Stem Cell and Apostosis 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 stem cell and apoptosis.
[Smith MH. **Stem Cell and Apostosis Literatures**. *Stem Cell* 2011;2(1):66-104] (ISSN 1545-4570). http://www.sciencepub.net/stem. 6

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

Stem cells and progenitor cells are necessary for repair and regeneration of injured renal tissue. Infiltrating or resident stem cells can contribute to the replacement of lost or damaged tissue. However, the regulation of circulating progenitor cells is not well understood. Many factors influence the stem cell growth in damaged kidney. For example, low levels of erythropoietin induce mobilization and differentiation of endothelial progenitor cells and erythropoietin ameliorates tissue injury. Full regeneration of renal tissue demands the existence of stem cells and an adequate local milieu, a so-called stem cell niche. It was reported that in the regenerating zone of the shark kidney, stem cells exist that can be induced by loss of renal tissue to form new glomeruli. Stem cell may eventually contribute to novel therapies of the kidney disease (Perin et al. 2008). This material collects some literatures on stem cell and apoptosis.

Literatures

Alenzi, F. Q., B. Q. Alenazi, et al. (2009). "The haemopoietic stem cell: between apoptosis and self renewal." Yale J Biol Med **82**(1): 7-18.

Self renewal and apoptosis of haemopoietic stem cells (HSC) represent major factors that determine the size of the haemopoietic cell mass. Changes in self renewal above or below the steady state value of 0.5 will result in either bone marrow expansion or aplasia, respectively. Despite the growing body of research that describes the potential role of HSC, there is still very little information on the mechanisms that govern HSC self renewal and apoptosis. Considerable insight into the role of HSC in many diseases has been gained in recent years. In light of their crucial importance, this article reviews recent developments in the understanding of the molecular, biological, and physiological characteristics of haemopoietic stem cells.

Allard, E. K. and K. Boekelheide (1996). "Fate of germ cells in 2,5-hexanedione-induced testicular injury. II. Atrophy persists due to a reduced stem cell mass and ongoing apoptosis." Toxicol Appl Pharmacol **137**(2): 149-56.

The Sertoli cell toxicant 2,5-hexanedione (2,5-HD) causes irreversible testicular atrophy in rats. After toxicant exposure, only Sertoli cells, stem cells, and a few spermatogonia remain in the seminiferous epithelium. In this study, the number, type, and fate of the remaining germ cells were determined. Male Sprague-Dawley rats were exposed to 1% 2,5-HD in drinking water for 5 weeks and then sacrificed 12 or 40 weeks after the start of exposure. Cell counts determined that the stem cell population was diminished in size, but made up a significant portion of the remaining germ cells. The remaining germ cells were primarily type A spermatogonia. Modeling of spermatogonial divisions suggested that most spermatogonia undergo degeneration at the level of type A3 spermatogonia after 2,5-HD-induced atrophy. Apoptosis was demonstrated to occur in the remaining germ cells by nuclear morphology and in situ analysis of DNA fragmentation. Quantitation indicated that apoptosis occurred in a majority of stem cell progeny. We conclude that the irreversibility of 2,5-HD-induced testicular injury results from the reduced size of the stem cell population as well as a block in germ cell development at the level of type A spermatogonia.

Andrew, D. and R. Aspinall (2001). "Il-7 and not stem cell factor reverses both the increase in apoptosis and

the decline in thymopoiesis seen in aged mice." J Immunol **166**(3): 1524-30.

Thymic atrophy is an age-associated decline in commitment to the T cell lineage considered to be associated with defective TCR beta-chain rearrangement. Both IL-7 and stem cell factor (SCF) have dominant roles at this stage of triple negative (TN) thymocyte development. Because there is no age-associated decrease in the number of CD44(+)CD25(-)CD3(-)CD4(-)CD8(-) cells, this study investigated whether alterations in apoptosis within the TN pathway accounted for diminishing thymocyte numbers with age. Here we show significant age-associated increases in apoptotic TN thymocytes, specifically within CD44(+)CD25(+) and CD44(-)CD25(+) subpopulations, known to be the location of TCR beta-chain rearrangement. IL-7 added to TN cultures established from old mice significantly both reduces apoptosis and increases the percentage of live cells within CD44(+)CD25(+) and CD44(-)CD25(+) subpopulations after 24 h, with prosurvival effects remaining after 5 days. SCF failed to demonstrate prosurvival effects in old or young cultures, and IL-7 and SCF together did not improve upon IL-7 alone. IL-7R expression did not decline with age, ruling out the possibility that the age-associated increase in apoptosis was attributed to reduced IL-7R expression. Compared with PBS, treatment of old mice with IL-7 produced significant increases in live TN cells. By comparison, treatment with SCF failed to increase live TN numbers, and IL-7 and SCF together failed to significantly improve thymopoiesis above that shown by IL-7 alone. Thus, treatment with IL-7 alone can reverse the age-associated defect in TN thymocyte development revealed by in vitro studies to be located at the stages of TCR beta-chain rearrangement.

Anthony, R. S., N. D. McKelvie, et al. (1998). "Flow cytometry using annexin V can detect early apoptosis in peripheral blood stem cell harvests from patients with leukaemia and lymphoma." Bone Marrow Transplant **21**(5): 441-6.

Quantifying progenitor cells in peripheral blood stem cell (PBSC) harvests by flow cytometric enumeration of CD34+ cells does not account for cell viability. Cell membrane asymmetry in early apoptosis exposes phosphatidylserine on the cell surface. This can be detected by staining with annexin V FITC. Apoptosis in 30 autologous PBSC harvests mobilised by cyclophosphamide + G-CSF or standard chemotherapy + G-CSF was analysed immediately after collection by dual-colour flow cytometry with CD34 PE and annexin V FITC. Harvests contained a median of 3.4 x 10(6)/kg (range 0.3-91.8) CD34+ cells. Of these 87.6% (range 30-96.5) were annexin V-. In 10% of harvests more than 50% of CD34+ cells were apoptotic. Differences in PBSC mobilisation or collection could not explain the variation in annexin V binding. Cyclophosphamide + G-CSF significantly increased the yield of CD34+ cells but did not increase apoptosis. Comparison of consecutive harvests showed no difference in the numbers of CD34+ cells collected but found a significant decrease in apoptotic CD34+ cells through multiple collections. Analysis of annexin V binding in PBSC harvests is a simple flow cytometry technique which gives additional information on the status of CD34+ progenitor cells.

Barcaui, C. B., A. M. Goncalves da Silva, et al. (2006). "Stem cell apoptosis in HIV-1 alopecia." J Cutan Pathol **33**(10): 667-71.

BACKGROUND: Diffuse alopecia occurs in almost 7% of HIV-1-infected patients. Telogen effluvium is the main pathogenic mechanism involved. Apoptotic keratinocytes in the outer root sheath at bulge level was described as the most characteristic histopathologic finding of this kind of hair loss. METHODS: A case-control study was conducted to investigate the occurrence of apoptosis of follicular stem cells at the bulge in diffuse alopecia of HIV-1 infection. We applied a double-staining procedure to transverse scalp sections from 15 HIV-1-infected patients and 12 controls, with the monoclonal antibody anticytokeratin 19 as stem cell marker and TUNEL technique to identify apoptosis. RESULTS: Eighty percent of cases and 25% of controls presented at least one double-stained follicle. The proportion of positive follicles per section was 48% (+/-7%) for cases and 26% (+/-13%) for controls. CONCLUSION: Our study demonstrated that diffuse alopecia related to HIV-1 infection represents a hair cycle disturbance and that part of the follicular stem cell population become apoptotic in a higher proportion than normal subjects. We found no cytotoxic folliculitis. Owing to its cell-cycle interaction and caspase-induction capacities, we propose HIV-1 viral protein R as a possible follicular stem cell apoptosis inductor.

Bertrand, J., G. Begaud-Grimaud, et al. (2009). "Cancer stem cells from human glioma cell line are resistant to Fas-induced apoptosis." Int J Oncol **34**(3): 717-27.

Glioblastoma is the most common primary brain tumor, characterized by its resistance to treatments. To define efficient therapy, the origin of tumor-forming cells needs to be elucidated in order to search for new therapeutic pathways. The objective of this study was to determine the different cell populations constituting a human glioblastoma cell

line, U-87 MG and their sensitivity to apoptosis induced through the activation of Fas, a membranous death receptor. By a cell sorting method, the sedimentation field flow fractionation, two major cell subpopulations were identified, a most differentiated cell fraction, containing large and adherent cells, sensitive to Fas-induced apoptosis and another one, characterized by small cells forming aggregates, expressing CD133, a marker of stem cells and more resistant to Fas-activated apoptosis. By using a selective method of culture, adapted for neural stem cell cultures, we have verified that the U-87 MG cell line contained cancer stem cells similar to the immature ones obtained by the cell sorting method. Interestingly, while these tumor stem cells, expressing CD133, were resistant to Fas-induced apoptosis, monomeric form of Fas protein was detected predominantly in these cells. In contrast, the most mature cells, responsive to Fas-activated apoptosis, collected in another cell fraction, contained oligomeric aggregates of Fas protein, a pre-signalling form of the Fas receptor, essential for the initiation of apoptosis through its activation. These results suggest that these immature stem cells in glioma could be an important factor of resistance to chemotherapy requiring apoptosis through Fas signalling system. Indeed, future strategies of treatment, inducing differentiation of these stem cells need to be considered to enhance therapeutic efficiency.

Bhakta, S., P. Hong, et al. (2006). "The surface adhesion molecule CXCR4 stimulates mesenchymal stem cell migration to stromal cell-derived factor-1 in vitro but does not decrease apoptosis under serum deprivation." Cardiovasc Revasc Med **7**(1): 19-24.

BACKGROUND: Bone marrow mesenchymal stem cells (MSCs) can be used for myocardial repair following myocardial infarction. Increased expression of stromal cell-derived factor-1 (SDF-1) by an ischemic myocardium attracts CXCR4+ stem cells toward it. CXCR4, the receptor for SDF-1, is important in the migration, homing, and survival of hematopoietic stem cells. Although low levels of CXCR4 expression were found in minor subpopulations of cultured MSCs, most MSCs do not express CXCR4. To optimize the migration and survival of human MSCs, we expressed the CXCR4 gene in these cells using retroviral transduction. MATERIALS AND METHODS: We isolated and cultured MSCs from healthy volunteers and transduced them with a retroviral vector containing either CXCR4 and green fluorescent protein (GFP; CXCR4/GFP vector) or GFP alone (control vector). Flow cytometry confirmed successful transduction and GFP and CXCR4 expression. We used a transwell migration system to study MSC migration to SDF-1. We used Annexin V and propidium iodide stains to assess cell survival before and after the survival challenge. RESULTS: Flow cytometry showed that, on average, 83.4+/-17.7% of transduced MSCs expressed CXCR4. Compared with control MSCs, MSCs transduced with CXCR4 showed significantly more migration toward SDF-1, threefold greater at 3 h and more than fivefold greater at 6 h. Mesenchymal stem cells transduced with CXCR4 showed no significant difference in survival under normal to serum-deprived growth conditions. CONCLUSION: Mesenchymal stem cells can be efficiently transduced to express CXCR4, and transduced MSCs migrate rapidly toward SDF-1. CXCR4 expression does not render survival advantage to MSCs under serum-deprived conditions.

Bieberich, E., J. Silva, et al. (2004). "Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants." J Cell Biol **167**(4): 723-34.

The formation of stem cell-derived tumors (teratomas) is observed when engrafting undifferentiated embryonic stem (ES) cells, embryoid body-derived cells (EBCs), or mammalian embryos and is a significant obstacle to stem cell therapy. We show that in tumors formed after engraftment of EBCs into mouse brain, expression of the pluripotency marker Oct-4 colocalized with that of prostate apoptosis response-4 (PAR-4), a protein mediating ceramide-induced apoptosis during neural differentiation of ES cells. We tested the ability of the novel ceramide analogue N-oleoyl serinol (S18) to eliminate mouse and human Oct-4(+)/PAR-4(+) cells and to increase the proportion of nestin(+) neuroprogenitors in EBC-derived cell cultures and grafts. S18-treated EBCs persisted in the hippocampal area and showed neuronal lineage differentiation as indicated by the expression of beta-tubulin III. However, untreated cells formed numerous teratomas that contained derivatives of endoderm, mesoderm, and ectoderm. Our results show for the first time that ceramide-induced apoptosis eliminates residual, pluripotent EBCs, prevents teratoma formation, and enriches the EBCs for cells that undergo neural differentiation after transplantation.

Bironaite, D., D. Baltriukiene, et al. (2009). "Role of MAP kinases in nitric oxide induced muscle-derived adult stem cell apoptosis." Cell Biol Int **33**(7): 711-9.

Apoptosis in heart failure has been intensively investigated in vitro and in vivo. Stem cells have therapeutic value in the direct treatment of diseases, including cardiovascular disease. The main

drawback of stem cell therapy is their poor survival in the diseased tissues. Since intracellular mitogen-activated protein kinases (MAPKs) actively participate in the regulation of cell survival and of proapoptotic signals, the ability to manipulate the mechanisms of MAPKs activation in myogenic stem cells might increase the survival of transplanted stem cells. Our results clearly demonstrate sustained activation of all three MAPKs, ERK, JNK and p38 in myogenic stem cells after exposure to the NO inducer, NOC-18. Inhibition of MAPKs phosphorylation by specific inhibitors revealed the anti-apoptotic role of MAPKs in myogenic stem cells.

Black, D., M. A. Bird, et al. (2004). "TNF alpha-induced hepatocyte apoptosis is associated with alterations of the cell cycle and decreased stem loop binding protein." Surgery **135**(6): 619-28.

BACKGROUND: Inhibition of nuclear factor kappa B (NF kappa B) during liver regeneration induces hepatocyte apoptosis associated with normal DNA synthesis but decreased mitosis, suggesting that inhibition of NF kappa B impairs progression from S-phase through the G(2)/M phase of the cell cycle. Our aim was to determine if inhibition of NF kappa B alters cell cycle characteristics in hepatocytes treated with tumor necrosis factor alpha (TNF alpha). METHODS: Primary hepatocytes from BALB/c mice were infected with adenoviruses expressing luciferase (control; AdLuc) or the I kappa B super-repressor (AdI kappa B) and treated with or without TNF alpha (30 ng/ml). Flow cytometry was performed (0 to 40 hours) to determine apoptosis and cell cycle progression. Reverse transcriptase-polymerase chain reaction and immunoblots assessed changes in cell cycle mediators and antiapoptotic factors. RESULTS: Primary hepatocytes treated with AdI kappa B and TNF alpha demonstrated significantly more S-phase cells (14% +/- 3% vs 6% +/- 2%, P<.05) at 14 hours compared with controls. Inhibition of NF kappa B with or without TNFalpha was associated with decreased expression of stem loop bind protein, a marker of cell cycle progression through S-phase. The NF kappa B-induced antiapoptotic proteins, iNOS and TRAF2, had decreased message at 9 and 12 hours, respectively, in TNF alpha- and AdI kappa B-treated cells. CONCLUSION: Inhibition of NF kappa B in TNF alpha-treated primary mouse hepatocytes is associated with increased S-phase cell cycle retention and decreased stem loop bind protein.

Borbolla-Escoboza, J. R., M. I. Leon, et al. (2004). "Induction of apoptosis and effect on CD20+ using rituximab on autologous peripheral blood stem cell harvests from patients with B cell lymphomas." Stem Cells Dev **13**(2): 193-6.

Purging of neoplastic cells for autologous stem cell transplantation is usually done in vivo by administering chemotherapy and/or other agents before harvesting. It is also possible to decrease malignant cells counts directly in the cell harvest. In this study, we ascertained the effect of anti-CD20 monoclonal antibody and rituximab administration on peripheral blood hematopoietic stem cells. Five samples of stem cell harvests from different patients with B cell lymphoma were obtained. Each sample was divided in two tubes with calcium gluconate (20 mEq/50 microl). Rituximab (1 mg/600,000 mononuclear cells) was added to one of the tubes. Using flow cytometry, CD19, CD20 (B cell markers), and CD95 (apoptosis marker), expression was measured at baseline and 24 h after the addition of rituximab. A one-sided t-test with equal variances was used to analyze the results. Immediately after rituximab addition, CD20 expression became null. No significant difference in variation of CD19 expression was detected after the addition of rituximab (-3.64% control vs. 0.63% rituximab, p = 0.69). Mean variations of percentage of CD95 expression were 2.9% (controls) and 10.52% (rituximab tubes) (p = 0.06). We conclude that rituximab is capable of initiating apoptosis in vitro. We found no decrease in the CD19+ cell count, used as a surrogate marker for CD20+ cells, meaning that, at least in 24 h, apoptosis activation is not capable of decreasing CD20+ cell numbers. In vitro purging of peripheral blood stem cells harvests with rituximab could be part of a broader therapeutic strategy to be offered to lymphoproliferative disorder patients.

Bourguignon, L. Y., C. C. Spevak, et al. (2009). "Hyaluronan-CD44 interaction with protein kinase C(epsilon) promotes oncogenic signaling by the stem cell marker Nanog and the Production of microRNA-21, leading to down-regulation of the tumor suppressor protein PDCD4, anti-apoptosis, and chemotherapy resistance in breast tumor cells." J Biol Chem **284**(39): 26533-46.

Multidrug resistance and disease relapse is a challenging clinical problem in the treatment of breast cancer. In this study, we investigated the hyaluronan (HA)-induced interaction between CD44 (a primary HA receptor) and protein kinase Cepsilon (PKCepsilon), which regulates a number of human breast tumor cell functions. Our results indicate that HA binding to CD44 promotes PKCepsilon activation, which, in turn, increases the phosphorylation of the stem cell marker, Nanog, in the breast tumor cell line MCF-7. Phosphorylated Nanog is then translocated from the cytosol to the nucleus and becomes associated with RNase III DROSHA and the RNA helicase p68. This process leads to

microRNA-21 (miR-21) production and a tumor suppressor protein (e.g. PDCD4 (program cell death 4)) reduction. All of these events contribute to up-regulation of inhibitors of apoptosis proteins (IAPs) and MDR1 (multidrug-resistant protein), resulting in anti-apoptosis and chemotherapy resistance. Transfection of MCF-7 cells with PKCepsilon or Nanog-specific small interfering RNAs effectively blocks HA-mediated PKCepsilon-Nanog signaling events, abrogates miR-21 production, and increases PDCD4 expression/eIF4A binding. Subsequently, this PKCepsilon-Nanog signaling inhibition causes IAP/MDR1 down-regulation, apoptosis, and chemosensitivity. To further evaluate the role of miR-21 in oncogenesis and chemoresistance, MCF-7 cells were also transfected with a specific anti-miR-21 inhibitor in order to silence miR-21 expression and inhibit its target functions. Our results indicate that anti-miR-21 inhibitor not only enhances PDCD4 expression/eIF4A binding but also blocks HA-CD44-mediated tumor cell behaviors. Thus, this newly discovered HA-CD44 signaling pathway should provide important drug targets for sensitizing tumor cell apoptosis and overcoming chemotherapy resistance in breast cancer cells.

Chiou, S. H., S. J. Chen, et al. (2006). "Fluoxetine up-regulates expression of cellular FLICE-inhibitory protein and inhibits LPS-induced apoptosis in hippocampus-derived neural stem cell." Biochem Biophys Res Commun **343**(2): 391-400.

Fluoxetine is a widely used antidepressant compound which inhibits the reuptake of serotonin in the central nervous system. Recent studies have shown that fluoxetine can promote neurogenesis and improve the survival rate of neurons. However, whether fluoxetine modulates the proliferation or neuroprotection effects of neural stem cells (NSCs) needs to be elucidated. In this study, we demonstrated that 20 microM fluoxetine can increase the cell proliferation of NSCs derived from the hippocampus of adult rats by MTT test. The up-regulated expression of Bcl-2, Bcl-xL and the cellular FLICE-inhibitory protein (c-FLIP) in fluoxetine-treated NSCs was detected by real-time RT-PCR. Our results further showed that fluoxetine protects the lipopolysaccharide-induced apoptosis in NSCs, in part, by activating the expression of c-FLIP. Moreover, c-FLIP induction by fluoxetine requires the activation of the c-FLIP promoter region spanning nucleotides -414 to -133, including CREB and SP1 sites. This effect appeared to involve the phosphatidylinositol-3-kinase-dependent pathway. Furthermore, fluoxetine treatment significantly inhibited the induction of proinflammatory factor IL-1beta, IL-6, and TNF-alpha in the culture medium of LPS-treated NSCs ($p<0.01$). The results of high performance liquid chromatography coupled to electrochemical detection further confirmed that fluoxentine increased the functional production of serotonin in NSCs. Together, these data demonstrate the specific activation of c-FLIP by fluoxetine and indicate the novel role of fluoxetine for neuroprotection in the treatment of depression.

Chung, I. J., C. Dai, et al. (2003). "Stem cell factor increases the expression of FLIP that inhibits IFNgamma -induced apoptosis in human erythroid progenitor cells." Blood **101**(4): 1324-8.

Interferon gamma (IFNgamma) acts on human erythroid colony-forming cells (ECFCs) to up-regulate Fas, without a demonstrable change of Fas ligand (FasL) or Fas-associated DD-containing protein (FADD) expression and activates caspase-8 plus caspase-3, which produce apoptosis. Our previous data showed that stem cell factor (SCF) reduced the inhibitory effect of IFNgamma on human ECFCs when both factors were present in the cultures. However, the mechanism by which SCF prevents IFNgamma-induced apoptosis in ECFCs is unclear. In this study we used highly purified human ECFCs to investigate the mechanism of the effect of SCF on IFNgamma-induced apoptosis. Because the binding of FasL to Fas is the first step of the apoptosis cascade and IFNgamma strongly up-regulates Fas expression, we added FasL (50 ng/mL) to the cultures with IFNgamma to accentuate the IFNgamma-induced activation of caspase-8 and caspase-3 plus subsequent apoptosis. SCF (100 ng/mL) clearly inhibited the activation of caspase-8 and caspase-3 induced by IFNgamma and/or FasL, and it also reduced apoptosis as measured by the terminal dUTP nick-end labeling (TUNEL) assay. SCF did not decrease the surface expression of Fas on the ECFCs. FADD-like interleukin 1 beta (IL-1beta)-converting enzyme (FLICE)-inhibitory protein (FLIP) has been reported to interact with FADD and/or caspase-8 at the death-inducing signaling complex (DISC) level following Fas stimulation and acts as a dominant-negative caspase-8. SCF increased FLIP mRNA and protein expression, concomitant with reduced apoptosis, whereas IFNgamma and/or FasL did not change FLIP expression. Reduction of FLIP expression with antisense oligonucleotides decreased the capacity of SCF to inhibit IFNgamma-induced apoptosis, demonstrating a definite role for FLIP in the SCF-induced protection of ECFCs from IFNgamma-initiated apoptosis.

Crisostomo, P. R., M. Wang, et al. (2007). "Gender differences in injury induced mesenchymal stem cell apoptosis and VEGF, TNF, IL-6 expression: role of

the 55 kDa TNF receptor (TNFR1)." J Mol Cell Cardiol **42**(1): 142-9.

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

Dai, C., I. J. Chung, et al. (2004). "In human immature BFU-E tumor necrosis factor-alpha not only downregulates CDK6 but also directly produces apoptosis which is prevented by stem cell factor." Exp Hematol **32**(10): 911-7.

OBJECTIVE: The aim of this study was to reveal the mechanisms by which tumor necrosis factor-alpha (TNF-alpha) inhibits immature human day-4 burst-forming units-erythroid (BFU-E) and the effect of stem cell factor (SCF) on this process. METHODS: Sequential density-gradient centrifugation, depletion of lymphocytes, removal of adherent cells, and negative selection with CD2, CD11b, CD16, and CD45 monoclonal antibodies were used to purify day-1 BFU-E, which were then incubated for 3 days to generate day-4 cells. The day-4 cells were incubated with TNF-alpha, and/or SCF, and the extent of apoptosis was gauged by morphologic observations, TUNEL assays, and Western blots. RESULTS: The cell number and the number and size of erythroid colonies were significantly reduced when day-4 cells were incubated with TNF-alpha. Apoptosis was observed in single-cell plasma clot assays. TUNEL assays showed 20% +/- 6% apoptotic cells with TNF-alpha while controls had 2.8% +/- 2.2%. Caspases 3 and 8 were strongly activated while the amount of CDK6 was reduced by TNF-alpha. When SCF, a potent stimulator of cell growth, was added with TNF-alpha, cell growth inhibition was reduced and the apoptotic cells decreased to 0.9% +/- 1.2%. The activations of caspase 3 and caspase 8 were almost completely blocked by SCF while CDK6 and the FLICE-inhibitory protein (FLIP) were increased. CONCLUSIONS: Our results indicate that in immature human BFU-E, TNF-alpha downregulates CDK6 but also directly produces apoptosis which is prevented by SCF.

de Boer, F., A. M. Drager, et al. (2002). "Early apoptosis largely accounts for functional impairment of CD34+ cells in frozen-thawed stem cell grafts." J Hematother Stem Cell Res **11**(6): 951-63.

Quality assessment of stem cell grafts is usually performed by flow cytometric CD34(+) enumeration or assessment of clonogenic output of fresh material. Previously, we identified the occurrence of early apoptosis, not detectable with the permeability marker 7-amino actinomycin D (7-AAD), in purified frozen-thawed CD34(+) cells, using the vital stain Syto16. Syto(high)/7-AAD(-) cells were defined as viable, Syto16(low)/7-AAD(-) cells as early apoptotic and Syto16(low)/7-AAD(+) as dead. This was confirmed in a subsequent study using frozen-thawed transplants of lymphoma patients. In the present study on grafts from multiple myeloma and lymphoma patients, we investigated the functional consequences of the early apoptotic process. The mean Syto16-defined viability was 41 and 42%, respectively, for both graft groups, compared to 78% and 72%, respectively, using 7-AAD only. The established early apoptosis marker annexin V missed roughly 50% of the early apoptosis detected with Syto16. In contrast, viability of CD34(+) cells in nonmanipulated whole blood transplants from a matched group of lymphoma patients, after 72 h of storage at 4 degrees C, was more than 90%, even with the Syto16 assay. CFU recovery (median 26-33%) after cryopreservation matched CD34(+) recovery

after Syto16, but not 7-AAD correction. In contrast, colony-forming unit (CFU) recovery in the whole blood transplant was close to 100%. Furthermore, early apoptotic CD34(+) cells had lost migratory ability toward stromal cell derived factor-1alpha (SDF-1alpha). The establishment of a Syto16(high)/7-AAD(-) proportion of CD34(+) cells offers a new approach for a more correct determination of the number of viable nonapoptotic CD34(+) cells in stem cell grafts. Further development of this assay should allow its incorporation into the routine CD34(+) assessment of post-thawed samples in clinical flow cytometry laboratories.

de Boer, F., A. M. Drager, et al. (2002). "Extensive early apoptosis in frozen-thawed CD34-positive stem cells decreases threshold doses for haematological recovery after autologous peripheral blood progenitor cell transplantation." Bone Marrow Transplant **29**(3): 249-55.

Stem cell doses necessary for engraftment after myelo-ablative therapy as defined for fresh transplants vary largely. Loss of CD34+ cell quality after cryopreservation might contribute to this variation. With a new early apoptosis assay including the vital stain Syto16, together with the permeability marker 7-AAD, CD34+ cell viability in leucapheresis samples of 49 lymphoma patients receiving a BEAM regimen was analysed. After freeze-thawing large numbers of non-viable, early apoptotic cells appeared, leading to only 42% viability compared to 72% using 7-AAD only. Based on this Syto16 staining in the frozen-thawed grafts, threshold numbers for adequate haematological recovery of 2.8-3.0 x 10(6) CD34+ cells/kg body weight determined for fresh grafts, now decreased to 1.2-1.3 x 10(6) CD34+ cells/kg. In whole blood transplantation of lymphoma patients (n = 45) receiving a BEAM-like regimen, low doses of CD34+ cells were sufficient for recovery (0.3-0.4 x 10(6)CD34+ cells/kg). In contrast to freeze-thawing of leucapheresis material, a high viability of CD34+ cells was preserved during storage for 3 days at 4 degrees C, leaving threshold doses for recovery unchanged. In conclusion, the Syto16 assay reveals the presence of many more non-functional stem cells in frozen-thawed transplants than presumed thus far. This led to a factor 2.3-fold adjustment downward of viable CD34+ threshold doses for haematological recovery.

Deng, X., Q. Luan, et al. (2009). "Nanosized zinc oxide particles induce neural stem cell apoptosis." Nanotechnology **20**(11): 115101.

Given the intensive application of nanoscale zinc oxide (ZnO) materials in our life, growing concerns have arisen about its unintentional health and environmental impacts. In this study, the neurotoxicity of different sized ZnO nanoparticles in mouse neural stem cells (NSCs) was investigated. A cell viability assay indicated that ZnO nanoparticles manifested dose-dependent, but no size-dependent toxic effects on NSCs. Apoptotic cells were observed and analyzed by confocal microscopy, transmission electron microscopy examination, and flow cytometry. All the results support the viewpoint that the ZnO nanoparticle toxicity comes from the dissolved Zn(2+) in the culture medium or inside cells. Our results highlight the need for caution during the use and disposal of ZnO manufactured nanomaterials to prevent the unintended environmental and health impacts.

Domen, J. (2001). "The role of apoptosis in regulating hematopoietic stem cell numbers." Apoptosis **6**(4): 239-52.

The importance of apoptosis, in combination with proliferation, in maintaining stable populations has become increasingly clear in the last decade. Perturbation of either of these processes can have serious consequences, and result in a variety of disorders. Moreover, as the players and pathways gradually emerge, it turns out that there are strong connections in the regulation of cell cycle progression and apoptosis. Apoptosis, proliferation, and the disorders resulting from aberrant regulation have been studied in a variety of cell types and systems. Hematopoietic stem cells (HSC) are defined as primitive mesenchymal cells that are capable of both self-renewal and differentiation into the various cell lineages that constitute the functioning hematopoietic system. Many (but certainly not all) mature hematopoietic cells are relatively short-lived, sometimes with a half-life in the order of days. Homeostasis requires the production of 10(8) (mouse) to 10(11) (human) cells each day. All of these cells are ultimately derived from HSC that mostly reside in the bone marrow in adult mammals. The study of the regulation of HSC numbers has focussed mainly on the choice between self-renewal and differentiation, symmetric and asymmetric cell divisions. Recently, however, it has been directly demonstrated that apoptosis plays an important role in the regulation of hematopoietic stem cells in vivo.

Dror, Y., M. Leaker, et al. (2000). "Mastocytosis cells bearing a c-kit activating point mutation are characterized by hypersensitivity to stem cell factor and increased apoptosis." Br J Haematol **108**(4): 729-36.

Mastocytosis is characterized by abnormal infiltration of mast cells into various organs. An activating mutation in c-kit, involving an A --> T

substitution at nucleotide 2648 has recently been described in some patients with mastocytosis. We describe a 12-year-old girl with this mutation in her bone marrow cells at diagnosis with a myelodysplastic syndrome (MDS) without evidence of mastocytosis, and then in peripheral blood mononuclear cells 1 year later after the emergence of mastocytosis. The role of the c-Kit receptor and its ligand stem cell factor (SCF) in the pathogenesis of the disease was analysed in marrow cell clonogenic assays. We show that the genetic abnormalities in the patient resulted in factor-independent growth and hypersensitivity of primitive progenitors to SCF, with increased production of mast cells. Increased apoptosis and cluster formation, consistent with the myelodysplastic nature of the disorder, accompanied accumulation of abnormal cells with increasing concentrations of SCF.

Drouet, M., F. Mourcin, et al. (2005). "Mesenchymal stem cells rescue CD34+ cells from radiation-induced apoptosis and sustain hematopoietic reconstitution after coculture and cografting in lethally irradiated baboons: is autologous stem cell therapy in nuclear accident settings hype or reality?" Bone Marrow Transplant **35**(12): 1201-9.

Autologous stem cell therapy (ACT) has been proposed to prevent irradiated victims from bone marrow (BM) aplasia by grafting hematopoietic stem and progenitor cells (HSPCs) collected early after damage, provided that a functional graft of sufficient size could be produced ex vivo. To address this issue, we set up a baboon model of cell therapy in which autologous peripheral blood HSPCs collected before lethal total body irradiation were irradiated in vitro (2.5 Gy, D0 1 Gy) to mimic the cell damage, cultured in small numbers for a week in a serum-free medium in the presence of antiapoptotic cytokines and mesenchymal stem cells (MSCs) and then cografted. Our study shows that baboons cografted with expanded cells issued from 0.75 and 1 x 10(6)/kg irradiated CD34+ cells and MSCs (n=2) exhibited a stable long-term multilineage engraftment. Hematopoietic recovery became uncertain when reducing the CD34+ cell input (0.4 x 10(6)/kg CD34+ cells; n=3). However, platelet recovery was accelerated in all surviving cografted animals, when compared with baboons transplanted with unirradiated, unmanipulated CD34+ cells (0.5-1 x 10(6)/kg, n=4). Baboons grafted with MSCs alone (n=3) did not recover. In all cases, the nonhematopoietic toxicity remained huge. This baboon study suggests that ACT feasibility is limited.

Ehtesham, M., P. Kabos, et al. (2002). "Induction of glioblastoma apoptosis using neural stem cell-mediated delivery of tumor necrosis factor-related apoptosis-inducing ligand." Cancer Res **62**(24): 7170-4.

Current therapies for gliomas fail to address their highly infiltrative nature. Standard treatments often leave behind microscopic neoplastic reservoirs, resulting in eventual tumor recurrence. Neural stem cells (NSCs) are capable of tracking disseminating glioma cells. To exploit this tropism to develop a therapeutic strategy that targeted tumor satellites, we inoculated human glioblastoma xenografts with tumor necrosis factor-related apoptosis-inducing ligand-secreting NSCs. This resulted in the dramatic induction of apoptosis in treated tumors and tumor satellites and was associated with significant inhibition of tumor growth. These results add credence to the potential of NSCs as therapeutically effective delivery vehicles for the treatment of intracranial glioma.

Endo, T., A. Odb, et al. (2001). "Stem cell factor protects c-kit+ human primary erythroid cells from apoptosis." Exp Hematol **29**(7): 833-41.

OBJECTIVE: It has been reported that stem cell factor (SCF) promotes cell survival in primary cultured human erythroid colony-forming cells (ECFC). Given the heterogeneous nature of ECFC, which may affect interpretation of the data, we purified c-kit+ ECFC and investigated the specificity and mechanisms of the anti-apoptotic effects of SCF on these cells. MATERIALS AND METHODS: Glycophorin A+ (GPA+) c-kit+ cells were purified from primary cultured ECFC derived from purified human CD34+ cells. The GPA+c-kit- and nonerythroid cells were generated from the same CD34+ cells. Apoptosis of ECFC was investigated in the absence or presence of SCF and erythropoietin (EPO) in serum-free medium. DNA fragmentation was measured with enzyme linked immunosorbent assay for oligonucleosome-sized DNA, gel electrophoresis, and annexin V labeling. Characterization of expanded cells and enriched cells was performed using multiparameter flow cytometry. For Akt assay, cells were lysed and the cleared lysates subjected to SDS-PAGE followed by Western blotting. RESULTS: In GPA+c-kit+ cells, deprivation of cytokine caused rapid DNA fragmentation within 4 hours that reached a maximum at 6 hours. This was partially but clearly prevented by SCF or EPO. In contrast, no significant DNA fragmentation was seen in GPA+c-kit- and nonerythroid cells within 24 hours. PP2, a specific Src family kinase inhibitor, but not its inactive analogue PP3, reversed the anti-apoptotic effects of SCF. PP2 also inhibited SCF-induced phosphorylation of Akt. CONCLUSION: These data indicate that SCF protects purified human GPA+c-kit+ cells from apoptosis and suggest that kit-

mediated Src kinase activation is involved in Akt activation and cell survival.

Esdar, C., S. Milasta, et al. (2001). "Differentiation-associated apoptosis of neural stem cells is effected by Bcl-2 overexpression: impact on cell lineage determination." Eur J Cell Biol **80**(8): 539-53.

Apoptosis is an integral part of neural development. To elucidate the importance of programmed cell death on cell lineage determination we utilized murine PCC7-Mzl cells, a model system for neural differentiation. Treatment of pluripotent PCC7-Mzl stem cells with 0.1 microM all-trans retinoic acid (RA) causes a cease of proliferation and an initiation of differentiation into neurons, glial cells and fibroblasts. Simultaneously, a fraction of the cell culture (ca. 25%) dies within 24 h by apoptosis. We transfected PCC7-Mzl cells with the human bcl-2 cDNA and generated PCC7-Mz-Bcl-2 cell lines expressing two- to tenfold higher levels of Bcl-2 than parental cells. Overexpression of Bcl-2 resulted in hypophosphorylation of the retinoblastoma (Rb) protein and consequently prolonged the doubling time of the culture from 18 h to 23 h. RA-induced apoptosis was drastically reduced to 3 to 15% depending on the level of Bcl-2 expression. RA-induced caspase activation, cytochrome c release from the mitochondria to the cytosol and DNA fragmentation was completely blocked. Furthermore, treating Bcl-2 cultures with ceramide (10 microM), a second messenger mediating the RA-initiated death signal in parental cells, no longer caused DNA laddering. Bcl-2 overexpression did not interfere with the potential of PCC7-Mz cells to develop into neurons, glial cells and fibroblasts. However, the relative distribution of cell types in the culture was shifted such that the fraction of neurons was reduced to half (from 60 to 30%) with a concomitant increase in the number of glial and fibroblastoid cells. Furthermore, Bcl-2-overexpressing neurons, but not neurons of parental or mock-transfected PCC7-Mzl cultures, were able to grow as single cells.

Ferrari, R., C. Ceconi, et al. (2009). "Mechanisms of remodelling: a question of life (stem cell production) and death (myocyte apoptosis)." Circ J **73**(11): 1973-82.

Remodeling myocytes show a typical switch between the embryonic and classical features of apoptosis and/or hypertrophy representing a signal of death (ie, apoptosis) and a signal of life (ie, hypertrophy). The adult myocyte, however, is a terminal cell; usually it is unable to reproduce and death is not genetically programmed (apoptosis), but occurs by necrosis. The reinstatement of apoptosis and development of hypertrophy during remodeling could be part of the switch forward to the embryonic phenotype with reinstatement of the early embryonic genetic program. Hypertrophy and apoptosis are "sons" of the same "mother": the local, tissue neuroendocrine-neurohumoral response to a mechanical stretch of the myocytes consequent to the geometric changes imposed on the viable myocytes by the necrotic ones. As expected, the life and death cycle is very closely regulated by several autocrine systems, one of which is linked to the interleukin-6 family via a regulatory protein named GP-130. Activation of the GP-130 slows down the death signals, thus favoring hypertrophy and reducing fibrosis.

Geng, Y. J. (2003). "Molecular mechanisms for cardiovascular stem cell apoptosis and growth in the hearts with atherosclerotic coronary disease and ischemic heart failure." Ann N Y Acad Sci **1010**: 687-97.

In the heart with atherosclerotic coronary disease, chronic ischemia causes progressive loss of cardiovascular cells and ultimately triggers myocardial dysfunctions or heart failure. Various types of stem cells from embryonic and adult tissues have potentials for regenerating functional cardiovascular cells in the heart undergoing ischemic injury. However, native or exogenous stem cells in the ischemic hearts are exposed to various proapoptotic or cytotoxic factors. Furthermore, during repopulation and differentiation, certain numbers of newly produced cells may die by apoptosis during neocardiovascular tissue remodeling and morphogenesis. Embryonic and adult stem cells may have different life spans, as being programmed genetically to apoptosis. The endogenous and environmental factors play important roles in regulation of stem cells, including inflammatory cytokines, growth factors, surface receptors, proteolytic enzymes, mitochondrial respiration, nuclear proteins, telomerase activities, hypoxia-responding proteins, and stem cell-host cell interaction. Clarification of the molecular mechanisms may help us understand and design stem cell therapies.

Germano, I. M., M. Uzzaman, et al. (2006). "Apoptosis in human glioblastoma cells produced using embryonic stem cell-derived astrocytes expressing tumor necrosis factor-related apoptosis-inducing ligand." J Neurosurg **105**(1): 88-95.

OBJECT: Embryonic stem (ES) cell-derived astrocytes have several theoretical and practical advantages as gene therapy vectors in the treatment of malignant gliomas. The aim of this study was to test the proapoptotic effects of ES cell-derived astrocytes

expressing transgenic tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in human malignant glioma cells. METHODS: Mouse ES cells containing a doxycycline-inducible transgene were engineered with human TRAIL (hTRAIL) and then directed to differentiate into astrocytes. The ES cell-derived-TRAIL-expressing astrocytes were cocultured with human malignant glioma cells. Reverse transcriptase polymerase chain reaction, immunocytochemistry, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling, and flow cytometry were used to quantify results. In vitro coculture of ES cell-derived astrocytes expressing hTRAIL with A172 human malignant glioma cells after doxycycline induction caused a significant decrease in cell viability from 85 +/- 2% at baseline to 8 +/- 2% posttreatment ($p < 0.001$). Labeling with apoptotic markers showed that cell death occurred by means of apoptosis. A significant increase in apoptotic rate (88 +/- 3%) from baseline (4 +/- 2%) was found in A172 cells after doxycycline induction ($p < 0.005$). This effect was superior to the apoptotic rate seen after treatment with recombinant TRAIL (57 +/- 2%). A decrease in cell viability and an increase in the apoptotic rate were not found in TRAIL-expressing-ES cell-derived astrocytes after induction with doxycycline or in A172 cells exposed to doxycycline alone. CONCLUSIONS: Engineering of transgenic hTRAIL by using ES cell-derived astrocytes induced apoptosis in human malignant glioma cells while sparing nontumor astrocytes. The apoptotic effects of transgenic hTRAIL are greater than those of recombinant hTRAIL. Analysis of these results suggests that hTRAIL-expressing-ES cell-derived astrocytes should be considered in the development of new in vivo strategies to treat malignant human gliomas.

Gonzalez, I., E. J. Andreu, et al. (2004). "Imatinib inhibits proliferation of Ewing tumor cells mediated by the stem cell factor/KIT receptor pathway, and sensitizes cells to vincristine and doxorubicin-induced apoptosis." Clin Cancer Res **10**(2): 751-61.

PURPOSE AND EXPERIMENTAL DESIGN: The stem cell factor/KIT receptor loop may represent a novel target for molecular-based therapies of Ewing tumor. We analyzed the in vitro impact of KIT blockade by imatinib in Ewing tumor cell lines. RESULTS: KIT expression was detected in 4 of 4 Ewing tumor cell lines and in 49 of 110 patient samples (44.5%) by immunohistochemistry and/or Western blot analysis. KIT expression was stronger in Ewing tumors showing EWS-FLI1 nontype 1 fusions. Despite absence of c-kit mutations, constitutive and ligand-inducible phosphorylation of KIT was found in all tumor cell lines, indicating an active receptor. Treatment with KIT tyrosine kinase inhibitor imatinib (0.5-20 micro M) induced down-regulation of KIT phosphorylation and dose response inhibition of cell proliferation (IC(50), 12-15 micro M). However, imatinib administered alone at doses close to IC(50) for growth inhibition (10 micro M) did not induce a significant increase in apoptosis. We then analyzed if blockade of KIT loop through imatinib (10 micro M) was able to increase the antitumor in vitro effect of doxorubicin (DXR) and vincristine (VCR), drugs usually used in Ewing tumor treatment. Addition of imatinib decreased in 15-20 and 15-36% of the proliferative rate of Ewing tumor cells exposed to DXR and VCR, respectively, and increased in 15 and 30% of the apoptotic rate of Ewing tumor cells exposed to the same drugs. CONCLUSIONS: Inhibition of Ewing tumor cell proliferation by imatinib is mediated through blockade of KIT receptor signaling. Inhibition of KIT increases sensitivity of these cells to DXR and VCR. This study supports a potential role for imatinib in the treatment of Ewing tumor.

Grullich, C., C. Ziegler, et al. (2009). "Rabbit anti T-lymphocyte globulin induces apoptosis in peripheral blood mononuclear cell compartments and leukemia cells, while hematopoetic stem cells are apoptosis resistant." Biol Blood Marrow Transplant **15**(2): 173-82.

Polyclonal anti-T-lymphocyte globulins (ATG) are used in allogeneic stem cell transplantation (SCT) for the prophylaxis of graft versus host disease (GVHD) by in vivo T cell depletion. In this study we investigated the complement independent induction of apoptosis by rabbit ATG in peripheral blood mononuclear cell (PBMNC) compartments and hematopoetic stem cells (HSC). We also detected antileukemic activity of ATG by measuring apoptosis in myeloid and lymphatic leukemia cell lines and primary leukemia cells. We found ATG to induce apoptosis in T-lymphocytes (CD4(+), CD8+), B-lymphocytes (CD20+), natural killer (NK)-cells (CD56(+)), and monocytes (CD14(+)). HSC, in contrast, were apoptosis resistant and could be growth stimulated by low-dose ATG in the presence of bystander cells. The human leukemia cell lines Jurkat, Daudi, DG-75 (lymphoblastic), and K562, HL-60, KG1, and U937 (myeloblastic) underwent ATG-induced apoptosis, whereas the NK-cell line YT was resistant. Primary leukemia cells from 6 investigated patients with acute lymphoblastic leukemia, 9 of 10 patients with chronic lymphocytic leukemia, and 4 of 8 patients with acute myeloblastic leukemia underwent ATG-induced apoptosis. We conclude apoptosis induction in all PBMNC compartments contributes to GVHD prophylaxis. ATG might

support engraftment. Finally, antileukemic activity of ATG could positively influence the transplantation outcome.

Guan, S., D. Ge, et al. (2009). "Protocatechuic acid promotes cell proliferation and reduces basal apoptosis in cultured neural stem cells." Toxicol In Vitro **23**(2): 201-8.

Protocatechuic acid (PCA), a phenolic compound isolated from the kernels of Alpinia oxyphylla, showed anti-oxidant neuroprotective property in our previous study. However, it is still unknown whether PCA have effects on the cultured neural stem cells (NSCs). In this study, we investigated the roles of PCA in the survival and apoptosis of rat NSCs under normal conditions. NSCs obtained from 13.5-day-old rat embryos were propagated as neurospheres and cultured under normal conditions with or without PCA for 4 and 7 days. The cell viability was determined by the cell counting kit-8 (CCK-8) test, while cell proliferation was assayed by bromodeoxyuridine (BrdU) labeling. PCA increased the cellular viability of NSCs and stimulated cell proliferation in a dose- and time-dependent manner. Apoptotic cells were detected after 4 days by observing the nuclear morphological changes and flow cytometric analysis. Compared with the control on both culture days, treatment with PCA effectively reduced the levels of apoptosis of NSCs. At the same time, the reactive oxygen species (ROS) level in NSCs was depressed. In addition, PCA also significantly decreased the activity of elevated caspase-3, indicating that PCA may inhibit apoptosis of NSCs via suppression of the caspase cascade. These results suggest that PCA may be a potential growth inducer and apoptosis inhibitor for NSCs.

Guo, Y., B. Graham-Evans, et al. (2006). "Murine embryonic stem cells secrete cytokines/growth modulators that enhance cell survival/anti-apoptosis and stimulate colony formation of murine hematopoietic progenitor cells." Stem Cells **24**(4): 850-6.

Stromal cell-derived factor (SDF)-1/CXCL12, released by murine embryonic stem (ES) cells, enhances survival, chemotaxis, and hematopoietic differentiation of murine ES cells. Conditioned medium (CM) from murine ES cells growing in the presence of leukemia inhibitory factor (LIF) was generated while the ES cells were in an undifferentiated Oct-4 expressing state. ES cell-CM enhanced survival of normal murine bone marrow myeloid progenitors (CFU-GM) subjected to delayed growth factor addition in vitro and decreased apoptosis of murine bone marrow c-kit(+)lin- cells. ES CM contained interleukin (IL)-1alpha, IL-10, IL-11, macrophage-colony stimulating factor (CSF), oncostatin M, stem cell factor, vascular endothelial growth factor, as well as a number of chemokines and other proteins, some of which are known to enhance survival/anti-apoptosis of progenitors. Irradiation of ES cells enhanced release of some proteins and decreased release of others. IL-6, FGF-9, and TNF-alpha, not detected prior to irradiation was found after ES cells were irradiated. ES cell CM also stimulated CFU-GM colony formation. Thus, undifferentiated murine ES cells growing in the presence of LIF produce/release a number of biologically active interleukins, CSFs, chemokines, and other growth modulatory proteins, results which may be of physiological and/or practical significance.

Hagiwara, M., B. Shen, et al. (2008). "Kallikrein-modified mesenchymal stem cell implantation provides enhanced protection against acute ischemic kidney injury by inhibiting apoptosis and inflammation." Hum Gene Ther **19**(8): 807-19.

Mesenchymal stem cells (MSCs) migrate to sites of tissue injury and serve as an ideal vehicle for cellular gene transfer. As tissue kallikrein has pleiotropic effects in protection against oxidative organ damage, we investigated the potential of kallikrein-modified MSCs (TK-MSCs) in healing injured kidney after acute ischemia/reperfusion (I/R). TK-MSCs secreted recombinant human kallikrein with elevated vascular endothelial growth factor levels in culture medium, and were more resistant to oxidative stress-induced apoptosis than control MSCs. Expression of human kallikrein was identified in rat glomeruli after I/R injury and systemic TK-MSC injection. Engrafted TK-MSCs exhibited advanced protection against renal injury by reducing blood urea nitrogen, serum creatinine levels, and tubular injury. Six hours after I/R, TK-MSC implantation significantly reduced renal cell apoptosis in association with decreased inducible nitric oxide synthase expression and nitric oxide levels. Forty-eight hours after I/R, TK-MSCs inhibited interstitial neutrophil and monocyte/macrophage infiltration and decreased myeloperoxidase activity, superoxide formation, p38 mitogen-activated protein kinase phosphorylation, and expression of tumor necrosis factor-alpha, monocyte chemoattractant protein-1, and intercellular adhesion molecule-1. In addition, tissue kallikrein and kinin significantly inhibited H2O2-induced apoptosis and increased Akt phosphorylation and cell viability in cultured proximal tubular cells. These results indicate that implantation of kallikrein-modified MSCs in the kidney provides advanced benefits in protection against ischemia-induced kidney injury by suppression of apoptosis and inflammation.

Hellstrom-Lindberg, E., J. Schmidt-Mende, et al. (2001). "Apoptosis in refractory anaemia with ringed sideroblasts is initiated at the stem cell level and associated with increased activation of caspases." Br J Haematol **112**(3): 714-26.

Treatment with granulocyte colony-stimulating factor plus erythropoietin may improve haemoglobin levels in patients with ringsideroblastic anaemia (RARS) and reduce bone marrow apoptosis. We studied bone marrow from 10 RARS patients, two of whom were also investigated after successful treatment. Mononuclear, erythroid and CD34+ cells were analysed with regard to proliferation, apoptosis, clonogenic capacity and oncoprotein expression, in the presence or absence of Fas-agonist, Fas-blocking antibody 2 and caspase-3 inhibitor. During culture, RARS bone marrow cells showed higher spontaneous apoptosis ($P < 0.05$) and caspase activity ($P < 0.05$)) than bone marrow cells from healthy donors. Eight out of nine patients had reduced growth of erythroid colony-forming units (CFU-E) (< 10% of control) and granulocyte-macrophage CFU (CFU-GM) (< 50% of control) from CD34+ cells. Fas ligation increased apoptosis and decreased colony growth equally in RARS and controls, but caused significantly more caspase activation in RARS ($P < 0.01$). Fas-blocking antibody showed no significant inhibitory effect on spontaneous apoptosis or ineffective haematopoiesis, as measured using phosphatidylserine exposure, the terminal deoxynucleotide transferase-mediated dUTP-biotin nick-end labelling technique, caspase activity or clonogenic growth. Caspase inhibition reduced apoptosis, increased proliferation and enhanced erythroid colony growth from CD34+ cells in RARS, but showed no effect on normal cells. CFU-E improved > 1000% after successful treatment. Thus, erythroid apoptosis in RARS is initiated at the CD34+ level and growth factor treatment may improve stem cell function. Enhanced caspase activation at the stem cell level, albeit not mediated through endogenous activation of the Fas receptor, contributes to the erythroid apoptosis in RARS.

Henkin, R. I. and J. D. Hoetker (2003). "Deficient dietary intake of vitamin E in patients with taste and smell dysfunctions: is vitamin E a cofactor in taste bud and olfactory epithelium apoptosis and in stem cell maturation and development?" Nutrition **19**(11-12): 1013-21.

OBJECTIVES: We reviewed dietary intake of several nutrients in a large group of patients with taste and smell dysfunction, compared intake of these nutrients with standard values, and recognized that intake of vitamin E was significantly less than that of most other nutrients. Based on this observation we attempted to develop an hypothesis of the possible role vitamin E might play in these sensory disorders. METHODS: Vitamin E intake was measured in 250 patients with taste and smell dysfunctions. RESULTS: Intake of the vitamin was 3.2 +/- 0.2 mg/d (mean +/- standard error of the mean), or 36 +/- 2% of the recommended daily allowance, an intake significantly below that considered adequate. This diminished intake occurred with normal intake of total calories; protein; fat; carbohydrate; several vitamins, including thiamin, niacin, and pyridoxine; and the trace metals zinc, copper, and iron. CONCLUSIONS: Although specific relations between vitamin E intake and smell and taste dysfunctions are unclear, the non-antioxidant roles of vitamin E indicate that it is a factor in apoptosis, cellular signaling, and growth of various cell lines, suggesting that this vitamin may play a role in growth and development of stem cells in taste buds and olfactory epithelium.

Hu, K. X., S. F. Zhao, et al. (2007). "[Effects of mesenchymal stem cells on cell cycle and apoptosis of hematopoietic tissue cells in irradiated mice]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **15**(6): 1226-30.

The aim of this study was to investigate the effect of mesenchymal stem cells (MSCs) on cell cycle and apoptosis of thymus, spleen and bone marrow cells in mice totally irradiated with sublethal dose, and to explore its mechanisms. BALB/c mice irradiated with 5.5 Gy 60Co gamma-ray were randomly divided into control group and MSC group. Mice in MSC group were infused with 0.4 ml containing 2.5x10(7)/kg of MSCs through tail vein at 1 hour after irradiation. Mice in control group were infused with 0.4 ml normal saline. The cell apoptosis and cell cycle of thymus, spleen and bone marrow cells were detected by flow cytometry at 6, 12, 24 and 72 hours after irradiation and the P53 protein expressions in thymus and bone marrow cells were assayed by immunohistochemistry at 12 hours after irradiation. The results showed that the arrest of cells in G0/G1 and G2/M phase, and decrease of cells in S phase appeared at 6 hours after irradiation, those reached peak respectively at 12 hours in thymus cells, 6 hours in spleen and 24 hours in bone marrow, then the cell counts in G0/G1 phase decreased and the cell counts in S and G2/M phases increased. At 72 hours the cell counts in G0/G1 phase were less than the normal level and the cell counts in S phase were more than the normal level. The above changes of cell cycle in thymus and spleen were more rapid in spleen and more obvious in amplitude than that in bone marrow, the change of cell cycle in MSC group was more rapid and obvious than those in control group. After irradiation the apoptosis cells increased from 6 hours, reached the highest level at 12 hours and decreased to

the normal level gradually after 24 hours in two groups; the apoptosis rates in spleen and thymus cells were higher than that in bone marrow cells. In comparison with the control group, the apoptosis rate in thymus cells at 12 hours, in spleen cells at 12 and 24 hours, and in bone marrow cells at 24 hours were fewer in MSC group. The cells expressing P53 protein in control group were more than that in MSC group. It is concluded that the MSCs accelerate the running of cell cycle in these hematopoietic tissue cells of irradiated mice, reduce the cell apoptosis and promote the recovery from injuries in hematopietic and immunological organs, thus protect the irradiated mice at early stage.

Hu, T., S. Liu, et al. (2008). "Octamer 4 small interfering RNA results in cancer stem cell-like cell apoptosis." Cancer Res **68**(16): 6533-40.

Octamer 4 (Oct4), a member of the POU family of transcription factors, plays a key role in the maintenance of pluripotency and proliferation potential of embryonic stem cells. Cancer stem cell-like cells (CSCLC) are reported to be a minor population in tumors or even in tumor cell lines which also express Oct4. The role of Oct4 in CSCLCs still remains to be defined. In our study, we show that, in vitro, almost all murine Lewis lung carcinoma 3LL cells and human breast cancer MCF7 cells express Oct4 at high levels. This expression of Oct4 is successfully reduced by small interfering RNA, which eventually results in cell apoptosis. The signal pathway Oct4/Tcl1/Akt1 has been observed to be involved in this event. The repression of Oct4 reduces Tcl1 expression and further down-regulates the level of p-Ser.473-Akt1. In vivo, only approximately 5% of tumor cells were detected to express Oct4 in established 3LL and MCF7 tumor models, respectively. Small interfering RNA against Oct4 successfully decreases the CSCLCs and markedly inhibits tumor growth. In summary, we show that Oct4 might maintain the survival of CSCLCs partly through Oct4/Tcl1/Akt1 by inhibiting apoptosis, which strongly indicates that targeting Oct4 may have important clinical applications in cancer therapy.

Huang, H. M., C. J. Huang, et al. (2000). "Mcl-1 is a common target of stem cell factor and interleukin-5 for apoptosis prevention activity via MEK/MAPK and PI-3K/Akt pathways." Blood **96**(5): 1764-71.

Stem cell factor (SCF) has been suggested as essential for optimal production of various hematopoietic lineages mainly because of its apoptosis prevention function when it costimulates with other cytokines. However, the underlying mechanism of this synergism of apoptosis prevention is largely unknown. The present study examined the expression of some Bcl-2 family members, including Bcl-2, Bcl-X(L), Mcl-1, and Bax, in response to cytokine stimulation in TF-1 and JYTF-1 cells in which SCF costimulation is differentially required for optimal proliferation. The results revealed that only the expression of Mcl-1 highly correlated with the antiapoptotic activity of interleukin-5 (IL-5) and the synergistic effect of SCF. In TF-1 cells, the defect of IL-5 in apoptosis suppression and Mcl-1 induction was associated with the incapability to highly phosphorylate Janus kinases (JAK1, JAK2), signal transducer and activator of transcription-5 (STAT5), mitogen-activated protein kinase (MAPK), and Akt/PKB, whereas SCF costimulation restored the potent phosphorylation of MAPK and Akt/PKB, but not STAT5. The importance of MAPK and Akt/PKB signaling pathways in regulating the expression of Mcl-1 and cell survival was further supported by the observation that inhibition of MEK by PD98059 or phosphatidylinositol-3 kinase (PI-3K) by LY294002 independently resulted in the reduction of Mcl-1 expression and loss of cell viability. Therefore, the data suggest that Mcl-1 is a common antiapoptotic target of both early-stage cytokine SCF and late-stage cytokine IL-5. Both MEK/MAPK and PI-3K/Akt signaling pathways are essential in the regulation of Mcl-1 expression and apoptosis prevention. (Blood. 2000;96:1764-1771)

Huang, S., M. Luca, et al. (1996). "Enforced c-KIT expression renders highly metastatic human melanoma cells susceptible to stem cell factor-induced apoptosis and inhibits their tumorigenic and metastatic potential." Oncogene **13**(11): 2339-47.

Expression of the tyrosine-kinase receptor encoded by the c-KIT proto-oncogene progressively decreases during local tumor growth and invasion of human melanomas. To provide direct evidence that c-KIT plays a role in metastasis of human melanoma, we transfected the c-KIT gene into the c-KIT negative highly metastatic human melanoma cell line A375SM and subsequently analysed its tumorigenic and metastatic potential. A375SM parental cells, A375SM-NOT (neo, control), and A375SM-KIT-positive cells were injected s.c. and i.v. into nude mice. A375SM-KIT cells produced significantly slower growing s.c. tumors and fewer lung metastases than control cells. Exposure of c-KIT-positive melanoma cells in vitro and in vivo to stem cell factor (SCF), the ligand for c-KIT, triggered apoptosis of these cells but not of c-KIT-negative melanoma cells or normal melanocytes. Since SCF is produced by keratinocytes and other dermal cells in the skin, these results suggest that the loss of c-KIT receptor expression may allow malignant melanoma cells to escape SCF/c-KIT-mediated apoptosis, hence

contributing to tumor growth and eventually metastasis. The antitumor and antimetastatic properties of SCF may be useful in treating human melanomas in early stages.

Iemura, A., M. Tsai, et al. (1994). "The c-kit ligand, stem cell factor, promotes mast cell survival by suppressing apoptosis." Am J Pathol **144**(2): 321-8.

Stem cell factor (SCF) and its receptor (SCFR), a member of the receptor tyrosine kinase III family that is encoded by the c-kit gene, critically regulate several complex biological programs including hematopoiesis, mast cell development, cutaneous pigmentation, and gametogenesis. We show herein that mouse mast cells die rapidly after the withdrawal of SCF in vivo or in vitro, and provide morphological evidence that such mast cells undergo programmed cell death or apoptosis. We also show that when in vitro-derived mouse mast cells maintained in SCF are removed from SCF-containing medium for only 5 or 6 hours, the cells' genomic DNA exhibits the ladder-like pattern of oligonucleosome-sized fragments typical of apoptosis. These findings demonstrate that SCF can regulate the survival of a cellular lineage which expresses the SCFR by suppressing apoptosis. They also identify a mechanism that can result in striking and rapid reductions in the size of tissue mast cell populations without histological evidence of the concomitant induction of a significant inflammatory response.

Ino, K., A. G. Ageitos, et al. (2001). "Activation-induced T cell apoptosis by monocytes from stem cell products." Int Immunopharmacol **1**(7): 1307-19.

We recently found that mobilized peripheral blood stem cell (PSC) products (from both cancer patients and normal donors) contain high levels of CD14+ monocytes, which can inhibit the proliferation of allogeneic and autologous T cells. We found in our studies that using CD14+ monocytes from mobilized PSC products (from normal and cancer patient donors), normal apheresis products or normal peripheral blood (PB) can affect lymphocyte function and apoptosis-dependent T cell activation. However, it appears that the apoptosis is dependent on the frequency of monocytes, which is increased by both mobilization and apheresis. Both phytohemagglutinin (PHA)- and interleukin (IL)-2-induced proliferation of steady-state peripheral blood mononuclear cells (PBMC) were markedly inhibited by co-culture with irradiated CD14+ monocytes, although inhibition was significantly greater with PHA than with IL-2 stimulation. IL-2 (predominately CD56+ NK cells) or anti-CD3 monoclonal antibody (mAb) and IL-2-expanded lymphocytes (activated T cells) were inhibited by PSC monocytes to a significantly greater level as compared to steady-state lymphocytes. Indeed, no inhibition of T cell proliferation was observed when lymphocytes were co-cultured in the absence of mitogenic or IL-2 stimulation. In contrast, an increased proliferation was observed in co-cultures of CD14+ monocytes and steady-state or activated lymphocytes without mitogenic stimulation. Cell cycle analysis by flow cytometry revealed a significant increase in hypodiploid DNA, in a time-dependent manner, following co-culture of monocytes and PBMC in PHA, suggesting that T cell apoptosis occurred during PHA-induced activation. These results demonstrate that PSC-derived monocytes inhibit T cell proliferation by inducing the apoptosis of activated T cells and NK cells, but not steady-state cells. This suggests a potential role for monocytes in the induction of peripheral tolerance following stem cell transplantation.

Inoue, T., K. Yoneda, et al. (2002). "Alteration of mast cell proliferation/apoptosis and expression of stem cell factor in the regression of mastocytoma--report of a case and a serial immunohistochemical study." J Cutan Pathol **29**(5): 305-12.

BACKGROUND: Spontaneous regression of solitary mastocytoma is a well-described phenomenon, but its mechanism is unknown. METHODS: Serial-section immunohistochemical analyses were performed on biopsies of a mastocytoma from a Japanese child during the proliferation stage (PS, 7 months of age) and the regression stage (RS, 5 years old). RESULTS: Mast cell (MC) density in RS was markedly decreased (406 cells/mm2) compared to that in PS (3554 cells/mm2). MCs in RS were larger than those in PS. With proliferative cell nuclear antigen (PCNA) staining, 1.7% MCs were positive in PS, whereas no positive MCs were seen in RS. TUNEL-labeling index (LI) in RS (2.8%) increased 1.5-fold in PS (1.9%). With stem cell factor (SCF) staining, 57% of lesional MCs in RS revealed strong cytoplasmic immunoreactivity, whereas only 9% of MCs were positive in PS. Epidermal SCF reactivity was found as intracellular and intercellular patterns in both PS and RS. CONCLUSIONS: Loss of MC proliferating activity, an increase in apoptotic MCs, and increased expression of SCF in remaining MCs in RS may play a role in the involution of mastocytomas.

Itakura, A., A. Tanaka, et al. (2002). "Ceramide and sphingosine rapidly induce apoptosis of murine mast cells supported by interleukin-3 and stem cell factor." Exp Hematol **30**(3): 272-8.

OBJECTIVE: Ceramide and sphingosine, generated by sphingomyelinase-mediated hydrolysis of sphingomyelin, which packs tightly in the bilayer

of the plasma membrane, have been proposed as intracellular mediators of apoptotic signals. However, precise function of endogenous sphingomyelin-cycle metabolites in mast cells has been unclear. Thus, we sought to define the involvement of ceramide and sphingosine in apoptotic pathways of mast cells. MATERIALS AND METHODS: We examined the effect of cell-permeable C(2)-ceramide, sphingosine, and sphingomyelinase on survival of murine bone marrow-derived cultured mast cells (BMCMC) supported by recombinant interleukin-3 (rIL-3) and/or recombinant stem cell factor (rSCF). Downstream signaling pathways of C(2)-ceramide and sphingosine were analyzed by using caspase inhibitors. RESULTS: C(2)-ceramide, sphingosine, and sphingomyelinase induced apoptosis in BMCMC in the presence of rIL-3 and/or rSCF, and Z-VAD-fmk (a broad caspase inhibitor), Z-DEVD-fmk (a caspase 3 inhibitor), and Z-IETD-fmk (a caspase 8 inhibitor) partially prevented apoptosis of BMCMC induced by C(2)-ceramide but not sphingosine. CONCLUSION: The present results suggest that ceramide and sphingosine may function as intracellular mediators of apoptotic signals in mast cells, which override survival signals from IL-3 and SCF. In addition, caspases may be partially involved in ceramide- but not sphingosine-mediated apoptosis of mast cells.

Ito, M., Y. Kawa, et al. (1999). "Removal of stem cell factor or addition of monoclonal anti-c-KIT antibody induces apoptosis in murine melanocyte precursors." J Invest Dermatol **112**(5): 796-801.

Previous findings indicate that the protein c-KIT and its ligand, stem cell factor (SCF) play a crucial role in the development of melanocytes from their precursors in the embryonic neural crest cells. Using a monoclonal anti-c-KIT antibody, ACK2, which is an antagonistic blocker of c-KIT function, we and colleagues demonstrated that mouse melanocytes disappeared with the injection of ACK2 during certain periods of embryonic and postnatal life. The precise mechanisms of this disappearance, however, remain unclear. Because melanocytes disappeared without any inflammation in these in vivo studies, we suspect that apoptosis was a main cause of their disappearance. In this study, to clarify the underlying mechanism, we studied whether ACK2 induces apoptosis in c-KIT-positive melanoblasts, which appear in mouse neural crest cells cultured with SCF from 9.5 d old mouse embryos. With an in situ apoptosis detection kit, a significant increase in apoptosis was detected after the removal of SCF, which further increased with the addition of ACK2 during SCF-dependent periods. The occurrence of apoptosis in the cultured cells was also demonstrated by a DNA analysis and electron microscopy. Immunohistochemical double staining confirmed that the apoptotic cells were c-KIT positive, and the electron microscopy showed that these apoptotic cells were melanocyte precursors. It was therefore demonstrated that apoptosis was induced in the SCF-dependent c-KIT-positive melanocytes in vitro when the SCF/c-KIT interaction was obstructed. These findings elucidate the mechanism of the regulation of melanocyte development, and the survival and proliferation of these precursor cells, by SCF/c-KIT interaction.

Jacobsen, F. W., T. Stokke, et al. (1995). "Transforming growth factor-beta potently inhibits the viability-promoting activity of stem cell factor and other cytokines and induces apoptosis of primitive murine hematopoietic progenitor cells." Blood **86**(8): 2957-66.

In contrast with the extensively characterized effects of transforming growth factor-beta (TGF-beta) on proliferation and differentiation of hematopoietic progenitors, little is known about the effects of TGF-beta on viability of normal hematopoietic progenitors. In the present report, we demonstrate that TGF-beta potently counteracts hematopoietic growth factor (HGF)-induced survival of individually cultured primitive Lin-Sca-1+ bone marrow progenitors. Specifically, 74% of single Lin-Sca-1+ cells cultured for 40 hours in the presence of stem cell factor (SCF) survived, whereas only 16% survived in the presence of SCF plus TGF-beta. Similarly, the enhanced survival of primitive hematopoietic progenitors in response to granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-1, IL-6, or IL-11 was also potently opposed by TGF-beta. Furthermore, it is demonstrated that neutralization of endogenous TGF-beta present in the cultures enhances survival of Lin-Sca-1+ progenitors in the absence, as well as in the presence, of HGFs such as SCF and IL-6. The reduced HGF-induced survival of primitive hematopoietic progenitors in the presence of TGF-beta was associated with increased apoptosis, as detected by an in situ terminal deoxynucleotidyl transferase (TdT) assay. After 16 hours of incubation in the absence of HGFs, 61% +/- 6% of the hematopoietic progenitors had DNA strand breaks characteristic of apoptosis. The presence of SCF reduced the frequency of apoptic cells to 27% +/- 5%, whereas 55% +/- 3% of the cells had signs of apoptosis in the presence of SCF plus TGF-beta.

Jalal Hosseinimehr, S., O. Inanami, et al. (2004). "Activation of c-kit by stem cell factor induces radioresistance to apoptosis through ERK-dependent expression of survivin in HL60 cells." J Radiat Res **45**(4): 557-61.

We investigated the effect of SCF, a c-kit ligand, on the radiosensitivity of HL60 cells. X-ray-induced apoptosis in HL60 cells was significantly lower in the presence of SCF than in the absence of SCF. This attenuation of X-ray-induced apoptosis by SCF was abolished by PD98059 (an ERK inhibitor), but not by wortmannin (a PI3-K inhibitor) or GF109203X (a PKC inhibitor). The expression of phospho-ERK1/2 (active form) and the ERK1/2-regulated expression of survivin were found to increase in cells treated with X irradiation and SCF. However, X irradiation alone induced down-regulation of the expression of phospho-ERK1/2. Our findings suggest that activation of c-kit by SCF confers radioresistance through up-regulation of ERK-dependent survivin expression in HL60 cells.

Kanbe, N., M. Kurosawa, et al. (2000). "Nerve growth factor prevents apoptosis of cord blood-derived human cultured mast cells synergistically with stem cell factor." Clin Exp Allergy **30**(8): 1113-20.

BACKGROUND: Stem cell factor (SCF) has been identified as a critical survival factor of human mast cells. Other cytokines which possess survival promotion activity on human mast cells are less known. OBJECTIVE: We examined the survival promotion activity of nerve growth factor (NGF) on cord blood-derived human cultured mast cells. METHODS: Expression and function of NGF receptors on the mast cells were examined by RT PCR, flowcytometric analysis, immunoprecipitaion and western blotting. The survival promotion activity of NGF to the mast cells was examined. To evaluate the proliferating activity of NGF on the human cultured mast cells, flow cytometric analysis with propidium iodide staining was applied. To confirm whether the human mast cell growth activity of NGF was caused by a suppression of apoptosis, the proportion of the cells containing in situ DNA fragmentation was counted. RESULTS: The human cultured mast cells expressed the high affinity receptor p140trk but not the low affinity receptor p75LNGFR. NGF induced the phosphorylation of p140trk. NGF alone could not support the survival of the mast cells, however, the addition of NGF to the culture medium containing recombinant SCF led to a significant increase of the number of survival mast cells. No significant changes of the cell cycle from G0/G1 phase to the S/G2 + M phases were observed by NGF. In contrast, the addition of NGF to the medium with SCF showed a significant inhibitory effect on the apoptosis of the mast cells. CONCLUSION: NGF may act as a key factor to promote the survival of human mast cells synergistically with SCF through the prevention of apoptosis.

Kearney, E. M., P. J. Prendergast, et al. (2008). "Mechanisms of strain-mediated mesenchymal stem cell apoptosis." J Biomech Eng **130**(6): 061004.

Mechanical conditioning of mesenchymal stem cells (MSCs) has been adopted widely as a biophysical signal to aid tissue engineering applications. The replication of in vivo mechanical signaling has been used in in vitro environments to regulate cell differentiation, and extracellular matrix synthesis, so that both the chemical and mechanical properties of the tissue-engineered construct are compatible with the implant site. While research in these areas contributes to tissue engineering, the effects of mechanical strain on MSC apoptosis remain poorly defined. To evaluate the effects of uniaxial cyclic tensile strain on MSC apoptosis and to investigate mechanotransduction associated with strain-mediated cell death, MSCs seeded on a 2D silicone membrane were stimulated by a range of strain magnitudes for 3 days. Mechanotransduction was investigated using the stretch-activated cation channel blocker gadolinium chloride, the L-type voltage-activated calcium channel blocker nicardipine, the c-jun NH(2)-terminal kinase (JNK) blocker D-JNK inhibitor 1, and the calpain inhibitor MDL 28170. Apoptosis was assessed through DNA fragmentation using the terminal deoxynucleotidyl transferase mediated-UTP-end nick labeling method. Results demonstrated that tensile strains of 7.5% or greater induce apoptosis in MSCs. L-type voltage-activated calcium channels coupled mechanical stress to activation of calpain and JNK, which lead to apoptosis through DNA fragmentation. The definition of the in vitro boundary conditions for tensile strain and MSCs along with a proposed mechanism for apoptosis induced by mechanical events positively contributes to the development of MSC biology, bioreactor design for tissue engineering, and development of computational methods for mechanobiology.

Kie, J. H., W. I. Yang, et al. (2002). "Decrease in apoptosis and increase in polyploidization of megakaryocytes by stem cell factor during ex vivo expansion of human cord blood CD34+ cells using thrombopoietin." Stem Cells **20**(1): 73-9.

Thrombopoietin (TPO) is widely used for ex vivo expansion of hematopoietic stem cells. Previously, we have reported that TPO induces a characteristic pattern of apoptosis, and the TPO-induced apoptosis is closely associated with megakaryocyte (MK) differentiation. In the present study, several cytokines, flt3-ligand, stem cell factor (SCF), interleukin-3 (IL-3), IL-6, IL-11, leukemia inhibitory factor, G-CSF, and erythropoietin, which

are known to affect megakaryocytopoiesis, have been evaluated to elucidate their effects on the TPO-induced apoptosis. Measurement of apoptosis by flow cytometry revealed that only SCF absolutely reduced the TPO-induced apoptosis in MK fractions, particularly in the late phase of ex vivo expansion. Platelet production was demonstrated by electron microscopy in a later phase when SCF was added. Simultaneous measurement of DNA contents with immunophenotyping demonstrated a significant increase in polyploidization in the CD41+ cell fraction when cultured with SCF. These results suggested that SCF not only inhibited premature senescence but also enhanced maturation of the differentiating cells of MK lineage during ex vivo expansion using TPO.

Koyanagi, M., J. Takahashi, et al. (2008). "Inhibition of the Rho/ROCK pathway reduces apoptosis during transplantation of embryonic stem cell-derived neural precursors." J Neurosci Res **86**(2): 270-80.

Rho-GTPase has been implicated in the apoptosis of many cell types, including neurons, but the mechanism by which it acts is not fully understood. Here, we investigate the roles of Rho and ROCK in apoptosis during transplantation of embryonic stem cell-derived neural precursor cells. We find that dissociation of neural precursors activates Rho and induces apoptosis. Treatment with the Rho inhibitor C3 exoenzyme and/or the ROCK inhibitor Y-27632 decreases the amount of dissociation-induced apoptosis (anoikis) by 20-30%. Membrane blebbing, which is an early morphological sign of apoptosis; cleavage of caspase-3; and release of cytochrome c from the mitochondria are also reduced by ROCK inhibition. These results suggest that dissociation of neural precursor cells elicits an intrinsic pathway of cell death that is at least partially mediated through the Rho/ROCK pathway. Moreover, in an animal transplantation model, inhibition of Rho and/or ROCK suppresses acute apoptosis of grafted cells. After transplantation, tumor necrosis factor-alpha and pro-nerve growth factor are strongly expressed around the graft. ROCK inhibition also suppresses apoptosis enhanced by these inflammatory cytokines. Taken together, these results indicate that inhibition of Rho/ROCK signaling may improve survival of grafted cells in cell replacement therapy.

Koyanagi-Katsuta, R., N. Akimitsu, et al. (2000). "Apoptosis of mouse embryonic stem cells induced by single cell suspension." Tissue Cell **32**(1): 66-70.

Embryonic stem cells (ES cells) are pluripotential, and are therefore used to construct gene knock-out mice. We found that the apoptosis of mouse ES cells was induced when the cells were dispersed as single cells, whereas this process was suppressed when they proliferated in aggregates. The apoptosis of ES cells was repressed when the cells were cultured on feeders prepared from STO cells, a cell line established from embryonic fibroblasts. Culture supernatants from STO cells did not block the apoptosis of ES cells, which suggests that a direct interaction between ES cells and STO cells is required for the suppression of apoptosis. The viability of ES cells examined by the trypan blue exclusion test or by the MTT ((3-4,5-dimethyithiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay decreased dramatically when the cells were dispersed in phosphate-buffered saline PBS. Cellular activity was restored by the addition of culture medium for ES cells. Glucose in the medium was found to be a major factor responsible for the restoration. Amino acids also restored the decrease in reduction of MTT. Suspension of the ES cells in PBS(-) caused leakage of the nucleosome into cytoplasm. Results indicate that the single cell suspension of ES cells leads to leakage of substrates for oxidative phosphorylation from the mitochondria, and that these cells finally become committed to apoptosis.

Laveder, F. and R. Marcolongo (1996). "Uncontrolled triggering of programmed cell death (apoptosis) in haematopoietic stem cells: a new hypothesis for the pathogenesis of aplastic anaemia." Immunol Cell Biol **74**(2): 159-62.

The cause of bone marrow failure in aplastic anaemia (AA) is still unknown; however, it is clear that acquired AA is a heterogeneous disease including basically different pathophysiological conditions. Causative agents, clinically associated with AA, possibly exert their action through restricted pathways. Some theoretical and experimental data show that programmed cell death (PCD) or apoptosis is physiologically important in normal haematopoiesis and could be involved in the pathophysiological events responsible for the development of AA. Therefore, it is intriguing to hypothesize that the pathogenetic mechanism underlying most cases of acquired AA could be represented by an excessive and/or uncontrolled triggering of PCD in haematopoietic stem cells. Investigations to test this hypothesis are proposed.

Lee, J. H., D. Schutte, et al. (2006). "Stem-cell protein Piwil2 is widely expressed in tumors and inhibits apoptosis through activation of Stat3/Bcl-XL pathway." Hum Mol Genet **15**(2): 201-11.

The genes of the piwi family are defined by conserved PAZ and Piwi domains and play important roles in stem-cell self-renewal, RNA silencing and translational regulation in various organisms. Both, mouse and human Piwil2 genes, members of the piwi

gene family, are specifically expressed in testis. We report here enhanced expression of the human Piwil2 gene in testicular seminomas, but not in testicular non-seminomatous tumors. Expression of the Piwil2 gene was also found in different tumors examined, including prostate, breast, gastrointestinal, ovarian and endometrial cancer of human and in breast tumors, rhabdomyosarcoma and medulloblastoma of mouse. Therefore, Piwil2 can be categorized as a novel member of cancer/testis antigens. To identify genes activated by Piwil2, RNA isolated from NIH-3T3 cells expressing constitutively Piwil2 were compared with RNA samples from control NIH-3T3 cells using a cancer gene array. Induction of high-level expression of the antiapoptotic gene Bcl-X(L) was observed in cells expressing Piwil2. Furthermore, increased Bcl-X(L) expression correlated with increase of signal transducer and activator of transcription 3 (Stat3) expression. Gene silencing of Piwil2 with its small interference RNA suppressed Stat3 and Bcl-X(L) expression and induced apoptosis. A causal link between Piwil2 expression and inhibition of apoptosis and enhanced proliferation was demonstrated in cells expressing Piwil2. Furthermore, results of soft agar assay indicated that Piwil2 overexpression induced transformation of fibroblast cells. In summary, our results demonstrate that Piwil2 is widely expressed in tumors and acts as an oncogene by inhibition of apoptosis and promotion of proliferation via Stat3/Bcl-X(L) signaling pathway. Expression of Piwil2 in a wide variety of tumors could be a useful prognostic factor that could have also diagnostic and therapeutic implications.

Lee, N. S., H. J. Cheong, et al. (2003). "Ex vivo purging of leukemia cells using tumor-necrosis-factor-related apoptosis-inducing ligand in hematopoietic stem cell transplantation." Leukemia **17**(7): 1375-83.

The aim of this study was to evaluate the potential of tumor-necrosis-factor-related apoptosis-inducing ligand TRAIL to eradicate leukemia cell lines, while sparing normal hematopoietic stem cells. Human Jurkat and Molt-4 cell lines were used to optimize the purging process in umbilical cord blood (UCB) mononuclear cells. The Jurkat cell line was TRAIL sensitive and TRAIL-resistant Molt-4 cell line became sensitive after being treated with TRAIL and a low dose of doxorubicin (0.1 micro M), but UCB mononuclear cells remained resistant. DR4 expression was increased when Jurkat cells were treated with TRAIL, and DR5 expression increased after exposing Molt-4 cells to TRAIL plus a low dose of doxorubicin for 24 h. The expression of DR4 and DR5 in UCB mononuclear cells was unchanged after treatment with TRAIL, a low-dose doxorubicin, or TRAIL plus a low dose of doxorubicin. In TRAIL-sensitive Jurkat cells, caspases 8, 9, 3, and 7 were activated by TRAIL treatment and activation of caspases was augmented by TRAIL plus a low dose of doxorubicin than TRAIL or a low dose of doxorubicin alone in Molt-4 cells. Experiments involving mixture of UCB mononuclear cells and Jurkat or Molt-4 cells showed a marked eradication of leukemia cells and the limiting dilution assay demonstrated an eradication rate of more than 4 logs after 24 h incubation with 100 ng/ml of TRAIL in Jurkat cells. In the case of Molt-4 cells, the eradication rate was about 3 logs when TRAIL was used in combination with a low dose of doxorubicin. No significant decrease in the number of granulocyte-macrophage colony-forming unit) (CFU-GM) colonies was detected when UCB mononuclear cells were treated with TRAIL in combination with a low dose of doxorubicin. These results suggest that TRAIL offers the possibility of being used as an ex vivo purging agent for autologous transplantation in hematologic malignancies.

Li, J. W., L. L. Li, et al. (2009). "Stem cell factor protects against neuronal apoptosis by activating AKT/ERK in diabetic mice." Braz J Med Biol Res **42**(11): 1044-9.

Neuronal apoptosis occurs in the diabetic brain due to insulin deficiency or insulin resistance, both of which reduce the expression of stem cell factor (SCF). We investigated the possible involvement of the activation of the MAPK/ERK and/or AKT pathways in neuroprotection by SCF in diabetes. Male C57/B6 mice (20-25 g) were randomly divided into four groups of 10 animals each. The morphology of the diabetic brain in mice treated or not with insulin or SCF was evaluated by H&E staining and TUNEL. SCF, ERK1/2 and AKT were measured by Western blotting. In diabetic mice treated with insulin or SCF, there was fewer structural change and apoptosis in the cortex compared to untreated mice. The apoptosis rate of the normal group, the diabetic group receiving vehicle, the diabetic group treated with insulin, and the diabetic group treated with SCF was 0.54 +/- 0.077%, 2.83 +/- 0.156%, 1.86 +/- 0.094%, and 1.78 +/- 0.095% (mean +/- SEM), respectively. SCF expression was lower in the diabetic cortex than in the normal cortex; however, insulin increased the expression of SCF in the diabetic cortex. Furthermore, expression of phosphorylated ERK1/2 and AKT was decreased in the diabetic cortex compared to the normal cortex. However, insulin or SCF could activate the phosphorylation of ERK1/2 and AKT in the diabetic cortex. The results suggest that SCF may protect the brain from apoptosis in diabetes and that the mechanism of this protection may, at least in part, involve activation of the ERK1/2 and AKT pathways.

These results provide insight into the mechanisms by which SCF and insulin exert their neuroprotective effects in the diabetic brain.

Liesveld, J. L., A. W. Harbol, et al. (1996). "Stem cell factor and stromal cell co-culture prevent apoptosis in a subculture of the megakaryoblastic cell line, UT-7." Leuk Res **20**(7): 591-600.

The megakaryoblastic cell line, UT-7, is dependent for its growth upon interleukin-3 (IL-3), erythropoietin, or granulocyte-macrophage colony stimulating factor (GM-CSF). A subculture of this line can be maintained in recombinant human c-kit ligand [stem cell factor (SCF)] at 100 ng/ml without requirement for other growth factors. Removal of this subculture from SCF results in rapid loss of viability and decreased proliferation. Cells grown in SCF also can be maintained in GM-CSF but not vice versa. In this work, we have characterized the SCF dependence of this UT-7 subculture. Stem cell factor removal results in apoptosis and a decline in viability which can be restored partially by re-addition of SCF, GM-CSF, or co-culture with adherent marrow stromal cells. Apoptosis in the factor-starved UT-7 population has been documented by light microscopy, electron microscopy and DNA analysis, showing the typical 180 base pair laddering characteristic of apoptosis. To quantitate the degree of apoptosis in the cell populations, and to assess whether apoptosis decreased with re-exposure of starved cells to growth factors or stroma, we utilized flow cytometry. This confirmed that exposure of previously factor-starved cells to stroma decreased the percentage of cells undergoing apoptosis. Co-culture with an SCF-deficient murine stromal cell line was also able to prevent apoptosis, suggesting contribution of other stromal cell factors. Experiments performed using trans-well inserts which do not allow cell passage, showed greatest viability of cells in contact with stroma, but viability was also improved in cells cultured in the presence of, but not in contact with, stromal cells compared to those cultured above plastic, suggesting a role for soluble stroma-produced substances. These data demonstrate that SCF alone can prevent apoptosis in cells dependent upon its presence for proliferation. Also, marrow stromal cells can serve as a partial substitute for growth factor in the prevention of apoptosis in these cells, probably due to constitutive presentation of SCF and other hematopoietic growth factors in both soluble and surface-bound forms.

Lin, Y. M., G. Z. Zhang, et al. (2006). "[Study of apoptosis gene expression in U937 cells induced by adhesion culture with mesenchymal stem cell]." Zhonghua Xue Ye Xue Za Zhi **27**(4): 249-53.

OBJECTIVE: To compare apoptosis gene expression profiling of U937 cells in suspension culture with that cultivated with mesenchymal stem cells (MSCs), and find out the relationship between drug resistance of leukemia cells and hemopoietic microenvironment. METHODS: U937 cells were cultivated in adhesion culture with MSCs and in suspension culture for 48 hours. Cell cycle was determined by flow cytometry and gene expression profiling by cDNA microarray. RESULTS: Compared with that in suspension, G(0)/G(1) fraction of U937 cells increased in adhesion culture (45.3 +/- 3.1)% vs (32.6 +/- 2.1)%, respectively ($P < 0.05$), whereas G(2)/M fraction and apoptosis rate were decreased. After 48 h twenty-eight differential expression genes were screened out in 487 apoptosis-related genes, among which 27 were up-regulated and were mainly apoptosis-suppressor genes, apoptosis-promoter genes, cell cycle positive control genes and cell cycle negative control genes. But Bcl-XL was up-regulated most obviously. The only one gene down-regulated was an apoptosis promoter gene. CONCLUSION: Adhesion culture with MSCs can lead to growth suppression and decrease natural apoptosis of U937 cells. The mechanism was multiple gene effects, but Bcl-XL may be of the most importance.

Lucas, T., B. Pratscher, et al. (2005). "The human orthologue of a novel apoptosis response gene induced during rat myelomonocytic stem cell apoptosis maps to 20q13.12." Stem Cells Dev **14**(5): 556-63.

Stem cell factor (SCF) stimulation of the receptor tyrosine kinase c-kit has effects on the proliferation, differentiation, and apoptotic regulation of hematopoietic progenitor cell populations. Rat bone marrow myelomonocytic stem cells (MSC) isolated in vitro by wheat germ agglutinin culture exclusively undergo self-renewal divisions when stimulated by SCF but bipotentially differentiate in the presence of dexamethasone or 1alpha,25-dihydroxyvitamin D(3) to granulocytes and macrophages, respectively. We show here that withdrawal of SCF from MSC induces rapid apoptosis in all stages of the cell cycle accompanied by development of an ultrastructural apoptotic morphology. To investigate immediate-early gene induction during MSC apoptosis, a differential display polymerase chain reaction (DD-PCR) screen coupled with rapid amplification of cDNA ends (RACE) PCR was performed. An immediate-early apoptosis response gene was isolated from growth factor-deprived MSC that was not expressed during self-renewal or differentiation induction cultures containing SCF. The protein contains a PEST region enriched in proline, glutamic acid, serine, and threonine residues common to proteins with a high

turnover and has a cytoplasmic, vesicular localization in apoptotic MSC shown by immunohistochemistry. The human orthologous gene, isolated by RACE PCR, shows 86% homology to the rat protein and high similarity with a human uncharacterized hypothalamus predicted protein (HSMNP1) localized to the long arm of chromosome 20. Because deletions in this region are a common occurrence in a wide range of myeloproliferative disorders characterized by treatment resistance to apoptosis, HSMNP1 expression may play a role in normal and pathological myeloid development.

Mariotti, J., J. Foley, et al. (2008). "Ex vivo rapamycin generates apoptosis-resistant donor Th2 cells that persist in vivo and prevent hemopoietic stem cell graft rejection." J Immunol **180**(1): 89-105.

Because ex vivo rapamycin generates murine Th2 cells that prevent Graft-versus-host disease more potently than control Th2 cells, we hypothesized that rapamycin would generate Th2/Tc2 cells (Th2/Tc2.R cells) that abrogate fully MHC-disparate hemopoietic stem cell rejection more effectively than control Th2/Tc2 cells. In a B6-into-BALB/c graft rejection model, donor Th2/Tc2.R cells were indeed enriched in their capacity to prevent rejection; importantly, highly purified CD4+ Th2.R cells were also highly efficacious for preventing rejection. Rapamycin-generated Th2/Tc2 cells were less likely to die after adoptive transfer, accumulated in vivo at advanced proliferative cycles, and were present in 10-fold higher numbers than control Th2/Tc2 cells. Th2.R cells had a multifaceted, apoptosis-resistant phenotype, including: 1) reduced apoptosis after staurosporine addition, serum starvation, or CD3/CD28 costimulation; 2) reduced activation of caspases 3 and 9; and 3) increased anti-apoptotic Bcl-xL expression and reduced proapoptotic Bim and Bid expression. Using host-versus-graft reactivity as an immune correlate of graft rejection, we found that the in vivo efficacy of Th2/Tc2.R cells 1) did not require Th2/Tc2.R cell expression of IL-4, IL-10, perforin, or Fas ligand; 2) could not be reversed by IL-2, IL-7, or IL-15 posttransplant therapy; and 3) was intact after therapy with Th2.R cells relatively devoid of Foxp3 expression. We conclude that ex vivo rapamycin generates Th2 cells that are resistant to apoptosis, persist in vivo, and effectively prevent rejection by a mechanism that may be distinct from previously described graft-facilitating T cells.

Maurer, M. and S. J. Galli (2004). "Lack of significant skin inflammation during elimination by apoptosis of large numbers of mouse cutaneous mast cells after cessation of treatment with stem cell factor." Lab Invest **84**(12): 1593-602.

We previously reported that subcutaneous (s.c.) administration of stem cell factor (SCF), the ligand for the c-Kit receptor, to the back skin of mice promotes marked local increases in the numbers of cutaneous mast cells (MCs), and that cessation of SCF treatment results in the rapid reduction of cutaneous MC populations by apoptosis. In the present study, we used the 125I-fibrin deposition assay, a very sensitive method for quantifying increased vascular permeability, to assess whether the clearance of large numbers of apoptotic MCs is associated with significant cutaneous inflammation. The s.c. injection of rrSCF164 (30 or 100 microg/kg/day) or rrSCF164-peg (polyethylene glycol-treated SCF, 30 or 100 microg/kg/day) for 23 days increased the numbers of dermal MCs at skin injection sites from 5.1+/-0.7 MCs/mm2 to 36.4+/-4.1, 34.7+/-9.7, 52.5+/-5.8, and 545+/-97 MCs/mm2, respectively. In contrast, MC numbers were markedly lower in mice that had been treated with SCF for 21 days, followed by 2 days of injection with the vehicle alone. Notably, when tested during the period of rapid reduction of skin MCs,125I-fibrin deposition in the skin was very similar to that in mice receiving continuous treatment with SCF or vehicle. We conclude that the rapid elimination of even very large populations of MCs by apoptosis, which also results in the clearance of the considerable quantities of proinflammatory products stored by these cells, does not lead to significant local cutaneous inflammatory responses.

Mekori, Y. A. and D. D. Metcalfe (1994). "Transforming growth factor-beta prevents stem cell factor-mediated rescue of mast cells from apoptosis after IL-3 deprivation." J Immunol **153**(5): 2194-203.

IL-3-dependent mast cells undergo apoptosis upon removal of IL-3, an event that is prevented by the addition of stem cell factor (SCF) acting through its receptor c-kit, suggesting that SCF provides a mechanism to allow mast cells to survive and to differentiate in tissues in the relative absence of IL-3. This observation is consistent with the thesis that the microenvironment, in part, controls mast cell number and viability by modulating SCF production and release. The purpose of the present study was to determine whether a second factor, TGF-beta 1, was capable of modifying the SCF-mediated survival pathway. TGF-beta 1 (1 and 10 ng/ml), known to be an important regulator of cell growth and function, did inhibit the SCF-mediated rescue from apoptosis in IL-3-deprived mast cells. TGF-beta 1 exerted its inhibitory effect on SCF-mediated rescue from apoptosis, even when added 4 h after the addition of SCF. In contrast, TGF-beta 1 had no substantial effect on the viability of mast cells that were grown in the presence of IL-3. TGF-beta 1 also had no noticeable

effect on viability and proliferation of a growth factor-independent mast cell line. The inhibitory effect of TGF-beta 1 was neutralized by specific anti-TGF-beta mAb. TGF-beta 1 did not affect the expression of c-kit, as determined by using flow cytometric analysis of mast cells labeled with FITC-conjugated anti-c-kit. These results demonstrate how SCF and TGF-beta may act in concert to regulate mast cell numbers under physiologic or pathologic conditions.

Mekori, Y. A., C. K. Oh, et al. (1995). "The role of c-Kit and its ligand, stem cell factor, in mast cell apoptosis." Int Arch Allergy Immunol **107**(1-3): 136-8.

The regulation of tissue mast cell number depends both on the rate of production of mast cell precursors and the length of survival of mature mast cells within tissues. Once mast cell precursors target to tissues, their survival may largely be dependent upon the local production of stem cell factor (SCF). Withdrawal of interleukin (IL)-3 results in mast cell apoptosis. The apoptotic changes following IL-3 deprivation are prevented by the addition of SCF which exerts its rescue effect upon interaction with its c-Kit tyrosine kinase receptor. Mast cells undergo apoptosis on withdrawal of IL-3 coincident with a decrease in endogenous bcl-2 mRNA; however, SCF does not induce expression of bcl-2 when added to these cells. When overexpressed, bcl-2 prolongs survival of bcl-2-transfected mast cells following IL-3 deprivation. Transforming growth factor-beta was found to specifically prevent this SCF-mediated rescue from apoptosis, probably by down-regulating the expression of c-Kit. Thus, microenvironmental factors play an important role in regulating mast cell numbers by effecting survival in the periphery.

Mentschel, J. and R. Claus (2003). "Increased butyrate formation in the pig colon by feeding raw potato starch leads to a reduction of colonocyte apoptosis and a shift to the stem cell compartment." Metabolism **52**(11): 1400-5.

Whereas butyrate is well known to induce apoptosis in transformed colon cells in vitro, evidence exists that it inhibits apoptosis of colon crypt cells in vivo. In this study, pigs were fed with resistant potato starch to increase microbial butyrate formation in the colon and to investigate its effects on mitosis and apoptosis. In addition, apoptosis regulating proteins were determined by immunocytochemistry, such as proapoptotic Bak, antiapoptotic Bcl-2, and the epidermal growth factor (EGF), which is synthesized by goblet cells and functions as a survival factor. Two groups of 6 barrows were both supplied with 381 g crude protein and 31 MJ metabolizable energy (ME) daily over a 19-day experimental period. The rations differed in the carbohydrate composition. The controls received gelatinized starch as the main carbohydrate, whereas the experimental group (butyrate group) received a ration with raw potato starch (low ileal digestibility). In the feces, butyrate concentration and pH were monitored daily. After killing the pigs, colon tissue was obtained for histologic and immunocytochemical evaluation, which was performed separately in the luminal, middle, and stem cell compartment of the crypts. In the butyrate group, the total number of apoptotic cells was reduced by 34% (P< or =.001) compared with controls, whereas the mitotic rate was not altered. The crypt depth was only moderately increased by 15%. Apoptosis in the luminal compartment of the butyrate group was reduced by 18.8%, but was increased by 21.7% in the stem cell compartment. The effect of butyrate on apoptosis was paralleled by an increased number of Bcl-2 positive cells mainly in the luminal compartment (butyrate: 2.6 cells; controls: 1.2 cells, P< or =.001), which was more pronounced compared with the number of Bak positive cells in the same compartment. Bak activity in the stem cell compartment was 3.4-fold increased compared with controls (P< or =.001). The size of EGF-positive stained mucus-droplets from the goblet cells was increased in the butyrate group (P< or =.001). We conclude that butyrate inhibits apoptosis of colonocytes in vivo. An excessive proliferation of crypts is counteracted by a shift of the remaining apoptosis towards the stem cell compartment.

Minamino, T., T. Yujiri, et al. (1999). "MEKK1 suppresses oxidative stress-induced apoptosis of embryonic stem cell-derived cardiac myocytes." Proc Natl Acad Sci U S A **96**(26): 15127-32.

A combination of in vitro embryonic stem (ES) cell differentiation and targeted gene disruption has defined complex regulatory events underlying oxidative stress-induced cardiac apoptosis, a model of postischemic reperfusion injury of myocardium. ES cell-derived cardiac myocytes (ESCM) having targeted disruption of the MEKK1 gene were extremely sensitive, relative to wild-type ESCM, to hydrogen peroxide-induced apoptosis. In response to oxidative stress, MEKK1-/- ESCM failed to activate c-Jun kinase (JNK) but did activate p38 kinase similar to that observed in wild-type ESCM. The increased apoptosis was mediated through enhanced tumor necrosis factor alpha production, a response that was positively and negatively regulated by p38 and the MEKK1-JNK pathway, respectively. Thus, MEKK1 functions in the survival of cardiac myocytes by inhibiting the production of a proapoptotic cytokine. MEKK1 regulation of the JNK pathway is a critical

response for the protection against oxidative stress-induced apoptosis in cardiac myocytes.

Muta, K. and S. B. Krantz (1993). "Apoptosis of human erythroid colony-forming cells is decreased by stem cell factor and insulin-like growth factor I as well as erythropoietin." J Cell Physiol **156**(2): 264-71.

To clarify the manner by which erythropoietin (EP), stem cell factor (SCF), or insulin-like growth factor I (IGF-I) regulate erythropoiesis, apoptosis of human erythroid progenitor cells was investigated. Human burst-forming units-erythroid (BFU-E) partially purified from peripheral blood were cultured for 6 days to generate erythroid colony-forming cells (ECFC), which consist mainly of colony-forming units-erythroid (CFU-E). The cells were labeled with [3H]thymidine, incubated in serum-free liquid media, at 37 degrees C, for 16 h, and the pattern of DNA breakdown was analyzed by agarose gel electrophoresis. When these cells were incubated without EP, 70% of the total cellular DNA was broken down into DNA fragments of less than 5 kilobases and nuclear condensation and fragmentation, characteristic of apoptosis, were evident. While EP greatly reduced the amount of DNA breakdown to 23%, SCF and IGF-I each reduced the amount of DNA breakdown to 38-46% and, when added together, to 24%. Dose-response experiments with SCF and IGF-I showed a dose-dependent reduction in DNA fragmentation at concentrations that stimulate colony formation in serum-free semisolid cultures. Finally, assays of ECFC performed by the plasma clot method, after serum-free liquid culture, at 37 degrees C, for 16 h, demonstrated marked protection of erythroid colony-forming capacity by SCF or IGF-I in the absence of EP, as well as by EP itself. These data indicate that human erythroid progenitor cells undergo apoptosis which is reduced by SCF and IGF-I as well as EP and suggest that the control of apoptosis by each of these factors has a prominent role in the regulation of erythropoiesis.

Napolitano, M. A., M. Cipollaro, et al. (2007). "Brg1 chromatin remodeling factor is involved in cell growth arrest, apoptosis and senescence of rat mesenchymal stem cells." J Cell Sci **120**(Pt 16): 2904-11.

Self-renewal, proliferation and differentiation properties of stem cells are controlled by key transcription factors. However, their activity is modulated by chromatin remodeling factors that operate at the highest hierarchical level. Studies on these factors can be especially important to dissect molecular pathways governing the biology of stem cells. SWI/SNF complexes are adenosine triphosphate (ATP)-dependent chromatin remodeling enzymes that have been shown to be required for cell cycle control, apoptosis and cell differentiation in several biological systems. The aim of our research was to investigate the role of these complexes in the biology of mesenchymal stem cells (MSCs). To this end, in MSCs we caused a forced expression of the ATPase subunit of SWI/SNF (Brg1 - also known as Smarca4) by adenoviral transduction. Forced Brg1 expression induced a significant cell cycle arrest of MSCs in culture. This was associated with a huge increase in apoptosis that reached a peak 3 days after transduction. In addition, we observed signs of senescence in cells having ectopic Brg1 expression. At the molecular level these phenomena were associated with activation of Rb- and p53-related pathways. Inhibition of either p53 or Rb with E1A mutated proteins allowed us to hypothesize that both Rb and p53 are indispensable for Brg1-induced senescence, whereas only p53 seems to play a role in triggering programmed cell death. We also looked at the effects of forced Brg1 expression on canonical MSC differentiation in adipocytes, chondrocytes and osteocytes. Brg1 did not induce cell differentiation per se; however, this protein could contribute, at least in part, to the adipocyte differentiation process. In conclusion, our results suggest that whereas some ATP-dependent chromatin remodeling factors, such as ISWI complexes, promote stem cell self-renewal and conservation of an uncommitted state, others cause an escape from 'stemness' and induction of differentiation along with senescence and cell death phenomena.

Nebel, A., E. Schaffitzel, et al. (2006). "Aging at the interface of stem cell renewal, apoptosis, senescence, and cancer." Sci Aging Knowledge Environ **2006**(9): pe14.

The aging-related research field has focused on the detection of genetic factors that affect the aging process, but more recently scientists have started to shift their attention to novel and more integrative ways of studying cellular and organismal function. Such approaches allow them to uncover and explore unexpected patterns and themes, resulting in a more comprehensive knowledge of the complex regulatory pathways and networks involved in aging and age-related diseases. Eventually, this knowledge will lead to a systems-level understanding of aging. The third "Functional Genomics of Aging" conference held in Palermo, Italy, in March/April 2006 highlighted some of the more exciting work in this area.

Nikolova, T., J. Czyz, et al. (2005). "Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells." Faseb J **19**(12): 1686-8.

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

Nishio, M., A. Oda, et al. (2001). "Stem cell factor prevents Fas-mediated apoptosis of human erythroid precursor cells with Src-family kinase dependency." Exp Hematol **29**(1): 19-29.

The Fas ligand (Fas-L) expressed on mature erythroblasts may induce apoptosis of more immature erythroid cells that express Fas, whereas stem cell factor (SCF) may prevent Fas-mediated cell death in hematopoietic progenitor cells. The manner in which SCF prevents Fas-mediated cell death still is unclear. Given the essential role of SCF and the potentially important involvement of the Fas/Fas-L system in the development of erythrocytes, we studied mechanisms related to SCF prevention of Fas-mediated apoptosis. We used primary cultured human erythroid colony-forming cells (ECFC) derived from CD34+ cells and enriched glycophorin A positive (GPA+) c-kit+ cells in ECFC. Apoptosis of ECFC was induced by an Fas-L mimetic monoclonal antibody CH11. DNA fragmentation and the activation of caspase-3 and caspase-8 were measured using commercially available kits. Characterization of expanded cells was performed using multiparameter flow cytometry. Lyn kinase activity was measured by enolase kinase assays. SCF inhibited the CH11-induced DNA fragmentation of ECFC as well as enriched GPA+ c-kit+ cells in ECFC, but not those of GPA+ c-kit- cells. SCF also inhibited the activation of caspase-3 and caspase-8, without downregulation of the surface expression of Fas, suggesting that SCF prevents apoptosis through uncoupling of Fas ligation from subsequent caspase activation. PP2, a specific inhibitor of Src-family kinases, antagonized the effects of SCF in preventing Fas-mediated apoptosis. We propose that SCF prevents Fas-mediated apoptosis of erythroid progenitor cells in a manner dependent on the activity of Src-family tyrosine kinases. We also identified active Lyn in erythroid cells. These data suggest the presence of a novel Src-family-dependent function of SCF in the development of erythrocytes.

Noda, S., A. Kosugi, et al. (1996). "Protection from anti-TCR/CD3-induced apoptosis in immature thymocytes by a signal through thymic shared antigen-1/stem cell antigen-2." J Exp Med **183**(5): 2355-60.

During T cell development in the thymus, the expression of thymic shared antigen-1 (TSA-1)/stem cell antigen-2 (Sca-2), a glycosylphosphatidylinositol (GPI)-anchored differentiation antigen, is developmentally regulated. The expression level of TSA-1 is the highest in most immature CD4- CD8- thymocytes, high in CD4+ CD8+ thymocytes, but barely detectable in mature CD4+ CD8- or CD4- CD8- thymocytes and peripheral T cells. We have previously shown that surface TSA-1 expression in peripheral T cells is induced upon activation and that anti-TSA-1 mAb inhibits the T cell receptor (TCR) signaling pathway in activated T cells. In the present study, we have analyzed a role of TSA-1 in thymic selection events, especially in TCR-mediated apoptosis. In in vitro experiments, anti-TSA-1 blocked anti-CD3-induced cell death of T cell hybridomas. When anti-TSA-1 was injected into newborn mice in vivo together with anti-CD3 epsilon or anti-TCR-beta, TCR/CD3-mediated apoptosis of thymocytes was almost completely blocked. The blockade of apoptosis was defined by the inhibition of, first, the decrease in total number of thymocytes; second, the decrease in percentages of CD4+ CD8+ thymocytes; and third, the induction of DNA fragmentation. However, anti-TSA-1 did not block either steroid- or radiation-induced apoptosis, indicating that a signal via TSA-1 does not inhibit a common pathway of thymocyte apoptosis. Since TCR-mediated apoptosis is pivotal in thymic ontogeny, these results suggest that TSA-

1/Sca-2 is an important cell surface molecule regulating the fate of a developing T cell.

Nomura, S., K. Ishii, et al. (2007). "Role of soluble tumor necrosis factor-related apoptosis-inducing ligand concentrations after stem cell transplantation." Transpl Immunol **18**(2): 115-21.

Although stem cell transplantation (SCT) is being used for hematopoietic reconstitution following high-dose chemotherapy for malignancy, it involves certain serious transplant-related complications such as graft-versus-host disease (GVHD). Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) plays important roles in regulating cell death, immune response, and inflammation. However, the role of soluble TRAIL (sTRAIL) after SCT is poorly understood. In this study, 42 patients underwent SCT; 22 patients received allogeneic SCT, while the remaining 20 received autologous SCT. In these patients, levels of sTRAIL, cytokines, and soluble factors were measured by enzyme-linked immunosorbent assay (ELISA). In addition, a basic study of the generation of endothelial cell-derived microparticle (EDMP) by TNF-alpha and soluble Fas ligand (sFasL) was conducted. sFasL and EDMP exhibited significant elevation in the early phase (2-3 weeks) after SCT. In addition, the elevation of IL-6, TNF-alpha, and sIL-2R after allogeneic SCT was observed. EDMP also exhibited changes similar to sFasL. The patients with high sTRAIL exhibited significant decrease of sFasL and EDMP as compared with those without high sTRAIL. TNF-alpha and sFasL induced an increase in procoagulant and apoptotic markers in endothelial cells, and EDMP shedding was observed. Furthermore, sTRAIL inhibited the EDMP elevation caused by TNF-alpha and sFasL. The apoptotic markers such as sFasL and sTRAIL exhibited particular changes after SCT. Our results suggest that sTRAIL generation after allogeneic SCT relates to the prevention of GVHD.

Oda, A., M. Nishio, et al. (2001). "Stem cell factor regulation of Fas-mediated apoptosis of human erythroid precursor cells." J Hematother Stem Cell Res **10**(5): 595-600.

Multiple cytokines regulate the development of erythrocytes. Increasing attention has been directed to the possible role of Fas and its cognate ligand (Fas-L), a subject of wide interest. Documentation of in vitro data supports the role of Fas and Fas-L in erythropoiesis. Several laboratories, including ours, investigated the opposing actions of erythropoietin (EPO) and stem cell factor (SCF) on Fas-mediated cell death of the erythroid cells. Only circumstantial in vivo evidence has accumulated concerning the issue. There are several reports suggesting that Fas-mediated cell death may have a role in some pathological conditions. Results of the accumulating findings and possible implications in clinical hematology are summarized in this review.

Ohta, H., S. Aizawa, et al. (2003). "Functional analysis of the p53 gene in apoptosis induced by heat stress or loss of stem cell factor signaling in mouse male germ cells." Biol Reprod **68**(6): 2249-54.

Apoptosis plays an important role in controlling germ cell numbers and restricting abnormal cell proliferation during spermatogenesis. The tumor suppressor protein, p53, is highly expressed in the testis, and is known to be involved in apoptosis, which suggests that it is one of the major causes of germ cell loss in the testis. Mice that are c-kit/SCF mutant (Sl/Sld) and cryptorchid show similar testicular phenotypes; they carry undifferentiated spermatogonia and Sertoli cells in their seminiferous tubules. To investigate the role of p53-dependent apoptosis in infertile testes, we transplanted p53-deficient spermatogonia that were labeled with enhanced green fluorescence protein into cryptorchid and Sl/Sld testes. In cryptorchid testes, transplanted p53-deficient spermatogonia differentiated into spermatocytes, but not into haploid spermatids. In contrast, no differentiated germ cells were observed in Sl/Sld mutant testes. These results indicate that the mechanism of germ cell loss in the c-kit/SCF mutant is not dependent on p53, whereas the apoptotic mechanism in the cryptorchid testis is quite different (i.e., although the early stage of differentiation of spermatogonia and the meiotic prophase is dependent on p53-mediated apoptosis, the later stage of spermatids is not).

Orlandi, A., A. Di Lascio, et al. (2008). "Stem cell marker expression and proliferation and apoptosis of vascular smooth muscle cells." Cell Cycle **7**(24): 3889-97.

Vascular endothelial Flt-1 and other stem cell markers are variably expressed in vascular smooth muscle cells (SMCs) during normal and pathological conditions, but their biological role remains uncertain. In normal rat aorta, rare flt-1+ and c-kit+ SMCs were detected. Fifteen days after injury, 61.8 +/- 3.8, 45.7 +/- 3% of the intimal cells resulted flt-1+ and c-kit+ and expressed low level of alpha-smooth muscle actin; CD133+ cells were 5.6 +/- 0.7%. BrDU+/flt-1+ largely predominated in the neointima, whereas BrDU+/CD133+ cells were rare. Forty-five and sixty days after injury, intimal proliferation such as BrDU+ cells was greatly reduced. After sixty days, intimal stem marker expression had almost disappeared whereas alpha-smooth muscle actin was restored. Flk-1 and Oct-4 SMC immunodection was consistently

negative. In vitro, intimal cells obtained fifteen days after injury exhibited an epithelioid phenotype and increased flt-1 and c-kit protein and mRNA and low smooth muscle markers compared to spindle-shaped medial and intimal SMCs obtained after sixty days. Epithelioid clones, independently from layer of origin, were similar in stem cell marker expression. The anti-flt-1 blocking antibody added to epithelioid SMC cultures reduced serum-deprived apoptosis and migration but not PDGF-BB-induced proliferation, and increased cell-populated collagen lattice contraction. In conclusion, vascular SMC stem marker expression was variable, chronologically modulated and prevalent in epithelioid populations and clones; among stem markers, flt-1 expression critically regulates intimal SMC response to microenviromental changes.

Oskeritzian, C. A., Z. Wang, et al. (1999). "Recombinant human (rh)IL-4-mediated apoptosis and recombinant human IL-6-mediated protection of recombinant human stem cell factor-dependent human mast cells derived from cord blood mononuclear cell progenitors." J Immunol **163**(9): 5105-15.

Although stem cell factor (SCF) appears to be the major growth factor for human mast cells, other factors undoubtedly play important roles in the development, survival, and function of these cells. The current study examined the effects of recombinant human (rh) IL-4 and rhIL-6 on rhSCF-dependent development and survival of human mast cells derived in vitro from cord blood progenitor cells. After 4-8 wk of culture with rhSCF and various amounts of rhIL-4, a dramatic decline in mast cell numbers was observed with rhIL-4, the EC50 being about 0.1 ng/ml. Numbers of other cell types remained high. Mast cells derived from cord blood progenitors after 7 wk of culture with rhSCF alone displayed an MCT phenotype and expressed Kit, FcepsilonRI, and IL-4R on their surface. Mast cells examined after purification by immunomagnetic sorting became apoptotic within hours after exposure to rhIL-4, a phenomenon blocked by anti-IL-4 Ab. Because rhIL-4-dependent apoptosis but not the loss of mitochondrial membrane potential was prevented by the pan-caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-(Z-VAD)-fluoromethylketone, mitochondrial perturbation most likely preceded caspase activation. Consistent with this conclusion was the observation that both apoptosis and loss of mitochondrial membrane potential (Deltapsim) were inhibited by cyclosporin A in combination with aristolochic acid. rhIL-6 protected cord blood mast cells from rhIL-4-induced apoptosis. Thus, IL-4 can cause both maturation and apoptosis of human mast cells, the latter effect being abrogated by IL-6.

Pesce, M., M. G. Farrace, et al. (1993). "Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis)." Development **118**(4): 1089-94.

Proliferating primordial germ cells (PGCs) isolated from mouse embryos soon after their arrival in the genital ridges would only survive in vitro at temperature of less than 30 degrees C (De Felici, M. and McLaren, A. (1983). Exp. Cell. Res. 144, 417-427; Wabik-Sliz, B. and McLaren, A. (1984). Exp. Cell. Res. 154, 530-536) or when co-cultured on cell feeder layers (Donovan, P. J., Stott, D., Godin, I., Heasman, J. and Wylie, C. C. (1986). Cell 44, 831-838; De Felici, M. and Dolci, S. (1991). Dev. Biol. 147, 281-284). In the present paper we report that mouse PGC death in vitro occurs with all the hallmarks of programmed cell death or apoptosis. We found that after 4-5 hours in culture many PGCs isolated from 12.5 dpc fetal gonads assumed a nuclear morphology and produced membrane bound fragments (apoptotic bodies) typical of apoptotic cells. In addition, PGCs in culture accumulated high level of tissue transglutaminase (tTGase; an enzyme that is induced and activated during apoptosis) and showed extensive degradation of DNA to oligonucleosomal fragments, which is characteristic of apoptosis. The physiological relevance of this mechanism of PGC death is supported by the finding that some PGCs undergoing apoptosis, as revealed by the high level of tTGase expression, were detected in the embryo. Most importantly, we show that the addition of stem cell factor (SCF) or leukemia inhibitory factor (LIF) to the culture medium, two cytokines known to favour PGC survival and/or proliferation in vitro, markedly reduced the occurrence of apoptosis in PGCs during the first hours in culture.(ABSTRACT TRUNCATED AT 250 WORDS)

Petrucci, M. T., L. De Felice, et al. (1996). "Stem cell factor and PIXY-321 in acute lymphoblastic leukemia: in vitro study on proliferative effects and apoptosis." Cytokines Mol Ther **2**(4): 225-30.

Management of acute lymphoblastic leukemia (ALL) patients may include growth factors (GFs) to reduce post-chemotherapy aplasia. A potential risk of GF administration is a stimulatory signal on the leukemic population. In the present study we investigated the proliferative and programmed cell death (PCD) effect of two cytokines that have recently entered clinical use, stem cell factor (SCF) and the granulocyte colony stimulating factor/IL-3 fusion molecule (PIXY-321), on 14 ALL samples. The activity of IL-7, a cytokine involved in the regulation of ALL cell proliferation, was also

tested alone and in combination with these two cytokines. Using the acridine orange flow cytometric technique and the clonogenic assay, we showed that none of these cytokines was capable of significantly increasing the mean percentage of S-phase cells and CFU-L number. A mean decrease of G0 cells from 60.6% to 52.6% ($p = 0.02$), coupled by a significant increase of G1 cells from 28.2% to 37.9% ($p = 0.003$) was demonstrated in the presence of PIXY-321. IL-7 alone and in combination with either PIXY-321 or SCF induced similar changes in the percentage of cells in G0 and G1. SCF showed no activity on G0 depletion. When each individual samples was analyzed separately, some heterogeneity was observed. An increase of S phase was recorded in a proportion of cases after SCF and PIXY-321 exposure. However, none of the cytokines evaluated by a clonogenic assay following liquid culture was capable of maintaining or promoting self-renewal of leukemic precursors, as determined by plating fresh cells at time 0. Detection of cytokine effects of apoptosis showed that SCF and PIXY-321 did not significantly reduce the mean percentage of cells in PCD, whereas a significant protective effect was observed in the presence of IL-7 ($p = 0.02$). We conclude that PIXY-321 and, to a further extent, SCF fail to induce leukemic lymphoid cell proliferation, and do not protect cells from entering apoptosis. These in vitro findings may be useful for ALL clinical trial design.

Piekorz, R. P., A. Hoffmeyer, et al. (2002). "The centrosomal protein TACC3 is essential for hematopoietic stem cell function and genetically interfaces with p53-regulated apoptosis." Embo J **21**(4): 653-64.

TACC3 is a centrosomal/mitotic spindle-associated protein that is highly expressed in a cell cycle-dependent manner in hematopoietic lineage cells. During embryonic development, TACC3 is expressed in a variety of tissues in addition to the hematopoietic lineages. TACC3 deficiency causes an embryonic lethality at mid- to late gestation involving several lineages of cells. Hematopoietic stem cells, while capable of terminal differentiation, are unable to be expanded in vitro or in vivo in reconstitution approaches. Although gross alterations in centrosome numbers and chromosomal segregation are not observed, TACC3 deficiency is associated with a high rate of apoptosis and expression of the p53 target gene, p21(Waf1/Cip1). Hematopoietic stem cell functions, as well as deficiencies in other cell lineages, can be rescued by combining the TACC3 deficiency with p53 deficiency. The results support the concept that TACC3 is a critical component of the centrosome/mitotic spindle apparatus and its absence triggers p53-mediated apoptosis.

Quinlan, L. R., S. Faherty, et al. (2003). "Phospholipase C and protein kinase C involvement in mouse embryonic stem-cell proliferation and apoptosis." Reproduction **126**(1): 121-31.

Activation of the phosphatidylinositol (PtdIns) signal transduction system involves stimulation of phospholipase C (PLC) by hormones and other agonists to produce two second messengers, the inositol phosphate, Ins(1,4,5)P3 which releases calcium from intracellular stores, and diacylglycerol which activates protein kinase C (PKC). This study, using activators or inhibitors of PLC and PKC and a calcium ionophore, examined the role of the PtdIns system in mouse embryonic stem (ES) cells. The PLC inhibitor, U-73122, inhibited ES-cell proliferation and also inhibited PLC activation as evidenced by a decrease in inositol phosphate formation in response to fetal calf serum stimulation. The two PKC activators, the diacylglycerol analogue 1,2, dioctanoyl-sn-glycerol (DOG) and the phorbol ester 12-O-tetra-decanoyl phorbol 13-acetate (TPA), increased cell proliferation in a dose-dependent manner, as did the calcium ionophore, ionomycin. However, co-stimulation with either ionomycin and DOG or ionomycin and TPA resulted in a reduced number of cells. The PKC inhibitor, bisindolylmaleimide II (Bis II), significantly decreased the number of ES cells, mainly due to increased apoptosis. The possible feedback effect of PKC on PLC was examined by preincubating ES cells with either the PKC inhibitor Bis II or the activator TPA before stimulation of inositol phosphate production with fetal calf serum; preincubation with Bis II increased inositol phosphate formation whereas preincubation with TPA decreased inositol formation. These results indicate that the PtdIns system is involved in the control of ES-cell proliferation and apoptosis.

Ramocki, N. M., H. R. Wilkins, et al. (2008). "Insulin receptor substrate-1 deficiency promotes apoptosis in the putative intestinal crypt stem cell region, limits Apcmin/+ tumors, and regulates Sox9." Endocrinology **149**(1): 261-7.

Reduced apoptosis of crypt stem/progenitor cells and elevated insulin and IGFs are linked to colon cancer risk. Insulin receptor substrate-1 (IRS-1) mediates the actions of insulin, IGF-I, and IGF-II, but the role of endogenous IRS-1 in crypt apoptosis and cancer is undefined. Using IRS-1(-/-), IRS-1(+/-), and IRS-1(+/+) mice, we tested the hypothesis that reduced IRS-1 expression increases apoptosis of intestinal crypt cells and protects against Apc(min/+)

(Min)/beta-catenin-driven intestinal tumors. Expression of Sox9, a transcriptional target of Tcf/beta-catenin and putative biomarker of crypt stem cells, was assessed in intestine of different IRS-1 genotypes and cell lines. Irradiation-induced apoptosis was significantly increased in the crypts and crypt stem cell region of IRS-1-deficient mice. Tumor load was significantly reduced by 31.2 +/- 14.6% in IRS-1(+/-)/Min and by 64.1 +/- 7.6% in IRS-1(-/-)/Min mice, with more prominent reductions in tumor number than size. Compared with IRS-1(+/+)/Min, IRS-1(-/-)/Min mice had fewer Sox9-positive cells in intestinal crypts and reduced Sox9 mRNA in intestine. IRS-1 overexpression increased Sox9 expression in an intestinal epithelial cell line. We conclude that even small reductions in endogenous IRS-1 increase apoptosis of crypt stem or progenitor cells, protect against beta-catenin-driven intestinal tumors, and reduce Sox9, a Tcf/beta-catenin target and putative stem/progenitor cell biomarker.

Re, R. N. and J. L. Cook (2009). "Senescence, apoptosis, and stem cell biology: the rationale for an expanded view of intracrine action." Am J Physiol Heart Circ Physiol **297**(3): H893-901.

Some extracellular-signaling peptides also at times function within the intracellular space. We have termed these peptides intracrines and have argued that intracrine function is associated with a wide variety of peptides/proteins including hormones, growth factors, cytokines, enzymes, and DNA-binding proteins among others. Here we consider the possibility that intracrines participate in the related phenomena of senescence, apoptosis, and stem cell regulation of tissue biology. Based on this analysis, we also suggest that the concept of intracrine action be expanded to include possible regulatory peptide transfer via exosomes/microvesicles and possibly by nanotubes. Moreover, the process of microvesicular and nanotube transfer of peptides and other biologically relevant molecules, which we inclusively term laterality, is explored. These notions have potentially important therapeutic implications, including implications for the therapy of cardiovascular disease.

Reipert, S., G. Bennion, et al. (1999). "Nucleolar segregation during apoptosis of haemopoietic stem cell line FDCP-Mix." Cell Death Differ **6**(4): 334-41.

The programmed elimination of cells during apoptosis is distinct from necrosis both morphologically and biochemically. Currently, the morphological description of apoptosis discriminates between the segregation of the nucleolus and the so called 'chromatin condensation'. The latter originates from observations of electron dense material adjacent to the nuclear envelope of apoptotic nuclei. Although there is ample evidence for an involvement of DNA in electron dense marginations, their true nature is still unknown. By studying apoptosis in FDCP-Mix, a pluripotent murine haemopoietic stem cell line, we found morphological and histochemical evidence that electron dense material at the nuclear envelope (NE) has emerged as a result of the segregation of nucleoli in association with the nuclear membrane. The remaining electron dense and homogenous bulk of the nucleolus labels for RNAse-gold, but even more intensely for DNAse-gold, and therefore could possibly be mistaken as 'condensed chromatin' in the light microscope. The labelling of the electron dense material for DNase-gold in FDCP-Mix could be explained by a migration of DNA into the bulk of the nucleoli at an early stage of cell death.

Richardson, S. J., G. F. Lemkine, et al. (2007). "Cell division and apoptosis in the adult neural stem cell niche are differentially affected in transthyretin null mice." Neurosci Lett **421**(3): 234-8.

Thyroid hormones (THs) are fundamental in regulation of growth and development, particularly of the brain. THs are required for full proliferative activity of neural stem cells in the subventricular zone (SVZ) of adult mouse brains, and also affect the normal fate of progenitor cells: apoptosis. Transthyretin (TTR) is a TH distributor protein in the blood and cerebrospinal fluid. TTR secretion by the choroid plexus is involved in transport of THs from blood into cerebrospinal fluid. We investigated the regulation of neural stem cell cycle in the SVZ of adult TTR null mice. Markers for neural stem cell mitosis that are reduced during hypothyroidism, did not differ between genotypes. However, in TTR null mice the level of apoptosis, the fate of most progenitor cells, was as low as that in brains of hypothyroid wildtype mice. Thus, lack of TTR results in reduced availability of TH to progenitor cells in the SVZ. We show that proliferation and apoptosis in the SVZ neural stem cell niche are differentially affected by the lack of TTR synthesis.

Sellge, G., A. Lorentz, et al. (2004). "Human intestinal fibroblasts prevent apoptosis in human intestinal mast cells by a mechanism independent of stem cell factor, IL-3, IL-4, and nerve growth factor." J Immunol **172**(1): 260-7.

In rodents, fibroblasts (FBs) mediate stem cell factor (SCF)-dependent growth of mast cells (MCs). In humans, SCF is mandatory for MC differentiation and survival. Other factors such as IL-3, IL-4, and nerve growth factor (NGF) act in synergism with SCF, thus enhancing proliferation and/or preventing apoptosis in MCs. In this study, we studied in vitro interactions between human MCs and

human FBs, both isolated from the intestine and purified to homogeneity. In coculture with FBs, MCs survived for up to 3 wk, whereas purified MCs cultured alone died within a few days. TNF-alpha and IL-1beta, which both did not affect MC survival directly, enhanced FB-dependent MC growth. We provide evidence that FB-derived MC growth factors are soluble, heat-sensitive molecules which down-regulate MC apoptosis without enhancing MC proliferation. However, only low amounts of SCF were measured in FB-conditioned medium (<0.2 ng/ml). Moreover, blocking of SCF/c-kit interaction by anti-SCF or anti-c-kit Abs and neutralization of IL-3, IL-4, and NGF did not affect MC survival in the coculture system. In conclusion, our data indicate that human FBs promote survival of human MCs by mechanisms independent of SCF, IL-3, IL-4, and NGF. Such interactions between MCs and FBs may explain why MCs accumulate at sites of inflammatory bowel disease and intestinal fibrosis.

Shimozato, O., J. R. Ortaldo, et al. (2002). "Impaired NK cell development in an IFN-gamma transgenic mouse: aberrantly expressed IFN-gamma enhances hematopoietic stem cell apoptosis and affects NK cell differentiation." J Immunol **168**(4): 1746-52.

Aberrant expression of IFN-gamma has been demonstrated to cause a wide variety of alterations in cell function and development. Previously we reported that constitutive expression of IFN-gamma in bone marrow (BM) and thymus results in a total absence of B cells and a substantial decrease in the number of hematopoietic progenitor cells. In this study, we demonstrate a severe deficiency of NK1.1(+)CD3(-) cells in this transgenic mouse model. Compared with normal control littermates, we found a pronounced reduction of NK cells in IFN-gamma transgenic mouse spleen and liver despite maintenance of normal function. In addition, we observed a reduced number of BM cells in the IFN-gamma transgenic mouse despite normal expression of hematopoietic growth factors in the BM. Interestingly, these cells were less responsive to stem cell factor (SCF) despite c-kit expression on hematopoietic stem cells (HSCs). We observed that addition of exogenous IFN-gamma inhibited proliferation of HSCs and differentiation of NK precursors from HSCs in normal mice in response to SCF, IL-7, fms-like tyrosine kinase 3 ligand, and IL-15. Furthermore, we found that HSCs express the IFN-gammaRalpha subunit and undergo apoptosis in response to exogenous IFN-gamma. Thus, we have demonstrated the occurrence of a severe deficiency of NK cells and lower numbers of BM cells in an IFN-gamma transgenic mouse model. Furthermore, because exogenous IFN-gamma affects the responsiveness to hematopoietic growth factors such as SCF in vitro, our results indicate that chronic expression of IFN-gamma in vivo leads to widespread immune system defects, including alterations in NK cell differentiation.

Singh, R. K., M. L. Varney, et al. (1999). "Fas-FasL-mediated CD4+ T-cell apoptosis following stem cell transplantation." Cancer Res **59**(13): 3107-11.

We report the preferential expression of Fas on CD4+ T cells and Fas ligand (FasL) on monocytes and their potential role in the selective loss of CD4+ T cells in breast cancer patients undergoing high-dose chemotherapy and peripheral blood stem cell transplantation (PSCT). A high frequency of apoptotic CD4+ T cells (28-51%) is observed during the first 100 days after PSCT concomitant with a significant increase in monocyte frequency and FasL expression (11.6-23%) on monocytes. The preferential expression of Fas on CD4+ T cells (73-92%) in the peripheral blood (PB) of these patients is associated with a significantly higher frequency of CD4+ T-cell apoptosis compared with CD8+ T cells (28-47%) and CD4+ T cells (46 +/- 5.7%) in normal PB. These data suggest that "primed" Fas+ CD4+ lymphocytes interact with activated monocytes that express FasL, resulting in apoptosis, leading to deletion of CD4+ T cells, an inversion in the CD4:CD8 T-cell ratio, and immune dysfunction. The prevention of CD4+ T-cell apoptosis and improved immune reconstitution by the manipulation of PB stem cell products, blockade of Fas-FasL interactions, or cytokine support after transplantation may be important adjuvant immunotherapeutic strategies in patients undergoing high-dose chemotherapy and PSCT.

Slinskey, A., D. Barnes, et al. (1999). "Simian virus 40 large T antigen J domain and Rb-binding motif are sufficient to block apoptosis induced by growth factor withdrawal in a neural stem cell line." J Virol **73**(8): 6791-9.

Serum-free mouse embryo (SFME) cells are a neural stem cell line that is dependent upon epidermal growth factor (EGF) for survival. Removal of EGF results in the G1 arrest and apoptosis of SFME cells. We have shown that the expression of simian virus 40 large T antigen in SFME cells blocks apoptosis and allows cell survival and division in the absence of EGF. Therefore the presence of T antigen abrogates the EGF requirement. The steady-state levels of p53, p21, and mdm-2 do not increase as SFME cells undergo apoptosis upon EGF withdrawal. Furthermore, the amino-terminal 136 amino acids (N136) of T antigen are sufficient to block death and to promote proliferation in the absence of EGF, while the carboxy-terminal fragment (C251-708), which

contains the p53 binding site, is unable to block death. Taken together, these data suggest that SFME cells deprived of EGF undergo p53-independent apoptosis. Mutations that disrupt either the J domain or Rb family binding abolish the ability of T antigen to block SFME cell apoptosis and to promote cell growth. We conclude that T antigen must act on one or more members of the Rb family to inhibit SFME cell apoptosis.

Su, L., B. Zhao, et al. (2007). "Safrole oxide is a useful tool for investigating the effect of apoptosis in vascular endothelial cells on neural stem cell survival and differentiation in vitro." Bioorg Med Chem Lett **17**(11): 3167-71.

Previously, we found safrole oxide could promote VEC apoptosis, however, it is not known whether it can induce NSC apoptosis. It is reported that neural stem cells (NSCs) are localized in a vascular niche. But the effects of apoptosis in vascular endothelial cells (VEC) on NSC growth and differentiation are not clear. To answer these questions, in this study, we co-cultured NSCs with VECs in order to imitate the situation in vivo, in which NSCs are associated with the endothelium, and treated the single-cultured NSCs and the co-cultured NSCs with safrole oxide. The results showed that safrole oxide (10-100 microg/mL) had no effects on NSC growth. Based on these results, we treated the co-culture system with this small molecule. The results showed that the NSCs differentiation, into neurons and gliacytes was induced by VECs untreated with safrole oxide. But in the co-culture system treated with safrole oxide, the NSCs underwent apoptosis. The data suggested that when VEC apoptosis occurred in the co-culture system, the NSC survival and differentiation could not be maintained, and NSCs died by apoptosis. Our finding provided a useful tool for investigating the effect of apoptosis in vascular endothelial cells on neural stem cell survival and differentiation in vitro.

Sugiyama, N., M. Obinata, et al. (2001). "Bcl-2 inhibits apoptosis of spermatogonia and growth of spermatogonial stem cells in a cell-intrinsic manner." Mol Reprod Dev **58**(1): 30-8.

The growth, differentiation, and death/survival of spermatogonia are precisely regulated for the proper production of spermatozoa. We have previously shown that Bcl-2 ectopically expressed in spermatogonia caused the inhibition of normal spermatogonial apoptosis and the subsequent failure of differentiation in transgenic mice. In addition, the growth of spermatogonial stem cells seemed to be temporally arrested in the transgenic mice. In the present study, we attempted to examine whether the abnormality of spermatogonia described above was caused by Bcl-2 misexpression in the spermatogonia or by an abnormal spermatogenic environment of the transgenic mice. We transplanted testicular cells of transgenic mice to seminiferous tubules of W/Wv mice in which transplanted normal testicular cells can undergo spermatogenesis. We found that the transplanted spermatogonia of the transgenic mice reproduced a series of abnormal changes including temporal growth arrest of spermatogonial stem cells and abnormal accumulation of spermatogonia in tubules, which were also observed in the testes of the transgenic mice. The results indicated that Bcl-2 inhibited apoptosis of spermatogonia and growth of spermatogonial stem cells in a cell-intrinsic manner. We also cultured testicular cells of transgenic mice and found that the spermatogonia of the transgenic mice were better able to survive than were those of wild-type mice but that their differentiation was not affected. The result suggested that failure of differentiation of the accumulated spermatogonia in the transgenic testes is not due to the abnormality of the bcl-2 misexpressing spermatogonia, but may be caused by extrinsic problems including improper interaction of spermatogonia with supporting cells.

Sui, X., S. B. Krantz, et al. (2000). "Stem cell factor and erythropoietin inhibit apoptosis of human erythroid progenitor cells through different signalling pathways." Br J Haematol **110**(1): 63-70.

Erythropoietin (EPO) and stem cell factor (SCF) are two important factors in human erythropoiesis. We have recently demonstrated that SCF and EPO synergistically activate mitogen-activated protein (MAP) kinase, thereby promoting growth of human erythroid colony-forming cells (ECFCs). In the present study, we have examined the intracellular mechanisms by which SCF and EPO maintain survival of these cells. In the absence of SCF and EPO, human ECFCs underwent rapid apoptosis. The process was significantly inhibited by addition of a single factor and was totally prevented in the presence of both factors. Treatment of ECFCs with wortmannin, a specific inhibitor of phosphoinositide 3-kinase (PI3K), inhibited the antiapoptotic effect of SCF but had no effect on that of EPO, indicating that SCF but not EPO inhibits apoptosis through the PI3K pathway. In contrast, treatment of ECFCs with PD98059, a specific inhibitor of MAP kinase/ERK kinase (MEK), inhibited cell growth but had no effect on the antiapoptotic activity of either SCF or EPO, suggesting that SCF and EPO prevent apoptosis of human ECFCs independent of the extracellular signal-regulated kinase (ERK) pathway. Interestingly, both EPO and SCF induced activation of PI3K. However,

through PI3K, SCF caused activation of protein kinase B (PKB), an anti-apoptosis signal, whereas EPO led to activation of ERKs. Furthermore, the SCF- and EPO-maintained expression of antiapoptotic protein Bcl-XL was correlated with the activation of ERKs and was inhibited by PD98059, suggesting that Bcl-XL may not have a major role in preventing apoptosis of human ECFCs. Phosphorylated BAD was not affected by SCF, EPO or wortmannin. Taken together with our previous results, the present study indicates that SCF and EPO support survival and growth of human ECFCs through different signalling pathways and that they transduce distinctly different signals through activation of PI3K.

Suzuki, R., T. S. Li, et al. (2007). "The reduction of hemodynamic loading assists self-regeneration of the injured heart by increasing cell proliferation, inhibiting cell apoptosis, and inducing stem-cell recruitment." J Thorac Cardiovasc Surg **133**(4): 1051-8.

OBJECTIVES: Mitotic cardiomyocytes and cardiac stem cells have been identified recently in adult hearts, and both have been found to be increased in acute infarcted myocardium. Although these findings suggest potential self-repair of the heart after injury, obvious self-regeneration of the injured heart has never been observed clinically. We hypothesized that hemodynamic loading impairs myocardial repair. METHODS: Myocardial infarction was induced in C57BL/6 mice by ligating the left anterior descending artery. After 60 minutes, either the infarcted heart was transplanted heterotopically into a healthy recipient C57BL/6 mouse to remove the ventricular hemodynamic loading (unloading group) or it was left as an infarcted heart under normal hemodynamic loading conditions in the same mouse (loading group). The infarcted hearts were dissected for histologic analysis after 3, 7, 14, and 28 days. RESULTS: Histologic analysis showed that the wall thickness of the infarcted left ventricle was significantly greater and the area of infarction was significantly smaller in the unloading group than in the loading group. Immunostaining analysis revealed significantly more Ki-67-positive cells and significantly fewer apoptotic cells in the infarcted myocardium in the unloading group than in the loading group. There were also significantly more c-kit- and Sca-1-positive stem cells in the infarcted myocardium in the unloading group than in the loading group. CONCLUSION: Our findings suggest that hemodynamic unloading assists self-regeneration of the injured heart by increasing cell proliferation, inhibiting cell apoptosis, and inducing stem-cell recruitment.

Tang, J., J. Wang, et al. (2008). "Adenovirus-mediated stromal cell-derived- factor-1alpha gene transfer induces cardiac preservation after infarction via angiogenesis of CD133+ stem cells and anti-apoptosis." Interact Cardiovasc Thorac Surg **7**(5): 767-70.

In our study, we found cardiocytes expressed CXCR4, and the number of cardiocytes apoptosis with SDF-1 treatment decreased obviously through SDF-1 induced the up-regulation of phosphorylated Akt. On day 7 after myocardial infarction, marked expression of SDF-1alpha, and the number of CD133(+) cells was the highest in the AdV-SDF-1 injection hearts. On day 28 post-treatment, blood vessel density in the AdV. SDF-1 group was higher in infracted zones. Infarct size and collagen accumulation in the infracted area decreased significantly, thickness of LV wall, vessels and cardiocytes' density increased obviously in the AdV-SDF-1 group than in control or Adv-LacZ group, and hemodynamics showed the improvement of left ventricle heart function in the AdV.SDF-1 group. Therefore, SDF-1alpha could improve cardiac structure and function through the combined effects of angiogenesis and anti-apoptosis.

Timeus, F., N. Crescenzio, et al. (1997). "Stem cell factor suppresses apoptosis in neuroblastoma cell lines." Exp Hematol **25**(12): 1253-60.

Stem cell factor (SCF) is a glycoprotein growth factor produced by marrow stromal cells that acts after binding to its specific surface receptor, which is the protein encoded by the protooncogene c-kit. SCF synergizes with specific lineage factors in promoting the proliferation of primitive hematopoietic progenitors, and has been administered to expand the pool of these progenitors in cancer patients treated with high-dose chemotherapy. SCF and its c-kit receptor are expressed by some tumor cells, including myeloid leukemia, breast carcinoma, small cell lung carcinoma, melanoma, gynecological tumors, and testicular germ cell tumors. Previous studies of SCF in neuroblastoma have produced conflicting conclusions. To explore the role of SCF in neuroblastoma, we studied five neuroblastoma lines (IMR-5, SK-N-SH, SK-N-BE, AF8, and SJ-N-KP) and the neuroepithelioma line CHP-100. All lines expressed mRNA for c-kit and c-kit protein at low intensity as measured by flow cytometry, and secreted SCF in medium culture as shown by ELISA. Exogenous SCF did not modify 3H thymidine uptake in the neuroblastoma and neuroepithelioma cell lines. After 6 days' culture in the presence of anti-c-kit, the number of viable neuroblastoma cells was significantly lower than the control, and terminal deoxynucleotidyl transferase assay showed a substantial increase of apoptotic cells: The percentage

of positive cells was 1-3% in the control lines, whereas in the presence of anti c-kit it varied from 29% of SK-N-BE to 92% of CHP-100. After 9 days' culture in the presence of anti-c-kit, no viable cells were detectable. These data indicate that SCF is produced by some neuroblastoma cell lines via an autocrine loop to protect them from apoptosis.

Tsiftsoglou, A. S., I. D. Bonovolias, et al. (2009). "Multilevel targeting of hematopoietic stem cell self-renewal, differentiation and apoptosis for leukemia therapy." Pharmacol Ther **122**(3): 264-80.

Human leukemias are considered clonal hematological malignancies initiated by chromosomal aberrations or epigenetic alterations occurring at the level of either pluripotent hematopoietic stem cells (HSCs) or early multipotent progenitors (MPPs). Leukemic cells are transformed, immortalized, actively proliferating cells that are still able to differentiate into cells resembling mature blood cells. Future therapies of leukemias require identification of molecular targets involved in hematopoiesis under normal and leukemic conditions and detailed understanding of the interactions between normal hematopoietic and leukemic cells within the bone marrow micro-environment. This review presents the basic aspects of hematopoiesis and highlights multilevel exploitable targets for leukemia therapy. These include HSC niche components, signaling pathways (SCF/c-kit-R, EPO-R-JAK2/STAT, Wnt, Notch, HOX), inducer-receptor interactions, superfine chromatin structure modifications, fused transcription factors, microRNAs and signaling of cell death through the Bcl-2 apoptotic switch (BH3-only proteins). The classes of therapeutics developed or being under development to eradicate human leukemias include novel antimetabolites, DNA hypomethylating agents, histone deacetylation inhibitors (HDACIs), retinoids and other inducers of differentiation, targeted monoclonal antibodies raised against cell surface proteins, pro-apoptotic receptor agonists (PARAs), BH3 peptidomimetics, cell cycle inhibitors, siRNAs and perhaps microRNAs. Some of these agents induce terminal differentiation while others promote cell cycle arrest and apoptosis in leukemia cells. At last but not least, this article describes the mechanisms of removal of damaged/harmful cells from organs since impairment in clearance of such cells can lead to autoimmune disorders by self-antigens.

Viswanathan, S., T. Benatar, et al. (2003). "Supplementation-dependent differences in the rates of embryonic stem cell self-renewal, differentiation, and apoptosis." Biotechnol Bioeng **84**(5): 505-17.

Although it is known that leukemia inhibitory factor (LIF) supports the derivation and expansion of murine embryonic stem (ES) cells, it is unclear whether this is due to inhibitory effects of LIF on ES cell differentiation or stimulatory effects on ES cell survival and proliferation. Using an ES cell line transgenic for green fluorescent protein (GFP) expression under control of the Oct4 promoter, we were able to simultaneously track the responses of live Oct4-GFP-positive (ES) and -negative (differentiated) fractions to LIF, serum, and other growth factors. Our findings show that, in addition to inhibiting differentiation of undifferentiated cells, the administration of LIF resulted in a distinct dose-dependent survival and proliferation advantage, thus enabling the long-term propagation of undifferentiated cells. Competitive responses from the differentiated cell fraction could only be elicited upon addition of serum, fibroblast growth factor-4 (FGF-4), or insulin-like growth factor-1 (IGF-1). The growth factors did not induce additional differentiation of ES cells, but rather they significantly improved the proliferation of already differentiated cells. Our analyses show that, by adjusting culture conditions, including the type and amount of growth factors or cytokines present, the frequency of media exchange, and the presence or absence of serum, we could selectively and specifically alter the survival, proliferation, and differentiation dynamics of the two subpopulations, and thus effectively control population outputs. Our findings therefore have important applications in engineering stem cell culture systems to predictably generate desired stem cells or their derivatives for various regenerative therapies.

Wang, C. H., S. Verma, et al. (2007). "Stem cell factor attenuates vascular smooth muscle apoptosis and increases intimal hyperplasia after vascular injury." Arterioscler Thromb Vasc Biol **27**(3): 540-7.

OBJECTIVE: Stem cell factor (SCF) through its cognate receptor, the tyrosine kinase c-kit, promotes survival and biological functions of hematopoietic stem cells and progenitors. However, whether SCF/c-kit interactions exacerbate intimal hyperplasia through attenuating VSMC apoptosis induced by vascular injury has not been thoroughly investigated. METHODS AND RESULTS: VSMCs were stimulated with serum deprivation and H2O2 to induce apoptosis. The transcription of c-kit mRNA and the expression of the c-kit protein by VSMCs were estimated by Q-polymerase chain reaction and Western blotting, respectively. The interactions of SCF and c-kit were investigated by in vitro and in vivo experiments. In vitro, H2O2 stimulation significantly induced apoptosis of VSMCs as evidenced by the 3- and 3.2-fold increases of cleaved

caspase-3 compared with those in the control group by Western blot and flow cytometric analyses, respectively (P<0.01). Stimulation of apoptosis also caused 3.5- and 9-fold increases in c-kit mRNA transcription and protein expression, respectively, by VSMCs compared with those in the control group. Administration of SCF (10 to 1000 ng/mL) significantly lowered the amount of cleaved caspase-3 in H2O2-treated VSMCs (P<0.01). Specifically, SCF exerted this effect through activating Akt, followed by increasing Bcl-2 and then inhibiting the release of cytochrome-c from the mitochondria to the cytosol. In vivo, the mouse femoral artery was injured with a wire in SCF mutant (Sl/Sl(d)), c-kit mutant (W/W(v)), and colony control mice. In colony control mice, confocal microscopy demonstrated that the wire-injury generated a remarkable activation of caspase-3 on medial VSMCs, coinciding with upregulation of c-kit expression. The wire-injury also caused an increase in the expression of SCF on surviving medial VSMCs and cells in the adventitia. The upregulated c-kit expression in the vessel wall also facilitated homing by circulating SCF+ cells. Compared with colony control mice, vascular injury in SCF mutant and c-kit mutant mice caused a higher number of apoptotic VSMCs on day 14 and a lower number of proliferating cells, and resulted in significantly less neointimal formation (P<0.01) on day 28. CONCLUSIONS: The interactions between SCF and the c-kit receptor play an important role in protecting VSMCs against apoptosis and in maintaining intimal hyperplasia after vascular injury.

Yamasaki, K., T. Setoguchi, et al. (2009). "Stem cell factor prevents neuronal cell apoptosis after acute spinal cord injury." Spine (Phila Pa 1976) **34**(4): 323-7.

STUDY DESIGN: A rat spinal cord injury (SCI) model and immunohistochemistry were used to examine the levels of expression of stem cell factor and c-kit. In addition, we examined whether intraperitoneal administration of stem cell factor could prevent neural cells apoptosis after acute SCI. OBJECTIVE: To evaluate the antiapoptotic effect of stem cell factor after SCI. SUMMARY OF BACKGROUND DATA: It is well known that the mode of delayed neuronal and glial cell death after SCI is apoptosis. Inhibition of apoptosis might thus promote neurologic improvement after SCI. Stem cell factor and its receptor c-kit exhibit pleiotropic effects in early hematopoiesis, and are also known to prevent hematopoietic progenitor cell apoptosis. Stem cell factor has recently been reported to be a survival factor for neural stem cells in vitro. We examined the levels of expression of stem cell factor and c-kit in normal and injured rat spinal cord. In addition, we examined whether stem cell factor prevents neural cell apoptosis after acute SCI. METHODS: We examined the expression of stem cell factor and c-kit in spinal cord after injury by quantitative RT-PCR and immunohistochemistry. Antiapoptotic effects of stem cell factor were examined using rats with SCI that received stem cell factor intraperitoneally, and were examined immunohistochemically with anticleaved PARP antibody and antiactive caspase-3 antibody between 1 and 3 days after injury. RESULTS: Upregulation of stem cell factor and c-kit expression occured after SCI. We also found that neurons express stem cell factor, and neurons and oligodendrocytes express c-kit after SCI. In addition, intraperitoneal administration of stem cell factor prevented spinal neural cells apoptosis after injury. CONCLUSION: These findings suggest the possibility that stem cell factor, a hematopoietic cytokine, could be useful as an agent for treatment of SCI.

Yan, W., J. Suominen, et al. (2000). "Involvement of Bcl-2 family proteins in germ cell apoptosis during testicular development in the rat and pro-survival effect of stem cell factor on germ cells in vitro." Mol Cell Endocrinol **165**(1-2): 115-29.

A large part of germ cells die apoptotically during testicular development in rodents. In the present study, a wave of germ cell apoptosis was observed between days 10 and 30 of postnatal life by in situ 3'-end labeling and DNA fragmentation analysis. To explore the potential involvement of Bcl-2 family members in this process, the expression and localization of some Bcl-2 family proteins (Bcl-2, Bcl-xL, Bcl-w, Bak, Bax, and Bad) and p53 were analyzed during testicular development in the rat by Western blotting and immunohistochemistry. The dynamic changes in the expression profiles of Bcl-2 family proteins are consistent with a model in which germ cells are primed for apoptosis during the first cycle of spermatogenesis by de novo expression of the death effectors Bax and Bad in a p53-dependent manner and these proteins are prevented from triggering further apoptosis after the first spermatogenic cycle has been set up by anti-apoptotic Bcl-2 family proteins Bcl-xL and Bcl-w. To examine whether the pro-survival effect of stem cell factor (SCF) on germ cells in vitro is mediated by Bcl-2 family proteins, the correlation between the pro-survival effect of SCF on germ cells and the expression of the above-mentioned apoptosis-related gene products in the seminiferous tubules at stage XII of the epithelial cycle were also investigated using a tubular culture system. The data suggest that SCF supports germ cell survival during spermatogenesis by up-regulating pro-survival Bcl-2 family proteins, Bcl-

w and Bcl-xL, and down-regulating pro-apoptosis Bcl-2 family proteins, e.g. Bax.

Yan, W., J. Suominen, et al. (2000). "Stem cell factor protects germ cells from apoptosis in vitro." J Cell Sci **113 (Pt 1)**: 161-8.

Stem cell factor (SCF) plays an important role in migration, adhesion, proliferation, and survival of primordial germ cells and spermatogonia during testicular development. However, the function of SCF in the adult testis is poorly described. We have previously shown that, in the presence of SCF, there were more type A spermatogonia incorporating thymidine at stage XII of rat seminiferous tubules cultured in vitro than in the absence of SCF, implying that the increased DNA synthesis might result from enhanced survival of spermatogonia. To explore the potential pro-survival function of SCF during spermatogenesis, the seminiferous tubules from stage XII were cultured in the presence or absence of SCF (100 ng/ml) for 8, 24, 48, and 72 hours, respectively, and apoptosis was analyzed by DNA laddering and in situ 3'-end labeling (ISEL) staining. Surprisingly, not only spermatogonia, but also spermatocytes and spermatids, were protected from apoptosis in the presence of SCF. Apoptosis took place much later and was less severe in the SCF-treated tubules than in the controls. Based on previous studies showing that FSH prevents germ cells from undergoing apoptosis in vitro, and that SCF level is increased dramatically in response to FSH stimulation, we also tested if the pro-survival effect of FSH is mediated through SCF by using a function-blocking monoclonal antibody, ACK-2, to block SCF/c-kit interaction. After 24 hours of blockade, the protective effect of FSH was partially abolished, as manifested by DNA laddering and ISEL analyses. The present study demonstrates that SCF acts as an important survival factor for germ cells in the adult rat testis and FSH pro-survival effect on germ cells is mediated partially through the SCF/c-kit pathway.

Yu, J., N. F. Huang, et al. (2009). "nAChRs mediate human embryonic stem cell-derived endothelial cells: proliferation, apoptosis, and angiogenesis." PLoS One **4**(9): e7040.

BACKGROUND: Many patients with ischemic heart disease have cardiovascular risk factors such as cigarette smoking. We tested the effect of nicotine (a key component of cigarette smoking) on the therapeutic effects of human embryonic stem cell-derived endothelial cells (hESC-ECs). METHODS AND RESULTS: To induce endothelial cell differentiation, undifferentiated hESCs (H9 line) underwent 4-day floating EB formation and 8-day outgrowth differentiation in EGM-2 media. After 12 days, CD31(+) cells (13.7+/-2.5%) were sorted by FACScan and maintained in EGM-2 media for further differentiation. After isolation, these hESC-ECs expressed endothelial specific markers such as vWF (96.3+/-1.4%), CD31 (97.2+/-2.5%), and VE-cadherin (93.7+/-2.8%), form vascular-like channels, and incorporated DiI-labeled acetylated low-density lipoprotein (DiI-Ac-LDL). Afterward, 5x10(6) hESC-ECs treated for 24 hours with nicotine (10(-8) M) or PBS (as control) were injected into the hearts of mice undergoing LAD ligation followed by administration for two weeks of vehicle or nicotine (100 microg/ml) in the drinking water. Surprisingly, bioluminescence imaging (BLI) showed significant improvement in the survival of transplanted hESC-ECs in the nicotine treated group at 6 weeks. Postmortem analysis confirmed increased presence of small capillaries in the infarcted zones. Finally, in vitro mechanistic analysis suggests activation of the MAPK and Akt pathways following activation of nicotinic acetylcholine receptors (nAChRs). CONCLUSIONS: This study shows for the first time that short-term systemic administrations of low dose nicotine can improve the survival of transplanted hESC-ECs, and enhance their angiogenic effects in vivo. Furthermore, activation of nAChRs has anti-apoptotic, angiogenic, and proliferative effects through MAPK and Akt signaling pathways.

Zha, Y. H., Y. W. Mei, et al. (2007). "[The advantages for Snail expression to promote cell migration and induce actin reorganization and to protect against the serum-deprivation-triggered apoptosis of bone marrow stem cells]." Sheng Wu Gong Cheng Xue Bao **23**(4): 645-51.

The Snail transcription factor has been described as a strong repressor of E-cadherin and its stable expression induces epithelial-mesenchymal transitions responsible for the acquisition of motile and invasive properties during tumor progression. A fascinating analogy that has been raised is the seemingly similar and shared characteristics of stem cells and tumorigenic cells, which prompted us to investigate whether the mechanisms of the acquisition of invasiveness during tumor progression are also involved in bone marrow stem cells (MSCs). In this study, we examined whether Snail gene expression acts in the mobility, cytoskeleton and anti-apoptosis of MSCs. Cell Transmigration Assay and Western Blotting were performed to evaluate the cell migratory capability and the related Signaling pathways in MSCs transfected with the Snail expression vector of pCAGGSneo-SnailHA (MSCs-Sna), compared with MSCs(MSCs-neo) transducted with the control vector(pCAGGSneo). Actin cytoskeleton by Immunofluorescence and Sub-G1 detection by a

FACScan flow cytometer were performed to analyze the cytoskeleton and antiapoptotic capability of MSCs-Sna. Compared with MSCs-neo, MSCs-Sna show significantly more migration in the transwell migration system ($P < 0.05$). And suppression of PI-3K activation by the specific PI-3K inhibitor, Wortmannin, brought on a reduction in Snail-mediated MSCs migration. In addition, we provide evidences that high expression of Snail inhibited the serum-deprivation triggered apoptosis and cytoskeleton changement of MSCs. These data suggest the possibility of facilitating MSCs migration to injured tissue and subsequent survival and maintenance in the local microenvironment after their transplantation, by investigating and increasing the advantage factors such as Snail high expression in MSCs.

Zhang, P., J. Li, et al. (2009). "Human neural stem cell transplantation attenuates apoptosis and improves neurological functions after cerebral ischemia in rats." Acta Anaesthesiol Scand **53**(9): 1184-91.

BACKGROUND: Neuroprotection is a major therapeutic approach for ischemic brain injury. We investigated the neuroprotective effects induced by transplantation of human embryonic neural stem cells (NSCs) into the cortical penumbra 24 h after focal cerebral ischemia. METHODS: NSCs were prepared from human embryonic brains obtained at 8 weeks of gestation. Focal cerebral ischemia was induced in adult rats by permanent occlusion of the middle cerebral artery. Animals were randomly divided into two groups: NSCs-grafted group and medium-grafted group (control). Infarct size was assessed 28 days after transplantation by hematoxylin and eosin staining. Neurological severity scores were evaluated before ischemia and at 1, 7, 14, and 28 days after transplantation. The terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay and immunohistochemical analysis of Bcl-2 and Bax were performed at 7, 14, and 28 days after transplantation. RESULTS: Physiological parameters of the two groups were comparable, but not significantly different. NSC transplantation significantly improved neurological function ($P<0.05$) but did not reduce the infarct size significantly ($P>0.05$). Compared with the control, NSC transplantation significantly reduced the number of TUNEL- and Bax-positive cells in the penumbra at 7 days. Interestingly, the number of Bcl-2-positive cells in the penumbra after NSC transplantation was significantly higher than that after medium transplantation ($P<0.05$). CONCLUSIONS: The results indicate that NSC transplantation has anti-apoptotic activity and can improve the neurological function; these effects are mediated by the up-regulation of Bcl-2 expression in the penumbra.

Zhang, X. M., G. W. Huang, et al. (2009). "Folate deficiency induces neural stem cell apoptosis by increasing homocysteine in vitro." J Clin Biochem Nutr **45**(1): 14-9.

Cellular events for neural progenitor cells, such as proliferation and differentiation, are regulated by multiple intrinsic and extrinsic cell signals. Folate plays a central role in central nervous system development, so folate, as an extrinsic signal, may affect neural stem cell (NSC) proliferation and differentiation. In the present study, we investigated the effects of folate deficiency on the cell proliferation, cell apoptosis and homocysteine concentrations in NSCs. NSCs were isolated from fetal rats and identified as NSCs by their expression of immunoreactive nestin. Cell proliferation was quantitated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Apoptotic cells were detected and confirmed by flow cytometric analysis. We measured homocysteine concentrations in NSCs by high performance liquid chromatography and detected the expression of caspase-3 by western blot method. Folate deficiency not only decreased cell proliferation, but also increased the apoptotic rate of NSCs as demonstrated by the increased expression of early apoptotic markers such as caspase-3, compared to control group ($p<0.05$). Furthermore, There was a statistically significant increase in homocysteine concentration during folate deficiency in NSCs ($p<0.05$). These data suggest that folate affects the cell proliferation, apoptosis and homocysteine generation in NSC cells.

Zhou, L., J. Opalinska, et al. (2007). "p38 MAP kinase regulates stem cell apoptosis in human hematopoietic failure." Cell Cycle **6**(5): 534-7.

Myelodysplastic syndromes (MDS) are clonal stem cell disorders that lead to ineffective hematopoiesis and are common causes of low blood counts in the elderly. The exact molecular mechanisms regulating increased stem apoptosis in these disorders are not well defined. p38 MAPK activation is important in regulating the growth inhibitory signals of TNF-alpha, TGF-beta and Interferons on human hematopoiesis. Our findings show that p38 MAPK is overactivated in myelodysplasia bone marrows and regulates hematopoietic stem cell apoptosis. Inhibition of p38 MAPK by genetic or pharmacologic means decreases apoptosis and stimulates in vitro hematopoiesis from primary MDS hematopoietic progenitors. These studies point to the potential efficacy of selective

p38alpha inhibitor, SCIO-469, in human bone marrow failure.

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11/12/2010