SP) were cocultured with C3H/He SP or BMC. Nonirradiated MSC did not inhibit the proliferation of alloantigen-stimulated BMC and SP. However, irradiated MSC suppressed the proliferation of alloantigen-stimulated SP at a

level comparable with that of immunosuppressive agents, and the suppression by MSC was reversed to a significant degree by interleukin 2. Lethally irradiated BALB/c mice received transplants of donor cells according to the following experimental groups (group A, BMC only; group B, BMC and SP; group C, BMC, SP, and MSC; group D, BMC and MSC). The survival rate in group D was higher than in the other groups ($P = .0057$), and the clinical GVHD scores and serum levels of interferon-gamma were low in group D. Our results suggest that cotransplantation of MSC in HSCT prevents lethal GVHD, possibly by immune modulation.

Cilloni, D., C. Carlo-Stella, et al. (2000). "Limited engraftment capacity of bone marrow-derived mesenchymal cells following T-cell-depleted hematopoietic stem cell transplantation." *Blood* **96**(10): 3637-43.

The engraftment capacity of bone marrow-derived mesenchymal cells was investigated in 41 patients who had received a sex-mismatched, T-cell-depleted allograft from human leukocyte antigen (HLA)-matched or -mismatched family donors. Polymerase chain reaction (PCR) analysis of the human androgen receptor (HUMARA) or the amelogenin genes was used to detect donor-derived mesenchymal cells. Only 14 marrow samples (34%) from 41 consenting patients generated a marrow stromal layer adequate for PCR analysis. Monocyte-macrophage contamination of marrow stromal layers was reduced below the levels of sensitivity of HUMARA and amelogenin assays (5% and 3%, respectively) by repeated trypsinizations and treatment with the leucyl-leucine (leu-leu) methyl ester. Patients who received allografts from 12 female donors were analyzed by means of the HUMARA assay, and in 5 of 12 cases a partial female origin of stromal cells was demonstrated. Two patients who received allografts from male donors were analyzed by amplifying the amelogenin gene, and in both cases a partial male origin of stromal cells was shown. Fluorescent in situ hybridization analysis using a Y probe confirmed the results of PCR analysis and demonstrated in 2 cases the existence of a mixed chimerism at the stromal cell level. There was no statistical difference detected between the dose of fibroblast progenitors (colony-forming unit-F [CFU-F]) infused to patients with donor- or host-derived stromal cells ($1.18 \pm 0.13 \times 10^4/\text{kg}$ vs $1.19 \pm 0.19 \times 10^4/\text{kg}$; $P > .97$). In conclusion, marrow stromal progenitors reinfused in patients receiving a T-cell-depleted allograft have a limited capacity of reconstituting marrow mesenchymal cells.

Colombat, P., P. Cornillet, et al. (2000). "Value of autologous stem cell transplantation with purged bone marrow as first-line therapy for follicular lymphoma with high tumor burden: a GOELAMS phase II study." *Bone Marrow Transplant* **26**(9): 971-7.

This prospective phase II study was undertaken to evaluate the efficacy and toxicity of early intensive therapy followed by purged autologous bone marrow transplantation (ABMT) in patients with follicular lymphoma with high tumor burden. All patients received the VCAP regimen (vindesine, cyclophosphamide, doxorubicin and prednisone) as conventional chemotherapy and DHAP as second-line therapy. Twenty-nine consecutive patients were included in the study. Twenty-seven patients were grafted, seven in first complete remission (CR) and 20 in first partial remission (PR). Preparative therapy consisted of cyclophosphamide and total body irradiation (TBI) in all the patients. With a median follow-up of 6 years, the actuarial overall survival is 64% and the actuarial event-free survival is 55%. Two treatment-related early deaths were observed. Eleven patients were informative for serial PCR analysis of minimal residual disease after ABMT: two relapsed, four remained disease-free with PCR positivity and five were disease-free with PCR negativity. These encouraging results lay the basis of future prospective randomized trials comparing autologous stem cell transplantation as front-line treatment with conventional chemotherapy for patients with bad prognostic factors.

Cui, Q., Y. Wang, et al. (2006). "Alcohol-induced adipogenesis in a cloned bone-marrow stem cell." *J Bone Joint Surg Am* **88 Suppl 3**: 148-54.

BACKGROUND: Alcohol has been shown to be associated with osteoporosis and osteonecrosis in patients and in animal models. Recent studies have demonstrated that alcohol contributes to abnormal lipid metabolism in the stromal cells of bone marrow, but the mechanisms have not been defined. The purpose of this study was to evaluate the effects of alcohol on the differentiation of a stem cell that was cloned from bone marrow. **METHODS:** D1 cells (cloned bone-marrow stem cells from a BALB/c mouse) were treated either with increasing concentrations of ethanol (0.09, 0.15, and 0.21 mol/L) or without alcohol to serve as controls. Morphologic features of the cells were monitored with use of a phase-contrast microscope. Alkaline phosphatase activity was determined with use of a colorimetric assay. The expression of genes that are indicators of adipogenesis [422(aP2), PPARgamma] and osteogenesis (osteocalcin) was evaluated using Northern blot and reverse transcription-polymerase chain reaction assays. **RESULTS:** The cells treated

with ethanol started to accumulate triglyceride vesicles at day seven. The number of adipocytes and the percentage of the area that contained the cells with fat vesicles increased significantly ($p < 0.05$), and the level of alkaline phosphatase activity diminished with longer durations of exposure to ethanol and with higher concentrations. Analysis of gene expression showed diminished expression of osteocalcin. This occurred without a significant increase in the expression of either the fat-cell-specific gene 422(aP2) or PPAR γ in cells treated with ethanol, suggesting that adipogenesis may occur at a point downstream in the fatty-acid-metabolism pathway. **CONCLUSIONS:** Alcohol treatment decreases osteogenesis while enhancing adipogenesis in a cloned bone-marrow stem cell, indicating that alcohol abuse may be one of the mechanisms leading to osteoporosis and osteonecrosis. This finding explains the clinical observation that there is increased adipogenesis in alcohol-induced osteoporosis and osteonecrosis. **CLINICAL RELEVANCE:** The inhibition of bone-marrow adipogenesis and the concomitant enhancement of osteogenesis may provide a novel approach to the prevention or treatment of osteonecrosis and osteoporosis.

Deng, X. L., C. P. Lau, et al. (2007). "Cell cycle-dependent expression of potassium channels and cell proliferation in rat mesenchymal stem cells from bone marrow." *Cell Prolif* **40**(5): 656-70.

OBJECTIVE: Recently, our team has demonstrated that voltage-gated delayed rectifier K(+) current (IK(DR)) and Ca(2+)-activated K(+) current (IKCa) are present in rat bone marrow-derived mesenchymal stem cells; however, little is known of their physiological roles. The present study was designed to investigate whether functional expression of IK(DR) and IKCa would change with cell cycle progression, and whether they could regulate proliferation in undifferentiated rat mesenchymal stem cells (MSCs). **MATERIALS AND METHODS:** Membrane potentials and ionic currents were recorded using whole-cell patch clamp technique, cell cycling was analysed by flow cytometry, cell proliferation was assayed with DNA incorporation method and the related genes were down-regulated by RNA interference (RNAi) and examined using RT-PCR. **RESULTS:** It was found that membrane potential hyperpolarized, and cell size increased during the cell cycle. In addition, IK(DR) decreased, while IKCa increased during progress from G(1) to S phase. RT-PCR revealed that the mRNA levels of Kv1.2 and Kv2.1 (likely responsible for IK(DR)) reduced, whereas the mRNA level of KCa3.1 (responsible for intermediate-conductance IKCa) increased with the cell cycle progression. Down-regulation of Kv1.2,

Kv2.1 or KCa3.1 with the specific RNAi, targeted to corresponding gene inhibited proliferation of rat MSCs. **CONCLUSION:** These results demonstrate that membrane potential, IK(DR) and IKCa channels change with cell cycle progression and corresponding alteration of gene expression. IK(DR) and intermediate-conductance IKCa play an important role in maintaining membrane potential and they participate in modulation of proliferation in rat MSCs.

Deng, Y. B., Q. T. Yuan, et al. (2005). "Functional recovery after rhesus monkey spinal cord injury by transplantation of bone marrow mesenchymal-stem cell-derived neurons." *Chin Med J (Engl)* **118**(18): 1533-41.

BACKGROUND: The treatment of spinal cord injury is still a challenge. This study aimed at evaluating the therapeutical effectiveness of neurons derived from mesenchymal stem cells (MSCs) for spinal cord injury. **METHODS:** In this study, rhesus MSCs were isolated and induced by cryptotanshinone in vitro and then a process of RT-PCR was used to detect the expression of glutamic acid decarboxylase (GAD) gene. The induced MSCs were tagged with Hoechst 33342 and injected into the injury site of rhesus spinal cord made by the modified Allen method. Following that, behavior analysis was made after 1 week, 1 month, 2 months and 3 months. After 3 months, true blue chloride retrograde tracing study was also used to evaluate the re-establishment of axons pathway and the hematoxylin-eosin (HE) staining and immunohistochemistry were performed after the animals had been killed. **RESULTS:** In this study, the expression of mRNA of GAD gene could be found in the induced MSCs but not in primitive MSCs and immunohistochemistry could also confirm that rhesus MSCs could be induced and differentiated into neurons. Behavior analysis showed that the experimental animals restored the function of spinal cord up to grade 2-3 of Tarlov classification. Retrograde tracing study showed that true blue chollide could be found in the rostral thoracic spinal cords, red nucleus and sensory-motor cortex. **CONCLUSIONS:** These results suggest that the transplantation is safe and effective.

Dolgachev, V. A., M. R. Ullenbruch, et al. (2009). "Role of stem cell factor and bone marrow-derived fibroblasts in airway remodeling." *Am J Pathol* **174**(2): 390-400.

Recent evidence suggests that bone marrow-derived fibroblasts are involved in airway remodeling in asthma, but the role and mechanism of recruitment of these fibroblasts remains unclear. Stem cell factor (SCF), a key factor in the propagation of hematopoietic stem cells, is important in the process

of airway remodeling as well. To test the hypothesis that SCF is involved in the recruitment and differentiation of bone marrow-derived progenitor cells, GFP-bone marrow chimeric mice were created. These mice were then sensitized and chronically challenged with cockroach antigen to induce chronic airway disease. Fluorescence microscopy revealed an influx of significant numbers of GFP-expressing fibroblasts in the airways of these mice, which was confirmed by flow cytometric analysis of cells co-expressing both GFP and collagen I. These cells preferentially expressed c-kit, interleukin-31 receptor, and telomerase reverse transcriptase when compared with control lung-derived fibroblasts. Interestingly, SCF stimulated interleukin-31 receptor expression in bone marrow cells, whereas interleukin-31 strongly induced telomerase reverse transcriptase expression in fibroblasts. Treatment with neutralizing antibodies to SCF significantly reduced airway remodeling and suppressed the recruitment of these bone marrow-derived cells to the lung. Thus SCF in conjunction with interleukin-31 may play a significant role in airway remodeling by promoting the recruitment of bone marrow-derived fibroblast precursors into the lung with the capacity to promote lung myofibroblast differentiation.

Dunbar, C. E., N. E. Seidel, et al. (1996). "Improved retroviral gene transfer into murine and Rhesus peripheral blood or bone marrow repopulating cells primed in vivo with stem cell factor and granulocyte colony-stimulating factor." *Proc Natl Acad Sci U S A* **93**(21): 11871-6.

In previous studies we showed that 5 days of treatment with granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) mobilized murine repopulating cells to the peripheral blood (PB) and that these cells could be efficiently transduced with retroviral vectors. We also found that, 7-14 days after cytokine treatment, the repopulating ability of murine bone marrow (BM) increased 10-fold. In this study we examined the efficiency of gene transfer into cytokine-primed murine BM cells and extended our observations to a nonhuman primate autologous transplantation model. G-CSF/SCF-primed murine BM cells collected 7-14 days after cytokine treatment were equivalent to post-5-fluorouracil BM or G-CSF/SCF-mobilized PB cells as targets for retroviral gene transfer. In nonhuman primates, CD34-enriched PB cells collected after 5 days of G-CSF/SCF treatment and CD34-enriched BM cells collected 14 days later were superior targets for retroviral gene transfer. When a clinically approved supernatant infection protocol with low-titer vector preparations was used, monkeys had up to 5% of circulating cells containing the vector for up to a year after

transplantation. This relatively high level of gene transfer was confirmed by Southern blot analysis. Engraftment after transplantation using primed BM cells was more rapid than that using steady-state bone marrow, and the fraction of BM cells saving the most primitive CD34+/CD38- or CD34+/CD38dim phenotype increased 3-fold. We conclude that cytokine priming with G-CSF/SCF may allow collection of increased numbers of primitive cells from both the PB and BM that have improved susceptibility to retroviral transduction, with many potential applications in hematopoietic stem cell-directed gene therapy.

Elmaagacli, A. H., D. W. Beelen, et al. (2000). "The detection of wt-1 transcripts is not associated with an increased leukemic relapse rate in patients with acute leukemia after allogeneic bone marrow or peripheral blood stem cell transplantation." *Bone Marrow Transplant* **25**(1): 91-6.

We studied the role of wt-1 as a minimal residual disease (MRD) marker in 46 patients with acute leukemia (AL) (1st CR n = 24; 2nd CR n = 9, in relapse n = 13) after allogeneic bone marrow or peripheral blood stem cell transplantation. Prior to allogeneic transplant, wt-1 transcripts were detected by PCR in 38 of 46 patients (83%) with AL. After transplant, in 14 of 38 patients (37%) wt-1 transcripts were detected in at least one PCR assay at a median of 12 months post transplant (range 1-89 months). Twelve of the 38 patients relapsed after transplant, but only seven of the 12 were wt-1 positive after transplant. In five relapsing patients the wt-1 test remained negative 0 to 3 months prior to relapse. On the other hand, only seven of 14 patients with a positive test for wt-1 after transplant, relapsed consecutively. In 17 of the 46 study patients chromosomal abnormalities had been found prior to transplant (AML-M4eo with inv16 n = 7, AML-M2 with t(8;21) n = 3, AML-M3 with t(15;17) n = 1, AML-M5 with t(4;11) n = 1, ALL with t(9;22) n = 5). In these 17 patients, we analyzed the wt-1 transcript simultaneously with a specific chimeric transcript characteristic for the corresponding chromosomal abnormality. In 32 of 45 samples (71%) the results for the MRD marker and wt-1 transcript were concordant, but differed in 13 patients. We conclude that detection of wt-1 transcripts does not predict leukemic relapse reliably and is therefore not a suitable MRD marker in patients with acute leukemia after allogeneic BM or PBSC transplantation. *Bone Marrow Transplantation* (2000) 25, 91-96.

Fischbach, N. A., S. Rozenfeld, et al. (2005). "HOXB6 overexpression in murine bone marrow immortalizes a myelomonocytic precursor in vitro and

causes hematopoietic stem cell expansion and acute myeloid leukemia in vivo." *Blood* **105**(4): 1456-66.

The HOX family of homeobox genes plays an important role in normal and malignant hematopoiesis. Dysregulated HOX gene expression profoundly affects the proliferation and differentiation of hematopoietic stem cells (HSCs) and committed progenitors, and aberrant activation of HOX genes is a common event in human myeloid leukemia. HOXB6 is frequently overexpressed in human acute myeloid leukemia (AML). To gain further insight into the role of HOXB6 in hematopoiesis, we overexpressed HOXB6 in murine bone marrow using retrovirus-mediated gene transfer. We also explored structure-function relationships using mutant HOXB6 proteins unable to bind to DNA or a key HOX-binding partner, pre-B-cell leukemia transcription factor-1 (PBX1). Additionally, we investigated the potential cooperative interaction with myeloid ecotropic viral integration site 1 homolog (MEIS1). In vivo, HOXB6 expanded HSCs and myeloid precursors while inhibiting erythropoiesis and lymphopoiesis. Overexpression of HOXB6 resulted in AML with a median latency of 223 days. Coexpression of MEIS1 dramatically shortened the onset of AML. Cytogenetic analysis of a subset of HOXB6-induced AMLs revealed recurrent deletions of chromosome bands 2D-E4, a region frequently deleted in HOXA9-induced AMLs. In vitro, HOXB6 immortalized a factor-dependent myelomonocytic precursor capable of granulocytic and monocytic differentiation. These biologic effects of HOXB6 were largely dependent on DNA binding but independent of direct interaction with PBX1.

Freitas, C. S. and S. R. Dalmau (2006). "Multiple sources of non-embryonic multipotent stem cells: processed lipoaspirates and dermis as promising alternatives to bone-marrow-derived cell therapies." *Cell Tissue Res* **325**(3): 403-11.

A body of evidence points to the existence of stem cell stores in adult tissues, in addition to the well-known hematopoietic stem cells from bone marrow. Many reports describe the ability of these multipotent cells (developmentally non-compromised with their organs of origin) to give rise to many different cell types in response to specific stimuli. This apparent plasticity provides new perspectives in tissue engineering and suggests the usefulness of these cells in future protocols of autologous transplantation, gene therapy, and tissue reconstitution in a number of pathological processes. Lipoaspirates and dermis represent accessible sources for obtaining such cells, with minimal discomfort to the donor, and might be promising candidates for cell therapy procedures once their features are experimentally accessed. The intention of the present work has been to gather

reports on the phenotypic characteristics, profile, and plastic potential of these stem cells.

Fukuda, K. (2003). "Regeneration of cardiomyocytes from bone marrow: Use of mesenchymal stem cell for cardiovascular tissue engineering." *Cytotechnology* **41**(2-3): 165-75.

We have isolated a cardiomyogenic cell line (CMG cell) from murine bone marrow mesenchymal stem cells. The cells showed a fibroblast-like morphology, but the morphology changed after 5-azacytidine exposure. They began spontaneous beating after 2 weeks, and expressed ANP and BNP. Electron microscopy revealed a cardiomyocyte-like ultrastructure. These cells had several types of action potentials; sinus node-like and ventricular cell-like action potentials. The isoform of contractile protein genes indicated that their muscle phenotype was similar to fetal ventricular cardiomyocytes. They expressed alpha(1A), alpha(1B), alpha(1D), beta(1), and beta(2) adrenergic and M(1) and M(2) muscarinic receptors. Stimulation with phenylephrine, isoproterenol and carbachol increased ERK phosphorylation and second messengers. Isoproterenol increased the beating rate, which was blocked with CGP20712A (beta(1)-selective blocker). These findings indicated that cell transplantation therapy for the patients with heart failure might possibly be achieved using the regenerated cardiomyocytes from autologous bone marrow cells in the near future.

Gindraux, F., Z. Selmani, et al. (2007). "Human and rodent bone marrow mesenchymal stem cells that express primitive stem cell markers can be directly enriched by using the CD49a molecule." *Cell Tissue Res* **327**(3): 471-83.

Bone marrow (BM) from human and rodent species contains a population of multipotential cells referred to as mesenchymal stem cells (MSCs). Currently, MSCs are isolated indirectly by using a culture step and then the generation of fibroblast colony-forming units (CFU-fs). Unprocessed or native BM MSCs have not yet been fully characterized. We have previously developed a direct enrichment method for the isolation of MSCs from human BM by using the CD49a protein (alpha1-integrin subunit). As the CD49a gene is highly conserved in mammals, we have evaluated whether this direct enrichment can be employed for BM cells from rodent strains (rat and mouse). We have also studied the native phenotype by using both immunodetection and immunomagnetic methods and have compared MSCs from mouse, rat and human BM. As is the case for human BM, we have demonstrated that all rodent multipotential CFU-fs are contained within the CD49a-positive cell population. However, in the mouse, the number of

CFU-fs is strain-dependent. Interestingly, all rat and mouse Sca-1-positive cells are concentrated within the CD49a-positive fraction and also contain all CFU-fs. In human, the colonies have been detected in the CD49a/CD133 double-positive population. Thus, the CD49a protein is a conserved marker that permits the direct enrichment of BM MSCs from various mammalian species; these cells have been phenotyped as true BM stem cells.

Goodell, M. A., K. A. Jackson, et al. (2001). "Stem cell plasticity in muscle and bone marrow." Ann N Y Acad Sci **938**: 208-18; discussion 218-20.

Recent discoveries have demonstrated the extraordinary plasticity of tissue-derived stem cells, raising fundamental questions about cell lineage relationships and suggesting the potential for novel cell-based therapies. We have examined this phenomenon in a potential reciprocal relationship between stem cells derived from the skeletal muscle and from the bone marrow. We have discovered that cells derived from the skeletal muscle of adult mice contain a remarkable capacity for hematopoietic differentiation. Cells prepared from muscle by enzymatic digestion and 5 day in vitro culture were harvested and introduced into each of six lethally irradiated recipients together with distinguishable whole bone marrow cells. Six and twelve weeks later, all recipients showed high-level engraftment of muscle-derived cells representing all major adult blood lineages. The mean total contribution of muscle cell progeny to peripheral blood was 56%, indicating that the cultured muscle cells generated approximately 10- to 14-fold more hematopoietic activity than whole bone marrow. Although the identity of the muscle-derived hematopoietic stem cells is still unknown, they may be identical to muscle satellite cells, some of which lack myogenic regulators and could respond to hematopoietic signals. We have also found that stem cells in the bone marrow can contribute to cardiac muscle repair and neovascularization after ischemic injury. We transplanted highly purified bone marrow stem cells into lethally irradiated mice that subsequently were rendered ischemic by coronary artery occlusion and reperfusion. The engrafted stem cells or their progeny differentiated into cardiomyocytes and endothelial cells and contributed to the formation of functional tissue.

Gregory, C. A., H. Singh, et al. (2003). "The Wnt signaling inhibitor dickkopf-1 is required for reentry into the cell cycle of human adult stem cells from bone marrow." J Biol Chem **278**(30): 28067-78.

Adult human mesenchymal stem cells from bone marrow stroma (hMSCs) differentiate into numerous mesenchymal tissue lineages and are

attractive candidates for cell and gene therapy. When early passage hMSCs are plated or replated at low density, the cultures display a lag phase of 3-5 days, a phase of rapid exponential growth, and then enter a stationary phase without the cultures reaching confluence. We found that as the cultures leave the lag phase, they secrete high levels of dickkopf-1 (Dkk-1), an inhibitor of the canonical Wnt signaling pathway. The addition of recombinant Dkk-1 toward the end of the lag period increased proliferation and decreased the cellular concentration of beta-catenin. The addition of antibodies to Dkk-1 in the early log phase decreased proliferation. Also, expression of Dkk-1 in hMSCs decreased during cell cycle arrest induced by serum starvation. The results indicated that high levels of Dkk-1 allow the cells to reenter the cell cycle by inhibiting the canonical Wnt/beta-catenin signaling pathway. Since antibodies to Dkk-1 also increased the lag phase of an osteosarcoma line that expressed the gene, Dkk-1 may have a similar role in some other cell systems.

Guardiola, P., G. Socie, et al. (1998). "Allogeneic stem cell transplantation for Fanconi Anaemia. Severe Aplastic Anaemia Working Party of the EBMT and EUFAR. European Group for Blood and Marrow Transplantation." Bone Marrow Transplant **21 Suppl 2**: S24-7.

Fanconi anaemia is a hereditary disorder characterised by chromosomal breaks increased by cross-linking agents. Bone marrow transplantation is the treatment of choice when a HLA identical sibling donor has been identified. The use of low-dose cyclophosphamide with thoraco-abdominal irradiation for the conditioning regimen of FA patients has led to a dramatic improvement of survival, with a long-term survival of 75% at our institution. However, if most patients are completely cured of their haematological disease, there is concern about an increased frequency of secondary tumours, mostly head and neck squamous cell carcinomas of poor prognosis. Results of BMT using alternative donors (HLA mismatched related and unrelated donors) have also improved during the last decade. A better selection of the donor via high-resolution techniques for class-II HLA matching, and more recently the use of T cell depleted grafts are probably the main explanations. Despite a short follow-up and the small number of patients analysed, transplants using HLA matched family cord blood give some promising results. On the other hand, first results with unrelated cord blood remind that this approach is clearly an experimental one that has to be evaluated through international registries and prospective studies. New approaches including autologous stem cell

transplantations and gene therapy are currently explored.

Guillaume, T., M. Sekhavat, et al. (1994). "Defective cytokine production following autologous stem cell transplantation for solid tumors and hematologic malignancies regardless of bone marrow or peripheral origin and lack of evidence for a role for interleukin-10 in delayed immune reconstitution." *Cancer Res* **54**(14): 3800-7.

A substantial body of evidence accumulated in recent years indicates a protracted delay in immune reconstitution following autologous stem cell transplantation. In order to investigate the cellular basis of this phenomenon, peripheral blood mononuclear cells were studied from recipients of autologous stem cell transplantation for solid tumors and hematological malignancies. On stimulation with phytohemagglutinin and phorbol 12-myristate 13-acetate, transplant-derived peripheral blood mononuclear cells demonstrate statistically significant depressed production of interleukin 3 (IL-3), IL-4, granulocyte-macrophage-colony-stimulating factor, and gamma-interferon as compared to normal controls, during the first 6 months following engraftment, which recover to normal levels 6 months or more posttransplant. When the overall group of transplant recipients is compared to the control group, there is a statistically significant lower production of IL-2. In addition, no differences were observed regardless of the source of the engrafted stem cells, whether from bone marrow alone (autologous bone marrow transplantation), from peripheral blood stem cells alone, or from a combination of autologous bone marrow transplantation and peripheral blood stem cells. The defect persisted past 6 months postengraftment. Transplant-derived peripheral blood mononuclear cells were stimulated with combinations of either phytohemagglutinin plus the calcium ionophore A23187, thereby circumventing the requirement for accessory cell function, or with phorbol 12-myristate 13-acetate plus anti-CD28 monoclonal antibody, mimicking the CD28-B7 cell surface-ligand interaction capable of triggering and stabilizing IL-2 gene transcription. In both situations, decreased production of IL-2 as compared to controls was observed in individuals within 6 months of transplantation. Quantitative polymerase chain reaction indicates that decreased transcription of IL-2 mRNA following transplantation is not due solely to a decrease in the absolute numbers of CD4⁺ T-cells but is secondary to reduced numbers of transcript copies per cell. Production of IL-10 was found to be decreased regardless of whether the autologous graft was of bone marrow or peripheral blood origin. These findings are consistent with the conclusion that: (a)

multiple dysregulations exist in the production of cytokines important in immune homeostasis; (b) a defect occurs at or prior to the level of transcription of IL-2 mRNA; (c) IL-10 does not play a direct role in the pathogenesis of posttransplantation immunosuppression; and (d) there is no evidence that peripheral blood stem cells may be superior to bone marrow-derived stem cells in accelerating immune reconstitution.

Haider, H., S. Jiang, et al. (2008). "IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair." *Circ Res* **103**(11): 1300-8.

We hypothesized that mesenchymal stem cells (MSCs) overexpressing insulin-like growth factor (IGF)-1 showed improved survival and engraftment in the infarcted heart and promoted stem cell recruitment through paracrine release of stromal cell-derived factor (SDF)-1alpha. Rat bone marrow-derived MSCs were used as nontransduced ((Norm)MSCs) or transduced with adenoviral-null vector ((Null)MSCs) or vector encoding for IGF-1 ((IGF-1)MSCs). (IGF-1)MSCs secreted higher IGF-1 until 12 days of observation ($P < 0.001$ versus (Null)MSCs). Molecular studies revealed activation of phosphoinositide 3-kinase, Akt, and Bcl.xL and inhibition of glycogen synthase kinase 3beta besides release of SDF-1alpha in parallel with IGF-1 expression in (IGF-1)MSCs. For in vivo studies, 70 μ L of DMEM without cells (group 1) or containing 1.5×10^6 (Null)MSCs (group 2) or (IGF-1)MSCs (group 3) were implanted intramyocardially in a female rat model of permanent coronary artery occlusion. One week later, immunoblot on rat heart tissue ($n=4$ per group) showed elevated myocardial IGF-1 and phospho-Akt in group 3 and higher survival of (IGF-1)MSCs ($P < 0.06$ versus (Null)MSCs) ($n=6$ per group). SDF-1alpha was increased in group 3 animal hearts (20-fold versus group 2), with massive mobilization and homing of ckit(+), MDR1(+), CD31(+), and CD34(+) cells into the infarcted heart. Infarction size was significantly reduced in cell transplanted groups compared with the control. Confocal imaging after immunostaining for myosin heavy chain, actinin, connexin-43, and von Willebrand factor VIII showed extensive angiomyogenesis in the infarcted heart. Indices of left ventricular function, including ejection fraction and fractional shortening, were improved in group 3 as compared with group 1 ($P < 0.05$). In conclusion, the strategy of IGF-1 transgene expression induced massive stem cell mobilization via SDF-1alpha

signaling and culminated in extensive angiomyogenesis in the infarcted heart.

Han, W., Y. Yu, et al. (2006). "Local signals in stem cell-based bone marrow regeneration." *Cell Res* 16(2): 189-95.

The cellular basis of bone marrow (BM) tissue development and regeneration is mediated through hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Local interplays between hematopoietic cells and BM stromal cells (BMSCs) determine the reconstitution of hematopoiesis after myelosuppression. Here we review the BM local signals in control of BM regeneration after insults. Hematopoietic growth factors (HGFs) and cytokines produced by BMSCs are primary factors in regulation of BM hematopoiesis. Morphogens which are critical to early embryo development in multiple species have been added to the family of HSCs regulators, including families of Wnt proteins, Notch ligands, BMPs, and Hedgehogs. Global gene expression analysis of HSCs and BMSCs has begun to reveal signature groups of genes for both cell types. More importantly, analysis of global gene expression coupled with biochemical and biological studies of local signals during BM regeneration have strongly suggested that HGFs and cytokines may not be the primary local regulators for BM recovery, rather chemokines (SDF-1, FGF-4) and angiogenic growth factors (VEGF-A, Ang-1) play instructive roles in BM reconstitution after myelosuppression. A new direction of management of BM toxicity is emerging from the identification of BM regenerative regulators.

Hardy, C. L. (2001). "Specificity of Hematopoietic Stem and Progenitor Cell Homing to Bone Marrow: A Perspective; Subject Heading." *Hematology* 5(5): 391-401.

Little is known about the hematopoietic stem and progenitor cell membrane recognition and adhesion molecules which mediate their specific patterns of movement into and out of the marrow compartment during steady state hematopoiesis and during pathological conditions. Implicit in the cellular targeting of these cells to marrow stroma, or "homing", is a high degree of molecular specificity. Identification of homing determinants and knowledge of their function in conferring specificity to these events may provide new insight into the localization of hematopoietic stem cells within the bone marrow, directly impacting clinical stem cell transplantation. In addition, a homing protein gene/promoter complex, or a stromal counter-receptor gene, may provide a valuable target for driving expression of gene constructs in early hematopoietic cells.

Haura, E. B. (2006). "Is repetitive wounding and bone marrow-derived stem cell mediated-repair an etiology of lung cancer development and dissemination?" *Med Hypotheses* 67(4): 951-6.

The prevailing view of lung cancer is multi-step progression of normal cells into cancer cells through gain of function oncogenes coupled with loss of tumor suppressor genes. This progression of genetic damage ultimately results in the hallmarks of cancer. This theory has strong support from studies finding genetic damage in early stage preneoplastic lesions in lung epithelial cells from current or former smokers. This paper discusses an alternate theory that lung cancer is a bone marrow stem cell derived disease. Chronic cigarette smoking results in lung inflammation and epithelial damage that activates a chronic wound repair program. Recent studies have demonstrated that ability of bone marrow derived stem cells to respond to epithelial wounding and contribute to epithelial repair. The identification of cancer stem cells that are distinct from the bulk tumor cells through their ability of self-renewal may suggest that such cells are important in the development of lung cancer. The evidence supporting the hypothesis along with its implications are discussed. Confirmation of the hypothesis would suggest that the transition time from a normal cell to overt cancer cell may be much shorter than that based on the multi-step cancer progression model. Additionally, if wounding in other organs is a beacon that attracts bone marrow derived tumor cells, efforts to ameliorate areas of epithelial injury and compensatory wounding may block bone marrow derived tumor cell homing, aberrant repair, and metastasis. Finally, a bone marrow derived lung cancer stem cell would require stem cell poisons for cure.

Hayakawa, J., M. Migita, et al. (2009). "Dextran sulfate and stromal cell derived factor-1 promote CXCR4 expression and improve bone marrow homing efficiency of infused hematopoietic stem cells." *J Nippon Med Sch* 76(4): 198-208.

Although the homing of hematopoietic stem cells (HSC) to the bone marrow (BM) is a crucial step in hematopoietic development and BM repopulation, the mechanisms underlying these processes have not been fully clarified. Recent studies suggest that interaction between the chemokine receptor CXCR4 and its ligand, stromal cell-derived factor 1 (SDF-1), plays a critical role in these processes. In addition, dextran sulfate increases plasma SDF-1 levels in mice and nonhuman primates. Thus, we examined the effects of preconditioning with SDF-1 and dextran sulfate on the homing efficiency of HSCs following BM transplantation in mice. We found that the

preconditioning of donor mice with either SDF-1 or dextran sulfate enhanced the homing efficiency of infused HSCs *in vivo*. The greatest effects were obtained with dextran sulfate. Moreover, reverse transcriptase polymerase chain reaction analysis demonstrated that SDF-1 and dextran sulfate increased transcription of a variety of homing-related genes, including those for CXCR4, lymphocyte function associated antigen-1, matrix metalloproteinase-9, very late antigen-4/5, and macrophage inflammatory protein-1. We suggest that whereas SDF-1 directly acts to upregulate CXCR4 expression in HSCs, dextran sulfate acts via multiple pathways involved in the induction of various homing-related molecules, in addition to SDF-1. Thus, preconditioning donors with dextran sulfate offers a novel clinical approach for improving the homing and engraftment of HSCs in the BM.

Hematti, P., S. Tuchman, et al. (2004). "Comparison of retroviral transduction efficiency in CD34+ cells derived from bone marrow versus G-CSF-mobilized or G-CSF plus stem cell factor-mobilized peripheral blood in nonhuman primates." *Stem Cells* **22**(6): 1062-9.

Hematopoietic stem cells (HSCs) are ideal targets for genetic manipulation in the treatment of several congenital and acquired disorders affecting the hematopoietic compartment. Although G-CSF-mobilized peripheral blood CD34(+) cells are the favored source of hematopoietic stem cells in clinical transplantation, this source of stem cells does not provide meaningful engraftment levels of genetically modified cells compared with G-CSF + stem cell factor (SCF)-mobilized cells in nonhuman primates. Furthermore, the use of G-CSF mobilization can have disastrous consequences in patients with sickle cell disease, a long-held target disorder for HSC-based gene therapy approaches. We therefore conducted a study to compare the levels of genetically modified cells attainable after retroviral transduction of CD34(+) cells collected from a bone marrow (BM) harvest with CD34(+) cells collected from a leukapheresis product after mobilization with G-CSF (n = 3) or G-CSF in combination with SCF (n = 3) in the rhesus macaque autologous transplantation model. Transductions were performed using retroviral vector supernatant on fibronectin-coated plates for 96 hours in the presence of stimulatory cytokines. BM was equal to or better than G-CSF-mobilized peripheral blood as a source of HSCs for retroviral transduction. Although the highest marking observed was derived from G-CSF + SCF-mobilized peripheral blood in two animals, marking in the third originated only from the BM fraction. These results demonstrate that steady-state BM is at least equivalent to G-CSF-mobilized

peripheral blood as a source of HSCs for retroviral gene transfer and the only currently available source for patients with sickle cell disease.

Henry, J. M., P. J. Sykes, et al. (1996). "Comparison of myeloma cell contamination of bone marrow and peripheral blood stem cell harvests." *Br J Haematol* **92**(3): 614-9.

It could be speculated for patients with myeloma and other lymphoproliferative disorders that peripheral blood stem cells may be preferable to bone marrow for autologous transplantation because they may be less contaminated by neoplastic cells. To test this possibility, the immunoglobulin heavy chain gene rearrangement and limiting dilution polymerase chain reaction were used to sensitively quantify myeloma cells in bone marrow and peripheral blood stem cell collections, taken at a similar time, from eight patients with multiple myeloma. Levels of residual disease in the peripheral blood stem cell harvests were variable and did not reflect the tumour burden in the marrow. Peripheral blood stem cells contained 1.7 to 23700-fold fewer myeloma cells compared with the bone marrow and would have resulted in reinfusion of 0.08 to 59480-fold fewer myeloma cells based on total reinfused CFU-GM and 0.24 to 24700-fold fewer myeloma cells based on total reinfused nucleated cells. Assuming that the proportion of clonogenic myeloma cells is equivalent, peripheral blood stem cells may be better than bone marrow as a source of hematopoietic stem cells for transplantation in multiple myeloma. The clinical followup suggested that patients transplanted with peripheral blood stem cells containing a low number of myeloma cells had better disease control than those transplanted with peripheral blood stem cells containing a high number.

Hermann, A., R. Gastl, et al. (2004). "Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells." *J Cell Sci* **117**(Pt 19): 4411-22.

Clonogenic neural stem cells (NSCs) are self-renewing cells that maintain the capacity to differentiate into brain-specific cell types, and may also replace or repair diseased brain tissue. NSCs can be directly isolated from fetal or adult nervous tissue, or derived from embryonic stem cells. Here, we describe the efficient conversion of human adult bone marrow stromal cells (hMSC) into a neural stem cell-like population (hmNSC, for human marrow-derived NSC-like cells). These cells grow in neurosphere-like structures, express high levels of early neuroectodermal markers, such as the proneural genes *NeuroD1*, *Neurog2*, *MSI1* as well as *otx1* and *nestin*, but lose the characteristics of mesodermal stromal cells. In the presence of selected growth factors,

hmNSCs can be differentiated into the three main neural phenotypes: astroglia, oligodendroglia and neurons. Clonal analysis demonstrates that individual hmNSCs are multipotent and retain the capacity to generate both glia and neurons. Our cell culture system provides a powerful tool for investigating the molecular mechanisms of neural differentiation in adult human NSCs. hmNSCs may therefore ultimately help to treat acute and chronic neurodegenerative diseases.

Hesdorffer, C., J. Ayello, et al. (1998). "Phase I trial of retroviral-mediated transfer of the human MDR1 gene as marrow chemoprotection in patients undergoing high-dose chemotherapy and autologous stem-cell transplantation." *J Clin Oncol* **16**(1): 165-72.

PURPOSE: Normal bone marrow cells have little or no expression of the MDR p-glycoprotein product and, therefore, are particularly susceptible to killing by MDR-sensitive drugs, such as vinca alkaloids, anthracyclines, podophyllins, and paclitaxel and its congeners. Here we report the results of a phase I clinical trial that tested the safety and efficacy of transfer of the human multiple drug resistance (MDR1, MDR) gene into hematopoietic stem cells and progenitors in bone marrow as a means of providing resistance of these cells to the toxic effects of cancer chemotherapy. **PATIENTS AND METHODS:** Up to one third of the harvested cells of patients who were undergoing autologous bone marrow transplantation as part of a high-dose chemotherapy treatment for advanced cancer were transduced with an MDR cDNA-containing retrovirus; these transduced cells were reinfused together with unmanipulated cells after chemotherapy. **RESULTS:** High-level MDR transduction of erythroid burst-forming unit (BFU-E) and colony-forming unit-granulocyte macrophage (CFU-GM) derived from transduced CD34+ cells was shown posttransduction and preinfusion. However, only two of the five patients showed evidence of MDR transduction of their marrow at a low level at 10 weeks and 3 weeks, respectively, posttransplantation. The cytokine-stimulated transduced cells may be out-competed in repopulation by unmanipulated normal cells that are reinfused concomitantly. The MDR retroviral supernatant that was used was shown to be free of replication-competent retrovirus (RCR) before use, and all tests of patients' samples posttransplantation were negative for RCR. In addition, no adverse events with respect to marrow engraftment or other problems related to marrow transplantation were encountered. **CONCLUSION:** These results indicate the feasibility and safety of bone marrow gene therapy with a potentially therapeutic gene, the MDR gene.

Inderbitzin, D., I. Avital, et al. (2005). "Functional comparison of bone marrow-derived liver stem cells: selection strategy for cell-based therapy." *J Gastrointest Surg* **9**(9): 1340-5.

Several distinct subpopulations of bone marrow-derived liver progenitor cells were recently described. However, there is inadequate information comparing these subpopulations from a liver-function point of view. This study was undertaken to compare two subpopulations of liver progenitors: beta(2)-microglobulin (beta(2)m)-negative/Thy-1-positive cells, and liver progenitors obtained from the non-adherent cell fraction after a panning procedure. The cells were cultured under several conditions including high- and low-dose hepatocyte growth factor, various cellular densities, and different media. Growth characteristics, liver-specific metabolic capacity, and liver regeneration-associated gene expression were studied. Both isolation procedures yielded cells that produced albumin and metabolized ammonia into urea. The study demonstrated that the beta(2)m-negative/Thy-1-positive cell fraction metabolized ammonia into urea more efficiently and produced a superior amount of albumin compared with the panned cell fraction. The beta(2)m-negative/Thy-1-positive cell fraction could be optimal for the development of novel cell-based treatment strategies for congenital or acquired liver diseases.

Irving, J. A., A. Lennard, et al. (1999). "Analysis of CD34 populations in mobilised peripheral blood stem cell harvests and in bone marrow by fluorescent in situ hybridisation for the bcr/abl gene fusion in patients with chronic granulocytic leukaemia." *Leukemia* **13**(6): 944-9.

For those patients ineligible for allogeneic bone marrow transplant and who are non-responsive to interferon, autotransplant with peripheral blood stem cells (PBSC) mobilised after intensive chemotherapy, may provide a novel approach to improve prognosis in patients with chronic granulocytic leukaemia. PBSC harvests are assessed for CD34-positive cell numbers, which serve as an indicator of engraftment potential, and are also analysed cytogenetically to ascertain tumour cell contamination. However, a more accurate assessment of PBSC harvest contamination requires investigation of the Philadelphia (Ph) status of the CD34pos population, in which the cells that provide long-term engraftment are contained. In this study, we have analysed these levels in mobilised PBSC and also in bone marrow (BM) harvests, taken several weeks prior to mobilising chemotherapy. Using fluorescent in situ hybridisation for the bcr/abl gene fusion, we have shown that the median number of Ph negative cells in CD34pos isolated populations was 14.95% in

BM compared to 79.05% in PBSC harvests and that in all PBSC samples tested, Ph positivity in CD34pos populations was always detectable either by FISH or one round PCR methods. In paired assessments of both PBSC and BM harvests, higher levels of Ph negative CD34pos cells ($> \text{ or } = 14\%$) isolated from BM harvests, taken prior to intensive chemotherapy, correlated with higher levels of Ph negative CD34pos cells ($> \text{ or } = 78.5\%$) in PBSC harvests. These data may aid in the selection of patients for whom PBSC harvesting, after mobilisation, is more likely to achieve an autograft product containing predominantly Ph negative CD34pos cells and may exclude those patients for whom the risk, morbidity and expense of stem cell harvesting may have no apparent benefit over a chronic phase BM harvest.

Ishizuka, T., K. Chayama, et al. (1999). "Mitogen-activated protein kinase activation through Fc epsilon receptor I and stem cell factor receptor is differentially regulated by phosphatidylinositol 3-kinase and calcineurin in mouse bone marrow-derived mast cells." *J Immunol* **162**(4): 2087-94.

Aggregation of high affinity FcR for IgE (Fc epsilon RI) on mast cells activates intracellular signal transduction pathways, including the activation of protein tyrosine kinases, phosphatidylinositol 3-kinase (PI3-kinase), and protein kinase C. Binding of stem cell factor (SCF) to its receptor (SCFR, c-Kit) on mast cells also induces increases in intrinsic tyrosine kinase activity and activation of PI3-kinase. Although ligation of both receptors induces Ras and Raf-1 activation, the downstream consequences of these early activation events are not well defined, except for the activation of extracellular signal-regulated kinases (ERK). Addition of Ag (OVA) to mouse bone marrow-derived mast cells (BMMC) sensitized with anti-OVA IgE triggers the activation of three members of the mitogen-activated protein (MAP) kinase family, c-Jun amino-terminal kinase (JNK), p38 MAP kinase (p38), and extracellular signal-regulated kinases. SCF similarly activates all three MAP kinases. Wortmannin, an inhibitor of PI3-kinase, inhibited both Fc epsilon RI- and SCFR-mediated JNK activation and partially inhibited Fc epsilon RI, but not SCFR-mediated p38 activation. Cyclosporin A inhibited Fc epsilon RI-mediated JNK and p38 activation, but did not affect the activation of these kinases when stimulated through the SCFR. Wortmannin and cyclosporin A inhibited Fc epsilon RI-mediated production of TNF-alpha and IL-4 in addition to serotonin release in BMMC. These results indicate that both PI3-kinase and calcineurin may contribute to the regulation of cytokine gene transcription and the degranulation response by modulating JNK activity in BMMC.

Jootar, S., N. Pornprasertsud, et al. (2006). "Bone marrow derived mesenchymal stem cells from chronic myeloid leukemia t(9;22) patients are devoid of Philadelphia chromosome and support cord blood stem cell expansion." *Leuk Res* **30**(12): 1493-8.

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of hematopoietic stem cells. It is characterized at cytogenetic level by the Philadelphia (Ph) chromosome and at the molecular level by the BCR/ABL gene rearrangement. Bone marrow derived mesenchymal stem cells (MSCs) are also pluripotent stem cells that can differentiate into several mesenchymal tissues. To date, no study has been performed to characterize whether MSCs from CML harbor the abnormal Ph chromosome similar to CML bone marrow cells. We isolated and characterized MSCs from diagnostic marrow samples (n=11) and showed that MSCs can be readily isolated from CML marrow and exhibit major expansion potential as well as intact osteogenic differentiation ability. Moreover, they do not harbor the Ph chromosome confirmed by fluorescence in situ hybridization (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR). Thus, we demonstrated that CML marrow is an abundant source of MSCs appearing through both FISH and RT-PCR not to be involved by the malignant process of CML. Furthermore, these MSCs from a CML patient could support in vitro cord blood expansion as those MSCs from a normal donor. Since MSCs are able to support engraftment of hematopoietic stem cells in stem cell transplantation (SCT) as well as suppress alloreactive T cells causing graft-versus-host disease, this current report thus provides evidence that in a SCT setting of CML patients, autologous MSCs could be a source of stem cell support in future cell therapy applications.

Kalberer, C. P., R. Pawliuk, et al. (2000). "Preselection of retrovirally transduced bone marrow avoids subsequent stem cell gene silencing and age-dependent extinction of expression of human beta-globin in engrafted mice." *Proc Natl Acad Sci U S A* **97**(10): 5411-5.

Transcriptional silencing of genes transferred into hematopoietic stem cells poses one of the most significant challenges to the success of gene therapy. If the transferred gene is not completely silenced, a progressive decline in gene expression as the mice age often is encountered. These phenomena were observed to various degrees in mouse transplant experiments using retroviral vectors containing a human beta-globin gene, even when cis-linked to locus control region derivatives. Here, we have investigated whether ex vivo preselection of retrovirally transduced stem cells on the basis of expression of the gene

fluorescent protein driven by the CpG island phosphoglycerate kinase promoter can ensure subsequent long-term expression of a cis-linked beta-globin gene in the erythroid lineage of transplanted mice. We observed that 100% of mice (n = 7) engrafted with preselected cells concurrently expressed human beta-globin and the green fluorescent protein in 20-95% of their RBC for up to 9.5 mo posttransplantation, the longest time point assessed. This expression pattern was successfully transferred to secondary transplant recipients. In the presence of beta-locus control region hypersensitive site 2 alone, human beta-globin mRNA expression levels ranged from 0.15% to 20% with human beta-globin chains detected by HPLC. Neither the proportion of positive blood cells nor the average expression levels declined with time in transplanted recipients. Although suboptimal expression levels and heterocellular position effects persisted, *in vivo* stem cell gene silencing and age-dependent extinction of expression were avoided. These findings support the further investigation of this type of vector for the gene therapy of human hemoglobinopathies.

Kiem, H. P., R. G. Andrews, et al. (1998). "Improved gene transfer into baboon marrow repopulating cells using recombinant human fibronectin fragment CH-296 in combination with interleukin-6, stem cell factor, FLT-3 ligand, and megakaryocyte growth and development factor." *Blood* **92**(6): 1878-86.

We have used a competitive repopulation assay in baboons to develop improved methods for hematopoietic stem cell transduction and have previously shown increased gene transfer into baboon marrow repopulating cells using a gibbon ape leukemia virus (GALV)-pseudotype retroviral vector (Kiem et al, *Blood* 90:4638, 1997). In this study using GALV-pseudotype vectors, we examined additional variables that have been reported to increase gene transfer into hematopoietic progenitor cells in culture for their ability to increase gene transfer into baboon hematopoietic repopulating cells. Baboon marrow was harvested after *in vivo* administration (priming) of stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF). CD34-enriched marrow cells were divided into two equal fractions to directly compare transduction efficiencies under different gene transfer conditions. Transduction by either incubation with retroviral vectors on CH-296-coated flasks or by cocultivation on vector-producing cells was studied in five animals; in one animal, transduction on CH-296 was compared with transduction on bovine serum albumin (BSA)-coated flasks. The highest level of gene transfer was obtained after 24 hours of prestimulation followed by 48 hours of incubation on CH-296 in vector-containing medium in the presence

of multiple hematopoietic growth factors (interleukin-6, stem cell factor, FLT-3 ligand, and megakaryocyte growth and development factor). Using these conditions, up to 20% of peripheral blood and marrow cells contained vector sequences for more than 20 weeks, as determined by both polymerase chain reaction and Southern blot analysis. Gene transfer rates were higher for cells transduced on CH-296 as compared with BSA or cocultivation. In one animal, we have used a vector expressing a cell surface protein (human placental alkaline phosphatase) and have detected 10% and 5% of peripheral blood cells expressing the transduced gene 2 and 4 weeks after transplantation as measured by flow cytometry. In conclusion, the conditions described here have resulted in gene transfer rates that will allow detection of transduced cells by flow cytometry to facilitate the evaluation of gene expression. The levels of gene transfer obtained with these conditions suggest the potential for therapeutic efficacy in diseases affecting the hematopoietic system.

Kim, D. H., K. H. Yoo, et al. (2005). "Gene expression profile of cytokine and growth factor during differentiation of bone marrow-derived mesenchymal stem cell." *Cytokine* **31**(2): 119-26.

Mesenchymal stem cells (MSCs), which are adherent stromal cells of a nonhematopoietic origin, have the ability to give rise to various differentiated cell types. MSCs regulate localization, self-renewal and differentiation of hematopoietic stem cells (HSCs) due to MSCs' secretion of cytokines and growth factors, the cell-to-cell interactions and the influence of the extracellular matrix proteins. Using RT-PCR analysis, we examined the expression levels of cytokines and growth factors from MSCs and their differentiated cell types, including osteoblasts, adipocytes and endothelial cells. Cytokine and growth factor genes, including IL-6, IL-8, IL-11, IL-12, IL-14, IL-15, LIF, G-CSF, GM-CSF, M-SCF, FL and SCF, were found to be expressed in the MSCs. In contrast, there was no IL-1alpha, IL-1beta, or IL-7 expression observed. The IL-12, IL-14, G-CSF, and GM-CSF mRNA expression levels either disappeared or decreased after the MSCs differentiated into osteoblasts, adipocytes, and endothelial cells. Among the differentiated cells derived from MSCs, osteoblasts, adipocytes, and endothelial cells expressed the osteopontin, aP2, and the VEGFR-2 gene, respectively. These profiles could help determine future clinical applications of MSCs and their derivatives for cell therapy.

Koide, Y., S. Morikawa, et al. (2007). "Two distinct stem cell lineages in murine bone marrow." *Stem Cells* **25**(5): 1213-21.

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.

Komatsu, F. and M. Kajiwara (1996). "Usefulness of lymphokine-activated killer cells generated from bone marrow mononuclear cells for the purging of residual tumor cells in peripheral blood stem cell graft." *Jpn J Cancer Res* **87**(2): 161-9.

Lymphokine-activated killer (LAK) cells were generated from bone marrow mononuclear cells (BM), and the usefulness of the BM-LAK for purging of residual tumor cells in autologous peripheral blood stem cell (PBSC) graft was determined. The BM and peripheral blood lymphocytes (PBL) were obtained from the same bone marrow donors. The BM-LAK and PBL-LAK were generated by incubation with interleukin-2 for 7 days. The BM-LAK demonstrated higher killer activity against a lymphoma cell line Raji than the PBL-LAK. The BM-LAK also had a higher percentage of CD4-CD8-CD16+ cells than the PBL-LAK, which suggests that their high killer activity is related to these cells. The BM-LAK did not show any

killer activity against the PBSC graft. However, they killed tumor cells which contaminated the PBSC graft, and in particular, killed chimeric bcr/abl messenger RNA-positive residual leukemic cells. These results suggest that the BM-LAK may be applicable for purging. As the BM-LAK possess higher killer activity than the PBL-LAK, they may be more useful than the PBL-LAK.

Koppula, P. R., L. K. Chelluri, et al. (2009). "Histocompatibility testing of cultivated human bone marrow stromal cells - a promising step towards pre-clinical screening for allogeneic stem cell therapy." *Cell Immunol* **259**(1): 61-5.

Mesenchymal stem cells (MSCs) lack major histocompatibility complex (MHC)-II and only show minimal MHC-I expression. Despite MSCs demonstrating T-cell anergy, there are no established methods to evaluate their suitability. It is crucial to evaluate the complete mismatch of MHC compatibility in view of the hypo-immunogenic nature and immunomodulatory properties of MSCs with respect to their proliferation potential (PP) and utility in terms of passage number. With bone marrow (BM) being the major source of MSCs, the use of these cells becomes even more complicated, due to many other receptors coming to fore and triggering alternative pathways. This prospective study included five BM aspirates for MSC cultures and five allogeneic peripheral blood mono nuclear cells (PBMNCs) from healthy volunteers. MHC compatibility was assessed by polymerase chain reaction-sequence specific primer (PCR-SSP). The PP and a T-cell response to MSCs was addressed in mixed cultures and evaluated on the basis of their stimulation index (SI). Allogeneic circulatory antibodies against the donor MSCs was performed by cytotoxicity assay. The PP of MSCs during interactions with PBMNCs (T-cells) demonstrated T-cell anergy and the response to circulatory antibodies was minimal, in consonance with other published reports. Although, the results are encouraging for potential clinical application of MSC transplantation, autologous is always preferable to allogeneic, at least until the long-term safety of these cells is established in clinical trials.

Krampera, M., A. Pasini, et al. (2005). "HB-EGF/HER-1 signaling in bone marrow mesenchymal stem cells: inducing cell expansion and reversibly preventing multilineage differentiation." *Blood* **106**(1): 59-66.

Epidermal growth factor receptor-1 (EGFR-1/HER-1/ErbB-1) regulates proliferation and cell fate during epidermal development. HER-1 is activated by several EGF-family ligands including heparin-binding epidermal growth factor-like growth factor (HB-EGF),

a mitogenic and chemotactic molecule that participates in tissue repair, tumor growth, and other tissue-modeling phenomena, such as angiogenesis and fibrogenesis. We found that mesenchymal stem cells (MSCs), the precursors of different mesenchymal tissues with a role in processes in which HB-EGF is often involved, normally express HER-1, but not HB-EGF itself. Under the effect of HB-EGF, MSCs proliferate more rapidly and persistently, without undergoing spontaneous differentiation. This effect occurs in a dose-dependent fashion, and is specific, direct, and HER-1 mediated, as it is inhibited by anti-HER-1 and anti-HB-EGF blocking antibodies. Moreover, HB-EGF reversibly prevents adipogenic, osteogenic, and chondrogenic differentiation induced with specific media. These data show that HB-EGF/HER-1 signaling is relevant to MSC biology, by regulating both proliferation and differentiation.

Krieg, A. M., M. F. Gourley, et al. (1991). "Association of murine lupus and thymic full-length endogenous retroviral expression maps to a bone marrow stem cell." *J Immunol* **146**(9): 3002-5.

Recent studies of thymic gene expression in murine lupus have demonstrated 8.4-kb (full-length size) modified polytropic (Mpmv) endogenous retroviral RNA. In contrast, normal control mouse strains do not produce detectable amounts of such RNA in their thymuses. Prior studies have attributed a defect in experimental tolerance in murine lupus to a bone marrow stem cell rather than to the thymic epithelium; in contrast, infectious retroviral expression has been associated with the thymic epithelium, rather than with the bone marrow stem cell. The present study was designed to determine whether the abnormal Mpmv expression associated with murine lupus mapped to thymic epithelium or to a marrow precursor. Lethally irradiated control and lupus-prone mice were reconstituted with T cell depleted bone marrow; one month later their thymuses were studied for endogenous retroviral RNA and protein expression. Recipients of bone marrow from nonautoimmune donors expressed neither 8.4-kb Mpmv RNA nor surface MCF gp70 in their thymuses. In contrast, recipients of bone marrow from autoimmune NZB or BXSb donors expressed thymic 8.4-kb Mpmv RNA and mink cell focus-forming gp70. These studies demonstrate that lupus-associated 8.4-kb Mpmv endogenous retroviral expression is determined by bone marrow stem cells.

Kubo, H., M. Shimizu, et al. (2009). "Identification of mesenchymal stem cell (MSC)-transcription factors by microarray and knockdown analyses, and signature molecule-marked MSC in bone marrow by immunohistochemistry." *Genes Cells* **14**(3): 407-24.

Although ex vivo expanded mesenchymal stem cells (MSC) have been used in numerous studies, the molecular signature and in vivo distribution status of MSC remain unknown. To address this matter, we identified numerous human MSC-characteristic genes--including nine transcription factor genes--using DNA microarray and real-time RT-PCR analyses: Most of the MSC-characteristic genes were down-regulated 24 h after incubation with osteogenesis-, chondrogenesis- or adipogenesis-induction medium, or 48-72 h after knockdown of the nine transcription factors. Furthermore, knockdowns of ETV1, ETV5, FOXP1, GATA6, HMGA2, SIM2 or SOX11 suppressed the self-renewal capacity of MSC, whereas those of FOXP1, SOX11, ETV1, SIM2 or PRDM16 reduced the osteogenic- and/or adipogenic potential. In addition, immunohistochemistry using antibodies for the MSC characteristic molecules--including GATA6, TRPC4, FLG and TGM2--revealed that MSC-like cells were present near the endosteum and in the interior of bone marrow of adult mice. These findings indicate that MSC synthesize a set of MSC markers in vitro and in vivo, and that MSC-characteristic transcription factors are involved in MSC stemness regulation.

Kubonishi, I., S. Takeuchi, et al. (1995). "Direct transplantation of chronic myelogenous leukemia cells into nude mice and establishment of a leukemic stem cell (Ph1+, CD34+) line dependent on mouse bone marrow stromal cells in vitro." *Jpn J Cancer Res* **86**(5): 451-9.

Peripheral blood cells from a female patient with Ph1-positive chronic myelogenous leukemia (CML) in blast crisis were serially transplanted in BALB/c nude mice for 16 passages. This in vivo cell line, designated CML-N-1, had Ph1 chromosome abnormality and BCR gene rearrangement. The cells expressed CD11b, CD13, CD33, CD34, CD38, and HLA-DR antigens until the 11th passage and subcutaneous tumors produced by these passages were composed of admixtures of immature and maturing cells that differentiated to basophils when cultured in vitro. From the 12th passage on, the tumors became composed mainly of immature cells expressing CD13, CD34, and HLA-DR, and no longer differentiated to basophils even upon in vitro culture. In contrast to the vigorous proliferation in vivo, CML-N-1 cells from any passage failed to proliferate in vitro under standard liquid culture conditions with or without growth factors, such as granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, monocyte colony-stimulating factor, interleukin 3, interleukin 6 and stem cell factor. However, a continuously growing cell line, designated CML-C-1, was established by culturing CML-N-1

cells on feeder layers of mouse bone marrow stromal cells. This mouse bone marrow stromal cell-dependent cell line showed immature cell morphology and expressed early myeloid phenotype positive for CD13, CD34, and HLA-DR. These results indicate that mouse bone marrow stromal cells provide a certain growth factor(s) active on human leukemia cells.

LaBarge, M. A. and H. M. Blau (2002). "Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury." *Cell* **111**(4): 589-601.

Adult bone marrow-derived cells (BMDC) are shown to contribute to muscle tissue in a step-wise biological progression. Following irradiation-induced damage, transplanted GFP-labeled BMDC become satellite cells: membrane-ensheathed mononucleate muscle stem cells. Following a subsequent exercise-induced damage, GFP-labeled multinucleate myofibers are detected. Isolated GFP-labeled satellite cells are heritably myogenic. They express three characteristic muscle markers, are karyotypically diploid, and form clones that can fuse into multinucleate cells in culture or into myofibers after injection into mouse muscles. These results suggest that two temporally distinct injury-related signals first induce BMDC to occupy the muscle stem cell niche and then to help regenerate mature muscle fibers. The stress-induced progression of BMDC to muscle satellite cell to muscle fiber results in a contribution to as many as 3.5% of muscle fibers and is due to developmental plasticity in response to environmental cues.

Lambert, J. F., M. Liu, et al. (2003). "Marrow stem cells shift gene expression and engraftment phenotype with cell cycle transit." *J Exp Med* **197**(11): 1563-72.

We studied the genetic and engraftment phenotype of highly purified murine hematopoietic stem cells (lineage negative, rhodamine-low, Hoechst-low) through cytokine-stimulated cell cycle. Cells were cultured in interleukin (IL)-3, IL-6, IL-11, and steel factor for 0 to 48 h and tested for engraftment capacity in a lethally irradiated murine competitive transplant model. Engraftment showed major fluctuations with nadirs at 36 and 48 h of culture and recovery during the next G1. Gene expression of quiescent (0 h) or cycling (48 h) stem cells was compared with lineage positive cells by 3' end PCR differential display analysis. Individual PCR bands were quantified using a 0 to 9 scale and results were visually compared using color-coded matrices. We defined a set of 637 transcripts expressed in stem cells and not expressed in lineage positive cells. Gene expression analyzed at 0 and 48 h showed a major shift from "stem cell genes" being highly expressed at

0 h and turned off at 48 h, while "cell division" genes were turned on at 48 h. These observations suggest stem cell gene expression shifts through cell cycle in relation to cell cycle related alterations of stem cell phenotype. The engraftment defect is related to a major phenotypic change of the stem cell.

Lavery, K., P. Swain, et al. (2008). "BMP-2/4 and BMP-6/7 differentially utilize cell surface receptors to induce osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells." *J Biol Chem* **283**(30): 20948-58.

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-beta superfamily of growth factors and are used clinically to induce new bone formation. The purpose of this study was to evaluate receptor utilization by BMP-2, BMP-4, BMP-6, and BMP-7 in primary human mesenchymal stem cells (hMSC), a physiologically relevant cell type that probably mediates the in vivo effects of BMPs. RNA interference-mediated gene knockdown revealed that osteoinductive BMP activities in hMSC are elicited through the type I receptors ACVR1A and BMPR1A and the type II receptors ACVR2A and BMPR2. BMPR1B and ACVR2B were expressed at low levels and were not found to play a significant role in signaling by any of the BMPs evaluated in this study. Type II receptor utilization differed significantly between BMP-2/4 and BMP-6/7. A greater reliance on BMPR2 was observed for BMP-2/4 relative to BMP-6/7, whereas ACVR2A was more critical to signaling by BMP-6/7 than BMP-2/4. Significant differences were also observed for the type I receptors. Although BMP-2/4 used predominantly BMPR1A for signaling, ACVR1A was the preferred type I receptor for BMP-6/7. Signaling by both BMP-2/4 and BMP-6/7 was mediated by homodimers of ACVR1A or BMPR1A. A portion of BMP-2/4 signaling also required concurrent BMPR1A and ACVR1A expression, suggesting that BMP-2/4 signal in part through ACVR1A/BMPR1A heterodimers. The capacity of ACVR1A and BMPR1A to form homodimers and heterodimers was confirmed by bioluminescence resonance energy transfer analyses. These results suggest different mechanisms for BMP-2/4- and BMP-6/7-induced osteoblastic differentiation in primary hMSC.

Lee, E., J. Yook, et al. (2005). "Induction of Ym1/2 in mouse bone marrow-derived mast cells by IL-4 and identification of Ym1/2 in connective tissue type-like mast cells derived from bone marrow cells cultured with IL-4 and stem cell factor." *Immunol Cell Biol* **83**(5): 468-74.

Mast cells play an important role in allergic inflammation by releasing various bioactive mediators. The function of mast cells is enhanced by various stimuli, partly due to the induction of specific genes and their products. Although many inducible genes have been identified, a significant number of genes remain to be identified. Therefore, this study used PCR-selected cDNA subtraction to establish the profile of induced genes in the connective tissue (CT) type-like mast cells derived from bone marrow cells cultured in the presence of IL-4 and stem cell factor. Two hundred and fifty cDNA clones were obtained from the CT type-like mast cells by PCR-selected cDNA subtraction. Among them, Ym1/2, a chitinase-like protein, is one of the most abundantly induced genes. Ym1 is produced by activated macrophages in a parasitic infection, whereas its isotype, Ym2, is highly upregulated in allergic lung disease. In order to differentiate which isotype is expressed in bone marrow cells, specific primers for bone marrow-derived mast cells (BMMC), and CT type-like mast cells were used for RT-PCR. The results showed that Ym1 was constitutively expressed in bone marrow cells and gradually decreased in the presence of IL-3, whereas Ym2 was induced only in the presence of IL-4. CT type-like mast cells from bone marrow cells expressed Ym1 throughout the culture period and Ym2 was induced only by the addition of IL-4 into BMMC, indicating that IL-4 is essential for the expression of Ym1/2 genes.

Lemieux, C., I. Cloutier, et al. (2008). "Estrogen-induced gene expression in bone marrow c-kit⁺ stem cells and stromal cells: identification of specific biological processes involved in the functional organization of the stem cell niche." *Stem Cells Dev* 17(6): 1153-63.

The recent interest in the role of bone marrow (BM)-derived endothelial progenitor cells (EPCs) and the benefits of estrogen on cardiovascular health brought us to evaluate if estrogen could affect cardiac repair more broadly by regulating biological processes involved in the functional organization of the BM stem cell (SC) niche. To assess such possibility, we evaluated gene expression profiles of BM c-kit⁺ SCs and CD44⁺ stromal cells (StroCs) after exposure to a physiological concentration of 17beta-estradiol (17betaE). Data analysis showed that 17betaE altered the expression (>1.5 fold) of 509 and 682 gene probes in c-kit⁺ SCs and CD44⁺ StroCs, respectively. Among them, 199 genes in c-kit⁺ SCs and 283 in CD44⁺ StroCs were associated to biological process categories of the Gene Ontology classification. Within processes highly regulated by 17betaE, we identified key factors involved in adhesion, migration, proteolysis, and signaling by

which 17betaE influences physiological regulation of the functional organization of the SC niche. Together, our results demonstrate that estrogen benefits on cardiovascular health could involve other BM-derived cells than EPCs and that this capacity of estrogen to influence the physiology of the BM SC niche deserves to be investigated clinically.

Leng, Q., Y. Nie, et al. (2008). "Elevated CXCL12 expression in the bone marrow of NOD mice is associated with altered T cell and stem cell trafficking and diabetes development." *BMC Immunol* 9: 51.

BACKGROUND: Type I diabetes (T1D) is an autoimmune disease resulting from destruction of the insulin-producing beta-cells by autoreactive T cells. Studies have shown that polymorphisms of chemokine CXCL12 gene are linked to T1D in humans. In non-obese diabetic (NOD) mice, which are predisposed to develop the disease, reduction of CXCL12 level leads to significant delays in the onset of diabetes. Despite these initial observations, however, how CXCL12 affects development of T1D has not been fully investigated. **RESULTS:** We found that the level of CXCL12 transcript is significantly elevated in the bone marrow of NOD mice as compared to Balb/c and C57BL/6 mice. Correspondingly, naive T cells, regulatory T cells and hematopoietic stem cells (HSC) accumulate in the bone marrow of NOD mice. Treatment of NOD mice with AMD3100, an antagonist for CXCL12's receptor CXCR4, mobilizes T cells and HSC from the bone marrow to the periphery, concomitantly inhibits insulinitis and delays the onset of diabetes. **CONCLUSION:** These results suggest that the elevated CXCL12 expression promotes T1D in NOD mice by altering T cell and hematopoietic stem cell trafficking. The findings highlight the potential usefulness of AMD3100 to treat or prevent T1D in humans.

Letchford, J., A. M. Cardwell, et al. (2006). "Isolation of C15: a novel antibody generated by phage display against mesenchymal stem cell-enriched fractions of adult human marrow." *J Immunol Methods* 308(1-2): 124-37.

Adult bone marrow stroma contains a source of mesenchymal stem cells (MSC) that have the capacity to self-renew and differentiate into multiple stromal lineages. These rare cells can be visualised indirectly by the formation of heterogeneous colonies, containing stem cells and their differentiated progeny in long-term culture. If MSC and their associated progenitor and precursor populations are to reach their full therapeutic potential, markers will be required to identify and characterize specific bone marrow stromal subsets. We sought to use phage display to

generate antibodies against bone marrow mononuclear cells (BMMNC) enriched for colony forming cells. Initially, we identified our target cell population by comparing the colony forming efficiency (CFE) of CD49a-positive, STRO-1-positive and CD45-negative BMMNC subpopulations with unseparated BMMNC. Selection with anti-CD49a gave the greatest enrichment (19-fold) of colony forming cells and in light of these findings, we generated phage antibodies against CD49a-positive BMMNC by simultaneous positive/negative selection. A dominant clone (C15), generated after 3 rounds of selection, has been isolated and sequenced, then characterized for cell and tissue specificity. Sequence analysis showed that the V(H) and V(L) gene segments of C15 aligned most closely to the VH26/DP-47 and IGLV3S1/DPL16 germline V segments found in the synthetic repertoire. C15 bound to 4% of freshly isolated BMMNC and localized to osteoblastic cells and proximal marrow cells in areas of active bone formation in sections of osteophyte. C15 binding was upregulated in cultured bone marrow stromal cells (BMSC) and was also detected on bone-derived cell lines. This report demonstrates that phage display is a powerful tool for the isolation of antibodies against rare cell populations, and provides a platform for the future application of this technology in the search for antigens on MSC and other rare cell populations.

Lin, P., L. Chen, et al. (2009). "Evaluation of stem cell differentiation in diabetic rats transplanted with bone marrow mesenchymal stem cells." *Transplant Proc* **41**(5): 1891-3.

OBJECTIVE: Bone marrow mesenchymal stem cell (BM-MSC) transplantation has generated a great deal of excitement as a promising therapeutic strategy for diabetes mellitus. However, the exact mechanisms of reversing hyperglycemia remain elusive. Our objective was to investigate whether stem cell differentiation determined therapeutic efficacy. **MATERIALS AND METHODS:** Wistar rats were rendered diabetic by an intraperitoneal injection of streptozotocin. BM-MSCs isolated from diabetic Wistar rats were analyzed for phenotype characteristics. Subsequently, BM-MSCs were transplanted into diabetic rats, followed by intravenous injection of recombinant lentiviruses encoding 2 different small hairpin RNAs (shRNAs) for specific interference with neurogenin 3 (Ngn3). We measured blood glucose levels and insulin and performed histological analysis of the pancreas. **RESULTS:** BM-MSCs lowered blood glucose by increasing beta-cell mass compared with sham-operated controls, but this effect was inhibited by interference with the Ngn3 gene. **CONCLUSION:** Differentiation of stem cells, including BM-MSCs and

endogenous pancreatic stem cells, plays a major role in the process of reversing hyperglycemia.

Linenberger, M. L., F. W. Jacobson, et al. (1995). "Stem cell factor production by human marrow stromal fibroblasts." *Exp Hematol* **23**(10): 1104-14.

To characterize the production of stem cell factor (SCF, the ligand for the c-kit receptor protein) and its regulation by inflammatory cytokines and glucocorticoids, primary marrow stromal fibroblasts were isolated from normal individuals and two patients with Diamond-Blackfan anemia. Unstimulated normal marrow stromal fibroblasts constitutively expressed a low level of SCF mRNA (9 +/- 2 copies/cell [mean +/- SEM]), continually secreted soluble SCF into the supernatant of 1- to 5-day-old cultures (0.16 +/- 0.02 to 0.73 +/- 0.04 ng/mL per 10(6) cells, respectively), and expressed membrane-bound SCF. Stimulation with interleukin-1 beta (IL-1 beta) only modestly increased SCF mRNA levels, soluble SCF production at 24 hours, and membrane-bound SCF. In comparison, hydrocortisone or tumor necrosis factor alpha (TNF-alpha) exposure increased SCF mRNA levels 3.5- to four-fold above controls, but with different kinetics. The peak TNF-alpha effect was at 6 hours, with return to near control levels at 24 hours, whereas hydrocortisone induced maximal mRNA increases at 12 to 18 hours, and the levels remained high at 24 hours. Similarly, a sustained increase in soluble SCF production was detected during 1 to 5 days of hydrocortisone exposure (0.27 +/- 0.03 to 1.10 +/- 0.08 ng/mL per 10(6) cells), while TNF-alpha stimulation modestly increased the production of soluble SCF in 24-hour cultures only. Unstimulated normal marrow fibroblasts expressed predominantly the long species of alternatively spliced SCF mRNA, and the relative amounts of long and short mRNAs did not change after stimulation with IL-1 beta, hydrocortisone, or TNF-alpha. SCF production by marrow stromal fibroblasts from a symptomatic patient with Diamond-Blackfan anemia was equivalent to simultaneously studied normal marrow fibroblasts. In contrast, marrow fibroblasts from a Diamond-Blackfan anemia patient in untreated hematologic remission constitutively expressed high levels of SCF mRNA (21 +/- 4 copies/cell) and soluble protein (0.40 ng/mL per 10(6) cells at 24 hours). Together, these observations suggest that SCF is constitutively produced by fibroblasts in the human marrow microenvironment and that hydrocortisone induces a modest but sustained increase in SCF gene expression and protein production, compared to only a transient increase induced by TNF-alpha. In addition, these findings support the hypothesis that endogenous or corticosteroid-induced increases in the production of

SCF could play a physiologic role in the clinical improvement of congenital anemia.

Lutz, M., M. Rosenberg, et al. (2008). "Local injection of stem cell factor (SCF) improves myocardial homing of systemically delivered c-kit + bone marrow-derived stem cells." *Cardiovasc Res* 77(1): 143-50.

AIMS: Recent studies have shown that stem cell therapy may alleviate the detrimental effects of myocardial infarction. Yet, most of these reports observed only modest effects on cardiac function, suggesting that there still is need for improvement before widespread clinical use. One potential approach would be to increase migration of stem cells to the heart. We therefore tested whether local administration of stem cell factor (SCF) improves myocardial homing of intravenously infused lin-/c-kit+ stem cells after myocardial infarction. **METHODS AND RESULTS:** Myocardial infarction was induced in mice via ligation of the left anterior descending artery and 2.5 microg of SCF were injected into the peri-infarct zone. Sham-operated mice and animals with intramyocardial injection of phosphate-buffered saline (PBS) served as controls. Twenty-four hours after myocardial infarction, lin-/c-kit+ stem cells were separated from murine bone marrow by magnetic cell sorting, labelled with the green fluorescent cell tracker CFDA or 111 Indium, and subsequently 750 000 labelled cells were systemically infused via the tail vein. Another 24 or 72 h later, respectively (i.e. 48 and 96 h after myocardial infarction), hearts were removed and analysed for myocardial homing of stem cells. Green fluorescent stem cells were exclusively detected in the peri-infarct zone of animals having prior SCF treatment. Radioactive measurements revealed that an intramyocardial SCF injection significantly amplified myocardial homing of lin-/c-kit+ stem cells compared to animals with PBS injections (3.58 +/- 0.53 vs. 2.28 +/- 0.23 cpm/mg/10(6)cpm, +60%, P < 0.05) and sham-operated mice without myocardial infarction (3.58 +/- 0.53 vs. 1.95 +/- 0.22 cpm/mg/10(6)cpm, +85%, P < 0.01). Similar results were obtained 72 h after stem cell injection. **CONCLUSION:** We demonstrate that intramyocardial administration of SCF sustainably directs more lin-/c-kit+ stem cells to the heart. Future studies will have to show whether higher levels of myocardial SCF (i.e. by virus-mediated gene transfer) can further improve homing of systemically delivered c-kit+ stem cells and thus favourably influence cardiac remodelling following myocardial infarction.

Ma, J., J. Ge, et al. (2005). "Time course of myocardial stromal cell-derived factor 1 expression

and beneficial effects of intravenously administered bone marrow stem cells in rats with experimental myocardial infarction." *Basic Res Cardiol* 100(3): 217-23.

OBJECTIVE: The chemokine stromal cell-derived factor-1 (SDF-1) has been implicated in homing of bone marrow cells to sites of injury. We investigated the time course of myocardial SDF-1 expression and effects of intravenously administered bone marrow mesenchymal stem cells (MSC) in rats with myocardial infarction (MI). **METHODS:** SDF-1 expression was measured by RT-PCR and Western blot in sham operated or infarcted hearts at 1/2, 1, 2, 4, 8 and 16 days post operation. MSCs from donor rats were labeled with BrdU. A total of 5 x 10(6) cells in 2.5 mL of PBS or equal volume PBS alone were injected through the tail vein at above mentioned time points. The number of the labeled MSCs in the infarcted hearts was counted 3 days post injection. Cardiac function and vessel numbers were assessed 28 days post injection. **RESULTS:** Myocardial SDF-1 expression increased and peaked at the first day and decreased thereafter post MI and remained unchanged in sham operated hearts. The MSCs enrichment and angiogenesis in the host hearts were more abundant in the 1 day transplantation group than in the other groups (P < 0.01). Cardiac function was only improved in rats received intravenous MSCs injection within 4 days post MI and not affected by PBS injection. **CONCLUSIONS:** Myocardial SDF-1 expression was increased only in the early phase post MI. MSCs intravenous infused at the early phase of MI were recruited to injured heart, enhanced angiogenesis and improved cardiac function.

Maciejewski, J. P., J. M. Liu, et al. (1992). "Expression of stem cell inhibitor (SCI) gene in patients with bone marrow failure." *Exp Hematol* 20(9): 1112-7.

Stem cell inhibitor (SCI) has been shown to inhibit the proliferation of primitive progenitors. The inhibitor, a product of bone marrow macrophages, activated lymphocytes, and monocytes, is identical to macrophage inflammatory protein (MIP-1 alpha). We report homologous (SCI/hMIP-1 alpha) sequences in freshly isolated lymphocytes, monocytes, and granulocytes and have found that SCI mRNA can be induced in monocytes by lipopolysaccharide (LPS) and interleukins 1, 2, and 6. In contrast, interferon gamma (IFN-gamma) decreases the expression of SCI/hMIP-1 alpha. Although only a low level expression of SCI/hMIP-1 alpha mRNA can be detected in normal human bone marrow nucleated cells (NCBM), very significant increases in the levels of SCI/hMIP-1 alpha RNA transcripts are observed in NCBM from patients with aplastic anemia (AA) and

myelodysplastic syndrome (MDS). These data suggest that the expression of SCI/hMIP-1 alpha in bone marrow may reflect dysregulated cytokine production and activation of the immune system that may possibly contribute to disease progression.

Maciejewski, J. P., C. O'Keefe, et al. (2007). "Immune-mediated bone marrow failure syndromes of progenitor and stem cells: molecular analysis of cytotoxic T cell clones." *Folia Histochem Cytobiol* **45**(1): 5-14.

The unique structure of the T cell receptor (TCR) enables molecular identification of individual T cell clones and provides a unique opportunity for the design of molecular diagnostic tests based on the structure of the rearranged TCR chain e.g., using the TCR CDR3 region. Initially, clonal T cell malignancies, including T cell large granular lymphocyte leukemia (T-LGL), mucosis fungoides and peripheral T cell lymphoma were targets for the TCR-based analytic assays such as detection of clonality by T-gamma rearrangement using γ -chain-specific PCR or Southern Blotting. Study of these disorders facilitated further analytic concepts and application of rational methods of TCR analysis to investigations of polyclonal T cell-mediated diseases. In hematology, such conditions include graft versus host disease (GvHD) and immune-mediated bone marrow failure syndromes. In aplastic anemia (AA), myelodysplastic syndrome (MDS) or paroxysmal nocturnal hemoglobinuria (PNH), cytotoxic T cell responses may be directed against certain antigens located on stem or more lineage-restricted progenitor cells in single lineage cytopenias. The nature of the antigenic targets driving polyclonal CTL responses remains unclear. Novel methods of TCR repertoire analysis, include VB flow cytometry, peptide-specific tetramer staining, in vitro stimulation assays and TCR CDR3-specific PCR. Such PCR assay can be either VB family-specific or multiplexed for all VB families. Amplified products can be characterized and quantitated to facilitate detection of the most immunodominant clonotypes. Such clonotypes may serve as markers for the global polyclonal T cell response. Identification of these clonotypes can be performed in blood and tissue biopsy material by various methods. Once immunodominant clonotypes corresponding to pathogenic CTL clones are identified they can serve as surrogate markers for the activity of the pathophysiologic process or even indicate the presence of specific antigens. The relevance of the individual clonotypes can be ascertained from clinical correlations with the activity of the disease. Quantitative clonotypic assays such as sequencing of multiple CDR3 clones or clonotypic Taqman PCR can be applied for the monitoring of the

immunosuppressive therapy and prediction of relapse. Future technologies may allow for the design of clonotypic microarrays or other more clinically applicable methods of clonotypic diagnostics. Similarly, identification of immunodominant clonotypes may facilitate targeting of autoimmune or malignant clones with vaccination and induction of anti-idiotypic responses.

Maina, N., Z. Han, et al. (2008). "Recombinant self-complementary adeno-associated virus serotype vector-mediated hematopoietic stem cell transduction and lineage-restricted, long-term transgene expression in a murine serial bone marrow transplantation model." *Hum Gene Ther* **19**(4): 376-83.

Although conventional recombinant single-stranded adeno-associated virus serotype 2 (ssAAV2) vectors have been shown to efficiently transduce numerous cells and tissues such as brain and muscle, their ability to transduce primary hematopoietic stem cells (HSCs) has been reported to be controversial. We have previously documented that among the ssAAV serotype 1 through 5 vectors, ssAAV1 vectors are more efficient in transducing primary murine HSCs, but that viral second-strand DNA synthesis continues to be a rate-limiting step. In the present studies, we evaluated the transduction efficiency of several novel serotype vectors (AAV1, AAV7, AAV8, and AAV10) and documented efficient transduction of HSCs in a murine serial bone marrow transplantation model. Self-complementary AAV (scAAV) vectors were found to be more efficient than ssAAV vectors, and the use of hematopoietic cell-specific enhancers/promoters, such as the human beta-globin gene DNase I-hypersensitive site 2 enhancer and promoter (HS2-beta_p) from the beta-globin locus control region (LCR), and the human parvovirus B19 promoter at map unit 6 (B19p6), allowed sustained transgene expression in an erythroid lineage-restricted manner in both primary and secondary transplant recipient mice. The proviral AAV genomes were stably integrated into progenitor cell chromosomal DNA, and did not lead to any overt hematological abnormalities in mice. These studies demonstrate the feasibility of the use of novel scAAV vectors for achieving high-efficiency transduction of HSCs as well as erythroid lineage-restricted expression of a therapeutic gene for the potential gene therapy of beta-thalassemia and sickle cell disease.

Marquez-Curtis, L., A. Jalili, et al. (2008). "Carboxypeptidase M expressed by human bone marrow cells cleaves the C-terminal lysine of stromal cell-derived factor-1alpha: another player in hematopoietic stem/progenitor cell mobilization?" *Stem Cells* **26**(5): 1211-20.

Carboxypeptidase M (CPM) is a membrane-bound zinc-dependent protease that cleaves C-terminal basic residues, such as arginine or lysine, from peptides/proteins. We examined whether CPM is expressed by hematopoietic and stromal cells and could degrade stromal cell-derived factor (SDF)-1alpha, a potent chemoattractant for hematopoietic stem/progenitor cells (HSPC). We found that (a) CPM transcript is expressed by bone marrow (BM) and mobilized peripheral blood CD34(+) cells, myeloid, erythroid, and megakaryocytic cell progenitors, mononuclear cells (MNC), polymorphonuclear cells (PMN), and stromal cells, including mesenchymal stem cells; and that (b) granulocyte-colony-stimulating factor (G-CSF) significantly increases its expression at the gene and protein levels in MNC and PMN. Moreover, we found that recombinant CPM cleaves full-length SDF-1alpha (1-68) rapidly, removing the C-terminal lysine and yielding des-lys SDF-1alpha (1-67). We demonstrated that such CPM treatment of SDF-1alpha reduced the in vitro chemotaxis of HSPC, which, however, was preserved when the CPM was exposed to the carboxypeptidase inhibitor dl-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid. Thus, we present evidence that CPM is expressed by cells occurring in the BM microenvironment and that the mobilizing agent G-CSF strongly upregulates it in MNC and PMN. We suggest that cleavage of the C-terminal lysine residue of SDF-1alpha by CPM leads to attenuated chemotactic responses and could facilitate G-CSF-induced mobilization of HSPC from BM to peripheral blood.

Marx, J. C., J. A. Allay, et al. (1999). "High-efficiency transduction and long-term gene expression with a murine stem cell retroviral vector encoding the green fluorescent protein in human marrow stromal cells." *Hum Gene Ther* **10**(7): 1163-73.

Bone marrow stromal cells (MSCs) are unique mesenchymal cells that have been utilized as vehicles for the delivery of therapeutic proteins in gene therapy protocols. However, there are several unresolved issues regarding their potential therapeutic applications. These include low transduction efficiency, attenuation of transgene expression, and the technical problems associated with drug-based selection markers. To address these issues, we have developed a transduction protocol that yields high-level gene transfer into human MSCs, employing a murine stem cell virus-based bicistronic vector containing the green fluorescent protein (GFP) gene as a selectable marker. Transduction of MSCs plated at low density for 6 hr per day for 3 days with high-titer viral supernatant resulted in a gene transfer efficiency of 80+/-6% (n = 10) as measured by GFP

fluorescence. Neither centrifugation nor phosphate depletion increased transduction efficiency. Assessment of amphotropic receptor (Pit-2) expression by RT-PCR demonstrated that all MSCs expressing the receptor were successfully transduced. Cell cycle distribution profiles measured by propidium iodide staining showed no correlation with the susceptibility of MSCs to transduction by the retroviral vector. Human MSCs sequentially transduced with an adenoviral vector encoding the ecotropic receptor and ecotropic retroviral vector encoding GFP demonstrated that all MSCs are susceptible to retroviral transduction. We further showed that both genes of bicistronic vector are expressed for at least 6 months in vitro and that transgene expression did not affect the growth or osteogenic differentiation potential of MSCs. Future studies will be directed toward the development of gene therapy protocols employing this strategy.

Matsuda-Hashii, Y., K. Takai, et al. (2004). "Hepatocyte growth factor plays roles in the induction and autocrine maintenance of bone marrow stromal cell IL-11, SDF-1 alpha, and stem cell factor." *Exp Hematol* **32**(10): 955-61.

OBJECTIVE: Bone marrow (BM) stroma provides the microenvironment required for long-term hematopoiesis, and this is supported by direct interaction between stromal cells and hematopoietic cells, mediated by adhesion molecules, and through cytokine releases from the BM stroma. In a previous study, we demonstrated that hepatocyte growth factor (HGF) is one of the cytokines constitutively produced from BM stromal cells, promoting hematopoiesis mainly in an indirect way. We also showed that stromal cells themselves express HGF receptor c-MET. It was therefore postulated that HGF exerts its effect on hematopoiesis and maintenance of the hematopoietic microenvironment in a paracrine and autocrine manner. **METHODS:** The effect of HGF on stromal cells was analyzed by neutralizing intrinsic HGF. **RESULTS:** Addition of neutralizing anti-HGF antibody inhibited the ability of BM stromal cells to support colony formation from CD34(+) cells and reduced production of significant cytokines from stromal cells, interleukin-11 (IL-11), stromal cell-derived factor-1 alpha (SDF-1 alpha), and to a lesser extent, stem cell factor (SCF). Furthermore, this neutralizing antibody reduced proliferation of stromal cells and inhibited adhesion of stromal cells to collagen type IV and fibronectin. Inhibition of adhesion to fibronectin was mediated by inhibition of alpha(5)beta(1)-integrin. **CONCLUSION:** These findings indicate that HGF constitutively produced from BM stromal cells is an autocrine regulator, which is able to maintain the hematopoietic

microenvironment through stimulating proliferation and adhesion to the extracellular matrix and promoting hematopoiesis through inducing constitutive production of IL-11, SDF-1 alpha, and SCF by stromal cells themselves.

Matzner, U., D. Hartmann, et al. (2002). "Bone marrow stem cell-based gene transfer in a mouse model for metachromatic leukodystrophy: effects on visceral and nervous system disease manifestations." *Gene Ther* **9**(1): 53-63.

Arylsulfatase A (ASA) knockout mice represent an animal model for the lysosomal storage disease metachromatic leukodystrophy (MLD). Stem cell gene therapy with bone marrow overexpressing the human ASA cDNA from a retroviral vector resulted in the expression of high enzyme levels in various tissues. Treatment partially reduces sulfatide storage in livers exceeding 18 ng ASA/mg tissue, while complete reduction was observed in livers exceeding 50 ng ASA/mg tissue. This corresponds to about 80% and 200% of normal enzyme activity. Similar values seem to apply for kidney. A partial correction of the lipid metabolism was detectable in the brain where the galactocerebroside/sulfatide ratio, which is diminished in ASA-deficient mice, increased upon treatment. This partial correction was accompanied by amelioration of neuropathology; axonal cross-sectional areas, which are reduced in deficient mice, were significantly increased in the saphenic and sciatic nerve but not in the optic nerve. Behavioral tests suggest some improvement of neuromotor abilities. The gene transfer did not delay the degeneration occurring in the acoustic ganglion of ASA-deficient animals. The limited success of the therapy appears to be due to the requirement of unexpected high levels of ASA for correction of the metabolic defect.

Matzner, U., F. Schestag, et al. (2001). "Bone marrow stem cell gene therapy of arylsulfatase A-deficient mice, using an arylsulfatase A mutant that is hypersecreted from retrovirally transduced donor-type cells." *Hum Gene Ther* **12**(9): 1021-33.

Arylsulfatase A (ASA)-deficient mice represent an animal model for the fatal lysosomal storage disease metachromatic leukodystrophy, which is characterized by widespread intralysosomal deposition of sulfatide. Bone marrow stem cell gene therapy in mice, using a retroviral vector mediating expression of wild-type human ASA, has the potential to ameliorate the visceral pathology, but improves the prevailing brain disease and neurologic symptoms only marginally. One factor that influences the efficacy of bone marrow transplantation therapy in lysosomal storage diseases is the secretion level of the

therapeutic enzyme from donor-type cells. Here we test the potential of a hypersecreted glycosylation variant of ASA. Although this mutant lacks mannose 6-phosphate residues it is taken up by cells by a mannose 6-phosphate receptor-independent pathway and causes partial metabolic correction of ASA-deficient mouse cells. Retrovirally mediated transfer of the mutant cDNA into ASA-deficient mice results in the sustained expression of the transgene. Serum levels argue for an increased secretion of the glycosylation mutant also in vivo. Tissue levels were reduced to 2% in liver and up to 40% in kidney compared with animals treated with the wild-type enzyme, indicating reduced endocytosis. Thus, the limited uptake of the variant enzyme outweighs the putative advantageous effect of improved supply. Although the mutant enzyme is able to correct the metabolic defect partially, histological examinations did not reveal any reduction of sulfatide storage in treated animals. Surprisingly, analysis of neurologic symptoms indicated a significant improvement of the gait pattern.

Maze, R., J. P. Carney, et al. (1996). "Increasing DNA repair methyltransferase levels via bone marrow stem cell transduction rescues mice from the toxic effects of 1,3-bis(2-chloroethyl)-1-nitrosourea, a chemotherapeutic alkylating agent." *Proc Natl Acad Sci U S A* **93**(1): 206-10.

The chloroethylnitrosourea (CNU) alkylating agents are commonly used for cancer chemotherapy, but their usefulness is limited by severe bone marrow toxicity that causes the cumulative depletion of all hematopoietic lineages (pancytopenia). Bone marrow CNU sensitivity is probably due to the inefficient repair of CNU-induced DNA damage; relative to other tissues, bone marrow cells express extremely low levels of the O6-methylguanine DNA methyltransferase (MGMT) protein that repairs cytotoxic O6-chloroethylguanine DNA lesions. Using a simplified recombinant retroviral vector expressing the human MGMT gene under control of the phosphoglycerate kinase promoter (PGK-MGMT) we increased the capacity of murine bone marrow-derived cells to repair CNU-induced DNA damage. Stable reconstitution of mouse bone marrow with genetically modified, MGMT-expressing hematopoietic stem cells conferred considerable resistance to the cytotoxic effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), a CNU commonly used for chemotherapy. Bone marrow harvested from mice transplanted with PGK-MGMT-transduced cells showed extensive in vitro BCNU resistance. Moreover, MGMT expression in mouse bone marrow conferred in vivo resistance to BCNU-induced pancytopenia and significantly reduced BCNU-induced mortality due to bone marrow

hypoplasia. These data demonstrate that increased DNA alkylation repair in primitive hematopoietic stem cells confers multilineage protection from the myelosuppressive effects of BCNU and suggest a possible approach to protecting cancer patients from CNU chemotherapy-related toxicity.

Mebius, R. E., S. van Tuijl, et al. (1998). "Transfer of primitive stem/progenitor bone marrow cells from LT alpha-/- donors to wild-type hosts: implications for the generation of architectural events in lymphoid B cell domains." *J Immunol* **161**(8): 3836-43.

To analyze whether the phenotypic abnormalities observed in lymphotoxin-alpha(-/-) (LT alpha-/-) mice are intrinsic to the hemolymphoid system itself or dependent on stromal elements, wild-type (WT) mice were reconstituted with bone marrow (BM) cells enriched for hemopoietic stem cells from LT alpha-/- animals. WT mice reconstituted with LT alpha-/- c-kit+ Lin- Sca-1+ BM cells do not maintain follicular dendritic cell (FDC) networks and do not form primary follicles, while clear segregation of B and T cells could be observed. Furthermore, IgM+ IgD- B cells, MOMA-1 (anti-metallophilic macrophages), ERTR-9 (anti-marginal zone macrophages), and MECA-367 (anti-MAdCAM-1) were all absent from the splenic marginal zone. Surprisingly, however, the expression of MOMA-1, ERTR-9, and MAdCAM-1 was normal in the lymph nodes of mice reconstituted with LT alpha-/- cells. In addition, peanut agglutinin-positive germinal centers were observed in both the spleen and mesenteric lymph nodes, although in the absence of detectable FDC. Furthermore, in animals reconstituted with a mixture of LT alpha-/- and WT c-kit+ Lin- Sca-1+, GC contained either predominantly LT alpha-/- B cells or WT B cells. These results suggest that although the formation of primary follicles, FDC networks, and the splenic marginal zone are all dependent on hemopoietically derived LT alpha, germinal center formation and the expression of MAdCAM-1, MOMA-1, and ERTR-9 in lymph nodes are not. Our results also suggest that the disturbed B-T cell separation in LT alpha-/- mice is unrelated to defects in the marginal zone.

Meirelles Lda, S. and N. B. Nardi (2003). "Murine marrow-derived mesenchymal stem cell: isolation, in vitro expansion, and characterization." *Br J Haematol* **123**(4): 702-11.

In spite of the attention given to the study of mesenchymal stem cells (MSCs) derived from the bone marrow (BM) of humans and other species, there is a lack of information about murine MSCs. We describe the establishment of conditions for the in vitro expansion of plastic-adherent cells from murine

BM for over 50 passages, and provide their characterization regarding morphology, surface marker profile and growth kinetics. These cells were shown to differentiate along osteogenic and adipogenic pathways, and to support the growth and differentiation of haematopoietic stem cells, and were thus operationally defined as murine mesenchymal stem cells (mMSCs). mMSCs were positive for the surface markers CD44, CD49e, CD29 and Sca-1, and exhibited a homogeneous, distinctive morphology. Their frequency in the BM of adult BALB/c and C57Bl/6 mice, normal or knockout for the alpha-L-iduronidase (IDUA) gene, was preliminarily estimated to be 1 per 11,300-27,000 nucleated cells. The emergence of a defined methodology for the culture of mMSCs, as well as a comprehensive understanding of their biology, will make the development of cellular and genetic therapy protocols in murine models possible, and provide new perspectives in the field of adult stem cells research.

Min, C. K., B. G. Kim, et al. (2007). "IL-10-transduced bone marrow mesenchymal stem cells can attenuate the severity of acute graft-versus-host disease after experimental allogeneic stem cell transplantation." *Bone Marrow Transplant* **39**(10): 637-45.

Recent data suggest that adult mesenchymal stem cells (MSCs) might enhance allogeneic hematopoietic engraftment and prevent graft-versus-host disease (GVHD) owing to their immunosuppressive nature. Using a murine model of acute GVHD, this study examined whether or not the immunosuppressive properties of MSCs could reduce the severity of experimental GVHD. The early injection of MSCs after transplant did not attenuate the severity of acute GVHD. Therefore, this study investigated whether or not the use of IL-10-transduced MSCs (IL-10 MSCs) could reduce the severity of acute GVHD. Lethally irradiated recipients were transplanted and injected with IL-10 MSCs, the MSC-expressing vector alone (vector MSCs), or the diluent (controls), respectively, on day +1. Compared with the vector MSCs or controls, there was a significantly lower mortality in the recipients of the IL-10 MSCs at day 50 after the transplant (percent survival, 0 or 10 vs 70%, $P=0.0004$ or 0.0064 , respectively). The decrease in mortality was confirmed by the semi-quantitative GVHD score ($P<0.05$), and was associated with decreased serum levels of the pro-inflammatory cytokines, IFN-gamma, on day +7 ($P=0.015$). Therefore, beneficial effects on GVHD were observed when MSCs were engineered to express the anti-inflammatory cytokine, IL-10.

Mohamadnejad, M., K. Alimoghaddam, et al. (2007). "Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis." *Arch Iran Med* 10(4): 459-66.

BACKGROUND: The standard treatment for decompensated liver cirrhosis is liver transplantation. However, it has several limitations. Recent animal studies suggest that bone marrow stem cell transplantation can lead to regression of liver fibrosis. The objective of this study was to determine the safety and feasibility of autologous bone marrow-mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. **METHODS:** In this phase 1 trial, four patients with decompensated liver cirrhosis were included. Their bone marrow was aspirated, mesenchymal stem cells were cultured, and a mean 31.73×10^6 mesenchymal stem cells were infused through a peripheral vein. Primary outcomes were evaluating the safety and feasibility of the work. Secondary outcomes were evaluating changes in the model for end-stage liver disease score, and the quality of life of the patients. **RESULTS:** There were no side-effects in the patients during follow-up. The model for end-stage liver disease scores of patients 1, and 4 improved by four and three points, respectively by the end of follow-up. Furthermore, the quality of life of all four patients improved by the end of follow-up. Using SF-36 questionnaire, the mean physical component scale increased from 31.44 to 65.19, and the mean mental component scale increased from 36.32 to 65.55. **CONCLUSION:** Mesenchymal stem cell transplantation seems to be feasible and safe in the treatment of decompensated liver cirrhosis.

Moore, M. A. (2002). "Cytokine and chemokine networks influencing stem cell proliferation, differentiation, and marrow homing." *J Cell Biochem Suppl* 38: 29-38.

The hematopoietic stem cell (HSC) is an attractive target for gene therapy of genetic diseases of the immune and hematopoietic system, and for drug-resistance strategies in which genes conferring resistance to a variety of chemotherapeutic agents can be transduced. Stem cells are relatively easy to obtain; e.g., by marrow aspiration or G-CSF mobilization into the peripheral blood, and can be enriched e.g., by the use of anti-CD34 + monoclonal antibody. For conventional retroviral transduction, normally quiescent HSC must be activated into the cell cycle by priming with appropriate cytokines, and it has been critical to identify cytokine combinations that preserve the self-renewal capacity of long-term repopulating HSC. It has become apparent that strategies designed to optimize HSC cycling and proviral integration can compromise the capacity of transduced HSC to

compete in vivo against endogenous HSC or HSC that have not been activated into cell cycle. Lentiviral vectors can integrate genes into non-cycling cells but there is an increased efficiency of transduction if Go HSC are activated into G1-phase of the cell cycle. This reduced efficiency of long-term engraftment of ex vivo cultured HSC may be due to impaired self-renewal capacity or reduced marrow homing efficiency. The latter may be attributed to down modulation of chemokine receptors necessary for chemotactic homing to the marrow. Alternatively, or in addition, there may be down modulation of (1) HSC adhesion molecules necessary for endothelial adhesion and egress from the circulation: (2) metalloproteinases secreted by HSC that facilitate their migration through extracellular matrix and promote release of critical soluble regulatory factors in the marrow microenvironment. A more controversial view is that cell death pathways, for example those involving FasR (CD95) may be activated in cycling HSC, resulting in their selective destruction upon transplantation and localization to sites rich in Fas ligand such as the liver.

Nagai, A., W. K. Kim, et al. (2007). "Multilineage potential of stable human mesenchymal stem cell line derived from fetal marrow." *PLoS One* 2(12): e1272.

Human bone marrow contains two major cell types, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). MSCs possess self-renewal capacity and pluripotency defined by their ability to differentiate into osteoblasts, chondrocytes, adipocytes and muscle cells. MSCs are also known to differentiate into neurons and glial cells in vitro, and in vivo following transplantation into the brain of animal models of neurological disorders including ischemia and intracerebral hemorrhage (ICH) stroke. In order to obtain sufficient number and homogeneous population of human MSCs, we have clonally isolated permanent and stable human MSC lines by transfecting primary cell cultures of fetal human bone marrow MSCs with a retroviral vector encoding v-myc gene. One of the cell lines, HM3.B10 (B10), was found to differentiate into neural cell types including neural stem cells, neurons, astrocytes and oligodendrocytes in vitro as shown by expression of genetic markers for neural stem cells (nestin and Musashi1), neurons (neurofilament protein, synapsin and MAP2), astrocytes (glial fibrillary acidic protein, GFAP) and oligodendrocytes (myelin basic protein, MBP) as determined by RT-PCR assay. In addition, B10 cells were found to differentiate into neural cell types as shown by immunocytochemical demonstration of nestin (for neural stem cells), neurofilament protein and beta-tubulin III (neurons) GFAP (astrocytes), and galactocerebroside (oligodendrocytes). Following brain transplantation in mouse ICH stroke model, B10

human MSCs integrate into host brain, survive, differentiate into neurons and astrocytes and induce behavioral improvement in the ICH animals. B10 human MSC cell line is not only a useful tool for the studies of organogenesis and specifically for the neurogenesis, but also provides a valuable source of cells for cell therapy studies in animal models of stroke and other neurological disorders.

Narita, Y., A. Yamawaki, et al. (2008). "Effects of transforming growth factor-beta 1 and ascorbic acid on differentiation of human bone-marrow-derived mesenchymal stem cells into smooth muscle cell lineage." *Cell Tissue Res* **333**(3): 449-59.

Bone-marrow-derived mesenchymal stem cells (MSCs) can differentiate into a variety of cell types including smooth muscle cells (SMCs). We have attempted to demonstrate that, following treatment with transforming growth factor-beta 1 (TGF-beta1) and ascorbic acid (AA), human bone-marrow-derived MSCs differentiate into the SMC lineage for use in tissue engineering. Quantitative polymerase chain reaction for SMC-specific gene (alpha smooth muscle actin, h1-calponin, and SM22alpha) expression was performed on MSCs, which were cultured with various concentrations of TGF-beta1 or AA. TGF-beta1 had a tendency to up-regulate the expression of SMC-specific genes in a dose-dependent manner. The expression of SM22alpha was significantly up-regulated by 30 microM AA. We also investigated the additive effect of TGF-beta1 and AA for differentiation into SMCs and compared this effect with that of other factors including platelet-derived growth factor BB (PDGF-BB). In addition to SMC-specific gene expression, SMC-specific proteins increased by two to four times when TGF-beta1 and AA were used together compared with their administration alone. PDGF did not increase the expression of SMC-specific markers. MSCs cultured with TGF-beta1 and AA did not differentiate into osteoblasts and adipocytes. These results suggest that a combination of TGF-beta1 and AA is useful for the differentiation of MSCs into SMCs for use in tissue engineering.

Neta, R., J. J. Oppenheim, et al. (1994). "Synergy of IL-1 and stem cell factor in radioprotection of mice is associated with IL-1 up-regulation of mRNA and protein expression for c-kit on bone marrow cells." *J Immunol* **153**(4): 1536-43.

Administration of IL-1 and stem cell factor (SCF) to mice 18 h before lethal 60Co whole-body irradiation resulted in synergistic radioprotection, as evidenced by increased numbers of mice surviving 1,200 to 1,300 cGy doses of radiation and the recovery of increased numbers of c-kit+ bone marrow

cells at 1 and 4 days after the lethal dose of 950 cGy. Anti-SCF Ab inhibited IL-1-induced radioprotection, indicating that endogenous production of SCF is necessary for radioprotection by IL-1. Conversely, radioprotection induced by SCF was reduced by anti-IL-1R Ab, indicating that endogenous IL-1 contributes to SCF radioprotection. SCF, unlike IL-1 does not induce hemopoietic CSFs and IL-6 or gene expression of a scavenging mitochondrial enzyme manganese superoxide dismutase in the bone marrow, suggesting that SCF and IL-1 radioprotect by distinct pathways. The mRNA expression for c-kit (by Northern blot analysis) and 125I-SCF binding on bone marrow cells was elevated within 2 and 4 h of IL-1 administration respectively. Four days after LD 100/30 radiation the recovery of c-kit+ bone marrow cells was increased sixfold in IL-1-treated mice, almost 20-fold in SCF-treated mice, and 40-fold in mice treated with the combination of the two cytokines. Thus, endogenous production of both IL-1 and SCF is required for resistance to lethal irradiation and the synergistic radioprotective effect of the two cytokines may, in part, depend on IL-1 and SCF-induced increases in numbers of c-kit+ hemopoietic stem and progenitor cells that survive lethal irradiation.

Niitsu, Y., Y. Takahashi, et al. (1998). "A proof of glutathione S-transferase-pi-related multidrug resistance by transfer of antisense gene to cancer cells and sense gene to bone marrow stem cell." *Chem Biol Interact* **111-112**: 325-32.

In order to directly prove the involvement of GST-pi in drug resistance, its antisense gene was transduced into human colorectal cancer cell line which has been shown to express high level of GST-pi and the sensitivity of this cell line to anticancer drugs were assessed. The transfectant showed higher sensitivity to adriamycin (3.3-fold), Cisplatinum (2.3-fold), Melphalan (2.2-fold), Etoposide (2.2-fold) than the parental cell, while the sensitivity to vincristine, mitomycin C, 5-fluorouracil was unchanged by transfection. When the transfectant and parental cells were inoculated in nude mice and treated with adriamycin, a significant suppression of tumor growth was observed with the transfectant as compared to the parental cell. On the basis of this observation, we then transduced sense GST-pi gene into human bone marrow stem cells (CD34+ cells) to protect them from toxicity of anticancer drug. The gene transduced CD34+ cells formed more CFU-GM than nontransduced CD34+ cell in the presence of adriamycin (30 ng/ml). Thus, the autotransplantation of GST-pi gene transduced cell into cancer patients to protect the bone marrow from subsequent highdose chemotherapy is considered to be a new strategy for cancer gene therapy.

Ogura, N., M. Kawada, et al. (2004). "Differentiation of the human mesenchymal stem cells derived from bone marrow and enhancement of cell attachment by fibronectin." *J Oral Sci* **46**(4): 207-13.

The ability of human mesenchymal stem cells (hMSC) to differentiate into osteoblasts was examined through the use of osteogenic induction medium (MSCOIM) cultures. hMSC first attached to the dish surface and exhibited fibroblast-like spindle shapes, and after proliferation, formed cuboidal shapes. Calcium assays and the use of von Kossa and alizarin red S staining showed that hMSC were capable of mineralization when cultured in MSCOIM. Gene expressions of Cbfa-1 and BMP-4, which are markers for osteogenic differentiation, were also increased during the hMSC differentiation into osteoblasts. When compared to albumin (Alb)-coated dishes, microscopic observation documented enhanced cell attachment and spreading when hMSC were cultured on fibronectin (FN)-coated dishes. Adherent cell numbers also exhibited a greater increase on the FN-coated dishes during earlier culture stages than that seen for the Alb-coated dishes. These findings suggest that hMSC have the capability to differentiate into osteoblasts and that FN can stimulate the attachment and spreading of the hMSC.

Okada, S., Z. Q. Wang, et al. (1994). "Mice lacking c-fos have normal hematopoietic stem cells but exhibit altered B-cell differentiation due to an impaired bone marrow environment." *Mol Cell Biol* **14**(1): 382-90.

Mice lacking c-fos develop severe osteopetrosis with deficiencies in bone remodeling and exhibit extramedullary hematopoiesis, thymic atrophy, and altered B-cell development. In this study, we have used these mice to characterize in detail the developmental potential of hematopoietic stem cells lacking c-fos and to analyze how the lymphoid differentiation is altered. In c-fos ^{-/-} mice, B-cell numbers are reduced in the spleen, lymph nodes, and the peripheral blood as a result of a marked reduction (> 90%) in the number of clonogenic B-cell precursors. In contrast, the number and lineage distribution of myeloid progenitor cells are not affected. The thymic defects observed in a large number of these mice correlate with their health status, suggesting that this may be an indirect effect of the c-fos mutation. In vitro differentiation and bone marrow reconstitution experiments demonstrated that hematopoietic stem cells lacking c-fos can give rise to all mature myeloid as well as lymphoid cells, suggesting that the observed B lymphopenia in the mutant mice is due to an altered environment. Transplantation of wild-type bone marrow cells into newborn mutant mice resulted in the establishment of

a bone marrow space and subsequent correction of the B-cell defect. These results demonstrate that hematopoietic stem cells lacking Fos have full developmental potential and that the observed defect in B-cell development is most likely due to the impaired bone marrow environment as a consequence of osteopetrosis.

Park, H., J. S. Temenoff, et al. (2007). "Injectable biodegradable hydrogel composites for rabbit marrow mesenchymal stem cell and growth factor delivery for cartilage tissue engineering." *Biomaterials* **28**(21): 3217-27.

We investigated the development of an injectable, biodegradable hydrogel composite of oligo(poly(ethylene glycol) fumarate) (OPF) with encapsulated rabbit marrow mesenchymal stem cells (MSCs) and gelatin microparticles (MPs) loaded with transforming growth factor-beta1 (TGF-beta1) for cartilage tissue engineering applications. Rabbit MSCs and TGF-beta1-loaded MPs were mixed with OPF, a poly(ethylene glycol)-diacrylate crosslinker and the radical initiators ammonium persulfate and N,N,N',N'-tetramethylethylenediamine, and then crosslinked at 37 degrees C for 8 min to form hydrogel composites. Three studies were conducted over 14 days in order to examine the effects of: (1) the composite formulation, (2) the MSC seeding density, and (3) the TGF-beta1 concentration on the chondrogenic differentiation of encapsulated rabbit MSCs. Bioassay results showed no significant difference in DNA amount between groups, however, groups with MPs had a significant increase in glycosaminoglycan content per DNA starting at day 7 as compared to controls at day 0. Chondrocyte-specific gene expression of type II collagen and aggrecan were only evident in groups containing TGF-beta1-loaded MPs and varied with TGF-beta1 concentration in a dose-dependent manner. Specifically, type II collagen gene expression exhibited a 161+/-49-fold increase and aggrecan gene expression a 221+/-151-fold increase after 14 days with the highest dose of TGF-beta1 (16 ng/ml). These results indicate that encapsulated rabbit MSCs remained viable over the culture period and differentiated into chondrocyte-like cells, thus suggesting the potential of OPF composite hydrogels as part of a novel strategy for localized delivery of stem cells and bioactive molecules.

Park, S. H., W. Y. Sim, et al. (2006). "An electromagnetic compressive force by cell exciter stimulates chondrogenic differentiation of bone marrow-derived mesenchymal stem cells." *Tissue Eng* **12**(11): 3107-17.

In this study, we present a biological micro-electromechanical system and its application to the

chondrogenic differentiation of rabbit bone marrow-derived mesenchymal stem cells (MSCs). Actuated by an electromagnetic force, the micro cell exciter was designed to deliver a cyclic compressive load (CCL) with various magnitudes. Two major parts in the system are an actuator and a cartridge-type chamber. The former has a permanent magnet and coil, and the latter is equipped with 7 sample dishes and 7 metal caps. Mixed with a 2.4% alginate solution, the alginate/MSC layers were positioned in the sample dishes; the caps contained chondrogenic defined medium without transforming growth factor-beta (TGF-beta). Once powered, the actuator coil-derived electromagnetic force pulled the metal caps down, compressing the samples. The cyclic load was given at 1-Hz frequency for 10 min twice a day. Samples in the dishes without a cap served as a control. The samples were analyzed at 3, 5, and 7 days after stimulation for cell viability, biochemical assays, histologic features, immunohistochemistry, and gene expression of the chondrogenic markers. Applied to the alginate/MSC layer, the CCL system enhanced the synthesis of cartilage-specific matrix proteins and the chondrogenic markers, such as aggrecan, type II collagen, and Sox9. We found that the micromechanically exerted CCL by the cell exciter was very effective in enhancing the chondrogenic differentiation of MSCs, even without using exogenous TGF-beta.

Petit, I., M. Szyper-Kravitz, et al. (2002). "G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4." *Nat Immunol* 3(7): 687-94.

Granulocyte colony-stimulating factor (G-CSF) induced hematopoietic stem cell mobilization is widely used for clinical transplantation; however, the mechanism is poorly understood. We report here that G-CSF induced a reduction of the chemokine stromal cell derived factor 1 (SDF-1) and an increase in its receptor CXCR4 in the bone marrow (BM), whereas their protein expression in the blood was less affected. The gradual decrease of BM SDF-1, due mostly to its degradation by neutrophil elastase, correlated with stem cell mobilization. Elastase inhibition reduced both activities. Human and murine stem cell mobilization was inhibited by neutralizing CXCR4 or SDF-1 antibodies, demonstrating SDF-1 CXCR4 signaling in cell egress. We suggest that manipulation of SDF-1 CXCR4 interactions may be a means with which to control the navigation of progenitors between the BM and blood to improve the outcome of clinical stem cell transplantation.

Pochampally, R. R., B. T. Neville, et al. (2004). "Rat adult stem cells (marrow stromal cells) engraft and

differentiate in chick embryos without evidence of cell fusion." *Proc Natl Acad Sci U S A* 101(25): 9282-5.

Cell fusion was recently reported to account for the plasticity of adult stem cells in vivo. Adult stem cells, referred to as mesenchymal stem cells or marrow stromal cells, from rat marrow, were infused into 1.5- to 2-day-old chick embryos. After 4 days, the rat cells had expanded 1.3- to 33-fold in one-third of surviving embryos. The cells engrafted into many tissues, and no multinuclear cells were detected. The most common site of engraftment was the heart, apparently because the cells were infused just above the dorsal aorta. Some of the cells in the heart expressed cardiotin, and alpha-heavy-chain myosin. GFP(+) cells reisolated from the embryos had a rat karyotype. Therefore, the cells engrafted and partially differentiated without evidence of cell fusion.

Povey, J., N. Weeratunge, 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): 4080-9.

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.

Pricola, K. L., N. Z. Kuhn, et al. (2009). "Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism." *J Cell Biochem* **108**(3): 577-88.

Adult human mesenchymal stem cells (MSCs) hold promise for an increasing list of therapeutic uses due to their ease of isolation, expansion, and multi-lineage differentiation potential. To maximize the clinical potential of MSCs, the underlying mechanisms by which MSC functionality is controlled must be understood. We have taken a deconstructive approach to understand the individual components in vitro, namely the role of candidate "stemness" genes. Our recent microarray gene expression profiling data suggest that interleukin-6 (IL-6) may contribute to the maintenance of MSCs in their undifferentiated state. In this study, we showed that IL-6 gene expression is significantly higher in undifferentiated MSCs as compared to their chondrogenic, osteogenic, and adipogenic derivatives. Moreover, we found that MSCs secrete copious amounts of IL-6 protein, which decreases dramatically during osteogenic differentiation. We further evaluated the role of IL-6 for maintenance of MSC "stemness," using a series of functional assays. The data showed that IL-6 is both necessary and sufficient for enhanced MSC proliferation, protects MSCs from apoptosis, inhibits adipogenic and chondrogenic differentiation of MSCs, and increases the rate of in vitro wound healing of MSCs. We further identified ERK1/2 activation as the key pathway through which IL-6 regulates both MSC proliferation and inhibition of differentiation. Taken together, these findings show for the first time that IL-6 maintains the proliferative and undifferentiated state of bone marrow-derived MSCs, an important parameter for the optimization of both in vitro and in vivo manipulation of MSCs.

Quesenberry, P. J. (2006). "The continuum model of marrow stem cell regulation." *Curr Opin Hematol* **13**(4): 216-21.

PURPOSE OF REVIEW: We review the continuum model of stem cell regulation. A series of studies on purified lineage negative rhodamine low Hoechst low murine stem cells driven through cell cycle by cytokine exposure have shown that many phenotypic features show reversible changes with cycle progression. **RECENT FINDINGS:** We and others have shown that purified murine marrow stem cells are a cycling population. Features that are labile with cycle progression are in-vivo engraftment, progenitor numbers, expression of adhesion proteins and cytokine receptors, global gene expression and differentiation into granulocytes and megakaryocytes. These observations have led to a theory that regulation of hematopoietic stem cells is on a continuum and not

in a hierarchy. Out-of-tissue plasticity in which marrow cells show a capacity to produce nonhematopoietic cells in non-marrow tissues also exists. We have shown 'robust' production of lung and skeletal muscle cells by marrow cells in the presence of appropriate tissue injury and demonstrated that the capacity of marrow cells to produce nonhematopoietic cells in the lung also varies reversibly with cell cycle status. **SUMMARY:** Thus, stem cells show a plastic plasticity and the continuum appears to hold for both nonhematopoietic and hematopoietic lineages.

Rameshwar, P. and P. Gascon (1995). "Substance P (SP) mediates production of stem cell factor and interleukin-1 in bone marrow stroma: potential autoregulatory role for these cytokines in SP receptor expression and induction." *Blood* **86**(2): 482-90.

Substance P (SP) is a neuropeptide widely distributed in the nervous system. Its release within the bone marrow (BM) can mediate bidirectional neurohematopoietic communication via specific receptors: neurokinin-1R (NK-1R), NK-2R, or NK-3R. We have previously reported that SP effects on hematopoiesis are mediated by an NK-1-type receptor, the BM stroma, and growth factors. Here, we have studied the induction of stem cell factor (SCF) and interleukin-1 (IL-1) by SP in stroma. At 10⁻⁹ mol/L SP, cytokine levels in supernatants were IL-1 alpha, 20 +/- 5 ng/mL; IL-1 beta, 40 +/- 10 ng/mL; and SCF, nondetectable; and the cell-associated levels were SCF, 21 +/- 2 ng/mL; IL-1 alpha, 90 +/- 6 ng/mL; and IL-1 beta, 45 +/- 3 ng/mL. Reverse transcriptase-polymerase chain reaction and ligand-binding studies with stroma stimulated by these two cytokines resulted in (1) NK-1-like receptor mRNA accumulation and (2) downregulation of SP binding sites (day 1) followed by an upregulation (day 3). Low numbers of high-affinity receptors were expressed by day 1 but not by day 3. The results indicate that SP induces IL-1 and SCF in stroma and that these cytokines have the potential to autoregulate NK-R.

Rameshwar, P., G. Zhu, et al. (2001). "The dynamics of bone marrow stromal cells in the proliferation of multipotent hematopoietic progenitors by substance P: an understanding of the effects of a neurotransmitter on the differentiating hematopoietic stem cell." *J Neuroimmunol* **121**(1-2): 22-31.

Communication within the hematopoietic-neuroendocrine-immune axis is partly mediated by neurotransmitters (e.g. substance P, SP) and cytokines. SP mediates neuromodulation partly through the stimulation of bone marrow (BM) progenitors. This study shows that SP, through the neurokinin-1 receptor, stimulates the proliferation of primitive hematopoietic progenitors: cobblestone-forming cells

(CAFC, CD34+). This effect is optimal when macrophage is included within the fibroblast support. Indirect induction of IL-1 could be important in the proliferation of CAFC colonies by SP. Phenotypic and functional studies suggest that SP might directly interact with the CD34+/CD45(dim) population. These studies indicate that SP can initiate a cascade of biological responses in the BM stroma and stem cells to stimulate hematopoiesis.

Ratajczak, M. Z., W. I. Kuczynski, et al. (1995). "Expression and physiologic significance of Kit ligand and stem cell tyrosine kinase-1 receptor ligand in normal human CD34+, c-Kit+ marrow cells." *Blood* **86**(6): 2161-7.

To determine the potential role of autocrine growth factor production in regulating primitive human hematopoietic cell development, we examined highly purified CD34+, c-Kit+ marrow mononuclear cells for expression of c-Kit ligand (KL) and stem cell tyrosine kinase 1 (stk1) ligand (STK1-L). Normal marrow mononuclear cells coexpressing CD34 and c-Kit were isolated by a combination of immunomagnetic bead isolation and fluorescence-activated cell sorting. Purified cells were then screened for expression of KL and stk1-L mRNA using a sensitive reverse transcription-polymerase chain reaction method. Using this approach, expression of both cytokine genes at the mRNA level was found in this highly enriched cell population. We then examined the functional significance of these mRNAs by inhibiting their expression with antisense (AS) oligodeoxynucleotides (ODN). In comparison to untreated or control ODN treated cells, inhibition of KL led to a 70% and 89% inhibition in burst-forming unit-erythroid (BFU-E) and colony-forming unit-Mix (CFU-Mix) colonies but had no significant effect on CFU-granulocyte-macrophage (CFU-GM) cloning efficiency. In contrast, inhibition of STK1-L alone had no effect on colony formation. However, when STK1-L AS ODN was combined with KL AS ODN, additive inhibition of CFU-GM and CFU-MIX but not of BFU-E colonies was observed. These findings, along with those of our previous studies showing inhibition of primitive hematopoietic cell growth with antisense ODN directed towards the stk1 receptor, suggest the possibility that both receptor/ligand axes regulate primitive hematopoietic cell growth via an autocrine growth loop.

Rider, D. A., T. Nalathamby, et al. (2007). "Selection using the alpha-1 integrin (CD49a) enhances the multipotentiality of the mesenchymal stem cell population from heterogeneous bone marrow stromal cells." *J Mol Histol* **38**(5): 449-58.

Bone marrow-derived mesenchymal stem cells consist of a developmentally heterogeneous population of cells obtained from colony forming progenitors. As these colonies express the alpha-1 integrin (CD49a), here we single-cell FACS sorted CD49a+ cells from bone marrow in order to create clones and then compared their colony forming efficiency and multilineage differentiation capacity to the unsorted cells. Following selection, 40% of the sorted CD49a+ cells formed colonies, whereas parental cells failed to form colonies following limited dilution plating at 1 cell/well. Following ex vivo expansion, clones shared a similar morphology to the parental cell line, and also demonstrated enhanced proliferation. Further analysis by flow cytometry using a panel of multilineage markers demonstrated that the CD49a+ clones had enhanced expression of CD90 and CD105 compared to unsorted cells. Culturing cells in adipogenic, osteogenic or chondrogenic medium for 7, 10 and 15 days respectively and then analysing them by quantitative PCR demonstrated that CD49a+ clones readily underwent multilineage differentiation into fat, bone and cartilage compared to unsorted cells. These results thus support the use of CD49a selection for the enrichment of mesenchymal stem cells, and describes a strategy for selecting the most multipotential cells from a heterogeneous pool of bone marrow mononuclear stem cells.

Rieger, K., O. Marinets, et al. (2005). "Mesenchymal stem cells remain of host origin even a long time after allogeneic peripheral blood stem cell or bone marrow transplantation." *Exp Hematol* **33**(5): 605-11.

OBJECTIVE: Plasticity of hematopoietic stem cells (HSC) has gained major interest in stem cell research. In order to investigate whether HSC may differentiate into mesenchymal stem cells (MSC), we assessed chimerism in peripheral blood (PB), mononuclear cell fractions (MNC) of bone marrow, and MSC derived from bone marrow (BM) from 27 up to 4225 days after allogeneic transplantation. **PATIENTS AND METHODS:** We applied fluorescence in situ hybridization using X/Y gene probes in sex-mismatched and STR-PCR in sex-matched patients. MSC could have been generated in 27 of 55 bone marrow samples derived from 20 patients. Fifteen patients received peripheral blood stem cell transplants (PBSCT), including CD34-selected PBSCT in two. Five patients received bone marrow. **RESULTS:** While all patients had chimerism in PB and MNC of the BM, in all but one patient BM-derived MSC were of recipient origin. This single patient showed reproducibly MSC of donor origin in a frequency of 1% after having received a CD34-selected PBSCT. Looking at graft collections, MSCs

were easily generated from BM specimens, while no MSC could be derived from PBSC samples. CONCLUSION: Even though HSC have been found to differentiate into a variety of nonhematological cell types, they usually do not differentiate into MSC after allogeneic transplantation.

Ritter, E., A. Perry, et al. (2008). "Breast cancer cell-derived fibroblast growth factor 2 and vascular endothelial growth factor are chemoattractants for bone marrow stromal stem cells." *Ann Surg* **247**(2): 310-4.

OBJECTIVE: Recent efforts by the scientific community to characterize the complex interplay between different cell types involved in the development of tumors have led us to investigate the roles of vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) in the development of breast cancer. **METHODS:** Using modified Boyden chamber assays, we measured the in vitro migration effect on murine mesenchymal stem cells (MSCs). Additionally, we assayed for the presence of receptors for these growth factors on MSCs, and for the presence of VEGF and FGF2 in breast cancer-conditioned media. We measured the change in migration of MSCs toward breast cancer when we depleted these growth factors from breast cancer-conditioned media. Further, we conducted a series of standard curve migration assays for basal media supplemented with physiologic concentrations of VEGF and FGF2. **RESULTS:** Analysis of gene expression and protein analysis demonstrated the expression of FGF2 and VEGF by the breast cancer cells, and the presence of VEGF (FLK1) and FGF2 receptors on the MSCs. We also demonstrated a reduction in migration when we antibody-depleted VEGF and FGF2 from breast cancer-conditioned media. Additionally, we found the physiologic concentrations of VEGF and FGF2 at 12 and 15 ng/mL, respectively. **CONCLUSIONS:** We demonstrate that VEGF and FGF2 induce migration of MSCs are secreted by breast cancer cells, their receptors are present on MSCs, and depletion of these growth factors reduces migration, and are therefore 2 relevant growth factors for MSC migration toward breast cancer cells.

Sarnowska, A., H. Braun, et al. (2009). "The neuroprotective effect of bone marrow stem cells is not dependent on direct cell contact with hypoxic injured tissue." *Exp Neurol* **215**(2): 317-27.

Bone marrow stem cells (BMSCs) are able to confer beneficial effects after transplantation into animals with ischemic brain injuries. This effect is probably mainly caused by the release of trophic factors, though the possibility of dead neural cells

being replaced by BMSCs cannot be excluded. The aim of this study was to determine whether the neuroprotective effects in question are dependent on direct cell-cell contacts between BMSCs and injured tissue. We therefore investigated that interplay in an in vitro model of hippocampal organotypic slice cultures (OHCs), in order to avoid the interference due to immunological rejection processes following transplantation in vivo. To perform ischemic injury in vitro, OHCs were made subject to oxygen-glucose deprivation (OGD). The possible direct or indirect neuroprotective effects induced by BMSCs were evaluated 24 h after injury by reference to two experimental paradigms using ischemic injured hippocampal slices: (i) cell transplantation on the top of OGD-treated OHC, (ii) co-cultivation of cell culture with OHC space separated for 24 h. In both paradigms, the BMSC treatment induced comparable and significant neuroprotection in OGD-injured OHCs. This effect increased after treatment with serum-deprived BMSCs, enriched with cells expressing nestin and GFAP. Comparing cell transplantation and cell co-cultivation with injured tissue, we concluded that the neuroprotective effect of BMSCs evoked shortly after ischemia (24 h) does not depend on cell-cell contacts. Additionally OGD-treated OHC was found to stimulate co-cultured BMSCs into expressing higher levels of bFGF and NGF. Finally, ischemic hippocampal slices increased the expression of nestin and GFAP in co-cultivated BMSCs, as well as changing their morphology.

Satoh, M., H. Mioh, et al. (1997). "Mouse bone marrow stromal cell line MC3T3-G2/PA6 with hematopoietic-supporting activity expresses high levels of stem cell antigen Sca-1." *Exp Hematol* **25**(9): 972-9.

The murine clonal preadipose cell line, MC3T3-G2/PA6 (PA6), has the ability to support in vitro proliferation of hematopoietic stem cells defined as colony-forming units in spleen (CFU-S). In order to ascertain the relationship between the hematopoietic-supporting activity of PA6 cells and their expression, we cultured a number of these cells for over 45 weeks and investigated the level at which they expressed several cell surface markers and membrane-bound growth factors. Besides expressing stem cell factor (SCF) and macrophage colony-stimulating factor (M-CSF), PA6 cells were found by flow cytometry analysis to express high levels of stem cell antigen-1 (Sca-1). The expression level of Sca-1 in PA6 cells correlated with the ability of the latter to support hematopoiesis, whereas no such correlation was observed in the case of SCF and M-CSF expression. A cDNA clone encoding the protein recognized by anti-Sca-1 antibody was isolated from PA6 cells by

expression cloning, so that its nucleotide sequence encoded the protein identical to mouse alloantigen Ly-6A.2. Genetically engineered COS-7 cells, transformed by the expression vector carrying the Ly-6A.2 gene, suppressed proliferation of murine lineage marker-negative (Lin) bone marrow cells by themselves and synergistically augmented proliferation of these cells in the presence of SCF. These results suggest that Ly-6A.2 regulates the proliferation of hematopoietic progenitor cells, and is one of the molecules organizing the hematopoietic microenvironment provided by stromal cells.

Schetelig, J., A. van Biezen, et al. (2008). "Allogeneic hematopoietic stem-cell transplantation for chronic lymphocytic leukemia with 17p deletion: a retrospective European Group for Blood and Marrow Transplantation analysis." *J Clin Oncol* **26**(31): 5094-100.

PURPOSE: Patients with chronic lymphocytic leukemia (CLL) and 17p deletion (17p-) have a poor prognosis. Although allogeneic hematopoietic stem-cell transplantation (HCT) has the potential to cure patients with advanced CLL, it is not known whether this holds true for patients with 17p-CLL. **PATIENTS AND METHODS:** Baseline data from patients, for whom information on the presence of 17p-CLL was available, were downloaded from the European Group for Blood and Marrow Transplantation database. Additional information on the course of CLL and follow-up was collected with a questionnaire. **RESULTS:** A total of 44 patients with 17p-CLL received allogeneic HCT between March 1995 and July 2006 from a matched sibling (n = 24) or an alternative donor (n = 20). 17p-CLL had been diagnosed by fluorescent in situ hybridization in 82% of patients and by conventional banding in 18% of patients. The median age was 54 years. Before HCT, a median of three lines of chemotherapy had been administered. At HCT, 53% of patients were in remission. Reduced-intensity conditioning was applied in 89% of patients. Acute, grade 2 to 4 graft-versus-host disease (GVHD) occurred in 43% of patients, and extensive chronic GVHD occurred in 53% of patients. At last follow-up, 19 patients were alive, with a median observation time of 39 months (range, 18 to 101 months). Three-year overall survival and progression-free survival rates were 44% and 37%, respectively. The cumulative incidence of progressive disease at 4 years was 34%. No late relapse occurred in nine patients with a follow-up longer than 4 years. **CONCLUSION:** Allogeneic HCT has the potential to induce long-term disease-free survival in patients with 17p-CLL.

Sekiya, K., M. Nishimura, et al. (2008). "Enhancement of osteogenesis by concanavalin A in human bone marrow mesenchymal stem cell cultures." *Int J Artif Organs* **31**(8): 708-15.

This study investigates concanavalin A (ConA) as a novel factor that may enhance osteogenesis of mesenchymal stem cells (MSCs) in vitro. Various factors, such as cytokine bone morphogenetic protein-2 (BMP-2), have been studied for their possible promotion of MSC osteogenesis in vivo and in vitro. However, the factor that might be safer, more effective, and less expensive than these has not been determined. ConA and BMP-2 enhanced calcification with comparable effectiveness. The combination of ConA and BMP-2 further enhanced calcification slightly but significantly. ConA also increased osteocalcin and BMP-2 protein levels in MSC culture medium. Furthermore, ConA increased osteocalcin, RUNX2, BMP-2, BMP-4, and BMP-6 mRNA expression levels. However, the gene expression pattern of ConA-stimulated MSCs was different from that of MSCs stimulated by BMP-2. Together, these results suggest that ConA and BMP-2 enhance MSC osteogenesis via different pathways. ConA-induced bone formation in MSC cultures may be useful in regenerative medicine or tissue engineering in clinical studies, as well as in basic research on bone formation.

Shimakura, Y., H. Kawada, et al. (2000). "Murine stromal cell line HESS-5 maintains reconstituting ability of Ex vivo-generated hematopoietic stem cells from human bone marrow and cytokine-mobilized peripheral blood." *Stem Cells* **18**(3): 183-9.

Human bone marrow (BM) or mobilized peripheral blood (mPB) CD34(+) cells have been shown to lose their stem cell quality during culture period more easily than those from cord blood (CB). We previously reported that human umbilical CB stem cells could effectively be expanded in the presence of human recombinant cytokines and a newly established murine bone marrow stromal cell line HESS-5. In this study we assessed the efficacy of this xenogeneic coculture system using human BM and mPB CD34(+) cells as materials. We measured the generation of CD34(+)CD38(-) cells and colony-forming units, and assessed severe-combined immunodeficient mouse-repopulating cell (SRC) activity using cells five days after serum-free cytokine-containing culture in the presence or the absence of a direct contact with HESS-5 cells. As compared with the stroma-free culture, the xenogeneic coculture was significantly superior on expansion of CD34(+)CD38(-) cells and colony-forming cells and on maintenance of SRC activity. The PKH26 study demonstrated that cell division was promoted faster in cells cocultured with

HESS-5 cells than in cells cultured without HESS-5 cells. These results indicate that HESS-5 supports rapid generation of primitive progenitor cells (PPC) and maintains reconstituting ability of newly generated stem cells during ex vivo culture irrespective of the source of samples. This xenogeneic coculture system will be useful for ex vivo manipulation such as gene transduction to promote cell division and the generation of PPC and to prevent loss of stem cell quality.

Sila-Asna, M., A. Bunyaratvej, et al. (2007). "Osteoblast differentiation and bone formation gene expression in strontium-inducing bone marrow mesenchymal stem cell." *Kobe J Med Sci* **53**(1-2): 25-35.

Osteoblastic differentiation from human mesenchymal stem cell (hMSCs) is an important step of bone formation. We studied the in vitro induction of hMSCs by using strontium ranelate, a natural trace amount in water, food and human skeleton. The mRNA synthesis of various osteoblast specific genes was assessed by means of reverse transcription polymerase chain reaction (RT-PCR). In the hMSCs culture, strontium ranelate could enhance the induction of hMSCs to differentiate into osteoblasts. Cbfa1 gene was earlier expressed on day 4 of cell culture (the control group, on day 14) and osteonectin on day 11 (control, on day 21). The early Cbfa1 expression indicates that strontium could enhance osteoblastic differentiation. The detection of osteonectin using strontium induction indicates the role of strontium in enhancing bone remodeling, bone structure stabilization of hydroxyapatite molecule and collagen fibril organization. The cultured hMSCs in the presence of strontium expressed genes of bone extracellular matrix: collagen type I, bone sialoprotein and osteocalcin on the same days as control (same medium with no strontium). Concentration of strontium ranelate has been recommended to be optimized in between 0.2107 - 21.07 microg/ml whereas the high concentration up to 210.7 microg/ml have delayed effect on osteoblastic differentiation with delayed expression on Cbfa1 and osteonectin, and inhibitory effect on bone sialoprotein expression. In addition, strontium could help cell expansion by maintaining cell proliferation rate of hMSCs and osteoblast lineage. We recommend that the strontium is an important factor for inducing mesenchymal stem cells to differentiate into osteoblasts with further enhancement on bone formation. This model might provide a useful cell source for tissue engineering and bone repair.

Song, S. U., C. S. Kim, et al. (2008). "Variations of clonal marrow stem cell lines established from human

bone marrow in surface epitopes, differentiation potential, gene expression, and cytokine secretion." *Stem Cells Dev* **17**(3): 451-61.

Bone marrow has been considered to contain many different types of progenitor or stem cells. This study aims to establish a new strategy that provides for the rapid establishment of human clonal marrow stem cell (hcMSC) lines with a relatively small amount of bone marrow aspirate and to characterize newly generated hcMSC lines for their cell phenotype, differentiation potential, lineage-specific gene expression, and cytokine secretion. Human cMSC lines were generated with human bone marrow aspirates using a new protocol, called the subfractionation culturing method. The newly established hcMSC lines were analyzed for their cell surface epitopes by fluorescence-activated cell sorting (FACS), differentiation potential by in vitro differentiation assays, lineage-specific gene expression by RT-PCR, and cytokine secretion by enzyme-linked immunoassay (ELISA). The overall profile of the cell-surface epitopes of the newly established hcMSC lines was similar to those of the known MSCs. These hcMSC lines were capable of differentiating into multilineages with some differences in differentiation capability. In addition, these hcMSC lines secrete high levels of transforming growth factor-beta1 (TGF-beta1), leukemia inhibitory factor (LIF), TGF-alpha, and interleukin-10 (IL-10), again with some variation in each cell line. The newly designed protocol may be an efficient method to establish hcMSC lines rapidly with a relatively small amount of bone marrow sample, and these newly established hcMSC lines possess stem cell characteristics and exhibit some differences in cell-surface epitopes, differentiation potential, lineage-specific gene expression, and cytokine secretion.

Stewart, F. M., D. Temeles, et al. (1993). "Post-5-fluorouracil human marrow: stem cell characteristics and renewal properties after autologous marrow transplantation." *Blood* **81**(9): 2283-9.

The effect of 5-fluorouracil (5-FU) pretreatment on human bone marrow (BM) progenitor/stem cells and recovery of hematopoiesis after autologous marrow transplant was studied. Twenty-one patients were treated with 5-FU (15 mg/kg to 45 mg/kg) intravenously (IV) for 1 to 3 days administered 6 to 22 days before BM harvest. Post-FU marrow was infused into 15 patients after high-dose cyclophosphamide, carmustine (BCNU), and VP-16 (CBV). Seventeen patients (historical controls) were treated with CBV and autologous BM transplantation but did not receive 5-FU before marrow harvest. The groups were comparable for diagnosis and prior therapy. In the 5-FU-treated group and control group,

median recovery times for platelet count to 50,000/mm³ were 20 and 30 days, respectively (P = .007), and for platelet count to 100,000/mm³, 23 and 38 days, respectively (P = .007), while neutrophil recovery was not significantly altered. In vitro cultures with 1 to 7 growth factors (interleukin-1 [IL-1], IL-3, IL-4, IL-6, colony-stimulating factor-1 [CSF-1], granulocyte-macrophage colony-stimulating factor [GM-CSF], and G-CSF) were performed. In 8 of 10 patients whose marrow was studied before and after 5-FU treatment, the numbers of CFU-C responsive to the combination of GM-CSF and IL-3 was increased 6.15-fold by 5-FU pretreatment. In 4 of these patients, thymidine suicide of GM-CSF- and IL-3-stimulated CFU-C ranged from 17% to 42%. High proliferative potential colony-forming cell (HPP-CFC) was observed in low frequency in normal marrow and patient's marrow before 5-FU treatment. In 11 of 16 patients pretreated with 5-FU, increased numbers of HPP-CFC were noted. GM-CSF and IL-3 interacted synergistically to stimulate HPP-CFC. Multifactor combinations, especially GM-CSF + G-CSF + IL-3 + IL-6 + IL-1 + CSF-1 did not increase total colony count or classic HPP-CFC but did result in altered morphology, producing huge, loose colonies. The marrow from patients pretreated with 5-FU is enriched with multifactor-responsive HPP-CFC, renews in vivo granulopoiesis in a manner comparable with marrow harvests without 5-FU pretreatment, and provides accelerated in vivo platelet recovery. This marrow may be an appropriate target marrow for gene insertion in gene-therapy protocols.

Takeda, K. and G. Dennert (1993). "The development of autoimmunity in C57BL/6 lpr mice correlates with the disappearance of natural killer type 1-positive cells: evidence for their suppressive action on bone marrow stem cell proliferation, B cell immunoglobulin secretion, and autoimmune symptoms." *J Exp Med* **177**(1): 155-64.

F1 hybrid mice are able to acutely reject parental marrow grafts, a phenomenon that is due to natural killer type 1-positive (NK1+) cells. Circumstantial evidence had suggested that the antigenic determinants recognized by these cells are self-antigens, leading to the hypothesis that the physiological role of NK1+ cells is a downregulatory or suppressive function on bone marrow stem cell proliferation and lymphocyte function. In analyzing this hypothesis it is shown here that in young mice there is a temporal correlation between appearance of NK1+ cells in the spleen and the ability to reject allogeneic marrow or to suppress endogenous stem cell proliferation. The reverse situation exists in mice expressing the homozygous lpr gene. Whereas in young mice cells with NK1+ phenotype are

demonstrable, these cells disappear with age, i.e., at the time autoimmunity develops. Concomitant with the disappearance of NK1+ cells, the ability to reject marrow grafts and to control endogenous stem cell proliferation also vanishes. The suggestion that the development of autoimmunity is causally related to the disappearance of NK1+ cells is supported by experiments in which NK1+ cells were either eliminated by antibody injection or increased by adoptively transferring cell populations enriched for NK1+ cells into lpr mice. It is shown that removal of cells enhances autoimmunity, whereas injection of NK1+ cells delays the onset of autoimmunity. In vitro assays are presented that demonstrate that suppression of autoantibody-secreting B cells is due to two NK1+ cell populations, one that expresses CD3 and causes specific suppression and one that lacks CD3 and causes nonspecific suppression.

Taylor, K. N., V. R. Shinde-Patil, et al. (2007). "Induction of FoxP3+CD4+25+ regulatory T cells following hemopoietic stem cell transplantation: role of bone marrow-derived facilitating cells." *J Immunol* **179**(4): 2153-62.

The establishment of donor cell lineages following allogeneic bone marrow transplantation is frequently associated with the development of graft-vs-host disease (GVHD). The identification of cell populations that are capable of supporting allogeneic stem cell (SC) engraftment and the induction of tolerance without inducing GVHD could expand the use of this therapy. CD8(+)TCR(-) facilitating cells (FC) have been shown to promote allogeneic SC engraftment with resulting transplantation tolerance across complete MHC barriers without inducing GVHD. Although donor reconstitution in SC plus FC recipients is associated with the induction of regulatory T cell-associated factors, it is not known whether an induction of regulatory T cells and subsequent tolerance is a direct effect of the FC. The current study demonstrates that 1) SC plus FC transplantation results in the induction of donor CD4(+)25(+) regulatory T cells and that FC are present in the spleen of recipients before the induction of these cells, 2) activation of FC with CpG-oligodeoxynucleotide promotes CD4(+)25(-) T cell differentiation into CD4(+)25(+) regulatory T cells in vitro, as demonstrated by cytokine and forkhead/winged helix transcription factor (FoxP3) gene and protein expression, and 3) direct contact between FC and CD4(+)25(-) T cells is required for FoxP3(+)CD4(+)25(+) regulatory T cell induction and is dependent on CD86 expression on FC. This is the first report to demonstrate a mechanism for FC in the induction of regulatory T cells following allogeneic SC plus FC transplantation. The transplantation of

donor FC may provide an alternative approach to permit clinical SC engraftment and induction of transplantation tolerance in the future.

Thorsteinsdottir, U., A. Mamo, et al. (2002). "Overexpression of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion." *Blood* **99**(1): 121-9.

Cytogenetic, genetic, and functional studies have demonstrated a direct link between deregulated Hoxa9 expression and acute myeloid leukemia (AML). Hoxa9 overexpression in mouse bone marrow cells invariably leads to AML within 3 to 10 months, suggesting the requirement for additional genetic events prior to AML. To gain further insight into how Hoxa9 affects hematopoietic development at the preleukemic stage, we have engineered its overexpression (1) in hematopoietic stem cells using retrovirus-mediated gene transfer and generated bone marrow transplantation chimeras and (2) in lymphoid cells using transgenic mice. Compared with controls, recipients of Hoxa9-transduced cells had an about 15-fold increase in transplantable lymphomyeloid long-term repopulating cells, indicating the capacity for this oncogene to confer a growth advantage to hematopoietic stem cells. In addition, overexpression of Hoxa9 in more mature cells enhanced granulopoiesis and partially blocked B lymphopoiesis at the pre-B-cell stage but had no detectable effect on T lymphoid development. Interestingly, despite specifically directing high expression of Hoxa9 in T and B lymphoid lineages, none of the Hoxa9 transgenic mice developed lymphoid malignancies for the observation period of more than 18 months.

Toksoz, D., K. M. Zsebo, et al. (1992). "Support of human hematopoiesis in long-term bone marrow cultures by murine stromal cells selectively expressing the membrane-bound and secreted forms of the human homolog of the steel gene product, stem cell factor." *Proc Natl Acad Sci U S A* **89**(16): 7350-4.

The maintenance and differentiation of hematopoietic stem cells is influenced by cells making up the hematopoietic microenvironment (HM), including bone marrow-derived stromal cells. We and several other investigators have recently demonstrated the molecular basis of abnormal HM observed in the steel mutant mouse and cloned the normal cDNA products of this gene (termed SCF, KL, or MCF). In this report, we focus on the human counterpart of the mouse Steel (Sl) gene. Alternative splicing of the human SCF pre-mRNA transcript results in secreted and membrane-bound forms of the protein. To investigate the role of these two forms of human SCF, we targeted an immortalized stromal cell line derived from fetal murine homozygous (Sl/SI) SCF-deficient

embryos for gene transfer of various human cDNAs encoding SCF. We report that stable stromal cell transfectants can differentially process the two forms of human SCF protein product. We also demonstrate that both soluble SCF and membrane-bound SCF are active in increasing the number of human progenitor cells in the context of stromal cell cultures, although in a qualitatively different manner. Hence, the membrane-bound form of SCF may play an important role in the cell-cell interactions observed between stromal and hematopoietic cells both in vitro and in vivo.

Tonti, G. A. and F. Mannello (2008). "From bone marrow to therapeutic applications: different behaviour and genetic/epigenetic stability during mesenchymal stem cell expansion in autologous and foetal bovine sera?" *Int J Dev Biol* **52**(8): 1023-32.

Bone marrow-derived mesenchymal stem cells are a multipotent adult cellular population endowed with broad differentiation potential. Their regeneration capability, ease to undergo gene modifications, and immuno-suppressive capacity makes them optimal tools for tissue engineering, gene- and immuno-therapy. Due to the ever-increasing number of studies on the clinical applications of mesenchymal stem cells in regenerative medicine, these cells have become attractive targets in clinical transplantation. However, the identification and definition of mesenchymal stem cell culture media for their clinical application in cell therapy is currently a matter of strong discussion. Up to now, clinical studies have been conducted with mesenchymal stem cells cultured in foetal calf serum, and the chance of contamination or immunological reaction towards xenogeneic compounds must be taken into consideration. On the other hand, a serum-free medium without the addition of growth factors is not able to expand these cells in vitro; so the evaluation of which is best, among foetal calf serum, human serum (whether autologous or allogeneic) and platelet-rich plasma, is a hot topic urgently needing further research efforts. The need for the establishment of standardized protocols for mesenchymal stem cell preparations, in order not to interfere with their self-renewal and differentiation processes, assuring durable engraftment and long-term therapeutic effects, is evidently crucial. Therefore, the search for optimal culture conditions for the effective clinical-scale production of vast numbers of mesenchymal stem cells for cellular therapy is of paramount importance and the need for a robust passage from basic to translational research is fundamental.

Tsinalovsky, O., A. O. Vik-Mo, et al. (2007). "Zebrafish kidney marrow contains ABCG2-

dependent side population cells exhibiting hematopoietic stem cell properties." Differentiation **75**(3): 175-83.

Zebrafish (*Danio rerio*) has emerged as a powerful genetic model for the study of vertebrate hematopoiesis. However, methods for detection and isolation of hematopoietic stem cells (HSCs) have not yet been reported. In mammals, the combination of Hoechst 33342 staining with flow cytometry can be used for separation of a bone marrow side population (SP), which is highly enriched for HSCs. We applied a similar procedure to hematopoietic kidney marrow cells from adult zebrafish, and identified a segregated cohort of SP cells, which demonstrate a set of features typical of stem cells. SP cells show extremely low scatter characteristics, and are small in size with a minimum of cytoplasm. Treatment of zebrafish kidney marrow cells with reserpine or fumitremorgin C, which inhibit the ABCG2 transporter responsible for Hoechst 33342 efflux, caused a clear reduction in the number of SP cells. Consistent with the quiescent state of HSCs, the SP cells are strongly resistant to the myelosuppressive agent 5-fluorouracil. In addition, SP cells specifically demonstrate higher expression of genes known to be markers of HSCs of mammals. Hence, our results show that the SP phenotype is conserved between mammals and teleosts, and the properties of the zebrafish SP cells indicate a significant enrichment for HSCs. These rapid flow cytometric methods for purification of HSCs from zebrafish may greatly facilitate genetic analysis of stem cells using the advantages of this vertebrate model.

Ueda, K., Y. Hanazono, et al. (2004). "High-level in vivo gene marking after gene-modified autologous hematopoietic stem cell transplantation without marrow conditioning in nonhuman primates." Mol Ther **10**(3): 469-77.

The successful engraftment of genetically modified hematopoietic stem cells (HSCs) without toxic conditioning is a desired goal for HSC gene therapy. To this end, we have examined the combination of intrabone marrow transplantation (iBMT) and in vivo expansion by a selective amplifier gene (SAG) in a nonhuman primate model. The SAG is a chimeric gene consisting of the erythropoietin (EPO) receptor gene (as a molecular switch) and c-Mpl gene (as a signal generator). *Cynomolgus* CD34+ cells were retrovirally transduced with or without SAG and returned into the femur and humerus following irrigation with saline without prior conditioning. After iBMT without SAG, 2-30% of colony-forming cells were gene marked over 1 year. The marking levels in the peripheral blood, however, remained low (<0.1%). These results indicate that

transplanted cells can engraft without conditioning after iBMT, but in vivo expansion is limited. On the other hand, after iBMT with SAG, the peripheral marking levels increased more than 20-fold (up to 8-9%) in response to EPO even at 1 year posttransplant. The increase was EPO-dependent, multilineage, polyclonal, and repeatable. Our results suggest that the combination of iBMT and SAG allows efficient in vivo gene transduction without marrow conditioning.

Vadala, G., R. K. Studer, et al. (2008). "Coculture of bone marrow mesenchymal stem cells and nucleus pulposus cells modulate gene expression profile without cell fusion." Spine (Phila Pa 1976) **33**(8): 870-6.

STUDY DESIGN: Changes in gene expression profile and cell fusion of mesenchymal stem cells (MSC) and nucleus pulposus cells (NPC) after coculture were analyzed. **OBJECTIVE:** To investigate the mechanisms of the interaction between NPC and MSC such as differentiation, stimulatory effect, and cell fusion. **SUMMARY OF BACKGROUND DATA:** Introduction of exogenous cells to supplement and replenish intervertebral disc cell population offers a potential approach to treat intervertebral disc degeneration (IDD). Recent evidences showed that intradiscal injection of MSC effectively alter the course of IDD in vivo, and the regenerative potential may result from up-regulated extracellular matrix protein synthesis mediated by MSC and NPC interaction. **METHODS:** Using a double labeling cell system and flow activated cell sorting, we quantitatively analyzed changes in the gene expression profile of human male MSC and female NPC after coculture in a 3-dimensional system that allows short distance paracrine interactions typical of the nucleus pulposus. Furthermore, we analyzed for cell fusion in the cell interaction by fluorescence in situ hybridization (FISH) for X and Y chromosomes, using a 3-dimensional culture system to allow cell-to-cell interactions conducive to cell fusion. **RESULTS:** Two weeks of coculture cell interaction in a 3-dimensional environment induces a change in MSCs towards a more chondrogenic gene expression profile indicating MSC differentiation, and NPC gene expression changes in matrix and chondrogenic genes demonstrating only a modest trophic effect of MSC on NPC. Moreover, FISH analysis demonstrated that cell fusion is not responsible for MSC plasticity in the interaction with NPCs. **CONCLUSION:** This study clarifies the mechanism of MSCs and NPCs interaction in a 3-dimensional environment, excluding cell fusion. These data support the use of undifferentiated MSC for stem cell therapy for IDD treatment.

Violini, S., P. Ramelli, et al. (2009). "Horse bone marrow mesenchymal stem cells express embryo stem cell markers and show the ability for tenogenic differentiation by in vitro exposure to BMP-12." *BMC Cell Biol* **10**: 29.

BACKGROUND: Mesenchymal stem cells (MSCs) have been recently investigated for their potential use in regenerative medicine. MSCs, in particular, have great potential, as in various reports they have shown pluripotency for differentiating into many different cell types. However, the ability of MSCs to differentiate into tendon cells in vitro has not been fully investigated. **RESULTS:** In this study, we show that equine bone marrow mesenchymal stem cells (BM-MSCs), defined by their expression of markers such as Oct4, Sox-2 and Nanog, have the capability to differentiate in tenocytes. These differentiated cells express tendon-related markers including tenomodulin and decorin. Moreover we show that the same BM-MSCs can differentiate in osteocytes, as confirmed by alkaline phosphatase and von Kossa staining. **CONCLUSION:** As MSCs represent an attractive tool for tendon tissue repair strategies, our data suggest that bone marrow should be considered the preferred MSC source for therapeutic approaches.

Walma, E. P., H. M. Vriesendorp, et al. (1987). "Engraftment of stem-cell-enriched bone marrow fractions in MHC-identical dogs after fractionated total-body irradiation." *Transplantation* **43**(6): 818-23.

Discontinuous albumin density gradients were used to obtain enrichment of hemopoietic stem cells and depletion of T lymphocytes in aspirated dog bone marrow. Colony forming units in agar (CFU-C) were determined to evaluate the degree of enrichment achieved. An average CFU-C concentration factor of 12.4 was obtained. All transplantations in the study were carried out between DLA-identical sibling combinations. The number of CFU-C administered varied from 0.2 to 5.5 X 10⁵/kg and the number of nucleated cells transfused varied from 0.1 to 1.0 X 10⁸/kg. Stem cell concentrates were found more difficult to engraft than unmodified bone marrow following standard conditioning with a single total-body irradiation (TBI) dose of 7.5 Gy. The efficacy of different TBI-fractionation schedules for obtaining sustained engraftment of CFU-C-enriched grafts in identical bone marrow transplantation (BMT) was determined. A total dose of 12 Gy TBI delivered in two equal fractions of 6.0 Gy (72-hr interval) resulted in sustained engraftment of stem cell grafts in 7 of 7 evaluable dogs. A TBI dose of 9 Gy in two fractions of 4.5 Gy (72-hr interval) resulted in sustained engraftment in 5 of 7 evaluable dogs. The two dogs with engraftment failure received low total cell

numbers (10⁷ cells/kg) and low CFU-C numbers. 9 Gy of TBI in two fractions of 4.5 Gy (24-hr interval) resulted in sustained engraftment in 11 of 12 evaluable dogs. A significant improvement of engraftment was obtained by increasing the total dose of TBI, which necessitates fractionation into two fractions of TBI. The lower-total-dose TBI (9 Gy) produced less early and late toxicity than the total high-dose (12 Gy) TBI. The incidence of engraftment was similar for the two dosages, however the recovery of peripheral leukocyte counts was slower after 9 Gy TBI. In the dog, optimal conditioning for lymphocyte-depleted hemopoietic stem cell grafts can be obtained by increasing the dose of TBI and concomitant fractionation.

Wang, L., H. O'Leary, et al. (2007). "Ph⁺/VE-cadherin⁺ identifies a stem cell like population of acute lymphoblastic leukemia sustained by bone marrow niche cells." *Blood* **110**(9): 3334-44.

Although leukemic stem cells (LSCs) show a symbiotic relationship with bone marrow microenvironmental niches, the mechanism by which the marrow microenvironment contributes to self-renewal and proliferation of LSCs remains elusive. In the present study, we identified a unique subpopulation of Philadelphia chromosome-positive (Ph⁺) acute lymphoblastic leukemia (ALL) cells coexpressing markers of endothelial cells (including VE-cadherin, PECAM-1, and Flk-1) and committed B-lineage progenitors. After long-term coculture with bone marrow stromal cells, tumor cells formed hematopoietic colonies and cords, expressed early stem-cell markers, and showed endothelial sprouting. Gene expression profiles of LSCs were altered in the presence of stromal cell contact. Stromal cell contact promoted leukemic cell VE-cadherin expression, stabilized beta-catenin, and up-regulated Bcr-abl fusion gene expression. Our study indicates that these specific tumor cells are uniquely positioned to respond to microenvironment-derived self-renewing and proliferative cues. Ph⁺/VE-cadherin⁺ tumor subpopulation circumvents the requirement of exogenous Wnt signaling for self-renewal through stromal cell support of leukemic cell VE-cadherin expression and up-regulated Bcr-abl tyrosine kinase activity. These data suggest that strategies targeting signals in the marrow microenvironment that amplify the Bcr-abl/VE-cadherin/beta-catenin axis may have utility in sensitizing drug-resistant leukemic stem cells.

Wang, Q., B. Sun, et al. (2008). "Murine bone marrow mesenchymal stem cells cause mature dendritic cells to promote T-cell tolerance." *Scand J Immunol* **68**(6): 607-15.

Bone mesenchymal stem cells (BMSC) are attractive not only in regenerative medicine, but also for the treatment of autoimmune diseases and graft-versus-host disease. BMSC also play a role in enabling alloantigen tolerance. An in-depth mechanistic understanding of this phenomenon of tolerance could lead to novel cell-based therapies for autoimmune disease. We demonstrate here that co-culture of mature dendritic cells (DC) with BMSC in a transwell system (BMSC-DC) downregulated expression of the maturation marker, CD83 and CD80/86 co-stimulatory molecules on DC, while increasing their endocytic activity. This resulted in defective antigen presentation and co-stimulatory capacity of mature DC. Functionally, BMSC-DC have impaired T-cell stimulatory activity in a mixed lymphocyte reaction and orchestrate a shift from predominantly pro-inflammatory T-helper (Th)-1 to anti-inflammatory Th2 cells. While the expression of MHC II, CD80 and CD86 were upregulated on BMSC co-cultured with DC, these BMSC lacked the ability to stimulate T-cell proliferation. Taken together, these data suggest that the interaction between BMSC and DC modulates the immunoregulatory function of these cells in a coordinated manner, effectively skewing the immune response towards T-cell tolerance.

Winter, A., S. Breit, et al. (2003). "Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells." *Arthritis Rheum* **48**(2): 418-29.

OBJECTIVE: To compare the chondrogenic potential of human bone marrow-derived mesenchymal stem cells (BMSC) and adipose tissue-derived stromal cells (ATSC), because the availability of an unlimited cell source replacing human chondrocytes could be strongly beneficial for cell therapy, tissue engineering, in vitro drug screening, and development of new therapeutic options to enhance the regenerative capacity of human cartilage. **METHODS:** Quantitative gene expression of common cartilage and cell interaction molecules was analyzed using complementary DNA array technology and reverse transcription-polymerase chain reaction during optimization of cell differentiation, in order to achieve a molecular phenotype similar to that of chondrocytes in cartilage. **RESULTS:** The multilineage potential of BMSC and ATSC was similar according to cell morphology and histology, but minor differences in marker gene expression occurred in diverse differentiation pathways. Although chondrogenic differentiation of BMSC and ATSC was indistinguishable in monolayer and remained partial, only BMSC responded (with improved chondrogenesis) to a shift to high-density 3-

dimensional cell culture, and reached a gene expression profile highly homologous to that of osteoarthritic (OA) cartilage. **CONCLUSION:** Hypertrophy of chondrocytes and high matrix-remodeling activity in differentiated BMSC spheroids and in OA cartilage may be the basis for the strong similarities in gene expression profiles between these samples. Differentiated stem cell spheroids represent an attractive tool for use in drug development and identification of drug targets in OA cartilage-like tissue outside the human body. However, optimization of differentiation protocols to achieve the phenotype of healthy chondrocytes is desired for cell therapy and tissue engineering approaches.

Xu, J., X. Deng, et al. (2008). "Factors released from cholestatic rat livers possibly involved in inducing bone marrow hepatic stem cell priming." *Stem Cells Dev* **17**(1): 143-55.

Previous studies have shown that bone marrow beta 2m(-)/Thy-1+ hepatic stem cells (BMHSCs) were able to engraft in vivo and differentiate into functioning hepatocytes in vitro. Our transcriptomic profiling on BMHSCs derived from rats subjected to common bile duct ligation (CBDL) demonstrated CBDL-derived beta 2m(-)/Thy-1+ BMHSCs expressed hepatocyte-like genes and shared more commonly expressed genes with hepatocytes, suggesting that an "on-site" priming of BMHSCs into hepatocyte lineage was initiated under the condition of CBDL. In this paper, transcriptomic profiling was carried out on livers from rats with CBDL to identify candidate factors released from cholestatic livers possibly involved in the priming of BMHSCs using Affymetrix Rat Genome U34A arrays. In CBDL rat livers, 1,091 probe sets were differentially expressed, of which 188 up-regulated probe sets were annotated as "extracellular" components. Gene ontology analysis showed many up-regulated genes belonged to cytokines, chemokines and growth factors, including Il1b, Il18, Ptn, Spp1, Grn, Ccl2, Cxcl1, Pf4, Tgfb, and Tgfb3. Cell differentiation and proliferation regulation factors such as Dmbt1, Efna1, Lgals1, Lep, Pmp2, and Gas6 were also induced in CBDL livers. Furthermore, many proteolysis and peptidolysis genes such as Mmp2, Mmp12, Mmp14, and Mmp23 were up-regulated in CBDL livers. Gene expression profiling showed that many cytokine-, chemokine-, growth factor- as well as certain extracellular protein-related genes were induced in CBDL livers, suggesting that these genes may be involved in hepatic BMHSCs priming.

Xu, J., X. Liu, et al. (2008). "MAPK/ERK signalling mediates VEGF-induced bone marrow stem cell

differentiation into endothelial cell." *J Cell Mol Med* **12**(6A): 2395-406.

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

Xu, W., H. Qian, et al. (2004). "A novel tumor cell line cloned from mutated human embryonic bone marrow mesenchymal stem cells." *Oncol Rep* **12**(3): 501-8.

A novel tumor cell line, denominated F6, was established from mutated human embryonic bone marrow mesenchymal stem cells (MSCs) which were induced by the GM-CSF and IL-4 in vitro. The characteristics of the F6 cell line, such as surface antigens, cell cycle, growth curve, gene expression, morphology, cytogenetics and tumor model were analyzed. The F6 cells were round and grew suspended in a plastic dish. The cell line has a strong self-renewal capability, was positive for CD13, CD29, CD44, but negative for CD1alpha, CD3, CD10, CD14, CD23, CD33, CD34, CD38, CD41, CD45, CD54 and HLA-DR. The surface antigens were lower than those of human embryonic MSCs. The karyotype of F6 cells

was abnormal. The cell cycle included: G0/G1 phase, 52.24%; G2/M phase, 8.00%; S phase, 41.76%. After the cells had been passaged serially for more than 17 months (62 passages), their characteristics were still retained. The F6 cells resulted in tumors in SCID nude mice in vivo (8/8) and caused metastasis (3/8). The pathologic examination revealed that the tumor cells extensively invaded surrounding normal tissues such as dermis, muscular tissue, nerve tissue, adipose tissue and lymphoid tissue. F6 cell line, tumor tissues derived from F6 cells and the MSCs expressed different levels of the nucleostemin gene. These findings suggested that F6 may be a novel tumor cell line. It may provide evidence for the theory that cancer originates from stem cells, and may be useful for the investigation on safety of human MSCs in the clinical application.

Xu, X., Z. Xu, et al. (2005). "Selective down-regulation of extracellular matrix gene expression by bone marrow derived stem cell transplantation into infarcted myocardium." *Circ J* **69**(10): 1275-83.

BACKGROUND: Myocardial fibrosis is a major component of ventricular remodeling after myocardial infarction (MI). The aim of the present study was to determine the outcome of transplantation into ischemic myocardium of bone marrow derived stem cell (MSC) on left ventricular (LV) function and remodeling, and to look closely at extracellular matrix gene expression. METHODS AND RESULTS: MI was induced by direct ligation of the left anterior descending coronary artery in rats, followed by MSC transplantation into the ischemic myocardium. Hemodynamic evaluations were performed at 7 and 28 days after coronary ligation. Changes in the mRNA expressions of collagen type I and type III, matrix metalloproteinase-1, tissue inhibitor of matrix metalloproteinase (TIMP)-1 and transforming growth factor (TGF)-beta(1) were investigated using reverse transcription polymerase chain reaction and in situ hybridization after MI. Heart/body weight ratio in the MI + MSC group decreased after coronary ligation. However, LV systolic pressure and LV peak velocities of contraction and relaxation significantly increased compared with the MI group ($p < 0.01$). There was marked up-regulation of the mRNA expressions of collagen types I and III, TIMP-1 and TGF-beta(1) in the MI rats, all of which were significantly attenuated by MSC transplantation. CONCLUSIONS: MSC transplantation could inhibit LV remodeling, improve heart function and reduce the expression of extracellular matrix genes.

Yang, L., L. Wang, et al. (2007). "Rho GTPase Cdc42 coordinates hematopoietic stem cell quiescence and

niche interaction in the bone marrow." Proc Natl Acad Sci U S A **104**(12): 5091-6.

Adult hematopoietic stem cells (HSCs) exist in a relatively quiescent state in the bone marrow (BM) microenvironment to fulfill long-term self-renewal and multilineage differentiation functions, an event that is tightly regulated by extrinsic and intrinsic cues. However, the mechanism coordinating the quiescent state of HSCs and their retention in the BM microenvironment remains poorly understood. In a conditional-knockout mouse model, we show that Cdc42(-/-) HSCs enter the active cell cycle, resulting in significantly increased number and frequency of the stem/progenitor cells in the BM. Cdc42 deficiency also causes impaired adhesion, homing, lodging, and retention of HSCs, leading to massive egress of HSCs from BM to distal organs and peripheral blood and to an engraftment failure. These effects are intrinsic to the HSCs and are associated with deregulated c-Myc, p21(Cip1), beta1-integrin, and N-cadherin expressions and defective actin organization. Thus, Cdc42 is a critical coordinator of HSC quiescence maintenance and interaction with the BM niche.

Yaroslavskiy, B., Y. Colson, et al. (1998). "Addition of a bone marrow "facilitating cell" population increases stem cell-derived cobblestone area formation in impaired long-term bone marrow culture stroma." Exp Hematol **26**(7): 604-11.

Treatment of mouse bone marrow (BM) with rabbit anti-mouse brain serum (RAMBS) plus complement (C') depletes several cell types, including T cells and facilitating cells (FCs), that is, cells that facilitate engraftment of sorted allogeneic stem cells (SCs) in vivo. In the present study, treatment of BM with RAMBS+C' resulted in the depletion of approximately half of the late cobblestone area (CA)-forming stem cells as assayed on irradiated long-term bone marrow culture (LTBMC) stroma. In addition, LTBMC of RAMBS+C'-treated BM produced functionally impaired stroma with reduced ability to support CA formation by nontreated exogenous SCs. This stromal impairment was not due to depletion of TCRalpha T cells in the BM, because BM cultures from TCR alpha-chain knockout mice supported normal numbers of exogenous CAs. Because CD8+/TCR- cells are enriched for FCs, we tested the effect of adding these cells back to the treated BM prior to culture. The sorted FCs alone did not produce CAs, but did improve the ability of the impaired stroma to support late CA formation by sorted SCs. These studies provide a new model for dissecting the roles of different cellular components of BM in producing functional stroma that supports CA formation by SCs, and show that the number of CAs formed depends on the "quality" of the stroma as well

as the number of SCs seeded. These findings further suggest that CD8+/TCR- BM cells may be important for the establishment of functional stroma.

Yeon Lim, J., S. S. Jeun, et al. (2006). "Multiple stem cell traits of expanded rat bone marrow stromal cells." Exp Neurol **199**(2): 416-26.

Bone marrow stromal cells (BMSC) exhibit many traits of a stem cell population. Knowing that BMSC have the ability to self-renew, proliferate and differentiate into a variety of cell types, questions may arise as to whether these traits differ between the cells that have different expansion times. In this study, we examined the stem cell potentiality of BMSC through their characterization, proliferative capacity and the ability to differentiate into multiple lineages in the cultured 2nd passage cells and 10th passage cells. The results were as follows: (1) the 10th passage cells had a larger and more flattened morphology than the 2nd passage cells and also exhibited a decreased labeling for BMSC-related antigens such as CD90, CD73. (2) The cell proliferative capacity was approximately 2 times greater in the 2nd passage cells, and the apoptosis phenomenon was detected in the 10th passage cells. (3) The ability to differentiate into mesodermal tissue (osteocytes, adipocytes), as well as into ectodermal tissue (neurons) was more effective in the 2nd passage cells. Taken together, early stage BMSC would be a valuable cell source for various in vitro applications, as well as cell therapy.

Zhang, H., P. Song, et al. (2007). "Injection of bone marrow mesenchymal stem cells in the borderline area of infarcted myocardium: heart status and cell distribution." J Thorac Cardiovasc Surg **134**(5): 1234-40.

OBJECTIVE: This study aimed to test the distribution of intramyocardially injected cells in variations in heart status in a porcine model of myocardial infarction. **METHODS:** Bone marrow-derived mesenchymal stem cells were obtained from male swine and labeled with iron oxide during culture. One week after creation of a myocardial infarction in female swine, the survivors were randomly divided into 4 groups. Cardiopulmonary bypass was set up to arrest the heart, and then labeled cells (1×10^8) were intramyocardially injected into the border zone of the infarcted zone in group 1 ($n = 6$). The same volume of cells was grafted into the beating heart in group 2 ($n = 6$). In groups 3 and 4, saline was injected in either the arresting or beating heart. Three days later, cell distribution was assessed by T2* change with magnetic resonance imaging and sex-determining region on Y-chromosome (SRY) with quantitative polymerase chain reaction. **RESULTS:** The cells were identified in the heart, spleen, lung, and liver. Most

injected cells were localized in the myocardium in groups 1 and 2; however, the amount of cells was much higher in group 1 (T2* change: 22.3 +/- 2.2 vs 17 +/- 0.84; SRY gene: 0.15 +/- 0.062 vs 0.072 +/- 0.003). CONCLUSIONS: Even after intramyocardial injection, many cells migrated to extracardiac organs, especially to the spleen. Our results indicated that injection in the arresting heart could favor retaining more cells in the myocardium. Thus, it was an optimal approach to deliver mesenchymal stem cells during open chest surgery.

Zhang, J. L., J. Cai, et al. (2005). "Competitive equality of donor cells expressing a disparate MHC antigen following stem cell-enriched bone marrow transplantation." *Transplantation* **79**(10): 1332-7.

INTRODUCTION: Bone marrow cells expressing foreign MHC antigens survive poorly after transplantation. Stable mixed hematopoietic chimerism requires reconstitution with a relatively large number of foreign bone marrow cells and intensive depletion of host cells. In addition, when foreign MHC-transduced autologous bone marrow cells are transplanted, prolonged hematopoietic transgene expression requires extensive host conditioning. We used a limiting-dilution competitive repopulation assay with cells from HLA-A2.1 transgenic mice to determine whether and to what extent foreign MHC antigen expression impairs engraftment in C57BL/6 hosts. Transplants were performed with Hoechst 33342 fluorescence-sorted side population (SP) cells, a subset of bone marrow enriched for stem cells. RESULTS.: Transplantation with 250 stem cell-enriched HLA-A2.1-transgenic side population cells successfully competed with nearly 5000 host C57BL/6 side population cells to produce stable long-term mixed chimerism. There was a direct relationship between the number of transplanted donor HLA-A2-expressing cells and the percentage of HLA-A2-expressing cells in the peripheral blood of reconstituted C57BL/6 mice ($r^2=0.1799$, $P=0.031$). This correlation was maintained in secondary transplant recipients. CONCLUSIONS: HLA-A2-expressing hematopoietic cells do not have an engraftment defect when transplanted into C57BL/6 hosts and immunologic resistance did not limit chimerism following lethal irradiation. These results may have relevance to understanding long-term gene expression after hematopoietic stem cell based gene therapy.

Zhao, Z., X. Tang, et al. (2006). "Assessment of bone marrow mesenchymal stem cell biological characteristics and support hematopoiesis function in patients with chronic myeloid leukemia." *Leuk Res* **30**(8): 993-1003.

Mesenchymal stem cells (MSCs) are of great therapeutic potential because of their ability to self-renew and differentiate into multiple tissues. Compare to allogeneic MSCs, autologous MSCs from patients needed cell-based therapy may be an ideal alternative stem cell source. However, characteristics of MSCs from a disease state are poorly understood. So, we have isolated and characterized MSCs from chronic myeloid leukemia (CML) patients and compared them with MSCs derived from normal adult bone marrow. Our results showed that CML derived MSCs are similar to normal MSCs in phenotype, morphology and multi-differentiation capacity. Moreover, CML derived MSCs did not express BCR/ABL gene and Ph chromosome, and had not the ability to development tumor in nude mice. At last, they could express hematopoietic cytokines, and possessed hematopoietic supportive ability. These findings indicate that MSCs derived from CML patients' bone marrow may be an attractive tool for clinical needs.

Zheng, H., J. A. Martin, et al. (2007). "Impact of aging on rat bone marrow-derived stem cell chondrogenesis." *J Gerontol A Biol Sci Med Sci* **62**(2): 136-48.

Damaged articular cartilage rarely heals or regenerates in middle-aged and elderly adults, suggesting that the chondrogenic potential of mesenchymal stem cells declines with age. To test this hypothesis, we measured the responses of rat bone marrow-derived mesenchymal stem cells (BMSCs) to chondrogenic induction in vitro. BMSCs from immature rats (1 week old), young adult rats (12 weeks old), and old adult rats (1 year old) were analyzed for cartilage extracellular matrix (ECM) production. Histologic analysis showed strong cartilage ECM formation by BMSCs from 1-week-old rats, but not by BMSCs from 12-week-old or 1-year-old rats. Real-time polymerase chain reaction revealed age-related declines in messenger RNA encoding type II collagen, aggrecan, and link protein, three major cartilage ECM components. Microarray analysis indicated significant age-related differences in the expression of genes that influence cartilage ECM formation. These findings support the hypothesis that the chondrogenic potential of mesenchymal stem cells declines with age.

Zhong, J. F., Y. Zhan, et al. (2002). "Murine hematopoietic stem cell distribution and proliferation in ablated and nonablated bone marrow transplantation." *Blood* **100**(10): 3521-6.

The engraftment of donor bone marrow (BM) cells in nonablated mice is inefficient. Niche availability has been thought to be the reason, and cytoablation with irradiation or cytotoxic agents is

routinely used with the belief that this frees the preoccupied niches in recipients. In this study, donor cell redistribution and proliferation in ablated and nonablated mice were compared by implanting donor cells directly into the femur cavity of sedated mice. The redistribution of Lin(-) donor cells into BM was similar between ablated and nonablated mice. Poor engraftment in nonablated mice was shown to be the result of inefficient donor cell proliferation rather than because of a lack of space. Competitive repopulation assays demonstrated that the donor hematopoietic stem cells (HSCs) were present in nonirradiated recipients for at least 6 months after transplantation, but that they did not expand as did their counterparts in lethally irradiated mice. This study suggests that efficient bone marrow transplantation in nonablated recipients may be possible as a result of better understanding of HSC proliferative regulation and appropriate *in vitro* manipulation.

Zhou, K., H. Zhang, et al. (2008). "Transplantation of human bone marrow mesenchymal stem cell ameliorates the autoimmune pathogenesis in MRL/lpr mice." *Cell Mol Immunol* 5(6): 417-24.

Recent evidence indicates that mesenchymal stem cells (MSC) possess immunosuppressive properties both *in vitro* and *in vivo*. We previously demonstrated the functional abnormality of bone marrow derived MSC in patients with systemic lupus erythematosus (SLE). In this study, we aimed to investigate whether transplantation of human bone marrow derived MSC affects the autoimmune pathogenesis in MRL/lpr mice. We found that human MSC from healthy donors reduced the proliferation of T lymphocytes from MRL/lpr mice in a dose-dependent fashion. Two weeks after *in vivo* transfer of MSC, we detected significantly reduced serum levels of anti ds-DNA antibodies and 24 hour proteinuria in MRL/lpr mice as compared with control groups without MSC transplantation. Moreover, flow cytometric analysis revealed markedly reduced number of CD4(+) T cells while increased Th1 subpopulation in MSC group and MSC + CTX group when compared with controls. Histopathological examination showed significantly reduced renal pathology in MSC-treated mice. Immunohistochemical studies further revealed reduced expression of TGF-beta, FN, VEGF and the deposition of complement C3 in renal tissue after MSC and MSC + CTX treatment. Taken together, we have demonstrated that transplantation of human MSC can significantly inhibit the autoimmune progression in MRL/lpr mice.

Zhu, W., W. Xu, et al. (2006). "Mesenchymal stem cells derived from bone marrow favor tumor cell growth *in vivo*." *Exp Mol Pathol* 80(3): 267-74.

Mesenchymal stem cells (MSCs) have generated a great deal of interest in clinical application because of their potential use in regenerative medicine and tissue engineering. However, the therapeutic application of MSCs still remain limited unless the favorable effect of MSCs for tumor growth *in vivo* and the long-term safety of the clinical applications of MSCs are better understood. In this study, MSCs derived from fetal bone marrow (FMSCs) and adult MSCs (AMSCs) alone or FMSCs and AMSCs with tumor cell line (F6 or SW480) together were transplanted subcutaneously into BALB/c-nu/nu mice to observe the outgrowth of tumor, and the characteristics of tumor cells were investigated by pathological and immunohistochemical methods, flow cytometry and real-time quantitative PCR. The results showed that both FMSCs and AMSCs could favor tumor growth *in vivo*. The pathologic examination revealed that tumor tissues had rich vessel distribution, extensive necrosis and invasion surrounding normal tissues, such as muscular tissue and subcutaneous tissue. In the immunohistochemical examination, tumor cells mixed with MSCs transplanted subcutaneously exhibited elevated capability of proliferation, rich angiogenesis in tumor tissues and highly metastatic ability. To understand whether MSCs affected the general properties of the tumor cells *in vivo*, the expression of some surface antigens and Bmi-1 gene of tumor tissue cells was detected in this study. The results indicated that these parameters were not affected after the interaction of MSCs with tumor cells *in vivo*. These findings suggested that MSCs could favor tumor growth *in vivo*. It is necessary to carry out a study for assurance of the long-term safety before MSCs were used as a therapy tools in regenerative medicine and tissue engineering.

Zipori, D. and F. Lee (1988). "Introduction of interleukin-3 gene into stromal cells from the bone marrow alters hemopoietic differentiation but does not modify stem cell renewal." *Blood* 71(3): 586-96.

Cloned endothelial-adipocytes from the stroma of mouse bone marrow (designated 14F1.1) induced growth of stem cells *in vitro*, accompanied by either pre-B lymphopoiesis or myelopoiesis. We examined the contribution of colony stimulating factors (CSF) to the process. mRNA for GM-CSF, interleukin 3 (IL3), G-CSF, and IL4 could not be detected in the stromal cells. Expression of IL3 gene, achieved by transfection of 14F1.1 cells with a plasmid carrying an IL3 cDNA, shifted the direction of differentiation but did not improve stem cell

maintenance. It is proposed that novel stromal cell factors, distinct from known CSFs, regulate stem cell renewal.

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