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Stem Cell

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Adult Stem Cell Research Literatures

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

Introduction

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

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

Abe, Y., et al. (2002). "Eradication of virus-infected T-cells in a case of adult T-cell leukemia/lymphoma by nonmyeloablative peripheral blood stem cell transplantation with conditioning consisting of low-dose total body irradiation and pentostatin." Int J Hematol **76**(1): 91-93.

We describe the case of a 55-year-old man with adult T-cell leukemia/lymphoma (ATL) in first remission who underwent nonmyeloablative allogeneic peripheral blood stem cell transplantation with conditioning consisting of 4 courses of pentostatin and low-dose total body irradiation. Complete chimerism in peripheral blood was achieved on day 42 without severe myelosuppression. Concomitantly, the proviral DNA load for human T-cell leukemia virus I (HTLV-I) in peripheral blood mononuclear cells decreased below detectable limits and was still undetectable on day 270. This fact indicates that eradication of ATL cells is feasible by induction of an alloimmune response without high-dose chemoradiotherapy.

Abuljadayel, I. S. (2003). "Induction of stem cell-like plasticity in mononuclear cells derived from unmobilised adult human peripheral blood." Curr Med Res Opin **19**(5): 355-375.

Undifferentiated pluripotent stem cells with flexible developmental potentials are not normally found in peripheral blood. However, such cells have recently been reported to reside in the bone marrow. Herein are reported methods of inducing pluripotency in cells derived from unmobilised adult human peripheral blood. In response to the inclusion of purified CR3/43 monoclonal antibody (mAb) to well-established culture conditions, mononuclear cells (MNC) obtained from a single blood donor are converted into pluripotent haematopoietic, neuronal and cardiomyogenic progenitor stem cells or undifferentiated stem cells. The haematopoietic stem cells are CD34+, clonogenic and have been shown to repopulate non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. The neuronal precursors transcribe the primitive stem cell markers OCT-4 and nestin, and on maturation, differentially stain positive for neuronal, glial or oligodendrocyte-specific antigens. The cardiomyogenic progenitor stem cells form large bodies of asynchronously beating cells and differentiate into mature cardiomyocytes which transcribe GATA-4. The undifferentiated stem cells do not express haematopoietic-associated markers, are negative for major histocompatibility complex (MHC) class I and II antigens, transcribe high levels of OCT-4

and form embryoid body (EB)-like structures. This induction of stem cell-like plasticity in MNC may have proceeded by a process of retrodifferentiation but, in any case, could have profound clinical and pharmacological implications. Finally, the flexibility and the speed by which a variety of stem cell classes can be generated ex vivo from donor blood could potentially transfer this novel process into a less invasive automated clinical procedure.

Abuljadayel, I. S., et al. (2012). "A novel autologous stem cell procedure for the treatment of aplastic anaemia using reprogrammed mature adult cells: a pilot study." Indian J Med Res **135**(6): 853-872.

BACKGROUND & OBJECTIVES: Aplastic anaemia is a life threatening rare bone marrow failure disorder. The underlying haematopoietic cellular deficit leads to haemorrhage, infection and severe anaemia. The treatment of choice for this haematological condition is allogeneic bone marrow transplantation from fully matched HLA sibling. Though this procedure is curative in the majority of young patients with aplastic anaemia, extending this benefit to older patients or those lacking a family donor remains a major challenge. Herein, the safety and efficacy of infusing autologous retrodifferentiated haematopoietic stem cells (RHSC) into four patients with aplastic anaemia without the use of any pre- or post-conditioning regimen including immunosuppression is described. METHODS: Un-mobilized, mononuclear cells were harvested from four patients with acquired aplastic anaemia by aphaeresis. Mononuclear cells of patients were cultured with purified monoclonal antibody against the monomorphic regions of the beta chain of MHC class II antigens (Clone CR3/43) for 3 h, to obtain autologous RHSC. Autologous RHSC were washed and infused into the four patients without the use of any pre- or post-conditioning regimen. Thereafter, the efficacy (engraftment) of autologous RHSC was assessed in these patients. RESULTS: Following single infusion of the autologous RHSC, two of the four patients with aplastic anaemia become transfusion independent for more than seven years. Karyotyping and G-banding analysis prior and post-procedure in all patients remained the same. INTERPRETATION & CONCLUSIONS: The findings of this pilot study demonstrated the functional utility of reprogrammed fully differentiated adult cells into pluripotent stem cells with extensive repopulation potentials in a human setting and without any pre- or post-conditioning regimen, including immunosuppression. This autologous approach of stem cell creation may broaden the curative potentials of stem cell therapy to a wider population of patients with aplastic anaemia, including many patients suffering from other haematological and non-haematological disorders.

Acquistapace, A., et al. (2011). "Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer." Stem Cells **29**(5): 812-824.

Because stem cells are often found to improve repair tissue including heart without evidence of engraftment or differentiation, mechanisms underlying wound healing are still elusive. Several studies have reported that stem cells can fuse with cardiomyocytes either by permanent or partial cell fusion processes. However, the respective physiological impact of these two processes remains unknown in part because of the lack of knowledge of the resulting hybrid cells. To further characterize cell fusion, we cocultured mouse fully differentiated cardiomyocytes with human multipotent adipose-derived stem (hMADS) cells as a model of adult stem cells. We found that heterologous cell fusion promoted cardiomyocyte reprogramming back to a progenitor-like state. The resulting hybrid cells expressed early cardiac commitment and proliferation markers such as GATA-4, myocyte enhancer factor 2C, Nkx2.5, and Ki67 and exhibited a mouse genotype. Interestingly, human bone marrow-derived stem cells shared similar reprogramming properties than hMADS cells but not human fibroblasts, which suggests that these features might be common to multipotent cells. Furthermore, cardiac hybrid cells were preferentially generated by partial rather than permanent cell fusion and that intercellular structures composed of f-actin and microtubule filaments were involved in the process. Finally, we showed that stem cell mitochondria were transferred into cardiomyocytes, persisted in hybrids and were required for somatic cell reprogramming. In conclusion, by providing new insights into previously reported cell fusion processes, our data might contribute to a better understanding of stem cell-mediated regenerative mechanisms and thus, the development of more efficient stem cell-based heart therapies.

Alexanian, A. R. and Y. W. Huang (2015). "Specific combinations of the chromatin-modifying enzyme modulators significantly attenuate glioblastoma cell proliferation and viability while exerting minimal effect on normal adult stem cells growth." Tumour Biol **36**(11): 9067-9072.

The discoveries of recent decade showed that all critical changes in cancer cells, such as silencing of tumor-suppressor genes and activation of oncogenes, are caused not only by genetic but also by epigenetic mechanisms. Although epigenetic changes are somatically heritable, in contrast to genetic changes, they are potentially reversible, making them good targets for therapeutic intervention. Covalent modifications of chromatin such as methylation and acetylation of histones and methylation of DNA are the important components of epigenetic machinery. In this

study, we investigated the effect of different modulators of DNA and histone covalent-modifying enzymes on the proliferation and viability of normal adult stem cells, such as human bone marrow mesenchymal stem cells (hMSCs), and on malignant tumor cells, such as glioblastoma (GB) D54 cells. Results demonstrated that specific combinations of histone methyltransferases and deacetylases inhibitors significantly attenuated D54 cells viability but having only a small effect on hMSCs growth. Taken together, these studies suggest that specific combinations of histone covalent modifiers could be an effective treatment option for the most aggressive type of primary brain tumors such as glioblastoma multiforme.

Alexanian, A. R. and S. N. Kurpad (2005). "Quiescent neural cells regain multipotent stem cell characteristics influenced by adult neural stem cells in co-culture." Exp Neurol **191**(1): 193-197.

The source of cells participating in central nervous system (CNS) tissue repair and regeneration is poorly defined. One possible source is quiescent neural cells that can persist in CNS in the form of dormant progenitors or highly specialized cell types. Under appropriate conditions, these quiescent cells may be capable of re-entering the mitotic cell cycle and contributing to the stem cell pool. The aim of this study was to determine whether in vitro differentiated neural stem cells (NSC) can regain their multipotent-like stem cell characteristics in co-culture with NSC. To this end, we induced neural differentiation by plating NSC, derived from the periventricular subependymal zone (SEZ) of ROSA26 transgenic mice in Neurobasal A/B27 medium in the absence of bFGF. Under these conditions, NSC differentiated into neurons, glia, and oligodendrocytes. While the level of Nestin expression was downregulated, persistence of dormant progenitors could not be ruled out. However, further addition of bFGF or bFGF/EGF with conditioned medium derived from adult NSC did not induce any noticeable cell proliferation. In another experiment, differentiated neural cells were cultured with adult NSC, isolated from the hippocampus of Balb/c mice, in the presence bFGF. This resulted in proliferating colonies of ROSA26 derived cells that mimicked NSC in their morphology, growth kinetics, and expressed NSC marker proteins. The average nuclear area and DAPI fluorescence intensity of these cells were similar to that of NSC grown alone. We conclude that reactivation of quiescent neural cells can be initiated by NSC-associated short-range cues but not by cell fusion.

Arnhold, S., et al. (2000). "Differentiation of green fluorescent protein-labeled embryonic stem cell-derived neural precursor cells into Thy-1-positive neurons and glia after transplantation into adult rat striatum." J Neurosurg **93**(6): 1026-1032.

OBJECT: The aim of this investigation was to assess new information concerning the capacity of transplanted embryonic stem cell (ESC)-derived neuronal cells to migrate into host brain and to evaluate these cells as a possible source for cell replacement therapy in neurodegenerative disorders such as Parkinson's disease (PD). METHODS: The authors investigated the ability of ESC-derived neural precursor cells to migrate and differentiate in a host striatum by using a D3-derived ESC clone that was transfected stably with a chicken beta-actin cytomegalovirus enhancer-driven green fluorescent protein (GFP)-labeled construct. This procedure allowed easy monitoring of all transplanted cells because of the green fluorescent labeling of donor cells. This approach also afforded easy estimation of cell integration and simultaneous observation of the entire transplanted cell population in relation to immunocytochemically identified neuronal and glial differentiation. After selection of nestin-positive neural precursor cells in a synthetic medium, they were implanted into the striatum of male adult Wistar rats. Their integration was analyzed on morphological studies performed 3 days to 4 weeks posttransplantation. CONCLUSIONS: The investigators found that after transplantation, a subpopulation of GFP-labeled cells differentiated into various neural morphological types that were positive for the mouse-specific Thy-1 antigen, which is known be expressed on neurons, as well as being positive for the astroglial marker glial fibrillary acidic protein. Moreover, GFP-expressing cells that were negative for either of these markers remained close to the injection site, presumably representing other derivatives of the neural lineage. Together, these findings contribute to basic research regarding future transplantation strategies in neurodegenerative diseases such as PD.

Arumugam, S. B., et al. (2011). "Detection of embryonic stem cell markers in adult human adipose tissue-derived stem cells." Indian J Pathol Microbiol **54**(3): 501-508.

BACKGROUND: Bone marrow transplantation is already an established therapy, which is now widely used in medicine to treat leukemia, lymphoma, and several inherited blood disorders. The culture of multilineage cells from easily available adipose tissue is another source of multipotent mesenchymal stem cells, and is referred to as adipose tissue-derived stem cells (ADSCs). While ADSCs are being used to treat various conditions, some lacuna exists regarding the specific proteins in these. It was therefore decided to analyze the specific proteins of embryonic cells in ADSCs. AIMS: To analyze the specific protein of embryonic stem cells (ESCs) in ADSCs. MATERIALS AND METHODS: Adult human adipose tissue-derived stem cells (ADSCs) were

harvested from 13 patients after obtaining patients' consent. The specific markers of ESCs included surface proteins CD10, CD13, CD44, CD59, CD105, and CD166, and further nucleostemin, (NS) NANOG, peroxisome proliferator-activated receptor-ggamma, collagen type 1 (Coll1), alkaline phosphate, (ALP) osteocalcin (OC), and core binding factor 1 (Cbfa1) were analyzed using by reverse transcription-polymerase chain reaction, (RT-PCR) immunofluorescence (IF), and western blot. RESULTS: All the proteins were expressed distinctly, except CD13 and OC. CD13 was found individually with different expressions, and OC expression was discernable. CONCLUSIONS: Although the ESC with its proven self-renewal capacity and pluripotency seems appropriate for clinical use, the recent work on ADSCs suggests that these adult stem cells would be a valuable source for future biotechnology, especially since there is a relative ease of procurement.

Askar, S. F., et al. (2013). "Engraftment patterns of human adult mesenchymal stem cells expose electrotonic and paracrine proarrhythmic mechanisms in myocardial cell cultures." Circ Arrhythm Electrophysiol **6**(2): 380-391.

BACKGROUND: After intramyocardial injection, mesenchymal stem cells (MSCs) may engraft and influence host myocardium. However, engraftment rate and pattern of distribution are difficult to control in vivo, hampering assessment of potential adverse effects. In this study, the role of the engraftment patterns of MSCs on arrhythmicity in controllable in vitro models is investigated. METHODS AND RESULTS: Cocultures of 4x10(5) neonatal rat cardiomyocytes and 7% or 28% adult human MSCs (hMSCs) in diffuse or clustered distribution patterns were prepared. Electrophysiological effects were studied by optical mapping and patch-clamping. In diffuse cocultures, hMSCs dose-dependently decreased neonatal rat cardiomyocyte excitability, slowed conduction, and prolonged action potential duration until 90% repolarization (APD90). Triggered activity (14% versus 0% in controls) and increased inducibility of re-entry (53% versus 6% in controls) were observed in 28% hMSC cocultures. MSC clusters increased APD90, slowed conduction locally, and increased re-entry inducibility (23%), without increasing triggered activity. Pharmacological heterocellular electric uncoupling increased excitability and conduction velocity to 133% in 28% hMSC cocultures, but did not alter APD90. Transwell experiments showed that hMSCs dose-dependently increased APD90, APD dispersion, inducibility of re-entry and affected specific ion channel protein levels, whereas excitability was unaltered. Incubation with hMSC-derived exosomes did not increase APD in neonatal rat cardiomyocyte cultures. CONCLUSIONS: Adult hMSCs affect arrhythmicity of neonatal rat cardiomyocyte cultures by heterocellular coupling leading to depolarization-induced conduction slowing and by direct release of paracrine factors that negatively affect repolarization rate. The extent of these detrimental effects depends on the number and distribution pattern of hMSCs. These results suggest that caution should be urged against potential adverse effects of myocardial hMSC engraftment.

Awaya, N., et al. (2002). "Failure of adult marrow-derived stem cells to generate marrow stroma after successful hematopoietic stem cell transplantation." Exp Hematol **30**(8): 937-942.

OBJECTIVE: The existence of adult, marrow-derived stem cells that retain the ability to generate various tissues is an appealing concept that has considerable therapeutic potential. The aim of this study was to test the extent of this proposed plasticity by defining the ability of adult marrow and peripheral blood stem cells to generate stromal cells of the marrow microenvironment. PATIENTS AND METHODS: We examined expanded populations of stromal cells from four patients 1 to 27 years after allogeneic, sex-mismatched marrow, or peripheral blood stem cell transplantation. The cultured stromal cells were stained by immunofluorescence and with nonspecific esterase (NSE) to detect macrophages, which can constitute a significant component of a primary long-term marrow culture. Fluorescence in situ hybridization (FISH) probes for chromosomes X and Y were applied to distinguish donor from host cells. RESULTS: FISH analysis of replicate slides indicated a good correlation between the number of NSE(+) cells and the number of donor-derived cells. By applying NSE and FISH to the same cells and capturing both bright-field and epifluorescence images, we confirmed that all donor signals were derived from NSE(+) macrophages. CONCLUSION: After successful allogeneic stem cell transplantation, the marrow stroma remains host in origin, even after 27 years of 100% donor hematopoiesis.

Baazm, M., et al. (2017). "Effects of different Sertoli cell types on the maintenance of adult spermatogonial stem cells in vitro." In Vitro Cell Dev Biol Anim **53**(8): 752-758.

Spermatongonial stem cells (SSCs) are unique testis cells that are able to proliferate, differentiate, and transmit genetic information to the next generation. However, the effect of different Sertoli cell types on the expression of specific SSC genes is not yet well understood. In this study, we compare the in vitro effect of adult Sertoli cells, embryonic Sertoli cells, and TM4 (a Sertoli cell line) as feeder layers on the expression of SSC genes. SSCs were isolated from the testis of adult male mice and purified by differential plating. Following enrichment, SSCs were cultivated

for 1 and 2 wk in the presence of various feeders. The expression of SSC-specific genes (Mvh, ZBTB, and c-kit) was evaluated by real-time polymerase chain reaction. Our results revealed that expression of the specific SSC genes was significantly higher in the embryonic Sertoli cells after 1 and 2 wk compared to the adult Sertoli cells and the TM4 group. Our finding suggest that co-culturing of SSCs with embryonic Sertoli cells is helpful for in vitro cultivation of SSCs and might improve the self-renewal of these stem cells.

Baek, S. H., et al. (2009). "Insulin withdrawal-induced cell death in adult hippocampal neural stem cells as a model of autophagic cell death." Autophagy **5**(2): 277-279.

The term "autophagic cell death" was coined to describe a form of cell death associated with the massive formation of autophagic vacuoles without signs of apoptosis. However, questions about the actual role of autophagy and its molecular basis in cell death remain to be elucidated. We recently reported that adult hippocampal neural stem (HCN) cells undergo autophagic cell death following insulin withdrawal. Insulin-deprived HCN cells exhibit morphological and biochemical markers of autophagy, including accumulation of Beclin 1 and the type II form of microtubule-associated protein 1 light chain 3 (LC3) without evidence of apoptosis. Suppression of autophagy by knockdown of Atg7 reduces cell death, whereas promotion of autophagy with rapamycin augments cell death in insulin-deficient HCN cells. These data reveal a causative role of autophagy in insulin withdrawal-induced HCN cell death. HCN cells have intact apoptotic capability despite the lack of apoptosis following insulin withdrawal. Our study demonstrates that autophagy is the default cell death mechanism in insulin-deficient HCN cells, and provides a genuine model of autophagic cell death in apoptosis-intact cells. Novel insight into molecular mechanisms of this underappreciated form of programmed cell death should facilitate the development of therapeutic methods to cope with human diseases caused by dysregulated cell death.

Baksh, D., et al. (2004). "Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy." J Cell Mol Med **8**(3): 301-316.

A considerable amount of retrospective data is available that describes putative mesenchymal stem cells (MSCs). However, there is still very little knowledge available that documents the properties of a MSC in its native environment. Although the precise identity of MSCs remains a challenge, further understanding of their biological properties will be greatly advanced by analyzing the mechanisms that govern their self-renewal and differentiation potential. This review begins with the current state of knowledge on the biology of MSCs, specifically with respect to their existence in the adult organism and postulation of their biological niche. While MSCs are considered suitable candidates for cell-based strategies owing to their intrinsic capacity to self-renew and differentiate, there is currently little information available regarding the molecular mechanisms that govern their stem cell potential. We propose here a model for the regulation of MSC differentiation, and recent findings regarding the regulation of MSC differentiation are discussed. Current research efforts focused on elucidating the mechanisms regulating MSC differentiation should facilitate the design of optimal in vitro culture conditions to enhance their clinical utility cell and gene therapy.

Balasubramanian, S., et al. (2009). "Non cell-autonomous reprogramming of adult ocular progenitors: generation of pluripotent stem cells without exogenous transcription factors." Stem Cells **27**(12): 3053-3062.

Direct reprogramming of differentiated cells to induced pluripotent stem (iPS) cells by ectopic expression of defined transcription factors (TFs) represents a significant breakthrough towards the use of stem cells in regenerative medicine (Takahashi and Yamanaka Cell 2006;126:663-676). However, the virus-mediated expression of exogenous transcription factors could be potentially harmful and, therefore, represents a barrier to the clinical use of iPS cells. Several approaches, ranging from plasmid-mediated TF expression to introduction of recombinant TFs (Yamanaka Cell 2009;137:13-17; Zhou, Wu, Joo et al. Cell Stem Cell 2009;4:381-384), have been reported to address the risk associated with viral integration. We describe an alternative strategy of reprogramming somatic progenitors entirely through the recruitment of endogenous genes without the introduction of genetic materials or exogenous factors. To this end, we reprogrammed accessible and renewable progenitors from the limbal epithelium of adult rat eye by microenvironment-based induction of endogenous iPS cell genes. Non cell-autonomous reprogramming generates cells that are pluripotent and capable of differentiating into functional neurons, cardiomyocytes, and hepatocytes, which may facilitate autologous cell therapy to treat degenerative diseases.

Ballas, C. B., et al. (2002). "Adult bone marrow stem cells for cell and gene therapies: implications for greater use." J Cell Biochem Suppl **38**: 20-28.

There is excitement generated almost daily about the possible uses of stem cells to treat human disease. Much of the interest of late is generated by embryonic stem cells (ESCs). As exciting as ESCs may be, they are quite controversial for moral reasons, given their source. They are also scientifically controversial since they are much less well understood than the original, long-standing, and clinically successful

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

Baptista, L. S., et al. (2018). "Adult Stem Cells Spheroids to Optimize Cell Colonization in Scaffolds for Cartilage and Bone Tissue Engineering." Int J Mol Sci **19**(5).

Top-down tissue engineering aims to produce functional tissues using biomaterials as scaffolds, thus providing cues for cell proliferation and differentiation. Conversely, the bottom-up approach aims to precondition cells to form modular tissues units (building-blocks) represented by spheroids. In spheroid culture, adult stem cells are responsible for their extracellular matrix synthesis, re-creating structures at the tissue level. Spheroids from adult stem cells can be considered as organoids, since stem cells recapitulate differentiation pathways and also represent a promising approach for identifying new molecular targets (biomarkers) for diagnosis and therapy. Currently, spheroids can be used for scaffold-free (developmental engineering) or scaffold-based approaches. The scaffold promotes better spatial organization of individual spheroids and provides a defined geometry for their 3D assembly in larger and complex tissues. Furthermore, spheroids exhibit potent angiogenic and vasculogenic capacity and serve as efficient vascularization units in porous scaffolds for bone tissue engineering. An automated combinatorial approach that integrates spheroids into scaffolds is starting to be investigated for macro-scale tissue biofabrication.

Barbosa, J. S., et al. (2016). "Single-cell in vivo imaging of adult neural stem cells in the zebrafish telencephalon." Nat Protoc **11**(8): 1360-1370.

Adult neural stem cells (aNSCs) in zebrafish produce mature neurons throughout their entire life span in both the intact and regenerating brain. An understanding of the behavior of aNSCs in their intact niche and during regeneration in vivo should facilitate the identification of the molecular mechanisms controlling regeneration-specific cellular events. A greater understanding of the process in regeneration-competent species may enable regeneration to be achieved in regeneration-incompetent species, including humans. Here we describe a protocol for labeling and repetitive imaging of aNSCs in vivo. We label single aNSCs, allowing nonambiguous re-identification of single cells in repetitive imaging sessions using electroporation of a red-reporter plasmid in Tg(gfap:GFP)mi2001 transgenic fish expressing GFP in aNSCs. We image using two-photon microscopy through the thinned skull of anesthetized and immobilized fish. Our protocol allows imaging every 2 d for a period of up to 1 month. This methodology allowed the visualization of aNSC behavior in vivo in their natural niche, in contrast to previously available technologies, which rely on the imaging of either dissociated cells or tissue slices. We used this protocol to follow the mode of aNSC division, fate changes and cell death in both the intact and injured zebrafish telencephalon. This experimental setup can be widely used, with minimal prior experience, to assess key factors for processes that modulate aNSC behavior. A typical experiment with data analysis takes up to 1.5 months.

Basford, C. L., et al. (2012). "The functional and molecular characterisation of human embryonic stem cell-derived insulin-positive cells compared with adult pancreatic beta cells." Diabetologia **55**(2): 358-371.

AIMS/HYPOTHESIS: Using a novel directed differentiation protocol, we recently generated up to 25% insulin-producing cells from human embryonic stem cells (hESCs) (insulin(+) cells). At this juncture, it was important to functionally and molecularly characterise these hESC-derived insulin(+) cells and identify key

differences and similarities between them and primary beta cells. METHODS: We used a new reporter hESC line with green fluorescent protein (GFP) cDNA targeted to the INS locus by homologous recombination (INS (GFP/w)) and an untargeted hESC line (HES2). INS (GFP/w) allowed efficient identification and purification of GFP-producing (INS:GFP(+)) cells. Insulin(+) cells were examined for key features of adult beta cells using microarray, quantitative PCR, secretion assays, imaging and electrophysiology. RESULTS: Immunofluorescent staining showed complete co-localisation of insulin with GFP; however, cells were often multihormonal, many with granules containing insulin and glucagon. Electrophysiological recordings revealed variable K(ATP) and voltage-gated Ca(2+) channel activity, and reduced glucose-induced cytosolic Ca(2+) uptake. This translated into defective glucose-stimulated insulin secretion but, intriguingly, appropriate glucagon responses. Gene profiling revealed differences in global gene expression between INS:GFP(+) cells and adult human islets; however, INS:GFP(+) cells had remarkably similar expression of endocrine-lineage transcription factors and genes involved in glucose sensing and exocytosis. CONCLUSIONS/INTERPRETATION: INS:GFP(+) cells can be purified from differentiated hESCs, providing a superior source of insulin-producing cells. Genomic analyses revealed that INS:GFP(+) cells collectively resemble immature endocrine cells. However, insulin(+) cells were heterogeneous, a fact that translated into important functional differences within this population. The information gained from this study may now be used to generate new iterations of functioning beta cells that can be purified for transplant.

Baxter, M., et al. (2015). "Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes." J Hepatol **62**(3): 581-589.

BACKGROUND & AIMS: Hepatocyte-like cells (HLCs), differentiated from pluripotent stem cells by the use of soluble factors, can model human liver function and toxicity. However, at present HLC maturity and whether any deficit represents a true fetal state or aberrant differentiation is unclear and compounded by comparison to potentially deteriorated adult hepatocytes. Therefore, we generated HLCs from multiple lineages, using two different protocols, for direct comparison with fresh fetal and adult hepatocytes. METHODS: Protocols were developed for robust differentiation. Multiple transcript, protein and functional analyses compared HLCs to fresh human fetal and adult hepatocytes. RESULTS: HLCs were comparable to those of other laboratories by multiple parameters. Transcriptional changes during differentiation mimicked human embryogenesis and showed more similarity to pericentral than periportal hepatocytes. Unbiased proteomics demonstrated greater proximity to liver than 30 other human organs or tissues. However, by comparison to fresh material, HLC maturity was proven by transcript, protein and function to be fetal-like and short of the adult phenotype. The expression of 81% phase 1 enzymes in HLCs was significantly upregulated and half were statistically not different from fetal hepatocytes. HLCs secreted albumin and metabolized testosterone (CYP3A) and dextrorphan (CYP2D6) like fetal hepatocytes. In seven bespoke tests, devised by principal components analysis to distinguish fetal from adult hepatocytes, HLCs from two different source laboratories consistently demonstrated fetal characteristics. CONCLUSIONS: HLCs from different sources are broadly comparable with unbiased proteomic evidence for faithful differentiation down the liver lineage. This current phenotype mimics human fetal rather than adult hepatocytes.

Bhandari, D. R., et al. (2011). "The simplest method for in vitro beta-cell production from human adult stem cells." Differentiation **82**(3): 144-152.

Diabetes mellitus is a challenging autoimmune disease. Biomedical researchers are currently exploring efficient and effective ways to solve this challenge. The potential of stem cell therapies for treating diabetes represents one of the major focuses of current research on diabetes treatment. Here, we have attempted to differentiate adult stem cells from umbilical cord blood-derived mesenchymal cells (UCB-MSC), Wharton's jelly-derived mesenchymal stem cells (WJ-MSC) and amniotic epithelial stem cells (AE-SC) into insulin-producing cells. The serum-free protocol developed in this study resulted in the differentiation of cells into definitive endoderm, pancreatic foregut, pancreatic endoderm and, finally, pancreatic endocrine cells, which expressed the marker genes SOX17, PDX1, NGN3, NKX6.1, INS, GCG, and PPY, respectively. Detection of the expression of the gap junction-related gene connexin-36 (CX36) using RT-PCR provided conclusive evidence for insulin-producing cell differentiation. In addition to this RT-PCR result, insulin and C-peptide protein were detected by immunohistochemistry and ELISA. Glucose stimulation test results showed that significantly greater amounts of C-peptide and insulin were released from differentiated cells than from undifferentiated cells. In conclusion, the methods investigated in this study can be considered an effective and efficient means of obtaining insulin-producing cells from adult stem cells within a week.

Bhartiya, D., et al. (2010). "Newer insights into premeiotic development of germ cells in adult human

testis using Oct-4 as a stem cell marker." J Histochem Cytochem **58**(12): 1093-1106.

The transcription factor octamer-binding transforming factor 4 (Oct-4) is central to the gene regulatory network responsible for self-renewal, pluripotency, and lineage commitment in embryonic stem (ES) cells and induced pluripotent stem cells (PSCs). This study was undertaken to evaluate differential localization and expression of two major transcripts of Oct-4, viz. Oct-4A and Oct-4B, in adult human testis. A novel population of 5- to 10-mum PSCs with nuclear Oct-4A was identified by ISH and immunolocalization studies. Besides Oct-4, other pluripotent markers like Nanog and TERT were also detected by RT-PCR. A(dark) spermatogonial stem cells (SSCs) were visualized in pairs and chains undergoing clonal expansion and stained positive for cytoplasmic Oct-4B. Quantitative PCR and Western blotting revealed both the transcripts, with higher expression of Oct-4B. It is proposed that PSCs undergo asymmetric cell division and give rise to A(dark) SSCs, which proliferate and initiate lineage-specific differentiation. The darkly stained nuclei in A(dark) SSCs may represent extensive nuclear reprogramming by epigenetic changes when a PSC becomes committed. Oct-4B eventually disappeared in mature germ cells, viz. spermatocytes, spermatids, and sperm. Besides maintaining normal testicular homeostasis, PSCs may also be implicated in germ cell tumors and ES-like colonies that have recently been derived from adult human testicular tissue.

Bjerknes, M. and H. Cheng (1981). "The stem-cell zone of the small intestinal epithelium. I. Evidence from Paneth cells in the adult mouse." Am J Anat **160**(1): 51-63.

Stem cells in the small intestinal epithelium are known to differentiate into columnar, mucous, enteroendocrine, and Paneth cells. However, the site of initiation of stem-cell differentiation has been unknown. To approach this problem we determined the site of stem-cell differentiation along the Paneth cell line, using light microscopic morphometry and radioautography. The smallest Paneth cells containing the smallest granules were in positions 6 and 7, while the largest ones containing the largest granules were in positions 1 and 2 at the base of the crypt. Paneth cell death was less prevalent above position 3 than it was in position 1. Since cell size, granule size, and cell death are indicators of Paneth cell age, it was deduced that there is a gradient of Paneth cell age in the crypt base, with the oldest Paneth cells at the bottom, and the youngest at the top. After single injection or continuous infusion of 3H-thymidine, the first labeled Paneth cells to appear were the highest Paneth cells in their crypt column. Later, labeled Paneth cells became more prevalent in lower positions, and, eventually, appeared in position 1. The size of granules in labeled Paneth cells increased with time. It was concluded that Paneth cells originate in position 5 or above and then migrate downward. These results are consistent with a stem-cell zone hypothesis, which proposes that stem cells in positions 1-4 receive no inducement to differentiate. Only those stem cells that migrate up out of the stem-cell zone into position 5 will be induced and then begin to differentiate.

Bjerknes, M. and H. Cheng (1981). "The stem-cell zone of the small intestinal epithelium. III. Evidence from columnar, enteroendocrine, and mucous cells in the adult mouse." Am J Anat **160**(1): 77-91.

In the first two articles of this series we demonstrated restriction of Paneth cell formation to positions 5 and above. Restriction was independent of the Paneth cell population-density gradient in the crypt base. We concluded that our results were consistent with the presence in the adult of a stem-cell zone in positions 1-4 in which stem cells received no inducement to differentiate. To further test the stem-cell zone hypothesis we determined the site of stem-cell differentiation along mucous, enteroendocrine, and columnar cell lines using radioautography with 3H-thymidine as a label. One hour after injection of 3H-thymidine, labeled mucous cells were not observed below position 5. Only later did they appear in lower positions and not until 4 days after injection were they observed in position 1. Labeled enteroendocrine cells first appeared above, and then were seen in the top of, and finally in the middle and bottom of the Paneth cell distribution. Thirty hours after injection there were two populations of labeled columnar cells in the crypt base, a heavily labeled population and a lightly labeled one. At this time interval the heavily labeled columnar cells were only observed in positions 5 and above, but they appeared in positions 1-4 by 66 hours after injection. The above evidence led us to conclude that all differentiated offspring of the common epithelial stem cell originate in positions 5 and above. Most columnar, mucous, and enteroendocrine cells originating in positions 5 and above migrate upward. However some of these cells migrate down. All differentiated cells found in positions 1-4 migrated down from their origin in position 5 or above. We also found that only stem cells proliferate in positions 1-4. We concluded that in the adult, there is a stem-cell zone in positions 1-4 where stem cells are not induced to differentiate and persist as stem cells throughout life.

Blazquez Fernandez, E. (2004). "[Therapeutic implications of the differentiation of adult stem cells into other cell types]." An R Acad Nac Med (Madr) **121**(4): 533-546; discussion 546-539.

During last years stem cells have generated a great interest because its potential therapeutic use. These are unspecialised cells, with ability to self-

renewal and to differentiate into other cell types, being embryo cells totipotent and of restrictive use for ethical reasons, while adult stem cells are multipotent and of potential clinical use . Between the latter the best studied are the cells of the bone marrow: hematopoietic producing blood cell lines and mesenchymal that may be transformed into hepatocytes, chondrocytes, osteocytes, adipocytes, cardiocytes and neural cells. Although these facts are promising many studies must be done until to get stem cells therapies, as well as verify these treatments in animal models. It is believed that in this century besides traditional therapies we will have to take into account cell therapies with stem cells, in whose development will be very important the scientific, clinical and ethical parameters.

Bocker, W., et al. (2002). "Common adult stem cells in the human breast give rise to glandular and myoepithelial cell lineages: a new cell biological concept." Lab Invest **82**(6): 737-746.

Breast biology and pathology are currently shaped by the two-cell concept that recognizes only glandular and myoepithelial cells. In the present study, we have visualized a previously unidentified cell population within the epithelial compartment of the breast, which displays the phenotypic characteristics of a committed stem cell. Immunofluorescence double labeling with digital image processing and Western blotting were applied to normal breast tissue as well as to noninvasive and invasive breast cancers using antibodies to basal cytokeratin 5 (Ck5), glandular cytokeratins 8/18 (Ck8/18/19), and smooth muscle alpha-actin (SMA) as markers for myoepithelial cells (SMA). A distinct population of cells was identified that expressed Ck5 in the absence of Ck8/18/19 or SMA. These cells differentiate toward glandular epithelial or myoepithelial Ck5-negative end cells passing through either Ck5/Ck8/18/19 or Ck5/SMA-positive intermediates. Our experiments clearly demonstrate a precursor or committed stem cell function of the Ck5-positive cell that is responsible for regeneration of the human adult breast epithelium. However, the observation that the vast majority of breast cancers display the glandular epithelial immunophenotype strongly suggests that the neoplastic cells derive from a late stage of the glandular epithelial differentiation pathway. The significance of this new cell biological model is that it might serve as a tool to unravel the regulatory mechanisms that govern regeneration and abnormal proliferation of breast epithelium at the cellular level.

Boecker, W. and H. Buerger (2003). "Evidence of progenitor cells of glandular and myoepithelial cell lineages in the human adult female breast epithelium: a new progenitor (adult stem) cell concept." Cell Prolif **36 Suppl 1**: 73-84.

Although experimental data clearly confirm the existence of self-renewing mammary stem cells, the characteristics of such progenitor cells have never been satisfactorily defined. Using a double immunofluorescence technique for simultaneous detection of the basal cytokeratin 5, the glandular cytokeratins 8/18 and the myoepithelial differentiation marker smooth muscle actin (SMA), we were able to demonstrate the presence of CK5+ cells in human adult breast epithelium. These cells have the potential to differentiate to either glandular (CK8/18+) or myoepithelial cells (SMA+) through intermediary cells (CK5+ and CK8/18+ or SMA+). We therefore proceeded on the assumption that the CK5+ cells are phenotypically and behaviourally progenitor (committed adult stem) cells of human breast epithelium. Furthermore, we furnish evidence that most of these progenitor cells are located in the luminal epithelium of the ductal lobular tree. Based on data obtained in extensive analyses of proliferative breast disease lesions, we have come to regard usual ductal hyperplasia as a progenitor cell-derived lesion, whereas most breast cancers seem to evolve from differentiated glandular cells. Double immunofluorescence experiments provide a new tool to characterize phenotypically progenitor (adult stem) cells and their progenies. This model has been shown to be of great value for a better understanding not only of normal tissue regeneration but also of proliferative breast disease. Furthermore, this model provides a new tool for unravelling further the regulatory mechanisms that govern normal and pathological cell growth.

Bombelli, S., et al. (2013). "PKH(high) cells within clonal human nephrospheres provide a purified adult renal stem cell population." Stem Cell Res **11**(3): 1163-1177.

The existence and identification of adult renal stem cells is a controversial issue. In this study, renal stem cells were identified from cultures of clonal human nephrospheres. The cultured nephrospheres exhibited the activation of stem cell pathways and contained cells at different levels of maturation. In each nephrosphere the presence of 1.12-1.25 cells mirroring stem cell properties was calculated. The nephrosphere cells were able to generate three-dimensional tubular structures in 3D cultures and in vivo. In clonal human nephrospheres a PKH(high) phenotype was isolated using PKH26 epifluorescence, which can identify quiescent cells within the nephrospheres. The PKH(high) cells, capable of self-renewal and of generating a differentiated epithelial, endothelial and podocytic progeny, can also survive in vivo maintaining the undifferentiated status. The PKH(high) status, together with a CD133(+)/CD24(-) phenotype, identified a homogeneous cell population displaying in vitro self-renewal and multipotency capacity. The

resident adult renal stem cell population isolated from nephrospheres can be used for the study of mechanisms that regulate self-renewal and differentiation in adult renal tissue as well as in renal pathological conditions.

Boras-Granic, K., et al. (2014). "Embryonic cells contribute directly to the quiescent stem cell population in the adult mouse mammary gland." Breast Cancer Res **16**(6): 487.

INTRODUCTION: Studies have identified multi-potent stem cells in the adult mammary gland. More recent studies have suggested that the embryonic mammary gland may also contain stem/progenitor cells that contribute to initial ductal development. We were interested in determining whether embryonic cells might also directly contribute to long-lived stem cells that support homeostasis and development in the adult mammary gland. METHODS: We used DNA-label retention to detect long label-retaining cells in the mammary gland. Mouse embryos were labeled with 5-ethynl-2'-deoxyuridine (EdU) between embryonic day 14.5 and embryonic day 18.5 and were subsequently sacrificed and examined for EdU retention at various intervals after birth. EdU retaining cells were co-stained for various lineage markers and identified after fluorescence activated cell sorting analysis of specific epithelial subsets. EdU-labeled mice were subjected to subsequent 5-bromo-2'-deoxyuridine administration to determine whether EdU-labeled cells could re-enter the cell cycle. Finally, EdU-labeled cells were grown under non-adherent conditions to assess their ability to form mammospheres. RESULTS: We demonstrate embryonically-derived, long label-retaining cells (eLLRCs) in the adult mammary gland. eLLRCs stain for basal markers and are enriched within the mammary stem cell population identified by cell sorting. eLLRCs are restricted to the primary ducts near the nipple region. Interestingly, long label retaining cells (labeled during puberty) are found just in front of the eLLRCs, near where the ends of the ducts had been at the time of DNA labeling in early puberty. A subset of eLLRCs becomes mitotically active during periods of mammary growth and in response to ovarian hormones. Finally, we show that eLLRCs are contained within primary and secondary mammospheres. CONCLUSIONS: Our findings suggest that a subset of proliferating embryonic cells subsequently becomes quiescent and contributes to the pool of long-lived mammary stem cells in the adult. eLLRCs can re-enter the cell cycle, produce both mammary lineages and self-renew. Thus, our studies have identified a putative stem/progenitor cell population of embryonic origin. Further study of these cells will contribute to an understanding of how quiescent stem cells are generated during development and how fetal exposures may alter future breast cancer risk in adults.

Broughton, K. M. and M. A. Sussman (2018). "Enhancement Strategies for Cardiac Regenerative Cell Therapy: Focus on Adult Stem Cells." Circ Res **123**(2): 177-187.

The idiom heart of the matter refers to the focal point within a topic and, with regard to health and longevity, the heart is truly pivotal for quality of life. Societal trends worldwide continue toward increased percent body fat and decreased physical activity with coincident increases in chronic diseases including cardiovascular disease as the top global cause of death along with insulin resistance, accelerated aging, cancer. Although long-term survival rates for cardiovascular disease patients are grim, intense research efforts continue to improve both prevention and treatment options. Pharmacological interventions remain the predominant interventional strategy for mitigating progression and managing symptoms, but cellular therapies have the potential to cure or even mediate remission of cardiovascular disease. Adult stem cells are the most studied cellular therapy in both preclinical and clinical investigation. This review will focus on the advanced therapeutic strategies to augment products and methods of delivery, which many think heralds the future of clinical investigations. Advanced preclinical strategies using adult stem cells are examined to promote synergism between preclinical and clinical research, streamline implementation, and improve this imminent matter of the heart.

Buaas, F. W., et al. (2004). "Plzf is required in adult male germ cells for stem cell self-renewal." Nat Genet **36**(6): 647-652.

Adult germline stem cells are capable of self-renewal, tissue regeneration and production of large numbers of differentiated progeny. We show here that the classical mouse mutant luxoid affects adult germline stem cell self-renewal. Young homozygous luxoid mutant mice produce limited numbers of normal spermatozoa and then progressively lose their germ line after birth. Transplantation studies showed that germ cells from mutant mice did not colonize recipient testes, suggesting that the defect is intrinsic to the stem cells. We determined that the luxoid mutant contains a nonsense mutation in the gene encoding Plzf, a transcriptional repressor that regulates the epigenetic state of undifferentiated cells, and showed that Plzf is coexpressed with Oct4 in undifferentiated spermatogonia. This is the first gene shown to be required in germ cells for stem cell self-renewal in mammals.

Bunk, E. C., et al. (2016). "Prox1 Is Required for Oligodendrocyte Cell Identity in Adult Neural Stem Cells of the Subventricular Zone." Stem Cells **34**(8): 2115-2129.

Adult neural stem cells with the ability to generate neurons and glia cells are active throughout

life in both the dentate gyrus (DG) and the subventricular zone (SVZ). Differentiation of adult neural stem cells is induced by cell fate determinants like the transcription factor Prox1. Evidence has been provided for a function of Prox1 as an inducer of neuronal differentiation within the DG. We now show that within the SVZ Prox1 induces differentiation into oligodendrocytes. Moreover, we find that loss of Prox1 expression in vivo reduces cell migration into the corpus callosum, where the few Prox1 deficient SVZ-derived remaining cells fail to differentiate into oligodendrocytes. Thus, our work uncovers a novel function of Prox1 as a fate determinant for oligodendrocytes in the adult mammalian brain. These data indicate that the neurogenic and oligodendrogliogenic lineages in the two adult neurogenic niches exhibit a distinct requirement for Prox1, being important for neurogenesis in the DG but being indispensable for oligodendrogliogenesis in the SVZ. Stem Cells 2016;34:2115-2129.

Byrne, J. A., et al. (2003). "Nuclei of adult mammalian somatic cells are directly reprogrammed to oct-4 stem cell gene expression by amphibian oocytes." Curr Biol **13**(14): 1206-1213.

Nuclear reprogramming by the transplantation of somatic cell nuclei to eggs (in second meiotic metaphase) is always followed by a phase of chromosome replication and cell division before new gene expression is seen. To help understand the mechanism of nuclear reprogramming, we have asked whether the nuclei of normal, nontransformed, nondividing, and terminally differentiated mammalian cells can be directly reprogrammed, without DNA replication, by Xenopus oocytes. We find that nuclei of adult mouse thymocytes and of adult human blood lymphocytes, injected into Xenopus oocytes, are induced to extinguish a differentiation marker and to strongly express oct-4, the most diagnostic mammalian stem cell/pluripotency marker. In the course of 2 days at 18 degrees C, the mammalian oct-4 transcripts are spliced to mature mRNA. We conclude that normal mammalian nuclei can be directly reprogrammed by the nucleus (germinal vesicle) of amphibian oocytes to express oct-4 at a rate comparable to that of oct-4 in mouse ES cells. To our knowledge, this is the first demonstration of a stem cell marker being induced in a differentiated adult human cell nucleus. This is an early step toward the long-term aim of developing a procedure for reprogramming readily accessible human adult cells for cell replacement therapy.

Carlin, S. M., et al. (2013). "T-cell potential of human adult and cord blood hemopoietic stem cells expanded with the use of aryl hydrocarbon receptor antagonists." Cytotherapy **15**(2): 224-230.

BACKGROUND AIMS: Expansion of hemopoietic stem cells (HSCs) in vitro is a potential strategy for improving transplant outcomes, but expansion methods tend to promote differentiation and loss of stem cell potential. Aryl hydrocarbon receptor antagonists (AhRAs) have recently been shown to protect HSC stemness during expansion; however, little is known of the T-cell regenerative capacity of AhRA-expanded HSCs. In this study, we confirm the protective effect of two commercially available AhRA compounds on HSCs from both cord blood (CB) and adult samples and assess the T-lymphocyte potential of the expanded cells. METHODS: Adult mobilized peripheral blood and CB samples were purified to CD34(+) cells, which were expanded in vitro with cytokines and AhRAs. After 14 d, CD34(+) cells were re-isolated and then grown on in OP9Delta co-culture under conditions that allow T-lymphocyte differentiation. Cells were monitored weekly for T-lineage markers by flow cytometry. RESULTS: Both AhRA compounds promoted maintenance of CD34 expression during 2 weeks of proliferation with growth factors, although adult cells proliferated markedly less than CB cells. AhRA-expanded CD34(+) cells from CB differentiated to T cells on OP9Delta co-culture with the same rate and time course as untreated cells. Adult cells, by contrast, had reduced differentiation to T cells, with donor-dependent variable responses. CONCLUSIONS: This study shows that whereas AhRA treatment is effective in CB samples, expansion of adult HSCs is less successful and reflects their inherent poor potential in T-cell generation.

Catacchio, I., et al. (2013). "Evidence for bone marrow adult stem cell plasticity: properties, molecular mechanisms, negative aspects, and clinical applications of hematopoietic and mesenchymal stem cells transdifferentiation." Stem Cells Int **2013**: 589139.

In contrast to the pluripotent embryonic stem cells (ESCs) which are able to give rise to all cell types of the body, mammalian adult stem cells (ASCs) appeared to be more limited in their differentiation potential and to be committed to their tissue of origin. Recently, surprising new findings have contradicted central dogmas of commitment of ASCs by showing their plasticity to differentiate across tissue lineage boundaries, irrespective of classical germ layer designations. The present paper supports the plasticity of the bone marrow stem cells (BMSCs), bringing the most striking and the latest evidences of the transdifferentiation properties of the bone marrow hematopoietic and mesenchymal stem cells (BMHSCs, and BMMSCs), the two BM populations of ASCs better characterized. In addition, we report the possible mechanisms that may explain these events, outlining the clinical importance of these phenomena and the relative problems.

Chiasson, B. J., et al. (1999). "Adult mammalian forebrain ependymal and subependymal cells demonstrate proliferative potential, but only subependymal cells have neural stem cell characteristics." J Neurosci **19**(11): 4462-4471.

The adult derivatives of the embryonic forebrain germinal zones consist of two morphologically distinct cell layers surrounding the lateral ventricles: the ependyma and the subependyma. Cell cycle analyses have revealed that at least two proliferating populations exist in this region, one that is constitutively proliferating and one that is relatively quiescent and thought to include the endogenous adult neural stem cells. Earlier studies demonstrated that specific dissection of the region surrounding the lateral ventricles was necessary for the in vitro isolation of multipotent, self-renewing neural stem cells. However, in these studies, the ependymal layer was not physically separated from the subependymal layer to identify the specific adult laminar localization of the neural stem cells around the lateral ventricles. To determine which cellular compartment in the adult forebrain contained the neural stem cells, we isolated and cultured the ependyma separately from the subependyma and tested for the presence of neural stem cells using the in vitro neurosphere assay. We demonstrate that the ependymal cells can proliferate in vitro to form sphere-like structures. However, the ependymal cells generating spheres do not have the ability to self-renew (proliferate to form secondary spheres after dissociation) nor to produce neurons, but rather only seem to generate glial fibrillary acidic protein-positive ependymal cells when plated under differentiation conditions in culture. On the other hand, a subpopulation of subependymal cells do possess the self-renewing and multipotential characteristics of neural stem cells. Therefore, the adult forebrain neural stem cell resides within the subependymal compartment.

Chicheportiche, A., et al. (2018). "Isolation of Neural Stem and Progenitor Cells from the Adult Brain and Live Imaging of Their Cell Cycle with the FUCCI System." Methods Mol Biol **1686**: 69-78.

Neural stem cells (NSCs) enter quiescence in early embryonic stages to create a reservoir of dormant NSCs able to enter proliferation and produce neuronal precursors in the adult mammalian brain. Various approaches of fluorescent-activated cell sorting (FACS) have emerged to allow the distinction between quiescent NSCs (qNSCs), their activated counterpart (aNSCs), and the resulting progeny. In this article, we review two FACS techniques that can be used alternatively. We also show that their association with transgenic Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI) mice allows an unprecedented overlook on the cell cycle dynamics of adult NSCs.

Choi, R. and B. J. Goldstein (2018). "Olfactory epithelium: Cells, clinical disorders, and insights from an adult stem cell niche." Laryngoscope Investig Otolaryngol **3**(1): 35-42.

Disorders causing a loss of the sense of smell remain a therapeutic challenge. Basic research has, however, greatly expanded our knowledge of the organization and function of the olfactory system. This review describes advances in our understanding of the cellular components of the peripheral olfactory system, specifically the olfactory epithelium in the nose. The article discusses recent findings regarding the mechanisms involved in regeneration and cellular renewal from basal stem cells in the adult olfactory epithelium, considering the strategies involved in embryonic olfactory development and insights from research on other stem cell niches. In the context of clinical conditions causing anosmia, the current view of adult olfactory neurogenesis, tissue homeostasis, and failures in these processes is considered, along with current and future treatment strategies. Level of Evidence: NA.

Chung, K. M., et al. (2016). "Mediation of Autophagic Cell Death by Type 3 Ryanodine Receptor (RyR3) in Adult Hippocampal Neural Stem Cells." Front Cell Neurosci **10**: 116.

Cytoplasmic Ca(2+) actively engages in diverse intracellular processes from protein synthesis, folding and trafficking to cell survival and death. Dysregulation of intracellular Ca(2+) levels is observed in various neuropathological states including Alzheimer's and Parkinson's diseases. Ryanodine receptors (RyRs) and inositol 1,4,5-triphosphate receptors (IP3Rs), the main Ca(2+) release channels located in endoplasmic reticulum (ER) membranes, are known to direct various cellular events such as autophagy and apoptosis. Here we investigated the intracellular Ca(2+)-mediated regulation of survival and death of adult hippocampal neural stem (HCN) cells utilizing an insulin withdrawal model of autophagic cell death (ACD). Despite comparable expression levels of RyR and IP3R transcripts in HCN cells at normal state, the expression levels of RyRs-especially RyR3-were markedly upregulated upon insulin withdrawal. While treatment with the RyR agonist caffeine significantly promoted the autophagic death of insulin-deficient HCN cells, treatment with its inhibitor dantrolene prevented the induction of autophagy following insulin withdrawal. Furthermore, CRISPR/Cas9-mediated knockout of the RyR3 gene abolished ACD of HCN cells. This study delineates a distinct, RyR3-mediated ER Ca(2+) regulation of autophagy and programmed cell death in neural stem cells. Our findings provide novel insights into the critical, yet understudied mechanisms underlying the

regulatory function of ER Ca(2+) in neural stem cell biology.

Chung, K. M., et al. (2015). "Calpain Determines the Propensity of Adult Hippocampal Neural Stem Cells to Autophagic Cell Death Following Insulin Withdrawal." Stem Cells **33**(10): 3052-3064.

Programmed cell death (PCD) has significant effects on the function of neural stem cells (NSCs) during brain development and degeneration. We have previously reported that adult rat hippocampal neural stem (HCN) cells underwent autophagic cell death (ACD) rather than apoptosis following insulin withdrawal despite their intact apoptotic capabilities. Here, we report a switch in the mode of cell death in HCN cells with calpain as a critical determinant. In HCN cells, calpain 1 expression was barely detectable while calpain 2 was predominant. Inhibition of calpain in insulin-deprived HCN cells further augmented ACD. In contrast, expression of calpain 1 switched ACD to apoptosis. The proteasome inhibitor lactacystin blocked calpain 2 degradation and elevated the intracellular Ca(2+) concentration. In combination, these effects potentiated calpain activity and converted the mode of cell death to apoptosis. Our results indicate that low calpain activity, due to absence of calpain 1 and degradation of calpain 2, results in a preference for ACD over apoptosis in insulin-deprived HCN cells. On the other hand, conditions leading to high calpain activity completely switch the mode of cell death to apoptosis. This is the first report on the PCD mode switching mechanism in NSCs. The dynamic change in calpain activity through the proteasome-mediated modulation of the calpain and intracellular Ca(2+) levels may be the critical contributor to the demise of NSCs. Our findings provide a novel insight into the complex mechanisms interconnecting autophagy and apoptosis and their roles in the regulation of NSC death.

Collins, C. A., et al. (2005). "Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche." Cell **122**(2): 289-301.

Satellite cells are situated beneath the basal lamina that surrounds each myofiber and function as myogenic precursors for muscle growth and repair. The source of satellite cell renewal is controversial and has been suggested to be a separate circulating or interstitial stem cell population. Here, we transplant single intact myofibers into radiation-ablated muscles and demonstrate that satellite cells are self-sufficient as a source of regeneration. As few as seven satellite cells associated with one transplanted myofiber can generate over 100 new myofibers containing thousands of myonuclei. Moreover, the transplanted satellite cells vigorously self-renew, expanding in number and repopulating the host muscle with new satellite cells. Following experimental injury, these cells proliferate extensively and regenerate large compact clusters of myofibers. Thus, within a normally stable tissue, the satellite cell exhibits archetypal stem cell properties and is competent to form the basal origin of adult muscle regeneration.

Conrad, S., et al. (2016). "Expression of Genes Related to Germ Cell Lineage and Pluripotency in Single Cells and Colonies of Human Adult Germ Stem Cells." Stem Cells Int **2016**: 8582526.

The aim of this study was to elucidate the molecular status of single human adult germ stem cells (haGSCs) and haGSC colonies, which spontaneously developed from the CD49f MACS and matrix-(collagen-/laminin+ binding-) selected fraction of enriched spermatogonia. Single-cell transcriptional profiling by Fluidigm BioMark system of a long-term cultured haGSCs cluster in comparison to human embryonic stem cells (hESCs) and human fibroblasts (hFibs) revealed that haGSCs showed a characteristic germ- and pluripotency-associated gene expression profile with some similarities to hESCs and with a significant distinction from somatic hFibs. Genome-wide comparisons with microarray analysis confirmed that different haGSC colonies exhibited gene expression heterogeneity with more or less pluripotency. The results of this study confirm that haGSCs are adult stem cells with a specific molecular gene expression profile in vitro, related but not identical to true pluripotent stem cells. Under ES-cell conditions haGSC colonies could be selected and maintained in a partial pluripotent state at the molecular level, which may be related to their cell plasticity and potential to differentiate into cells of all germ layers.

Conrad, S., et al. (2018). "Single-Cell Expression Profiling and Proteomics of Primordial Germ Cells, Spermatogonial Stem Cells, Adult Germ Stem Cells, and Oocytes." Adv Exp Med Biol.

The mammalian germ cells, cell assemblies, tissues, and organs during development and maturation have been extensively studied at the tissue level. However, to investigate and understand the fundamental insights at the molecular basis of germ and stem cells, their cell fate plasticity, and determination, it is of most importance to analyze at the large scale on the single-cell level through different biological windows. Here, modern molecular techniques optimized for single-cell analysis, including single fluorescence-activated cell sorting (FACS) and single-cell RNA sequencing (scRNA-seq) or microfluidic high-throughput quantitative real-time polymerase chain reaction (qRT-PCR) for single-cell gene expression and liquid chromatography coupled to tandem mass spectrometry (LC-MSMS) for protein profiling, have been established and are still getting optimized.This review aims on describing and discussing recent single-cell expression profiling and

proteomics of different types of human germ cells, including primordial germ cells (PGCs), spermatogonial stem cells (SSCs), human adult germ stem cells (haGSCs), and oocytes.

Cornelison, D. and E. Perdiguero (2017). "Muscle Stem Cells: A Model System for Adult Stem Cell Biology." Methods Mol Biol **1556**: 3-19.

Skeletal muscle stem cells, originally termed satellite cells for their position adjacent to differentiated muscle fibers, are absolutely required for the process of skeletal muscle repair and regeneration. In the last decade, satellite cells have become one of the most studied adult stem cell systems and have emerged as a standard model not only in the field of stem cell-driven tissue regeneration but also in stem cell dysfunction and aging. Here, we provide background in the field and discuss recent advances in our understanding of muscle stem cell function and dysfunction, particularly in the case of aging, and the potential involvement of muscle stem cells in genetic diseases such as the muscular dystrophies.

Crouch, E. E., et al. (2015). "Regional and stage-specific effects of prospectively purified vascular cells on the adult V-SVZ neural stem cell lineage." J Neurosci **35**(11): 4528-4539.

Adult neural stem cells reside in specialized niches. In the ventricular-subventricular zone (V-SVZ), quiescent neural stem cells (qNSCs) become activated (aNSCs), and generate transit amplifying cells (TACs), which give rise to neuroblasts that migrate to the olfactory bulb. The vasculature is an important component of the adult neural stem cell niche, but whether vascular cells in neurogenic areas are intrinsically different from those elsewhere in the brain is unknown. Moreover, the contribution of pericytes to the neural stem cell niche has not been defined. Here, we describe a rapid FACS purification strategy to simultaneously isolate primary endothelial cells and pericytes from brain microregions of nontransgenic mice using CD31 and CD13 as surface markers. We compared the effect of purified vascular cells from a neurogenic (V-SVZ) and non-neurogenic brain region (cortex) on the V-SVZ stem cell lineage in vitro. Endothelial and pericyte diffusible signals from both regions differentially promote the proliferation and neuronal differentiation of qNSCs, aNSCs, and TACs. Unexpectedly, diffusible cortical signals had the most potent effects on V-SVZ proliferation and neurogenesis, highlighting the intrinsic capacity of non-neurogenic vasculature to support stem cell behavior. Finally, we identify PlGF-2 as an endothelial-derived mitogen that promotes V-SVZ cell proliferation. This purification strategy provides a platform to define the functional and molecular contribution of vascular cells to stem cell niches and other brain regions under different physiological and pathological states.

Das, M., et al. (2013). "Adult mesenchymal stem cells and their potency in the cell-based therapy." J Stem Cells **8**(1): 1-16.

Recent advances in the field of regenerative medicines manifested the unique properties of stem cells including the ability of self-renewal and differentiation to make them available for their replacement in tissue injury. Mesenchymal stem cells (MSCs) are important tools in treating immune disorders and in tissue repair due to their multipotency, immunosuppressive properties, and production of cytokines or growth factors. MSC-mediated therapy is a fast-growing field that has proven safe and effective in the treatment of various degenerative diseases and tissue injuries. Generation of induced pluripotent stem (iPS) cells holds a great promise for regenerative medicine and other aspects of clinical applications. The mechanisms governing multipotency in MSCs are not well understood. This review mainly throws light on the biology of MSCs, including their efficiency in treating several diseases and also the progress of the use of iPSC-derived MSC widely in the clinic.

Davis, R. J., et al. (2017). "The Developmental Stage of Adult Human Stem Cell-Derived Retinal Pigment Epithelium Cells Influences Transplant Efficacy for Vision Rescue." Stem Cell Reports **9**(1): 42-49.

Age-related macular degeneration (AMD) is a common cause of central visual loss in the elderly. Retinal pigment epithelial (RPE) cell loss occurs early in the course of AMD and RPE cell transplantation holds promise to slow disease progression. We report that subretinal transplantation of RPE stem cell (RPESC)-derived RPE cells (RPESC-RPE) preserved vision in a rat model of RPE cell dysfunction. Importantly, the stage of differentiation that RPESC-RPE acquired prior to transplantation influenced the efficacy of vision rescue. Whereas cells at all stages of differentiation tested rescued photoreceptor layer morphology, an intermediate stage of RPESC-RPE differentiation obtained after 4 weeks of culture was more consistent at vision rescue than progeny that were differentiated for 2 weeks or 8 weeks of culture. Our results indicate that the developmental stage of RPESC-RPE significantly influences the efficacy of RPE cell replacement, which affects the therapeutic application of these cells for AMD.

Daynac, M., et al. (2015). "Cell Sorting of Neural Stem and Progenitor Cells from the Adult Mouse Subventricular Zone and Live-imaging of their Cell Cycle Dynamics." J Vis Exp(103).

Neural stem cells (NSCs) in the subventricular zone of the lateral ventricles (SVZ) sustain olfactory neurogenesis throughout life in the mammalian brain. They successively generate transit amplifying cells (TACs) and neuroblasts that differentiate into neurons

once they integrate the olfactory bulbs. Emerging fluorescent activated cell sorting (FACS) techniques have allowed the isolation of NSCs as well as their progeny and have started to shed light on gene regulatory networks in adult neurogenic niches. We report here a cell sorting technique that allows to follow and distinguish the cell cycle dynamics of the above-mentioned cell populations from the adult SVZ with a LeX/EGFR/CD24 triple staining. Isolated cells are then plated as adherent cells to explore in details their cell cycle progression by time-lapse video microscopy. To this end, we use transgenic Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI) mice in which cells are red-fluorescent during G1 phase due to a G1 specific red-Cdt1 reporter. This method has recently revealed that proliferating NSCs progressively lengthen their G1 phase during aging, leading to neurogenesis impairment. This method is easily transposable to other systems and could be of great interest for the study of the cell cycle dynamics of brain cells in the context of brain pathologies.

Del Debbio, C. B., et al. (2013). "Adult ciliary epithelial stem cells generate functional neurons and differentiate into both early and late born retinal neurons under non-cell autonomous influences." BMC Neurosci **14**: 130.

BACKGROUND: The neural stem cells discovered in the adult ciliary epithelium (CE) in higher vertebrates have emerged as an accessible source of retinal progenitors; these cells can self-renew and possess retinal potential. However, recent studies have cast doubt as to whether these cells could generate functional neurons and differentiate along the retinal lineage. Here, we have systematically examined the pan neural and retinal potential of CE stem cells. RESULTS: Molecular and cellular analysis was carried out to examine the plasticity of CE stem cells, obtained from mice expressing green fluorescent protein (GFP) under the influence of the promoter of the rod photoreceptor-specific gene, Nrl, using the neurospheres assay. Differentiation was induced by specific culture conditions and evaluated by both transcripts and protein levels of lineage-specific regulators and markers. Temporal pattern of their levels were examined to determine the expression of genes and proteins underlying the regulatory hierarchy of cells specific differentiation in vitro. Functional attributes of differentiation were examined by the presence of current profiles and pharmacological mobilization of intracellular calcium using whole cell recordings and Fura-based calcium imaging, respectively. We demonstrate that stem cells in adult CE not only have the capacity to generate functional neurons, acquiring the expression of sodium and potassium channels, but also respond to specific cues in culture and preferentially differentiate along the lineages of retinal ganglion cells (RGCs) and rod photoreceptors, the early and late born retinal neurons, respectively. The retinal differentiation of CE stem cells was characterized by the temporal acquisition of the expression of the regulators of RGCs and rod photoreceptors, followed by the display of cell type-specific mature markers and mobilization of intracellular calcium. CONCLUSIONS: Our study demonstrates the bonafide retinal potential of adult CE stem cells and suggests that their plasticity could be harnessed for clinical purposes once barriers associated with any lineage conversion, i.e., low efficiency and fidelity is overcome through the identification of conducive culture conditions.

Devitt, S. M., et al. (2015). "Successful isolation of viable adipose-derived stem cells from human adipose tissue subject to long-term cryopreservation: positive implications for adult stem cell-based therapeutics in patients of advanced age." Stem Cells Int **2015**: 146421.

We examined cell isolation, viability, and growth in adipose-derived stem cells harvested from whole adipose tissue subject to different cryopreservation lengths (2-1159 days) from patients of varying ages (26-62 years). Subcutaneous abdominal adipose tissue was excised during abdominoplasties and was cryopreserved. The viability and number of adipose-derived stem cells isolated were measured after initial isolation and after 9, 18, and 28 days of growth. Data were analyzed with respect to cryopreservation duration and patient age. Significantly more viable cells were initially isolated from tissue cryopreserved <1 year than from tissue cryopreserved >2 years, irrespective of patient age. However, this difference did not persist with continued growth and there were no significant differences in cell viability or growth at subsequent time points with respect to cryopreservation duration or patient age. Mesenchymal stem cell markers were maintained in all cohorts tested throughout the duration of the study. Consequently, longer cryopreservation negatively impacts initial live adipose-derived stem cell isolation; however, this effect is neutralized with continued cell growth. Patient age does not significantly impact stem cell isolation, viability, or growth. Cryopreservation of adipose tissue is an effective long-term banking method for isolation of adipose-derived stem cells in patients of varying ages.

Dihazi, H., et al. (2011). "Multipotent adult germline stem cells and embryonic stem cells functional proteomics revealed an important role of eukaryotic initiation factor 5A (Eif5a) in stem cell differentiation." J Proteome Res **10**(4): 1962-1973.

Multipotent adult germline stem cells (maGSCs) are pluripotent cells that can be differentiated into somatic cells of the three primary germ layers. To highlight the protein profile changes

associated with stem cell differentiation, retinoic acid (RA) treated mouse stem cells (maGSCs and ESCs) were compared to nontreated stem cells. 2-DE and DIGE reference maps were created, and differentially expressed proteins were further processed for identification. In both stem cell types, the RA induced differentiation resulted in an alteration of 36 proteins of which 18 were down-regulated and might be potential pluripotency associated proteins, whereas the other 18 proteins were up-regulated. These might be correlated to stem cell differentiation. Surprisingly, eukaryotic initiation factor 5A (Eif5a), a protein which is essential for cell proliferation and differentiation, was significantly down-regulated under RA treatment. A time-dependent investigation of Eif5a showed that the RA treatment of stem cells resulted in a significant up-regulation of the Eif5a in the first 48 h followed by a progressive down-regulation thereafter. This effect could be blocked by the hypusination inhibitor ciclopirox olamine (CPX). The alteration of Eif5a hypusination, as confirmed by mass spectrometry, exerts an antiproliferative effect on ESCs and maGSCs in vitro, but does not affect the cell pluripotency. Our data highlights the important role of Eif5a and its hypusination for stem cell differentiation and proliferation.

Dihne, M., et al. (2006). "Embryonic stem cell-derived neuronally committed precursor cells with reduced teratoma formation after transplantation into the lesioned adult mouse brain." Stem Cells **24**(6): 1458-1466.

The therapeutic potential of embryonic stem (ES) cells in neurodegenerative disorders has been widely recognized, and methods are being developed to optimize culture conditions for enriching the cells of interest and to improve graft stability and safety after transplantation. Whereas teratoma formation rarely occurs in xenogeneic transplantation paradigms of ES cell-derived neural progeny, more than 70% of mice that received murine ES cell-derived neural precursor cells develop teratomas, thus posing a major safety problem for allogeneic and syngeneic transplantation paradigms. Here we introduce a new differentiation protocol based on the generation of substrate-adherent ES cell-derived neural aggregates (SENAs) that consist predominantly of neuronally committed precursor cells. Purified SENAs that were differentiated into immature but postmitotic neurons did not form tumors up to four months after syngeneic transplantation into the acutely degenerated striatum and showed robust survival.

Dollet, P. E., et al. (2016). "Comprehensive Screening of Cell Surface Markers Expressed by Adult-Derived Human Liver Stem/Progenitor Cells Harvested at Passage 5: Potential Implications for Engraftment." Stem Cells Int **2016**: 9302537.

Mesenchymal stromal cells (MSCs) are known to have potential therapeutic benefits for a number of diseases. However, many studies report low engraftment levels, regardless of the target organ. One possible explanation could be that MSCs do not express the necessary receptors for engraftment. Indeed, MSCs appear to use a similar mechanism to leukocytes to engraft into injured organs, relying on various receptors for rolling, firm adhesion, and transmigration. In this study, we conducted an extensive surface molecule screening of adult-derived human liver stem/progenitor cells (ADHLSC) in an attempt to shed some light on this subject. We observed that ADHLSCs lack expression of most of the costimulatory molecules tested. Furthermore, study of the adhesion molecule profile of ADHLSCs revealed that they do not express selectin ligands or LFA-1 which are, respectively, involved in the rolling process and the firm adhesion. In addition, ADHLSCs slightly express VLA-4 and lose expression of CXCR4 altogether on their surface during culture expansion. However, ADHLSCs express all the integrin couples and matrix metalloproteinases needed to bind and integrate the extracellular matrix once the endothelial barrier is crossed. Collectively, these results suggest that binding to the endothelium may be the critical weak point in the engraftment process.

Dor, Y., et al. (2004). "Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation." Nature **429**(6987): 41-46.

How tissues generate and maintain the correct number of cells is a fundamental problem in biology. In principle, tissue turnover can occur by the differentiation of stem cells, as is well documented for blood, skin and intestine, or by the duplication of existing differentiated cells. Recent work on adult stem cells has highlighted their potential contribution to organ maintenance and repair. However, the extent to which stem cells actually participate in these processes in vivo is not clear. Here we introduce a method for genetic lineage tracing to determine the contribution of stem cells to a tissue of interest. We focus on pancreatic beta-cells, whose postnatal origins remain controversial. Our analysis shows that pre-existing beta-cells, rather than pluripotent stem cells, are the major source of new beta-cells during adult life and after pancreatectomy in mice. These results suggest that terminally differentiated beta-cells retain a significant proliferative capacity in vivo and cast doubt on the idea that adult stem cells have a significant role in beta-cell replenishment.

Dreyfus, P. A., et al. (2004). "Adult bone marrow-derived stem cells in muscle connective tissue and satellite cell niches." Am J Pathol **164**(3): 773-779.

Skeletal muscle includes satellite cells, which reside beneath the muscle fiber basal lamina and

mainly represent committed myogenic precursor cells, and multipotent stem cells of unknown origin that are present in muscle connective tissue, express the stem cell markers Sca-1 and CD34, and can differentiate into different cell types. We tracked bone marrow (BM)-derived stem cells in both muscle connective tissue and satellite cell niches of irradiated mice transplanted with green fluorescent protein (GFP)-expressing BM cells. An increasing number of GFP+ mononucleated cells, located both inside and outside of the muscle fiber basal lamina, were observed 1, 3, and 6 months after transplantation. Sublaminal cells expressed unambiguous satellite cell markers (M-cadherin, Pax7, NCAM) and fused into scattered GFP+ muscle fibers. In muscle connective tissue there were GFP+ cells located close to blood vessels that expressed the ScaI or CD34 stem-cell antigens. The rate of settlement of extra- and intralaminal compartments by BM-derived cells was compatible with the view that extralaminal cells constitute a reservoir of satellite cells. We conclude that both muscle satellite cells and stem cell marker-expressing cells located in muscle connective tissue can derive from BM in adulthood.

Dumble, M. L., et al. (2001). "Hepatoblast-like cells populate the adult p53 knockout mouse liver: evidence for a hyperproliferative maturation-arrested stem cell compartment." Cell Growth Differ **12**(5): 223-231.

Although p53 regulates the cell cycle and apoptosis, gross embryonic development is normal in the p53 knockout (-/-) mouse. In this study, we comprehensively assessed liver development in p53 -/- mice (from embryonic day 15 to adult) for evidence of a cell cycle-induced perturbation in differentiation. Liver cell proliferation in the embryo and newborn is similar in p53 -/- and +/+ mice; in contrast, -/- adult hepatocytes divide at twice the rate of wild types. Developmental expression patterns of liver-specific markers that are up-regulated (e.g., phosphoenolpyruvate carboxykinase and aldolase B) and down-regulated (e.g., alpha-fetoprotein) are similar. Therefore, embryonic and perinatal liver development is normal in the absence of p53. However, the p53 -/- adult liver displays small blast-like cells, the majority being hepatic and some lymphoid. These cells appear in periportal regions and can infiltrate the parenchyma. The hepatic blast-like cells express both mature and immature liver markers, suggesting that differentiation of the liver stem cell compartment is blocked.

Dumont, C. M., et al. (2017). "Factors Released from Endothelial Cells Exposed to Flow Impact Adhesion, Proliferation, and Fate Choice in the Adult Neural Stem Cell Lineage." Stem Cells Dev **26**(16): 1199-1213.

The microvasculature within the neural stem cell (NSC) niche promotes self-renewal and regulates lineage progression. Previous work identified endothelial-produced soluble factors as key regulators of neural progenitor cell (NPC) fate and proliferation; however, endothelial cells (ECs) are sensitive to local hemodynamics, and the effect of this key physiological process has not been defined. In this study, we evaluated adult mouse NPC response to soluble factors isolated from static or dynamic (flow) EC cultures. Endothelial factors generated under dynamic conditions significantly increased neuronal differentiation, while those released under static conditions stimulated oligodendrocyte differentiation. Flow increases EC release of neurogenic factors and of heparin sulfate glycosaminoglycans that increase their bioactivity, likely underlying the enhanced neuronal differentiation. Additionally, endothelial factors, especially from static conditions, promoted adherent growth. Together, our data suggest that blood flow may impact proliferation, adhesion, and the neuron-glial fate choice of adult NPCs, with implications for diseases and aging that reduce flow.

Dutta, R., et al. (2011). "A comparative study on efficiency of adult fibroblast, putative embryonic stem cell and lymphocyte as donor cells for production of handmade cloned embryos in goat and characterization of putative ntES cells obtained from these embryos." Theriogenology **76**(5): 851-863.

The main purpose of the experiment was to compare the efficiency of three cell types, namely adult fibroblast, putative embryonic stem (ES) cell, and lymphocyte, as donor cells for somatic cell nuclear transfer by handmade cloning in goats. The outcome clearly shows that putative embryonic stem cells, with a cleavage and blastocyst production rate of 74.69% +/- 3.92 and 39.75% +/- 3.86, respectively, performs better in comparison to adult fibroblast cell and lymphocyte. Between adult fibroblast cell and lymphocyte no statistically significant difference exists at $P < 0.05$. An overall cleavage and blastocyst formation rate of 67.41% +/- 3.92 and 26.96% +/- 3.86 was obtained using adult fibroblast donor cells. The study establishes beyond doubt the reprogrammability of lymphocyte by handmade cloning (HMC) protocol with a cleavage and blastocyst production rate of 56.47% +/- 3.92 and 24.70% +/- 3.86, respectively. PCR analysis of highly polymorphic 286 bp fragment of MHC II DRB genes of cloned embryos and three donor cells were performed to verify the cloned embryos. The amplified PCR products were subjected to SSCP to confirm their genetic identity. The karyotyping of the cloned embryos showed normal chromosomal status as expected in goat. Significantly, in the second stage of the experiment, the produced cloned embryos were successfully used to derive ntES-like cells. The rate of primary colony formation rate was 62.50% +/- 4.62 for fibroblast donor cell derived embryos. The same was 60.60% +/- 4.62 for putative ES donor cell derived

embryos and 66.66% +/- 4.62 for lymphocyte donor cell derived embryos, respectively. The putative ntES colonies were positively characterized for alkaline phosphatase, Oct-4, TRA-1-60, TRA-1-81, Sox-2, and Nanog by Immunocytochemistry and Reverse Transcription PCR. To further validate the stem ness, the produced putative ntES colonies were differentiated to embryoid bodies. Immunocytochemistry revealed that embryoid bodies expressed NESTIN specific for ectodermal lineage; GATA-4 for endodermal lineage and smooth muscle actin-I, and troponin-I specific for mesodermal lineage. The study has established an efficient protocol for putative ntES cell derivation from HMC embryos. It could be of substantial significance as patient specific ntES cells have proven therapeutic significance.

Ema, H., et al. (2006). "Adult mouse hematopoietic stem cells: purification and single-cell assays." Nat Protoc **1**(6): 2979-2987.

Mouse hematopoietic stem cells (HSCs) are the best-studied stem cells because functional assays for mouse HSCs were established earliest and purification techniques for mouse HSCs have progressed furthest. Here we describe our current protocols for the purification of CD34-/lowc-Kit+Sca-1+lineage marker- (CD34-KSL) cells, the HSC population making up approximately 0.005% of bone marrow cells in adult C557BL/6 mice. Purified HSCs have been characterized at cellular and molecular levels. Since clonal analysis is essential for the study of self-renewal and lineage commitment in HSCs, here we present our single-cell colony assay and single-cell transplantation procedures. We also introduce our immunostaining procedures for small numbers of HSCs, which are useful for signal transduction analysis. The purification of CD34-KSL cells requires approximately 6 h. Initialization of single-cell culture requires approximately 1 h. Single-cell transplantation requires approximately 6 h. Single-cell immunostaining requires approximately 2 d.

Faast, R., et al. (2006). "Use of adult mesenchymal stem cells isolated from bone marrow and blood for somatic cell nuclear transfer in pigs." Cloning Stem Cells **8**(3): 166-173.

Mesenchymal stem cells (MSCs) isolated from bone marrow were used to examine the hypothesis that a less differentiated cell type could increase adult somatic cell nuclear transfer (SCNT) efficiencies in the pig. SCNT embryos were produced using a fusion before activation protocol described previously and the rate at which these developed to the blastocyst stage compared with that using fibroblasts obtained from ear tissue from the same animal. The use of bone marrow MSCs did not increase cleavage rates compared with adult fibroblasts. However, the percentage of embryos that developed to the blastocyst stage was almost doubled, providing support for the hypothesis that a less differentiated cell can increase cloning efficiencies. As MSCs are relatively difficult to isolate from the bone marrow of live animals, a second experiment was undertaken to determine whether MSCs could be isolated from the peripheral circulation and used for SCNT. Blood MSCs were successfully isolated from four of the five pigs sampled. These cells had a similar differentiation capacity and marker profile to those isolated from bone marrow but did not result in increased rates of development. This is the first study to our knowledge, to report that MSCs can be derived from peripheral blood and used for SCNT for any species. These cells can be readily obtained under relatively sterile conditions compared with adult fibroblasts and as such, may provide an alternative cell type for cloning live animals.

Farioli-Vecchioli, S., et al. (2014). "Running rescues defective adult neurogenesis by shortening the length of the cell cycle of neural stem and progenitor cells." Stem Cells **32**(7): 1968-1982.

Physical exercise increases the generation of new neurons in adult neurogenesis. However, only few studies have investigated the beneficial effects of physical exercise in paradigms of impaired neurogenesis. Here, we demonstrate that running fully reverses the deficient adult neurogenesis within the hippocampus and subventricular zone of the lateral ventricle, observed in mice lacking the antiproliferative gene Btg1. We also evaluated for the first time how running influences the cell cycle kinetics of stem and precursor subpopulations of wild-type and Btg1-null mice, using a new method to determine the cell cycle length. Our data show that in wild-type mice running leads to a cell cycle shortening only of NeuroD1-positive progenitor cells. In contrast, in Btg1-null mice, physical exercise fully reactivates the defective hippocampal neurogenesis, by shortening the S-phase length and the overall cell cycle duration of both neural stem (glial fibrillary acidic protein(+) and Sox2(+)) and progenitor (NeuroD1(+)) cells. These events are sufficient and necessary to reactivate the hyperproliferation observed in Btg1-null early-postnatal mice and to expand the pool of adult neural stem and progenitor cells. Such a sustained increase of cell proliferation in Btg1-null mice after running provides a long-lasting increment of proliferation, differentiation, and production of newborn neurons, which rescues the impaired pattern separation previously identified in Btg1-null mice. This study shows that running positively affects the cell cycle kinetics of specific subpopulations of newly generated neurons and suggests that the plasticity of neural stem cells without cell cycle inhibitory control is reactivated by running, with implications for the long-term modulation of neurogenesis.

Fernando, R. N., et al. (2011). "Cell cycle restriction by histone H2AX limits proliferation of adult neural stem cells." Proc Natl Acad Sci U S A **108**(14): 5837-5842.

Adult neural stem cell proliferation is dynamic and has the potential for massive self-renewal yet undergoes limited cell division in vivo. Here, we report an epigenetic mechanism regulating proliferation and self-renewal. The recruitment of the PI3K-related kinase signaling pathway and histone H2AX phosphorylation following GABA(A) receptor activation limits subventricular zone proliferation. As a result, NSC self-renewal and niche size is dynamic and can be directly modulated in both directions pharmacologically or by genetically targeting H2AX activation. Surprisingly, changes in proliferation have long-lasting consequences on stem cell numbers, niche size, and neuronal output. These results establish a mechanism that continuously limits proliferation and demonstrates its impact on adult neurogenesis. Such homeostatic suppression of NSC proliferation may contribute to the limited self-repair capacity of the damaged brain.

Filip, S., et al. (2003). "Adult stem cells and their importance in cell therapy." Folia Biol (Praha) **49**(1): 9-14.

For their unique properties stem cells promise to be of universal use in clinical medicine, especially in regeneration of many organs and tissues in the human body. This attractive subject receives an ever growing attention of specialists from different branches of science and, no doubt, will present one of the most studied trends in medicine in the new millennium. In this communication, the authors discuss two main sources of human stem cells potentially suitable for cell-based therapy. The first are the cells obtained from embryonic tissues--embryonic stem cells, the second are the cells derived from adult tissues--adult stem cells. Presently, harvesting and therapeutic use of embryonic stem cells are associated with many problems both methodical and ethical. Utilization of adult stem cells in cell-based therapy is a certain solution in the current state of replacement therapy. Still, we have to be aware that this is not a compromise but one of the most prospective ways to treat a variety of serious diseases. To date, it is not yet clear which way would be more suitable and it is up to us which way we choose for the benefit of millions of patients. Considering the current state of knowledge, it is impossible yet to predict which stem cells--embryonic or adult--or therapeutic approaches would yield the best results. Much research is to be done and verified in practice and, at the same time, ethical problems must be resolved.

Flachsbarth, K., et al. (2014). "Neural stem cell-based intraocular administration of ciliary neurotrophic factor attenuates the loss of axotomized ganglion cells in adult mice." Invest Ophthalmol Vis Sci **55**(11): 7029-7039.

PURPOSE: To analyze the neuroprotective effect of intravitreally grafted neural stem (NS) cells genetically modified to secrete ciliary neurotrophic factor (CNTF) on intraorbitally lesioned retinal ganglion cells (RGCs) in adult mice. METHODS: Adherently cultivated NS cells were genetically modified to express a secretable variant of mouse CNTF together with the fluorescent reporter protein Venus. Clonal CNTF-secreting NS cell lines were established using fluorescence activated cell sorting, and intravitreally grafted into adult mice 1 day after an intraorbital crush of the optic nerve. Brn-3a-positive RGCs were counted in flat-mounted retinas at different postlesion intervals to evaluate the neuroprotective effect of the CNTF-secreting NS cells on the axotomized RGCs. Anterograde axonal tracing experiments were performed to analyze the regrowth of the injured RGC axons in CNTF-treated retinas. RESULTS: Intravitreally grafted NS cells preferentially differentiated into astrocytes that survived in the host eyes, stably expressed CNTF, and significantly attenuated the loss of the axotomized RGCs over a period of at least 4 months, the latest postlesion time point analyzed. Depending on the postlesion interval analyzed, the number of RGCs in eyes with grafted CNTF-secreting NS cells was 2.8-fold to 6.4-fold higher than in eyes with grafted control NS cells. The CNTF-secreting NS cells additionally induced long-distance regrowth of the lesioned RGC axons. CONCLUSIONS: Genetically modified clonal NS cell lines may serve as a useful tool for preclinical studies aimed at evaluating the therapeutic potential of a sustained cell-based intravitreal administration of neuroprotective factors in mouse models of glaucoma.

Florek, M., et al. (2005). "Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer." Cell Tissue Res **319**(1): 15-26.

Human prominin-1/CD133 has been reported to be expressed in neural and hematopoietic stem/progenitor cells and in embryonic, but not adult, epithelia. This lack of detection of human prominin-1, as defined by its glycosylation-dependent AC133 epitope, is surprising given the expression of the murine ortholog in adult epithelia. Here, we demonstrate, by using a novel prominin-1 antiserum (alphahE2), that the decrease of AC133 immunoreactivity observed during differentiation of the colonic adenocarcinoma-derived Caco-2 cells is not paralleled by a down-regulation of prominin-1. We have also shown that alphahE2 immunoreactivity, but not AC133 immunoreactivity, is present in several adult human tissues, such as kidney proximal tubules and the parietal layer of Bowman's capsule of

juxtamedullary nephrons, and in lactiferous ducts of the mammary gland. These observations suggest that only the AC133 epitope is down-regulated upon cell differentiation. Furthermore, alphahE2 immunoreactivity has been detected in several kidney carcinomas derived from proximal tubules, independent of their grading. Interestingly, in one particular case, the AC133 epitope, which is restricted to stem cells in normal adult tissue, was up-regulated in the vicinity of the tumor. Our data thus show that (1) in adults, the expression of human prominin-1 is not limited to stem and progenitor cells, and (2) the epitopes of prominin-1 might be useful for investigating solid cancers.

Forster, R., et al. (2014). "Human intestinal tissue with adult stem cell properties derived from pluripotent stem cells." Stem Cell Reports **2**(6): 838-852.

Genetically engineered human pluripotent stem cells (hPSCs) have been proposed as a source for transplantation therapies and are rapidly becoming valuable tools for human disease modeling. However, many applications are limited due to the lack of robust differentiation paradigms that allow for the isolation of defined functional tissues. Here, using an endogenous LGR5-GFP reporter, we derived adult stem cells from hPSCs that gave rise to functional human intestinal tissue comprising all major cell types of the intestine. Histological and functional analyses revealed that such human organoid cultures could be derived with high purity and with a composition and morphology similar to those of cultures obtained from human biopsies. Importantly, hPSC-derived organoids responded to the canonical signaling pathways that control self-renewal and differentiation in the adult human intestinal stem cell compartment. This adult stem cell system provides a platform for studying human intestinal disease in vitro using genetically engineered hPSCs.

Fujimaki, S., et al. (2013). "Intrinsic ability of adult stem cell in skeletal muscle: an effective and replenishable resource to the establishment of pluripotent stem cells." Stem Cells Int **2013**: 420164.

Adult stem cells play an essential role in mammalian organ maintenance and repair throughout adulthood since they ensure that organs retain their ability to regenerate. The choice of cell fate by adult stem cells for cellular proliferation, self-renewal, and differentiation into multiple lineages is critically important for the homeostasis and biological function of individual organs. Responses of stem cells to stress, injury, or environmental change are precisely regulated by intercellular and intracellular signaling networks, and these molecular events cooperatively define the ability of stem cell throughout life. Skeletal muscle tissue represents an abundant, accessible, and replenishable source of adult stem cells. Skeletal muscle contains myogenic satellite cells and muscle-derived stem cells that retain multipotent differentiation abilities. These stem cell populations have the capacity for long-term proliferation and high self-renewal. The molecular mechanisms associated with deficits in skeletal muscle and stem cell function have been extensively studied. Muscle-derived stem cells are an obvious, readily available cell resource that offers promise for cell-based therapy and various applications in the field of tissue engineering. This review describes the strategies commonly used to identify and functionally characterize adult stem cells, focusing especially on satellite cells, and discusses their potential applications.

Fujio, K., et al. (1994). "Expression of stem cell factor and its receptor, c-kit, during liver regeneration from putative stem cells in adult rat." Lab Invest **70**(4): 511-516.

BACKGROUND: Stem cell factor (SCF) and its receptor, c-kit, are known to play important roles in hematopoiesis, melanogenesis, and gametogenesis. The biologic effects of the SCF/c-kit system are believed to involve survival, proliferation, and migration of early stem cell progeny. Although SCF and c-kit receptor are widely expressed during normal embryonic development, their expression in the adult is limited. EXPERIMENTAL DESIGN: The expression of SCF and c-kit genes was examined during liver regeneration via the oval cell compartment utilizing partial hepatectomy (PH) combined with the administration of a noncarcinogenic dose of 2-acetylaminofluorene (AAF) for 8 days (AAF/PH model). RESULTS: Both the ligand and the receptor genes were expressed during the early stages of oval cell proliferation after partial hepatectomy in the AAF/PH model, while neither simple partial hepatectomy nor AAF administration alone induced a noticeable expression of the SCF/c-kit system. The level of SCF mRNA increased within 12 hours after partial hepatectomy and reached a peak around day 4. Thus, the expression of SCF preceded the major expansion of the oval cell compartment. The level of c-kit transcripts gradually increased from the 12-hour time point and stayed elevated until day 11, when a large proportion of the oval cells differentiated into small basophilic hepatocytes. Separation of liver cells at day 3 in the AAF/PH model into parenchymal and nonparenchymal fractions demonstrated that the expression of both SCF and c-kit receptor genes was restricted to the nonparenchymal cells. Furthermore, in situ hybridization revealed that the c-kit transcripts were restricted to oval cells, whereas the SCF transcripts were expressed in both oval cells and Ito cells. CONCLUSIONS: The transcripts for the c-kit receptor are expressed in the early progeny of the hepatic stem cells. The SCF/c-kit system may, possibly in combination with other growth factor/receptor systems,

be involved in the early activation of the hepatic stem cells as well as in the expansion and differentiation of oval cells.

Funakoshi, N., et al. (2011). "Comparison of hepatic-like cell production from human embryonic stem cells and adult liver progenitor cells: CAR transduction activates a battery of detoxification genes." Stem Cell Rev **7**(3): 518-531.

In vitro production of human hepatocytes is of primary importance in basic research, pharmacotoxicology and biotherapy of liver diseases. We have developed a protocol of differentiation of human embryonic stem cells (ES) towards hepatocyte-like cells (ES-Hep). Using a set of human adult markers including CAAT/enhancer binding protein (C/EBPalpha), hepatocyte nuclear factor 4/7 ratio (HNF4alpha1/HNF4alpha7), cytochrome P450 7A1 (CYP7A1), CYP3A4 and constitutive androstane receptor (CAR), and fetal markers including alpha-fetoprotein, CYP3A7 and glutathione S-transferase P1, we analyzed the expression of a panel of 41 genes in ES-Hep comparatively with human adult primary hepatocytes, adult and fetal liver. The data revealed that after 21 days of differentiation, ES-Hep are representative of fetal hepatocytes at less than 20 weeks of gestation. The glucocorticoid receptor pathway was functional in ES-Hep. Extending protocols of differentiation to 4 weeks did not improve cell maturation. When compared with hepatocyte-like cells derived from adult liver non parenchymal epithelial (NPE) cells (NPE-Hep), ES-Hep expressed several adult and fetal liver makers at much greater levels (at least one order of magnitude), consistent with greater expression of liver-enriched transcription factors Forkhead box A2, C/EBPalpha, HNF4alpha and HNF6. It therefore seems that ES-Hep reach a better level of differentiation than NPE-Hep and that these cells use different lineage pathways towards the hepatic phenotype. Finally we showed that lentivirus-mediated expression of xenoreceptor CAR in ES-Hep induced the expression of several detoxification genes including CYP2B6, CYP2C9, CYP3A4, UDP-glycosyltransferase 1A1, solute carriers 21A6, as well as biotransformation of midazolam, a CYP3A4-specific substrate.

Galvin, K. A. and D. G. Jones (2002). "Adult human neural stem cells for cell-replacement therapies in the central nervous system." Med J Aust **177**(6): 316-318.

Human neural stem cells (HNSCs) can be isolated from both the developing and adult central nervous system (CNS). HNSCs can be successfully grown in culture, are self-renewable, and can generate mature neuronal and glial progeny. Embryonic HNSCs can be induced to differentiate into specific neuronal phenotypes. HNSCs successfully integrate into the host environment after transplantation into the developing or adult CNS. HNSCs transplanted into animal models of Parkinson's disease and spinal cord injury have induced functional recovery. The risks associated with stem cell transplantation trials are difficult to assess, but have not become overtly apparent throughout preclinical investigations. Major hurdles remain to be overcome before human clinical trials can be embarked upon.

Galvin, K. A. and D. G. Jones (2006). "Adult human neural stem cells for autologous cell replacement therapies for neurodegenerative disorders." NeuroRehabilitation **21**(3): 255-265.

Neural stem cells residing in the adult human brain have the potential to provide a source of tissue for self-to-self cell replacement strategies for the treatment of neurodegenerative diseases. Adult human neural stem cells (NSCs) are self renewable in culture and can generate mature neural progeny which display the characteristics of functional neurons and glia. Despite this, a number of concerns remain regarding their current suitability for treating neurodegenerative disorders. It must be demonstrated that desired neuronal types can be generated in clinically significant quantities, and can induce long-lasting functional improvements in well-characterised animal models of neurodegenerative disorders. Furthermore, the risks to patients in terms of tumour formation and side effects must be adequately assessed. Due to the paucity of data on adult human NSCs, a move from preclinical studies to clinical trials in human patients in the foreseeable future is unlikely. If clinical trials with autologous NSCs are pursued as a treatment option for neurodegenerative diseases, then lessons and insights from many years of clinical trials with fetal neural transplantation for Parkinson's and Huntington's diseases will be invaluable, and should be heeded. Issues include experimental versus therapeutic research, standardisation of methodologies, and minimisation of risks and maximisation of benefits.

Gangemi, R. M., et al. (2004). "Regulatory genes controlling cell fate choice in embryonic and adult neural stem cells." J Neurochem **89**(2): 286-306.

Neural stem cells are the most immature progenitor cells in the nervous system and are defined by their ability to self-renew by symmetric division as well as to give rise to more mature progenitors of all neural lineages by asymmetric division (multipotentiality). The interest in neural stem cells has been growing in the past few years following the demonstration of their presence also in the adult nervous system of several mammals, including humans. This observation implies that the brain, once thought to be entirely post-mitotic, must have at least a limited capacity for self-renewal. This raises the possibility that the adult nervous system may still have the necessary plasticity to undergo repair of inborn defects

and acquired injuries, if ways can be found to exploit the potential of neural stem cells (either endogenous or derived from other sources) to replace damaged or defective cells. A full understanding of the molecular mechanisms regulating generation and maintenance of neural stem cells, their choice between different differentiation programmes and their migration properties is essential if these cells are to be used for therapeutic applications. Here, we summarize what is currently known of the genes and the signalling pathways involved in these mechanisms.

Garcia-Fuster, M. J., et al. (2011). "Decreased proliferation of adult hippocampal stem cells during cocaine withdrawal: possible role of the cell fate regulator FADD." Neuropsychopharmacology **36**(11): 2303-2317.

The current study uses an extended access rat model of cocaine self-administration (5-h session per day, 14 days), which elicits several features manifested during the transition to human addiction, to study the neural adaptations associated with cocaine withdrawal. Given that the hippocampus is thought to have an important role in maintaining addictive behavior and appears to be especially relevant to mechanisms associated with withdrawal, this study attempted to understand how extended access to cocaine impacts the hippocampus at the cellular and molecular levels, and how these alterations change over the course of withdrawal (1, 14, and 28 days). Therefore, at the cellular level, we examined the effects of cocaine withdrawal on cell proliferation (Ki-67+ and NeuroD+ cells) in the DG. At the molecular level, we employed a 'discovery' approach with gene expression profiling in the DG to uncover novel molecules possibly implicated in the neural adaptations that take place during cocaine withdrawal. Our results suggest that decreased hippocampal cell proliferation might participate in the adaptations associated with drug removal and identifies 14 days as a critical time-point of cocaine withdrawal. At the 14-day time-point, gene expression profiling of the DG revealed the dysregulation of several genes associated with cell fate regulation, highlighting two new neurobiological correlates (Ascl-1 and Dnmt3b) that accompany cessation of drug exposure. Moreover, the results point to Fas-Associated protein with Death Domain (FADD), a molecular marker previously associated with the propensity to substance abuse and cocaine sensitization, as a key cell fate regulator during cocaine withdrawal. Identifying molecules that may have a role in the restructuring of the hippocampus following substance abuse provides a better understanding of the adaptations associated with cocaine withdrawal and identifies novel targets for therapeutic intervention.

Garcia-Verdugo, J. M., et al. (1998). "Architecture and cell types of the adult subventricular zone: in search of the stem cells." J Neurobiol **36**(2): 234-248.

Neural stem cells are maintained in the subventricular zone (SVZ) of the adult mammalian brain. Here, we review the cellular organization of this germinal layer and propose lineage relationships of the three main cell types found in this area. The majority of cells in the adult SVZ are migrating neuroblasts (type A cells) that continue to proliferate. These cells form an extensive network of tangentially oriented pathways throughout the lateral wall of the lateral ventricle. Type A cells move long distances through this network at high speeds by means of chain migration. Cells in the SVZ network enter the rostral migratory stream (RMS) and migrate anteriorly into the olfactory bulb, where they differentiate into interneurons. The chains of type A cells are ensheathed by slowly proliferating astrocytes (type B cells), the second most common cell type in this germinal layer. The most actively proliferating cells in the SVZ, type C, form small clusters dispersed throughout the network. These foci of proliferating type C cells are in close proximity to chains of type A cells. We discuss possible lineage relationships among these cells and hypothesize which are the neural stem cells in the adult SVZ. In addition, we suggest that interactions between type A, B, and C cells may regulate proliferation and initial differentiation within this germinal layer.

Gasparetto, M., et al. (2012). "Varying levels of aldehyde dehydrogenase activity in adult murine marrow hematopoietic stem cells are associated with engraftment and cell cycle status." Exp Hematol **40**(10): 857-866 e855.

Aldehyde dehydrogenase (ALDH) activity is a widely used marker for human hematopoietic stem cells (HSCs), yet its relevance and role in murine HSCs remain unclear. We found that murine marrow cells with a high level of ALDH activity as measured by Aldefluor staining (ALDH(br) cells) do not contain known HSCs or progenitors. In contrast, highly enriched murine HSCs defined by the CD48(-)EPCR(+) and other phenotypes contain two subpopulations, one that stains dimly with Aldefluor (ALDH(dim)) and one that stains at intermediate levels (ALDH(int)). The CD48(-)EPCR(+)ALDH(dim) cells are virtually all in G(0) and yield high levels of engraftment via both intravenous and intrabone routes. In contrast the CD48(-)EPCR(+)ALDH(int) cells are virtually all in G(1), have little intravenous engraftment potential, and yet can engraft long-term after intrabone transplantation. These data demonstrate that Aldefluor staining of unfractionated murine marrow does not identify known HSCs or progenitors. However, varying levels of Aldefluor staining when combined with CD48

and EPCR detection can identify novel populations in murine marrow including a highly enriched population of resting HSCs and a previously unknown HSC population in G(1) with an intravenous engraftment defect.

Gayraud-Morel, B., et al. (2012). "Myf5 haploinsufficiency reveals distinct cell fate potentials for adult skeletal muscle stem cells." J Cell Sci **125**(Pt 7): 1738-1749.

Skeletal muscle stem cell fate in adult mice is regulated by crucial transcription factors, including the determination genes Myf5 and Myod. The precise role of Myf5 in regulating quiescent muscle stem cells has remained elusive. Here we show that most, but not all, quiescent satellite cells express Myf5 protein, but at varying levels, and that resident Myf5 heterozygous muscle stem cells are more primed for myogenic commitment compared with wild-type satellite cells. Paradoxically however, heterotypic transplantation of Myf5 heterozygous cells into regenerating muscles results in higher self-renewal capacity compared with wild-type stem cells, whereas myofibre regenerative capacity is not altered. By contrast, Pax7 haploinsufficiency does not show major modifications by transcriptome analysis. These observations provide a mechanism linking Myf5 levels to muscle stem cell heterogeneity and fate by exposing two distinct and opposing phenotypes associated with Myf5 haploinsufficiency. These findings have important implications for how stem cell fates can be modulated by crucial transcription factors while generating a pool of responsive heterogeneous cells.

Ghosn, E. E., et al. (2012). "Distinct B-cell lineage commitment distinguishes adult bone marrow hematopoietic stem cells." Proc Natl Acad Sci U S A **109**(14): 5394-5398.

The question of whether a single hematopoietic stem cell (HSC) gives rise to all of the B-cell subsets [B-1a, B-1b, B-2, and marginal zone (MZ) B cells] in the mouse has been discussed for many years without resolution. Studies here finally demonstrate that individual HSCs sorted from adult bone marrow and transferred to lethally irradiated recipients clearly give rise to B-2, MZ B, and B-1b, but does not detectably reconstitute B-1a cells. These findings place B-2, MZ, and B-1b in a single adult developmental lineage and place B-1a in a separate lineage derived from HSCs that are rare or missing in adults. We discuss these findings with respect to known developmental heterogeneity in other HSC-derived lymphoid, myeloid, and erythroid lineages, and how HSC developmental heterogeneity conforms to the layered model of the evolution of the immune system that we proposed some years ago. In addition, of importance to contemporary medicine, we consider the implications that HSC developmental heterogeneity may have for selecting HSC sources for human transplantation.

Gioviale, M. C., et al. (2013). "Beyond islet transplantation in diabetes cell therapy: from embryonic stem cells to transdifferentiation of adult cells." Transplant Proc **45**(5): 2019-2024.

Exogenous insulin is, at the moment, the therapy of choice of diabetes, but does not allow tight regulation of glucose leading to long-term complications. Recently, pancreatic islet transplantation to reconstitute insulin-producing beta cells, has emerged as an alternative promising therapeutic approach. Unfortunately, the number of donor islets is too low compared with the high number of patients needing a transplantation leading to a search for renewable sources of high-quality beta-cells. This review, summarizes more recent promising approaches to the generation of new beta-cells from embryonic stem cells for transdifferentiation of adult cells, particularly a critical examination of the seminal work by Lumelsky et al.

Gong, S. P., et al. (2010). "Embryonic stem cell-like cells established by culture of adult ovarian cells in mice." Fertil Steril **93**(8): 2594-2601, 2601 e2591-2599.

OBJECTIVE: To suggest an alternative strategy for deriving histocompatible stems cells without undertaking genetic manipulation. DESIGN: Prospective approach using an animal model. SETTING: Stem cell and bioevaluation laboratory, Seoul National University. ANIMAL(S): F1 (C57BL6 X DBA2) and outbred (ICR) mice. INTERVENTION(S): Ovarian stroma cells of less than 40 mum in diameter were subcultured with fibroblast monolayer, and colony-forming cells were characterized. MAIN OUTCOME MEASURE(S): Stemness, genotype, and imprinted gene methylation. RESULT(S): Two-lines of colony-forming cells were established, which expressed markers specific for embryonic stem cells (ESC) and formed embryoid bodies and teratomas. Complete matching of microsatellite markers with the cell donor strain confirmed their establishment from ovarian tissue, and identification of both homozygotic and heterozygotic chromosomes raised the possibility of their derivation from parthenogenetic oocytes. However, the use of cells smaller than mature oocytes for primary culture, the difference in imprinted gene methylation compared with parthenogenetic ESCs, and failure to establish the ESC-like cells by primary follicle culture collectively suggested the irrelevancy to gametes. CONCLUSION(S): Coculture of adult ovarian cells with somatic fibroblasts can yield colony-forming cells having ESC-like activity, which may provide an alternative for establishing autologous stem cells from adults that can be obtained without genetic manipulation.

Gothert, J. R., et al. (2005). "In vivo fate-tracing studies using the Scl stem cell enhancer: embryonic hematopoietic stem cells significantly contribute to adult hematopoiesis." Blood **105**(7): 2724-2732.

Evidence for the lineage relationship between embryonic and adult hematopoietic stem cells (HSCs) in the mouse is primarily indirect. In order to study this relationship in a direct manner, we expressed the tamoxifen-inducible Cre-ER(T) recombinase under the control of the stem cell leukemia (Scl) stem-cell enhancer in transgenic mice (HSC-SCL-Cre-ER(T)). To determine functionality, HSC-SCL-Cre-ER(T) transgenics were bred with Cre reporter mice. Flow cytometric and transplantation studies revealed tamoxifen-dependent recombination occurring in more than 90% of adult long-term HSCs, whereas the targeted proportion within mature progenitor populations was significantly lower. Moreover, the transgene was able to irreversibly tag embryonic HSCs on days 10 and 11 of gestation. These cells contributed to bone marrow hematopoiesis 5 months later. In order to investigate whether the de novo HSC generation is completed during embryogenesis, HSC-SCL-Cre-ER(T)-marked fetal liver cells were transplanted into adult recipients. Strikingly, the proportion of marked cells within the transplanted and the in vivo-remaining HSC compartment was not different, implying that no further HSC generation occurred during late fetal and neonatal stages of development. These data demonstrate for the first time the direct lineage relationship between midgestation embryonic and adult HSCs in the mouse. Additionally, the HSC-SCL-Cre-ER(T) mice will provide a valuable tool to achieve temporally controlled genetic manipulation of HSCs.

Goulas, S., et al. (2012). "The Par complex and integrins direct asymmetric cell division in adult intestinal stem cells." Cell Stem Cell **11**(4): 529-540.

The adult Drosophila midgut is maintained by intestinal stem cells (ISCs) that generate both self-renewing and differentiating daughter cells. How this asymmetry is generated is currently unclear. Here, we demonstrate that asymmetric ISC division is established by a unique combination of extracellular and intracellular polarity mechanisms. We show that Integrin-dependent adhesion to the basement membrane induces cell-intrinsic polarity and results in the asymmetric segregation of the Par proteins Par-3, Par-6, and aPKC into the apical daughter cell. Cell-specific knockdown and overexpression experiments suggest that increased activity of aPKC enhances Delta/Notch signaling in one of the two daughter cells to induce terminal differentiation. Perturbing this mechanism or altering the orientation of ISC division results in the formation of intestinal tumors. Our data indicate that mechanisms for intrinsically asymmetric cell division can be adapted to allow for the flexibility in lineage decisions that is required in adult stem cells.

Grandel, H., et al. (2006). "Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate." Dev Biol **295**(1): 263-277.

Lifelong neurogenesis in vertebrates relies on stem cells producing proliferation zones that contain neuronal precursors with distinct fates. Proliferation zones in the adult zebrafish brain are located in distinct regions along its entire anterior-posterior axis. We show a previously unappreciated degree of conservation of brain proliferation patterns among teleosts, suggestive of a teleost ground plan. Pulse chase labeling of proliferating populations reveals a centrifugal movement of cells away from their places of birth into the surrounding mantle zone. We observe tangential migration of cells born in the ventral telencephalon, but only a minor rostral migratory stream to the olfactory bulb. In contrast, the lateral telencephalic area, a domain considered homologous to the mammalian dentate gyrus, shows production of interneurons and migration as in mammals. After a 46-day chase, newborn highly mobile cells have moved into nuclear areas surrounding the proliferation zones. They often show HuC/D immunoreactivity but importantly also more specific neuronal identities as indicated by immunoreactivity for tyrosine hydroxylase, serotonin and parvalbumin. Application of a second proliferation marker allows us to recognize label-retaining, actively cycling cells that remain in the proliferation zones. The latter population meets two key criteria of neural stem cells: label retention and self renewal.

Gregory, C. A., et al. (2003). "The Wnt signaling inhibitor dickkopf-1 is required for reentry into the cell cycle of human adult stem cells from bone marrow." J Biol Chem **278**(30): 28067-28078.

Adult human mesenchymal stem cells from bone marrow stroma (hMSCs) differentiate into numerous mesenchymal tissue lineages and are attractive candidates for cell and gene therapy. When early passage hMSCs are plated or replated at low density, the cultures display a lag phase of 3-5 days, a phase of rapid exponential growth, and then enter a stationary phase without the cultures reaching confluence. We found that as the cultures leave the lag phase, they secrete high levels of dickkopf-1 (Dkk-1), an inhibitor of the canonical Wnt signaling pathway. The addition of recombinant Dkk-1 toward the end of the lag period increased proliferation and decreased the cellular concentration of beta-catenin. The addition of antibodies to Dkk-1 in the early log phase decreased proliferation. Also, expression of Dkk-1 in hMSCs decreased during cell cycle arrest induced by serum starvation. The results indicated that high levels of

Dkk-1 allow the cells to reenter the cell cycle by inhibiting the canonical Wnt/beta-catenin signaling pathway. Since antibodies to Dkk-1 also increased the lag phase of an osteosarcoma line that expressed the gene, Dkk-1 may have a similar role in some other cell systems.

Griffiths, D. S., et al. (2011). "LIF-independent JAK signalling to chromatin in embryonic stem cells uncovered from an adult stem cell disease." Nat Cell Biol **13**(1): 13-21.

Activating mutations in the tyrosine kinase Janus kinase 2 (JAK2) cause myeloproliferative neoplasms, clonal blood stem cell disorders with a propensity for leukaemic transformation. Leukaemia inhibitory factor (LIF) signalling through the JAK-signal transducer and activator of transcription (STAT) pathway enables self-renewal of embryonic stem (ES) cells. Here we show that mouse ES cells carrying the human JAK2V617F mutation were able to self-renew in chemically defined conditions without cytokines or small-molecule inhibitors, independently of JAK signalling through the STAT3 or phosphatidylinositol-3-OH kinase pathways. Phosphorylation of histone H3 tyrosine 41 (H3Y41) by JAK2 was recently shown to interfere with binding of heterochromatin protein 1alpha (HP1alpha). Levels of chromatin-bound HP1alpha were lower in JAK2V617F ES cells but increased following inhibition of JAK2, coincident with a global reduction in histone H3Y41 phosphorylation. JAK2 inhibition reduced levels of the pluripotency regulator Nanog, with a reduction in H3Y41 phosphorylation and concomitant increase in HP1alpha levels at the Nanog promoter. Furthermore, Nanog was required for factor independence of JAK2V617F ES cells. Taken together, these results uncover a previously unrecognized role for direct signalling to chromatin by JAK2 as an important mediator of ES cell self-renewal.

Guo, J., et al. (2013). "The increased number of Leydig cells by di(2-ethylhexyl) phthalate comes from the differentiation of stem cells into Leydig cell lineage in the adult rat testis." Toxicology **306**: 9-15.

The objective of the present study is to determine whether di(2-ethylhexyl) phthalate (DEHP) exposure at adulthood increases rat Leydig cell number and to investigate the possible mechanism. 90-day-old Long-Evans rats were randomly divided into 3 groups, and were gavaged with the corn oil (control) or 10 or 750 mg/kg DEHP daily for 7 days, and then received an intraperitoneal injection of 75 mg/kg ethane dimethanesulfonate (EDS) to eliminate Leydig cells. Serum testosterone concentrations were assessed by RIA, and the mRNA levels of Leydig cell genes were measured by qPCR. EDS eliminated all Leydig cells in the control testis on day 4 post-EDS, as judged by undetectable serum testosterone level and no 3beta-hydroxysteroid dehydrogenase positive (3beta-HSD(pos)) cells in the interstitium. However, in DEHP-treated groups, there were detectable serum testosterone concentrations and some oval-shaped 3beta-HSD(pos) cells in the interstitium. These 3beta-HSD(pos) cells were not stained by the antibody against 11beta-hydroxysteroid dehydrogenase 1 (11beta-HSD1), a marker for Leydig cells at a more advanced stage. The disappearance of mRNAs of Leydig cell biomarkers including Lhcgr, Cyp11a1, Cyp17a1, Insl3 and Hsd11b1 in the control testis was observed on day 4 post-EDS. However, there were detectable concentrations of Lhcgr, Cyp11a1 and Cyp17a1 mRNAs but undetectable concentrations of Insl3, Hsd17b3 and Hsd11b1 in the DEHP-treated testes, indicating that these 3beta-HSD(pos) cells were newly formed progenitor Leydig cells. The mRNA level for nestin (Nes, biomarker for stem Leydig cells) was significantly increased in the control testis on day 4 post-EDS, but not in the DEHP treated testes, suggesting that these nestin positive stem cells were differentiated into progenitor Leydig cells in the DEHP-treated testes. The present study suggests that DEHP increases the differentiation of stem cells into progenitor Leydig cells.

Ha, S., et al. (2017). "Phosphorylation of p62 by AMP-activated protein kinase mediates autophagic cell death in adult hippocampal neural stem cells." J Biol Chem **292**(33): 13795-13808.

In the adult brain, programmed death of neural stem cells is considered to be critical for tissue homeostasis and cognitive function and is dysregulated in neurodegeneration. Previously, we have reported that adult rat hippocampal neural (HCN) stem cells undergo autophagic cell death (ACD) following insulin withdrawal. Because the apoptotic capability of the HCN cells was intact, our findings suggested activation of unique molecular mechanisms linking insulin withdrawal to ACD rather than apoptosis. Here, we report that phosphorylation of autophagy-associated protein p62 by AMP-activated protein kinase (AMPK) drives ACD and mitophagy in HCN cells. Pharmacological inhibition of AMPK or genetic ablation of the AMPK alpha2 subunit by clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 genome editing suppressed ACD, whereas AMPK activation promoted ACD in insulin-deprived HCN cells. We found that following insulin withdrawal AMPK phosphorylated p62 at a novel site, Ser-293/Ser-294 (in rat and human p62, respectively). Phosphorylated p62 translocated to mitochondria and induced mitophagy and ACD. Interestingly, p62 phosphorylation at Ser-293 was not required for staurosporine-induced apoptosis in HCN cells. To the best of our knowledge, this is the first report on the direct phosphorylation of p62 by AMPK. Our data

suggest that AMPK-mediated p62 phosphorylation is an ACD-specific signaling event and provide novel mechanistic insight into the molecular mechanisms in ACD.

Ha, S., et al. (2015). "Regulation of autophagic cell death by glycogen synthase kinase-3beta in adult hippocampal neural stem cells following insulin withdrawal." Mol Brain **8**: 30.

BACKGROUND: Neural stem cells (NSCs) hold great potential for the treatment of neurodegenerative diseases. However, programmed cell death (PCD) provoked by the harsh conditions evident in the diseased brain greatly undermines the potential of NSCs. Currently, the mechanisms of PCD that effect NSCs remain largely unknown. RESULTS: We have previously reported that hippocampal neural stem (HCN) cells derived from the adult rat brain undergo autopahgic cell death (ACD) following insulin withdrawal without hallmarks of apoptosis despite their normal apoptotic capabilities. In this study, we demonstrate that glycogen synthase kinase 3beta (GSK-3beta) induces ACD in insulin-deprived HCN cells. Both pharmacological and genetic inactivation of GSK-3beta significantly decreased ACD, while activation of GSK-3beta increased autophagic flux and caused more cell death without inducing apoptosis following insulin withdrawal. In contrast, knockdown of GSK-3alpha barely affected ACD, lending further support to the critical role of GSK-3beta. CONCLUSION: Collectively, these data demonstrate that GSK-3beta is a key regulator of ACD in HCN cells following insulin withdrawal. The absence of apoptotic indices in GSK-3beta-induced cell death in insulin-deprived HCN cells corroborates the notion that HCN cell death following insulin withdrawal represents the genuine model of ACD in apoptosis-intact mammalian cells and identifies GSK-3beta as a key negative effector of NSC survival downstream of insulin signaling.

Hachem, L. D., et al. (2016). "Glutamate Increases In Vitro Survival and Proliferation and Attenuates Oxidative Stress-Induced Cell Death in Adult Spinal Cord-Derived Neural Stem/Progenitor Cells via Non-NMDA Ionotropic Glutamate Receptors." Stem Cells Dev **25**(16): 1223-1233.

Traumatic spinal cord injury (SCI) leads to a cascade of secondary chemical insults, including oxidative stress and glutamate excitotoxicity, which damage host neurons and glia. Transplantation of exogenous neural stem/progenitor cells (NSPCs) has shown promise in enhancing regeneration after SCI, although survival of transplanted cells remains poor. Understanding the response of NSPCs to the chemical mediators of secondary injury is essential in finding therapies to enhance survival. We examined the in vitro effects of glutamate and glutamate receptor agonists on adult rat spinal cord-derived NSPCs. NSPCs isolated from the periventricular region of the adult rat spinal cord were exposed to various concentrations of glutamate for 96 h. We found that glutamate treatment (500 muM) for 96 h significantly increased live cell numbers, reduced cell death, and increased proliferation, but did not significantly alter cell phenotype. Concurrent glutamate treatment (500 muM) in the setting of H2O2 exposure (500 muM) for 10 h increased NSPC survival compared to H2O2 exposure alone. The effects of glutamate on NSPCs were blocked by the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist GYKI-52466, but not by the N-methyl-D-aspartic acid receptor antagonist MK-801 or DL-AP5, or the mGluR3 antagonist LY-341495. Furthermore, treatment of NSPCs with AMPA, kainic acid, or the kainate receptor-specific agonist (RS)-2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propanoic acid mimicked the responses seen with glutamate both alone and in the setting of oxidative stress. These findings offer important insights into potential mechanisms to enhance NSPC survival and implicate a potential role for glutamate in promoting NSPC survival and proliferation after traumatic SCI.

Hackett, C. H., et al. (2012). "Comparison of gene-specific DNA methylation patterns in equine induced pluripotent stem cell lines with cells derived from equine adult and fetal tissues." Stem Cells Dev **21**(10): 1803-1811.

Cellular pluripotency is associated with expression of the homeobox transcription factor genes NANOG, SOX2, and POU5F1 (OCT3/4 protein). Some reports suggest that mesenchymal progenitor cells (MPCs) may express increased quantities of these genes, creating the possibility that MPCs are more "pluripotent" than other adult cell types. The objective of this study was to determine whether equine bone marrow-derived MPCs had gene expression or DNA methylation patterns that differed from either early fetal-derived or terminally differentiated adult cells. Specifically, this study compared DNA methylation of the NANOG and SOX2 promoter regions and concurrent gene expression of NANOG, SOX2, and POU5F1 in equine induced pluripotent stem (iPS) cells, fetal fibroblasts, fetal brain cells, adult chondrocytes, and MPCs. Results indicate that NANOG and POU5F1 were not detectable in appreciable quantities in tissues other than the equine iPS cell lines. Equine iPS cells expressed large quantities of all three genes examined. Significantly increased quantities of SOX2 were noted in iPS cells and both fetal-derived cell types compared with adult cells. MPCs and adult chondrocytes expressed equivalent, low quantities of SOX2. Further, NANOG and SOX2 expression inversely correlated with the DNA methylation pattern in the promoter

region, such that as gene expression increased, DNA methylation decreased. The equine iPS cell lines examined demonstrated DNA methylation and gene expression patterns that were consistent with pluripotency features described in other species. Results do not support previous reports that NANOG, SOX2, and POU5F1 are poised for increased activity in MPCs compared with other adult cells.

Hafizi, M., et al. (2012). "Exploring the enkephalinergic differentiation potential in adult stem cells for cell therapy and drug screening implications." In Vitro Cell Dev Biol Anim **48**(9): 562-569.

Stem cell therapy is one of the most promising treatments in neuroregenerative medicine. Considering the role of the endogenous opioid system in controlling the pathophysiology of neurological disorders and behavioral aberrations, current studies have focused on enkephalins as a part of the opioid system. Due to high capability of unrestricted somatic stem cells (USSCs) and human mesenchymal stem cells (hMSCs) for cell therapy and transplantation; here, we examined their enkephalinergic differentiation potential through Ikaros-related pathways in order to develop in vitro models to help drug screening and stem cell therapy for the opioid-related disorders. The authenticity of the stem cells was verified by differentiation experiments along with flow cytometry for surface markers. Later, we confirmed their neurogenic differentiation with semiquantitative and quantitative transcriptional and translational evaluations of the enkephalinergic-related genes such as proenkephalin, CREBZF, Ikaros, and prodynorphin. Our findings supported the enkephalinergic differentiation of these stem cells. Noteworthy, USSCs showed higher potential for differentiating into enkephalinergic neurons under Ikaros activation than hMSCs, which makes them appropriate for neurological therapeutic applications. In conclusion, this study suggests a powerful in vitro model for neurogenesis that may help clarification of enkephalinergic differentiation and related signaling networks along with neural drug screening. Such investigations may be beneficial to ameliorate the neural-related therapeutic approaches.

Hallmann, A. L., et al. (2016). "Comparative transcriptome analysis in induced neural stem cells reveals defined neural cell identities in vitro and after transplantation into the adult rodent brain." Stem Cell Res **16**(3): 776-781.

Reprogramming technology enables the production of neural progenitor cells (NPCs) from somatic cells by direct transdifferentiation. However, little is known on how neural programs in these induced neural stem cells (iNSCs) differ from those of alternative stem cell populations in vitro and in vivo. Here, we performed transcriptome analyses on murine iNSCs in comparison to brain-derived neural stem cells (NSCs) and pluripotent stem cell-derived NPCs, which revealed distinct global, neural, metabolic and cell cycle-associated marks in these populations. iNSCs carried a hindbrain/posterior cell identity, which could be shifted towards caudal, partially to rostral but not towards ventral fates in vitro. iNSCs survived after transplantation into the rodent brain and exhibited in vivo-characteristics, neural and metabolic programs similar to transplanted NSCs. However, iNSCs vastly retained caudal identities demonstrating cell-autonomy of regional programs in vivo. These data could have significant implications for a variety of in vitro- and in vivo-applications using iNSCs.

Hamanoue, M., et al. (2016). "Cell-permeable p38 MAP kinase promotes migration of adult neural stem/progenitor cells." Sci Rep **6**: 24279.

Endogenous neural stem/progenitor cells (NPCs) can migrate toward sites of injury, but the migration activity of NPCs is insufficient to regenerate damaged brain tissue. In this study, we showed that p38 MAP kinase (p38) is expressed in doublecortin-positive adult NPCs. Experiments using the p38 inhibitor SB203580 revealed that endogenous p38 participates in NPC migration. To enhance NPC migration, we generated a cell-permeable wild-type p38 protein (PTD-p38WT) in which the HIV protein transduction domain (PTD) was fused to the N-terminus of p38. Treatment with PTD-p38WT significantly promoted the random migration of adult NPCs without affecting cell survival or differentiation; this effect depended on the cell permeability and kinase activity of the fusion protein. These findings indicate that PTD-p38WT is a novel and useful tool for unraveling the roles of p38, and that this protein provides a reasonable approach for regenerating the injured brain by enhancing NPC migration.

Hamazaki, Y. (2015). "Adult thymic epithelial cell (TEC) progenitors and TEC stem cells: Models and mechanisms for TEC development and maintenance." Eur J Immunol **45**(11): 2985-2993.

The thymus is the primary lymphoid organ for generating self-restricted and self-tolerant functional T cells. Its two distinct anatomical regions, the cortex and the medulla, are involved in positive and negative selection, respectively. Thymic epithelial cells (TECs) constitute the framework of this tissue and function as major stromal components. Extensive studies for more than a decade have revealed how TECs are generated during organogenesis; progenitors specific for medullary TECs (mTECs) and cortical TECs (cTECs) as well as bipotent progenitors for both lineages have been identified, and the signaling pathways required for the development and maturation of mTECs have been elucidated. However, little is known about the initial commitment of mTECs and cTECs during ontogeny, and how regeneration of both lineages is sustained in

the postnatal/adult thymus. Recently, stem cell activities in TECs have been demonstrated, and TEC progenitors have been identified in the postnatal thymus. In this review, recent advances in studying the development and maintenance of TECs are summarized, and the possible mechanisms of thymic regeneration and involution are discussed.

Han, J. W., et al. (2013). "Cell therapy for diabetic neuropathy using adult stem or progenitor cells." Diabetes Metab J **37**(2): 91-105.

Diabetic neuropathy (DN) is the most common and disabling complication of diabetes that may lead to foot ulcers and limb amputations. Despite widespread awareness of DN, the only effective treatments are glucose control and pain management. A growing body of evidence suggests that DN is characterized by reduction of vascularity in peripheral nerves and deficiency in neurotrophic and angiogenic factors. Previous studies have tried to introduce neurotrophic or angiogenic factors in the form of protein or gene for therapy, but the effect was not significant. Recent studies have shown that bone marrow (BM)-derived stem or progenitor cells have favorable effects on the repair of cardiovascular diseases. Since these BM-derived stem or progenitor cells contain various angiogenic and neurotrophic factors, these cells have been attempted for treating experimental DN, and turned out to be effective for reversing various manifestations of experimental DN. These evidences suggest that cell therapy, affecting both vascular and neural components, can represent a novel therapeutic option for treatment of clinical DN.

Harari-Steinberg, O., et al. (2011). "Selecting the optimal cell for kidney regeneration: fetal, adult or reprogrammed stem cells." Organogenesis **7**(2): 123-134.

Chronic kidney disease (CKD) is a progressive loss in renal function over a period of months or years. End-stage renal disease (ESRD) or stage 5 CKD ensues when renal function deteriorates to under 15% of the normal range. ESRD requires either dialysis or, preferentially, a kidney organ allograft, which is severely limited due to organ shortage for transplantation. To combat this situation, one needs to either increase supply of organs or decrease their demand. Two strategies therefore exist: for those that have completely lost their kidney function (ESRD), we will need to supply new kidneys. Taking into account the kidneys' extremely complex structure, this may prove to be impossible in the near future. In contrast, for those patients that are in the slow progression route from CKD to ESRD but still have functional kidneys, we might be able to halt progression by introducing stem cell therapy to diseased kidneys to rejuvenate or regenerate individual cell types. Multiple cell compartments that fall into three categories are likely to be worthy targets for cell repair: vessels, stroma (interstitium) and nephron epithelia. Different stem/progenitor cells can be linked to regeneration of specific cell types; hematopoietic progenitors and hemangioblastic cell types have specific effects on the vascular niche (vasculogenesis and angiogenesis). Multipotent stromal cells (MSC), whether derived from the bone marrow or isolated from the kidney's non-tubular compartment, may, in turn, heal nephron epithelia via paracrine mechanisms. Nevertheless, as we now know that all of the above lack nephrogenic potential, we should continue our quest to derive genuine nephron (epithelial) progenitors from differentiated pluripotent stem cells, from fetal and adult kidneys and from directly reprogrammed somatic cells.

Haridass, D., et al. (2009). "Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice." Am J Pathol **175**(4): 1483-1492.

Fetal liver progenitor cell suspensions (FLPC) and hepatic precursor cells derived from embryonic stem cells (ES-HPC) represent a potential source for liver cell therapy. However, the relative capacity of these cell types to engraft and repopulate a recipient liver compared with adult hepatocytes (HC) has not been comprehensively assessed. We transplanted mouse and human HC, FLPC, and ES-HPC into a new immunodeficient mouse strain (Alb-uPA(tg(+/-))Rag2(-/-)gamma(c)(-/-) mice) and estimated the percentages of HC after 3 months. Adult mouse HC repopulated approximately half of the liver mass (46.6 +/- 8.0%, 1 x 10(6) transplanted cells), whereas mouse FLPC derived from day 13.5 and 11.5 post conception embryos generated only 12.1 +/- 3.0% and 5.1 +/- 1.1%, respectively, of the recipient liver and smaller cell clusters. Adult human HC and FLPC generated overall less liver tissue than mouse cells and repopulated 10.0 +/- 3.9% and 2.7 +/- 1.1% of the recipient livers, respectively. Mouse and human ES-HPC did not generate HC clusters in our animal model. We conclude that, in contrast to expectations, adult HC of human and mouse origin generate liver tissue more efficiently than cells derived from fetal tissue or embryonic stem cells in a highly immunodeficient Alb-uPA transgenic mouse model system. These results have important implications in the context of selecting the optimal strategy for human liver cell therapies.

Hasebe, T., et al. (2013). "Thyroid hormone-induced cell-cell interactions are required for the development of adult intestinal stem cells." Cell Biosci **3**(1): 18.

The mammalian intestine has long been used as a model to study organ-specific adult stem cells, which are essential for organ repair and tissue regeneration throughout adult life. The establishment

of the intestinal epithelial cell self-renewing system takes place during perinatal development when the villus-crypt axis is established with the adult stem cells localized in the crypt. This developmental period is characterized by high levels of plasma thyroid hormone (T3) and T3 deficiency is known to impair intestinal development. Determining how T3 regulates adult stem cell development in the mammalian intestine can be difficult due to maternal influences. Intestinal remodeling during amphibian metamorphosis resembles perinatal intestinal maturation in mammals and its dependence on T3 is well established. A major advantage of the amphibian model is that it can easily be controlled by altering the availability of T3. The ability to manipulate and examine this relatively rapid and localized formation of adult stem cells has greatly assisted in the elucidation of molecular mechanisms regulating their formation and further revealed evidence that supports conservation in the underlying mechanisms of adult stem cell development in vertebrates. Furthermore, genetic studies in Xenopus laevis indicate that T3 actions in both the epithelium and the rest of the intestine, most likely the underlying connective tissue, are required for the formation of adult stem cells. Molecular analyses suggest that cell-cell interactions involving hedgehog and BMP pathways are critical for the establishment of the stem cell niche that is essential for the formation of the adult intestinal stem cells.

Hassan, N. T. and N. A. AbdelAziz (2018). "Oral Mucosal Stem Cells, Human Immature Dental Pulp Stem Cells and Hair Follicle Bulge Stem Cells as Adult Stem Cells Able to Correct Limbal Stem Cell Deficiency." Curr Stem Cell Res Ther **13**(5): 356-361.

BACKGROUND: Adult stem cells are somatic stem cells distributed all over the body. They represent a promising future for regenerative medicine because of their multiple advantages as they are widely available, accessible, easily stored and manipulated to a wide range of cells and with minimal invasive extraction. OBJECTIVE: This review describes three examples of adult stem cells: oral mucosal epithelial stem cells, human immature dental pulp stem cells and hair follicle bulge stem cells that show an ability to correct limbal stem cell deficiency, their isolation and cultivation methods, feeder layers, carriers, markers expressed, successfulness to regenerate the ocular surface and mimic the corneal function in LSCD. CONCLUSION: Although hair follicle bulge stem cells and human immature dental pulp stem cells are a promising adult stem cell source to correct limbal stem cell deficiency, but the published research evaluating this ability is limited. Therefore, this article emphasize further research in this area.

Hausburg, F., et al. (2018). "Protocol for MicroRNA Transfer into Adult Bone Marrow-derived Hematopoietic Stem Cells to Enable Cell Engineering Combined with Magnetic Targeting." J Vis Exp(136).

While CD133(+) hematopoietic stem cells (SCs) have been proven to provide high potential in the field of regenerative medicine, their low retention rates after injection into injured tissues as well as the observed massive cell death rates lead to very restricted therapeutic effects. To overcome these limitations, we sought to establish a non-viral based protocol for suitable cell engineering prior to their administration. The modification of human CD133(+) expressing SCs using microRNA (miR) loaded magnetic polyplexes was addressed with respect to uptake efficiency and safety as well as the targeting potential of the cells. Relying on our protocol, we can achieve high miR uptake rates of 80-90% while the CD133(+) stem cell properties remain unaffected. Moreover, these modified cells offer the option of magnetic targeting. We describe here a safe and highly efficient procedure for the modification of CD133(+) SCs. We expect this approach to provide a standard technology for optimization of therapeutic stem cell effects and for monitoring of the administered cell product via magnetic resonance imaging (MRI).

Henriksson, H. B., et al. (2012). "Support of concept that migrating progenitor cells from stem cell niches contribute to normal regeneration of the adult mammal intervertebral disc: a descriptive study in the New Zealand white rabbit." Spine (Phila Pa 1976) **37**(9): 722-732.

STUDY DESIGN: Descriptive experimental study performed in rabbits of 2 age groups. OBJECTIVE: To study and investigate presence of prechondrocytic cells and cell migration routes (MR) in the intervertebral disc (IVD) region to gain knowledge about the normal IVD regeneration pattern. SUMMARY OF BACKGROUND DATA: Disc degeneration is thought to play a major role in patients with chronic lumbar pain. Regeneration processes and cell migration within the IVD have been sparsely described. Therefore, it is of interest to increase knowledge of these processes in order to understand pathological conditions of the IVD. METHODS: At the beginning of the experiment, 5-bromo-2-deoxyuridine (BrdU) in vivo labeling was performed in 2 groups of rabbits, 3 and 9 months old (total 27 rabbits). BrdU is incorporated into DNA during mitosis, and then it is gradually diluted with each cell division until it finally disappears. Incorporation of BrdU was then visualized by immunohistochemistry (IHC) at different time points providing cell division pattern and presence of slow-cycling cells in the IVD region. IVD tissue was investigated by IHC for growth and differentiation factor-5 (GDF5), SOX9 (chondrogenic lineage markers), SNAIL homolog 1 (SNAI1), SNAIL homolog 2 (SLUG) (migration markers), and beta1-

INTEGRIN (cellular adhesion marker). In addition, GDF5, SOX9, and BMPRIB expression were investigated on genetic level. RESULTS: BrdU cells were observed in early time points in the IVD niche, adjacent to the epiphyseal plate, at later time points mainly in outer region of the annulus fibrosus for both age groups of rabbits, indicating a gradual migration of cells. The presence of SLUG, SNAI1, GDF5, SOX9, and beta1-INTEGRIN was found in same regions. CONCLUSION: The results suggest a cellular MR from the IVD stem cell niche toward the annulus fibrosus and the inner parts of the IVD. These findings may be of importance for understanding IVD regenerative mechanisms and for future development of biological treatment strategies.

Hermann, A., et al. (2004). "Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells." J Cell Sci **117**(Pt 19): 4411-4422.

Clonogenic neural stem cells (NSCs) are self-renewing cells that maintain the capacity to differentiate into brain-specific cell types, and may also replace or repair diseased brain tissue. NSCs can be directly isolated from fetal or adult nervous tissue, or derived from embryonic stem cells. Here, we describe the efficient conversion of human adult bone marrow stromal cells (hMSC) into a neural stem cell-like population (hmNSC, for human marrow-derived NSC-like cells). These cells grow in neurosphere-like structures, express high levels of early neuroectodermal markers, such as the proneural genes NeuroD1, Neurog2, MSl1 as well as otx1 and nestin, but lose the characteristics of mesodermal stromal cells. In the presence of selected growth factors, hmNSCs can be differentiated into the three main neural phenotypes: astroglia, oligodendroglia and neurons. Clonal analysis demonstrates that individual hmNSCs are multipotent and retain the capacity to generate both glia and neurons. Our cell culture system provides a powerful tool for investigating the molecular mechanisms of neural differentiation in adult human NSCs. hmNSCs may therefore ultimately help to treat acute and chronic neurodegenerative diseases.

Hishizawa, M., et al. (2010). "Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study." Blood **116**(8): 1369-1376.

Allogeneic hematopoietic stem cell transplantation (HSCT) is increasingly used as a curative option for adult T-cell leukemia (ATL), an intractable mature T-cell neoplasm causally linked with human T-cell leukemia virus type I (HTLV-I). We compared outcomes of 386 patients with ATL who underwent allogeneic HSCT using different graft sources: 154 received human leukocyte antigen (HLA)-matched related marrow or peripheral blood; 43 received HLA-mismatched related marrow or peripheral blood; 99 received unrelated marrow; 90 received single unit unrelated cord blood. After a median follow-up of 41 months (range, 1.5-102), 3-year overall survival for entire cohort was 33% (95% confidence interval, 28%-38%). Multivariable analysis revealed 4 recipient factors significantly associated with lower survival rates: older age (> 50 years), male sex, status other than complete remission, and use of unrelated cord blood compared with use of HLA-matched related grafts. Treatment-related mortality rate was higher among patients given cord blood transplants; disease-associated mortality was higher among male recipients or those given transplants not in remission. Among patients who received related transplants, donor HTLV-I seropositivity adversely affected disease-associated mortality. In conclusion, allogeneic HSCT using currently available graft source is an effective treatment in selected patients with ATL, although greater effort is warranted to reduce treatment-related mortality.

Hussenet, T., et al. (2010). "An adult tissue-specific stem cell molecular phenotype is activated in epithelial cancer stem cells and correlated to patient outcome." Cell Cycle **9**(2): 321-327.

Recent studies have shown that embryonic stem cell-like molecular phenotypes are commonly activated in human epithelial primary tumors and are linked to adverse patient prognosis.(1,2) However it remains unclear whether these correlations to outcome are linked to the differentiation status of the human primary tumors(1) or represent molecular reminiscences of epithelial cancer stem cells.(2) In addition, while it has been demonstrated that leukemic cancer stem cells re-acquire an embryonic stem cell-like phenotype,(3,4) the molecular basis of stem cell function in epithelial cancer stem cells has not been investigated. Here we show that a normal adult tissue-specific stem cell molecular phenotype is commonly activated in epithelial cancer stem cells and for the first time provide evidence that enrichment in cancer stem cells-specific molecular signatures are correlated to highly aggressive tumor phenotypes in human epithelial cancers.

Iguchi, H., et al. (2011). "cAMP response element-binding protein (CREB) is required for epidermal growth factor (EGF)-induced cell proliferation and serum response element activation in neural stem cells isolated from the forebrain subventricular zone of adult mice." Endocr J **58**(9): 747-759.

Neurogenesis, which occurs not only in the developing brain but also in restricted regions in the adult brain including the forebrain subventricular zone (SVZ), is regulated by a variety of environmental factors, extracellular signals, and intracellular signal transduction pathways. We investigated whether the

transcription factor cAMP response element (CRE)-binding protein (CREB) is involved in the regulation of cell proliferation of neural stem cells (NSCs) isolated from the SVZ of adult mice. Treatment of NSCs with the protein kinase A (PKA) inhibitors H89 and KT5720 inhibited epidermal growth factor (EGF)-stimulated NSC proliferation. Similar inhibition was observed when a dominant-negative mutant of CREB (MCREB) was expressed. EGF treatment increased CRE-mediated transcriptional activity, but this increase was much less than that caused by treatment with the adenylate cyclase activator forskolin, which changed neither basal nor EGF-stimulated proliferation of NSCs. Neither PKA inhibitors nor MCREB expression blocked EGF-induced phosphorylation of extracellular signal-regulated kinase (ERK), a protein kinase mediating EGF's mitogenic action. However, MCREB suppressed EGF-induced expression of several immediately early genes including c-fos, c-jun, jun-B, and fra-1 and subsequent AP-1 transcriptional activation. MCREB expression also inhibited the ability of EGF to stimulate transcriptional activation mediated by the serum response element (SRE), a promoter sequence regulating c-fos gene expression. These results suggest that basal activity of CREB is required for the mitogenic signaling of EGF in NSCs at a level between ERK activation and SRE-mediated transcriptional activation.

Jeong, J. H. (2008). "Adipose stem cells as a clinically available and effective source of adult stem cell therapy." Int J Stem Cells **1**(1): 43-48.

The greatest advantage of adipose-derived stem cells (ASCs) over other types of adult stem cells is its large number when we harvest primarily. The number of ASCs within adipose tissue reaches more than hundreds of times compared with BMSCs contained in the same amount of bone marrow. The major role of 'regenerative medicine' in 21st century is based on cell therapy and ASC is going to take the core position. It is important to know the characteristics of ASCs for successful clinical application. There are several unique features of ASCs which is known common characteristic of mesenchymal stem cells (MSCs). Cellular plasticity is one of the most important features of ASCs as in other adult stem cells and the cells also have a special function of immune modulation and immunosuppression. Strong angiogenic potential is another important nature of ASCs. In many reports, ASCs are known not only to be differentiated into osteoblasts, chondrocytes, vascular endothelial cells, but also to be cardiomyocytes and neuronal cells. In conclusion, the new knowledge of ASCs is going to impact on the regenerative medicine. To take the advantage of this new type of cells and utilize the cells, we need to understand the function of ASCs and future possibilities of ASCs. We plastic surgeons continue to stimulate the our curiosity and creativity, as well as our clinical inspiration.

Jung, Y. H., et al. (2010). "Glial cell line-derived neurotrophic factor alters the growth characteristics and genomic imprinting of mouse multipotent adult germline stem cells." Exp Cell Res **316**(5): 747-761.

This study evaluated the essentiality of glial cell line-derived neurotrophic factor (GDNF) for in vitro culture of established mouse multipotent adult germline stem (maGS) cell lines by culturing them in the presence of GDNF, leukemia inhibitory factor (LIF) or both. We show that, in the absence of LIF, GDNF slows the proliferation of maGS cells and result in smaller sized colonies without any change in distribution of cells to different cell-cycle stages, expression of pluripotency genes and in vitro differentiation potential. Furthermore, in the absence of LIF, GDNF increased the expression of male germ-line genes and repopulated the empty seminiferous tubule of W/W(v) mutant mouse without the formation of teratoma. GDNF also altered the genomic imprinting of Igf2, Peg1, and H19 genes but had no effect on DNA methylation of Oct4, Nanog and Stra8 genes. However, these effects of GDNF were masked in the presence of LIF. GDNF also did not interfere with the multipotency of maGS cells if they are cultured in the presence of LIF. In conclusion, our results suggest that, in the absence of LIF, GDNF alters the growth characteristics of maGS cells and partially impart them some of the germline stem (GS) cell-like characteristics.

Kamat, V., et al. (2014). "MicroRNA screen of human embryonic stem cell differentiation reveals miR-105 as an enhancer of megakaryopoiesis from adult CD34+ cells." Stem Cells **32**(5): 1337-1346.

MicroRNAs (miRNAs) can control stem cell differentiation by targeting mRNAs. Using 96-well plate electroporation, we screened 466 human miRNA mimics by four-color flow cytometry to explore differentiation of common myeloid progenitors (CMP) derived from human embryonic stem cells (hESCs). The transfected cells were then cultured in a cytokine cocktail that supported multiple hematopoietic lineages. At 4-5 days post-transfection, flow cytometry of erythroid (CD235(+)CD41(-)), megakaryocyte (CD41(+)CD42(+)), and myeloid (CD18(+)CD235(-)) lineages revealed miR-105 as a novel enhancer of megakaryocyte production during in vitro primitive hematopoiesis. In hESC-derived CMPs, miR-105 caused a sixfold enhancement in megakaryocyte production. miR-513a, miR-571, and miR-195 were found to be less potent megakaryocyte enhancers. We confirmed the relevance of miR-105 in adult megakaryopoiesis by demonstrating increased megakaryocyte yield and megakaryocyte colony forming potential in human adult CD34(+) cells derived from peripheral blood. In addition, adult

CD34(+) cells express endogenous miR-105 during megakaryocyte differentiation. siRNA knockdown of the hematopoietic transcription factor c-Myb caused a similar enhancement of megakaryocyte production as miR-105. Finally, a luciferase/c-Myb-3'UTR construct and Western blot analysis demonstrated that the hematopoietic transcription factor c-Myb mRNA was a target of miR-105. We report a novel hESC-based miR screening platform and demonstrate that miR-105 is an enhancer of megakaryopoiesis in both primitive and definitive hematopoiesis.

Kaushik, A. and D. Bhartiya (2018). "Pluripotent Very Small Embryonic-Like Stem Cells in Adult Testes - An Alternate Premise to Explain Testicular Germ Cell Tumors." Stem Cell Rev.

Developmental exposure to endocrine disruptors has resulted in the increased incidence of infertility and testicular germ cell tumors (T2GCT) in young men residing in developed countries. Unlike T1GCT (infants and young children) and T3GCT (aged men), T2GCT arise from CIS/GCNIS that develops from pre-CIS. Pre-CIS represents undifferentiated, growth-arrested gonocytes that persist in fetal testes due to endocrine disruption. However, whether pre-CIS truly exist, do CIS develop into T2GCT, why no CIS in T1GCT/T3GCT, why germ cell tumors (GCT) also occur along midline at extra-gonadal sites, why T1GCT show partial erasure and T2GCT show complete erasure of genomic imprints are open questions that are awaiting answers. We propose that rather than pre-CIS, pluripotent, very small embryonic-like stem cells (VSELs) get affected by exposure to endocrine disruption. Since VSELs are developmentally equivalent to primordial germ cells (PGCs), T2GCT cells show complete erasure of genomic imprints and CIS represents growth-arrested clonally expanding stem/progenitor cells. PGCs/VSELs migrate along the midline to various organs and this explains why GCT occur along the midline, T1GCT show partial erasure of imprints as they develop from migrating PGCs. T3GCT possibly reflects effects of aging due to compromised differentiation and expansion of pre-meiotic spermatocytes. Absent spermatogenesis in pre-pubertal and aged testes explains absence of CIS in T1GCT and T3GCT. Endocrine disruptors possibly alter epigenetic state of VSELs and thus rather than maintaining normal tissue homeostasis, VSELs undergo increased proliferation and compromised differentiation resulting in reduced sperm count, infertility and TGCT. This newly emerging understanding offers alternate premise to explain TGCT and warrants further exploration.

Kawase, Y., et al. (2004). "Characterization of multipotent adult stem cells from the skin: transforming growth factor-beta (TGF-beta) facilitates cell growth." Exp Cell Res **295**(1): 194-203.

Recently, adult stem cells have been isolated from the skin and designated as skin-derived precursors (SKPs). These SKPs, cultured in vitro, can give rise to neurons, glia, smooth muscle cells, and adipocytes. In the current study, we confirmed the clonal expansion of SKPs using a sphere-forming culture system in a medium containing methylcellulose. Among the growth factors, only transforming growth factor-beta (TGF-beta) was revealed to uniquely facilitate the sphere formation and proliferation of the SKPs in combination with EGF and bFGF. In addition, TGF-beta did not alter phenotypical characteristics of the SKPs under sphere-forming conditions. The effect of TGF-beta on sphere formation was not observed in neural stem cells, which expressed a different set of cell surface markers from SKPs, suggesting that SKPs have distinct features. Although the number of SKPs decreased with age, TGF-beta increased the sphere colony formation and proliferation in all ages. These results suggest that SKPs maintained in the presence of TGF-beta during culture are of potential use in cell-replacement therapies employing adult tissue sources.

Kayo, H., et al. (2007). "Stem cell properties and the side population cells as a target for interferon-alpha in adult T-cell leukemia/lymphoma." Biochem Biophys Res Commun **364**(4): 808-814.

The cancer stem cell theory suggests that chemoresistance and recurrence of tumors are often due to the similarity of stem cell properties between normal and cancer cells. Adult T-cell leukemia/lymphoma (ATLL) has poor prognosis, suggesting that ATLL cells possess common stem cell properties. We analyzed side population (SP), a characteristic stem cell phenotype, and CD markers in ATLL cell lines. We found that several lines contained SP with expressions of some hematopoietic stem cell markers. On the other hand, treatment with interferon (IFN)-alpha is sometimes effective in ATLL, particularly combined with other drugs. We examined its effect on ATLL cells and found that IFN-alpha significantly reduced the SP proportion. Moreover, CD25-positive cells and phosphorylation of STAT1/5 and ERK were upregulated during this process. These data suggest that their stem cell properties render ATLL cells therapy-resistant, and IFN-alpha exerts its clinical effect through a reduction of the SP cell population.

Kelly, A. M., et al. (2016). "Glucocorticoid Cell Priming Enhances Transfection Outcomes in Adult Human Mesenchymal Stem Cells." Mol Ther **24**(2): 331-341.

Human mesenchymal stem cells (hMSCs) are one of the most widely researched stem cell types with broad applications from basic research to therapeutics, the majority of which require introduction of exogenous DNA. However, safety and scalability

issues hinder viral delivery, while poor efficiency hinders nonviral gene delivery, particularly to hMSCs. Here, we present the use of a pharmacologic agent (glucocorticoid) to overcome barriers to hMSC DNA transfer to enhance transfection using three common nonviral vectors. Glucocorticoid priming significantly enhances transfection in hMSCs, demonstrated by a 3-fold increase in efficiency, 4-15-fold increase in transgene expression, and prolonged transgene expression when compared to transfection without glucocorticoids. These effects are dependent on glucocorticoid receptor binding and caused in part by maintenance of normal metabolic function and increased cellular (5-fold) and nuclear (6-10-fold) DNA uptake over hMSCs transfected without glucocorticoids. Results were consistent across five human donors and in cells up to passage five. Glucocorticoid cell priming is a simple and effective technique to significantly enhance nonviral transfection of hMSCs that should enhance their clinical use, accelerate new research, and decrease reliance on early passage cells.

Kelsh, R. N., et al. (2017). "Zebrafish adult pigment stem cells are multipotent and form pigment cells by a progressive fate restriction process: Clonal analysis identifies shared origin of all pigment cell types." Bioessays **39**(3).

Skin pigment pattern formation is a paradigmatic example of pattern formation. In zebrafish, the adult body stripes are generated by coordinated rearrangement of three distinct pigment cell-types, black melanocytes, shiny iridophores and yellow xanthophores. A stem cell origin of melanocytes and iridophores has been proposed although the potency of those stem cells has remained unclear. Xanthophores, however, seemed to originate predominantly from proliferation of embryonic xanthophores. Now, data from Singh et al. shows that all three cell-types derive from shared stem cells, and that these cells generate peripheral neural cell-types too. Furthermore, clonal compositions are best explained by a progressive fate restriction model generating the individual cell-types. The numbers of adult pigment stem cells associated with the dorsal root ganglia remain low, but progenitor numbers increase significantly during larval development up to metamorphosis, likely via production of partially restricted progenitors on the spinal nerves.

Kempermann, G., et al. (1998). "[New nerve cells for the adult brain. Adult neurogenesis and stem cell concepts in neurologic research]." Nervenarzt **69**(10): 851-857.

A growing branch of neuroscience is investigating conditions that permit neurogenesis in the adult brain. Partially, this aims at using the neuroectodermal stem or precursor cells that persist in the adult brain to induce neuroregenerative processes in the treatment for neurologic disorders. In ex vivo approaches, isolated precursor cells are implanted into the host brain, while in vivo concepts favor a stimulation of precursor cells in situ.

Kennedy, E., et al. (2014). "Adult vascular smooth muscle cells in culture express neural stem cell markers typical of resident multipotent vascular stem cells." Cell Tissue Res **358**(1): 203-216.

Differentiation of resident multipotent vascular stem cells (MVSCs) or de-differentiation of vascular smooth muscle cells (vSMCs) might be responsible for the SMC phenotype that plays a major role in vascular diseases such as arteriosclerosis and restenosis. We examined vSMCs from three different species (rat, murine and bovine) to establish whether they exhibit neural stem cell characteristics typical of MVSCs. We determined their SMC differentiation, neural stem cell marker expression and multipotency following induction in vitro by using immunocytochemistry, confocal microscopy, fluorescence-activated cell sorting analysis and quantitative real-time polymerase chain reaction. MVSCs isolated from rat aortic explants, enzymatically dispersed rat SMCs and rat bone-marrow-derived mesenchymal stem cells served as controls. Murine carotid artery lysates and primary rat aortic vSMCs were both myosin-heavy-chain-positive but weakly expressed the neural crest stem cell marker, Sox10. Each vSMC line examined expressed SMC differentiation markers (smooth muscle alpha-actin, myosin heavy chain and calponin), neural crest stem cell markers (Sox10(+), Sox17(+)) and a glia marker (S100beta(+)). Serum deprivation significantly increased calponin and myosin heavy chain expression and decreased stem cell marker expression, when compared with serum-rich conditions. vSMCs did not differentiate to adipocytes or osteoblasts following adipogenic or osteogenic inductive stimulation, respectively, or respond to transforming growth factor-beta1 or Notch following gamma-secretase inhibition. Thus, vascular SMCs in culture express neural stem cell markers typical of MVSCs, concomitant with SMC differentiation markers, but do not retain their multipotency. The ultimate origin of these cells might have important implications for their use in investigations of vascular proliferative disease in vitro.

Khuu, D. N., et al. (2007). "Epithelial cells with hepatobiliary phenotype: is it another stem cell candidate for healthy adult human liver?" World J Gastroenterol **13**(10): 1554-1560.

AIM: To investigate the presence and role of liver epithelial cells in the healthy human adult liver. METHODS: Fifteen days after human hepatocyte primary culture, epithelial like cells emerged and started proliferating. Cell colonies were isolated and

subcultured for more than 160 d under specific culture conditions. Cells were analyzed for each passage using immunofluorescence, flow cytometry and reverse transcription-polymerase chain reaction (RT-PCR). RESULTS: Flow cytometry analysis demonstrated that liver epithelial cells expressed common markers for hepatic and stem cells such as CD90, CD44 and CD29 but were negative for CD34 and CD117. Using immunofluorescence we demonstrated that liver epithelial cells expressed not only immature (alpha-fetoprotein) but also differentiated hepatocyte (albumin and CK-18) and biliary markers (CK-7 and 19), whereas they were negative for OV-6. RT-PCR analysis confirmed immunofluorescence data and revealed that liver epithelial cells did not express mature hepatocyte markers such as CYP2B6, CYP3A4 and tyrosine amino-transferase. Purified liver epithelial cells were transplanted into SCID mice. One month after transplantation, albumin positive cell foci were detected in the recipient mouse parenchyma. CONCLUSION: According to their immature and bipotential phenotype, liver epithelial cells might represent a pool of precursors in the healthy human adult liver other than oval cells.

Kilcoyne, K. R., et al. (2014). "Fetal programming of adult Leydig cell function by androgenic effects on stem/progenitor cells." Proc Natl Acad Sci U S A **111**(18): E1924-1932.

Fetal growth plays a role in programming of adult cardiometabolic disorders, which in men, are associated with lowered testosterone levels. Fetal growth and fetal androgen exposure can also predetermine testosterone levels in men, although how is unknown, because the adult Leydig cells (ALCs) that produce testosterone do not differentiate until puberty. To explain this conundrum, we hypothesized that stem cells for ALCs must be present in the fetal testis and might be susceptible to programming by fetal androgen exposure during masculinization. To address this hypothesis, we used ALC ablation/regeneration to identify that, in rats, ALCs derive from stem/progenitor cells that express chicken ovalbumin upstream promoter transcription factor II. These stem cells are abundant in the fetal testis of humans and rodents, and lineage tracing in mice shows that they develop into ALCs. The stem cells also express androgen receptors (ARs). Reduction in fetal androgen action through AR KO in mice or dibutyl phthalate (DBP) -induced reduction in intratesticular testosterone in rats reduced ALC stem cell number by approximately 40% at birth to adulthood and induced compensated ALC failure (low/normal testosterone and elevated luteinizing hormone). In DBP-exposed males, this failure was probably explained by reduced testicular steroidogenic acute regulatory protein expression, which is associated with increased histone methylation (H3K27me3) in the proximal promoter. Accordingly, ALCs and ALC stem cells immunoexpressed increased H3K27me3, a change that was also evident in ALC stem cells in fetal testes. These studies highlight how a key component of male reproductive development can fundamentally reprogram adult hormone production (through an epigenetic change), which might affect lifetime disease risk.

Kim, K., et al. (2008). "Adult stem cell-like tubular cells reside in the corticomedullary junction of the kidney." Int J Clin Exp Pathol **1**(3): 232-241.

The existence of adult renal stem cells has long been suspected because the kidney is capable of regeneration in response to injury, such as acute tubular necrosis (ATN), but their location, or niche, has not been fully defined yet. The aim of this study was to identify the niche of adult renal stem cells responsible for the tubular regeneration. The location of label-retaining cells (LRCs) was studied in adult mouse kidneys after administration of a pulse of bromodeoxyuridine (BrdU) during embryonic period. To study regional participation in renal tubular regeneration, the expression of the proliferation marker Ki-67 was examined after induction of unilateral ATN in mouse kidneys. Regional colony-forming capacity was examined using cultured cells derived from normal mouse and human kidneys and their multipotency was examined in human kidneys. LRCs in adult mouse kidneys were mostly tubular epithelial cells and concentrated constantly in the outer stripe of the corticomedullary junction (CMJ). In the ATN model, Ki-67 positive cells were concentrated in the tubular epithelial cells of the outer stripe, not only in the ATN kidneys but also in the contralateral non-ATN kidneys. High colony-forming capacity was noted in the CMJ of mouse and human kidneys. Cultured cells derived from a single human CMJ cell revealed multipotency, differentiating not only into tubular cells but also into glomerular podocytes. These results demonstrate that the CMJ of the kidney contains label-retaining, renal-repairing, highly colony-forming multipotent stem cell-like tubular cells, suggesting the CMJ as the niche of adult renal stem cells.

Kimura, H., et al. (2005). "Transplantation of embryonic stem cell-derived neural stem cells for spinal cord injury in adult mice." Neurol Res **27**(8): 812-819.

AIMS: To investigate the efficacy of embryonic stem cell-derived neural stem cells (NSCs) for spinal cord injury (SCI) in mice and whether a combination treatment with thyroid hormone provides a more effective ES cell-based therapy. METHODS: Nestin-positive NSCs were induced from undifferentiated mouse ES cells by a step-by-step culture and used as grafts. Thirty-six mice were subjected to an SCI at Th10 and divided into three

groups of 12. Graft cells were transplanted into the injury site 10 days after injury. Group 1 mice were left under observation without receiving graft cells, while mice in Group 2 received 2 x 104 graft cells, and those in Group 3 received 2 x 104 graft cells and were treated with a continuous intraperitoneal injection of thyroxin using osmotic mini-pumps. Behavioral improvement was assessed by a scoring system throughout the experimental period until post-transplantation day (PD) 28. RESULTS: Mice in Groups 2 and 3 demonstrated an improved behavioral function, as compared to those in Group 1 after PD 14. There was no significant difference in behavioral recovery between Groups 2 and 3. CONCLUSIONS: Transplantation of ES-NSCs into the injury site was effective for SCI, while thyroxine did not deliver additional effectiveness.

Kishigami, S., et al. (2006). "Cloned mice and embryonic stem cell establishment from adult somatic cells." Hum Cell **19**(1): 2-10.

Cloning methods are now well described and becoming routine. Yet the frequency at which cloned offspring are produced remains below 2% irrespective of nucleus donor species or cell type. Especially in the mouse, few laboratories can make clones from adult somatic cells, and most mouse strains never succeed to produce cloned mice. On the other hand, nuclear transfer can be used to generate embryonic stem (ntES) cell lines from a patient's own somatic cells. We have shown that ntES cells can be generated relatively easily from a variety of mouse genotypes and cell types of both sexes, even though it may be more difficult to generate clones directly. Several reports have already demonstrated that ntES cells can be used in regenerative medicine in order to rescue immune deficient or infertile phenotypes. However, it is unclear whether ntES cells are identical to fertilized embryonic stem (ES) cells. In general, ntES cell techniques are expected to be applicable to regenerative medicine, however, these techniques can also be used for the preservation of the genetic resources of mouse strains instead of preserving such resources in embryos, oocytes or spermatozoa. This review seeks to describe the phenotype, application, and possible abnormalities of cloned mice and ntES cell lines.

Kitada, M., et al. (2018). "Intracellular signaling similarity reveals neural stem cell-like properties of ependymal cells in the adult rat spinal cord." Dev Growth Differ **60**(6): 326-340.

Proliferation of ependymal cells of the adult spinal cord (SCEp cells) in the intact condition has been considered as a quite rare event. To visualize proliferating/proliferated SCEp cells, we used the intensive 5-bromo-2'-deoxyuridine (BrdU) administration method to find that about two cells in the ependymal layer incorporated BrdU in a 10-mum-thick section. Because these two cells were not considered to undergo further proliferation, we analyzed the positioning and motility of two neighboring BrdU-incorporated proliferated cells and elucidated the tendency of the movement of SCEp cells to the outer side inside the ependymal layer. Even if it was rare, one of the proliferated cells in the ependymal layer differentiated into astrocytes. Gene introduction of Notch intracellular domain (NICD), a constitutively active form of Notch1, into SCEp cells demonstrated both increase in proliferation and induction of differentiation into astrocytes. Overexpression of Sox2 promoted proliferation in SCEp cells. The reaction of gene introduction of NICD and Sox2 indicates the similarity of intracellular signaling between SCEp cells and neural stem cells. Also, considering the fact that SCEp cells express these two factors in the intact condition, Notch and Sox2 are important for the cell fate control of SCEp cells in the intact condition.

Knoll, A., et al. (2013). "Adult Rat Bone Marrow-Derived Stem Cells Promote Late Fetal Type II Cell Differentiation in a Co-Culture Model." Open Respir Med J **7**: 46-53.

Bronchopulmonary dysplasia develops in preterm infants due to a combination of lung immaturity and lung injury. Cultured pluripotent bone marrow stem cells (BMSC) are known to reduce injury and induce repair in adult and in immature lungs, possibly through paracrine secretion of soluble factors. The paracrine relationship between BMSC and primary fetal lung epithelial type II cells is unknown. We determined the effects of BMSC on type II cell and fibroblast behavior using an in vitro co-culture model. Rat BMSC were isolated and co-cultured with primary fetal E21 rat type II cells or lung fibroblasts in a Transwell((R)) system without direct cell contact. Effects of BMSC conditioned media (CM) on type II cell and fibroblast proliferation and on type II cell surfactant phospholipid (DSPC) synthesis and mRNA expression of surfactant proteins B and C (sftpb and sftpc) were studied. We also determined the effect of fibroblast and type II cell CM on BMSC proliferation and surface marker expression. Co-culture with BMSC significantly decreased type II cell and fibroblast proliferation to 72.5% and 83.7% of controls, respectively. Type II cell DSPC synthesis was significantly increased by 21% and sftpb and sftpc mRNA expressions were significantly induced (2.1 fold and 2.4 fold, respectively). BMSC proliferation was significantly reduced during the co-culture. Flow cytometry confirmed that BMSC retained the expression of undifferentiated stem cell markers despite their exposure to fetal lung cell CM. We conclude that BMSC induce fetal type II cell differentiation through paracrine release of soluble factors. These studies provide clues for how BMSC may act in promoting alveolar repair following injury.

Kobayashi, H., et al. (2009). "Thy-1(+) cells isolated from adult human testicular tissues express human embryonic stem cell genes OCT3/4 and NANOG and may include spermatogonial stem cells." Reprod Med Biol **8**(2): 71-77.

Purpose: Spermatogonial stem cells (SSCs) are self-renewing cells whose progeny are committed to differentiate into spermatozoa; this is a life-long process in male mammals. There are several methods for obtaining enriched populations of mouse SSCs, and immunological separation using surface antigens is a commonly used technique. The study of human SSCs is much less advanced. Methods: We used biopsied human testicular tissues [obstructive azoospermia patients (n = 5) and patients who underwent a testis biopsy as part of an evaluation for infertility (n = 7)] to obtain Thy-1(+) cells. Thy-1-a glycosyl phosphatidylinositol-anchored surface antigen-is a marker uniquely expressed on SSCs that is used to isolate SSC-enriched cell populations in mice. The Thy-1(+) cells from human testicular tissues were cultured in a basic system consisting of serum-free medium and mitotically inactivated STO (SIM mouse embryo-derived thioguanine- and ouabain-resistant) cell feeders with added growth factors: glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), and GDNF-family receptor alpha1 (GFRalpha-1). Results: The Thy-1(+) cells were maintained in vitro using this system for 1 week. The Thy-1(+) cells expressed OCT3/4 and alkaline phosphatase, like mouse SSCs. They also expressed NANOG. Thy-1(+) cells injected into nude mice did not cause tumor formation over a period of at least 6 months. Conclusions: These results support the possibility that the Thy-1(+) cell population included human SSCs, and that Thy-1 may be a marker for human SSCs.

Korhonen, L., et al. (2003). "Tumor suppressor gene BRCA-1 is expressed by embryonic and adult neural stem cells and involved in cell proliferation." J Neurosci Res **71**(6): 769-776.

BRCA-1 is a tumor suppressor gene that plays a role in DNA repair and cellular growth control. Here we show that BRCA-1 mRNA is expressed by embryonic rat brain and is localized to the neuroepithelium containing neuronal precursor cells. The expression of BRCA-1 decreases during rat brain development, but BRCA-1 is expressed postnatally by proliferating neuronal precursor cells in the developing cerebellum. Neural stem cells (NSC) prepared from embryonic rat brain and cultured in the presence of epidermal growth factor were positive for BRCA-1. Induction of NSC differentiation resulted in down-regulation of BRCA-1 expression as shown by RNA and protein analyses. In addition to embryonic cells, BRCA-1 is also present in NSC prepared from adult rat brain. In adult rats, BRCA1 was expressed by cells in the walls of brain ventricles and in choroid plexus. The results show that BRCA-1 is present in embryonic and adult rat NSC and that the expression is linked to NSC proliferation.

Kruse, C., et al. (2006). "Adult pancreatic stem/progenitor cells spontaneously differentiate in vitro into multiple cell lineages and form teratoma-like structures." Ann Anat **188**(6): 503-517.

Cells isolated from pancreas have a remarkable potential for self-renewal and multilineage differentiation. We here present a comprehensive characterisation of stem/progenitor cells derived from exocrine parts of the adult rat pancreas. Using purified cells from either single colonies or even single-cell clones, we specifically demonstrate: (i) the cells contain the typical stem/progenitor cell markers alkaline phophatase, SSEA-1, Oct-4, CD9, Nestin, Pax6, CD44, a-Fetoprotein and Brachyury, demonstrated by immunocytochemistry and RT-PCR; (ii) the cells have the potential to differentiate into lineages of all three germ layers in vitro; (iii) a clonal analysis revealed that even cell lines derived from a single cell have stem/progenitor cell properties such as self-renewal and spontaneous differentiation into various cell lineages; (iv) the cells have the propensity to form three-dimensional, teratoma-like structures in vitro, which contain cells of different lineages; and (v) external stimuli can activate the generation of certain cell types. For instance, cells treated with retinoic acid show an increased expression of alpha-smooth muscle actin. These results suggest that exocrine glands, such as pancreas may be a potential source of adult stem/progenitor cells, suitable for cell therapy of degenerative diseases.

Lang, H., et al. (2015). "Neural stem/progenitor cell properties of glial cells in the adult mouse auditory nerve." Sci Rep **5**: 13383.

The auditory nerve is the primary conveyor of hearing information from sensory hair cells to the brain. It has been believed that loss of the auditory nerve is irreversible in the adult mammalian ear, resulting in sensorineural hearing loss. We examined the regenerative potential of the auditory nerve in a mouse model of auditory neuropathy. Following neuronal degeneration, quiescent glial cells converted to an activated state showing a decrease in nuclear chromatin condensation, altered histone deacetylase expression and up-regulation of numerous genes associated with neurogenesis or development. Neurosphere formation assays showed that adult auditory nerves contain neural stem/progenitor cells (NSPs) that were within a Sox2-positive glial population. Production of neurospheres from auditory nerve cells was stimulated by acute neuronal injury and hypoxic conditioning. These

results demonstrate that a subset of glial cells in the adult auditory nerve exhibit several characteristics of NSPs and are therefore potential targets for promoting auditory nerve regeneration.

Lao, C. L., et al. (2013). "Dopamine D3 receptor activation promotes neural stem/progenitor cell proliferation through AKT and ERK1/2 pathways and expands type-B and -C cells in adult subventricular zone." Glia **61**(4): 475-489.

The neurotransmitter dopamine acts on the subventricular zone (SVZ) to regulate both prenatal and postnatal neurogenesis, in particular through D(3) receptor (D(3) R) subtype. In this study, we explored the cellular mechanism(s) underlying D(3) R-mediated cell proliferation and tested if systemic delivery of a D(3) R agonist would induce SVZ multipotent neural stem/precursor cell (NSC/NPC) proliferation in vivo. We found that treatment with the D(3) R agonist, 7-OH-DPAT, enhances cell proliferation in a dose-dependent manner in cultured SVZ neurospheres from wild-type, but not D(3) R knock-out mice. Furthermore, D(3) R activation also stimulates S-phase and enhances mRNA and protein levels of cyclin D1 in wild-type neurospheres, a process which requires cellular Akt and ERK1/2 signaling. Moreover, chronic treatment with low dose 7-OH-DAPT in vivo increases BrdU(+) cell numbers in the adult SVZ, but this effect was not seen in D(3) R KO mice. Additionally, we probed the cell type specificity of D(3) R agonist-mediated cell proliferation. We found that in adult SVZ, GFAP(+) astrocytes, type-B GFAP(+) /nestin(+) and type-C EGF receptor (EGFR(+))/nestin(+) cells express D(3) R mRNA, but type-A Doublecortin (Dcx)(+) neuroblasts do not. Using flow cytometry and immunofluorescence, we demonstrated that D(3) R activation increases GFAP(+) type-B and EGFR(+) type-C cell numbers, and the newly divided Dcx(+) type-A cells. However, BrdU(+) /Dcx(+) cell numbers were decreased in D(3) R KO mice compared to wildtype, suggesting that D(3) R maintains constitutive NSC/NPCs population in the adult SVZ. Overall, we demonstrate that D(3) R activation induces NSC/NPC proliferation through Akt and ERK1/2 signaling and increases the numbers of type-B and -C NSC/NPCs in the adult SVZ.

Lapter, S., et al. (2007). "Structure and implied functions of truncated B-cell receptor mRNAs in early embryo and adult mesenchymal stem cells: Cdelta replaces Cmu in mu heavy chain-deficient mice." Stem Cells **25**(3): 761-770.

Stem cells exhibit a promiscuous gene expression pattern. We show herein that the early embryo and adult MSCs express B-cell receptor component mRNAs. To examine possible bearings of these genes on the expressing cells, we studied immunoglobulin mu chain-deficient mice. Pregnant mu chain-deficient females were found to produce a higher percentage of defective morulae compared with control females. Structure analysis indicated that the mu mRNA species found in embryos and in mesenchyme consist of the constant region of the mu heavy chain that encodes a recombinant 50-kDa protein. In situ hybridization localized the constant mu gene expression to loose mesenchymal tissues within the day-12.5 embryo proper and the yolk sac. In early embryo and in adult mesenchyme from mu-deficient mice, delta replaced mu chain, implying a possible requirement of these alternative molecules for embryo development and mesenchymal functions. Indeed, overexpression of the mesenchymal-truncated mu heavy chain in 293T cells resulted in specific subcellular localization and in G(1) growth arrest. The lack of such occurrence following overexpression of a complete, rearranged form of mu chain suggests that the mesenchymal version of this mRNA may possess unique functions.

Lardon, J., et al. (2008). "Stem cell marker prominin-1/AC133 is expressed in duct cells of the adult human pancreas." Pancreas **36**(1): e1-6.

OBJECTIVES: Many efforts are spent in identifying stem cells in adult pancreas because these could provide a source of beta cells for cell-based therapy of type 1 diabetes. Prominin-1, particularly its specific glycosylation-dependent AC133 epitope, is expressed on stem/progenitor cells of various human tissues and can be used to isolate them. We, therefore, examined its expression in adult human pancreas. METHODS: To detect prominin-1 protein, monoclonal antibody CD133/1 (AC133 clone), which recognizes the AC133 epitope, and the alphahE2 antiserum, which is directed against the human prominin-1 polypeptide, were used. Prominin-1 RNA expression was analyzed by real-time polymerase chain reaction. RESULTS: We report that all duct-lining cells of the pancreas express prominin-1. Most notably, the cells that react with the alphahE2 antiserum also react with the AC133 antibody. After isolation and culture of human exocrine cells, we found a relative increase in prominin-1 expression both at protein and RNA expression level, which can be explained by an enrichment of cells with ductal phenotype in these cultures. CONCLUSIONS: Our data show that pancreatic duct cells express prominin-1 and surprisingly reveal that its particular AC133 epitope is not an exclusive stem and progenitor cell marker.

Lawrence, J. M., et al. (2007). "MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics." Stem Cells **25**(8): 2033-2043.

Growing evidence suggests that glial cells may have a role as neural precursors in the adult central nervous system. Although it has been shown that Muller cells exhibit progenitor characteristics in the

postnatal chick and rat retinae, their progenitor-like role in developed human retina is unknown. We first reported the Muller glial characteristics of the spontaneously immortalized human cell line MIO-M1, but recently we have derived similar cell lines from the neural retina of several adult eye donors. Since immortalization is one of the main properties of stem cells, we investigated whether these cells expressed stem cell markers. Cells were grown as adherent monolayers, responded to epidermal growth factor, and could be expanded indefinitely without growth factors under normal culture conditions. They could be frozen and thawed without losing their characteristics. In the presence of extracellular matrix and fibroblast growth factor-2 or retinoic acid, they acquired neural morphology, formed neurospheres, and expressed neural stem cell markers including betaIII tubulin, Sox2, Pax6, Chx10, and Notch 1. They also expressed markers of postmitotic retinal neurons, including peripherin, recoverin, calretinin, S-opsin, and Brn3. When grafted into the subretinal space of dystrophic Royal College of Surgeons rats or neonatal Lister hooded rats, immortalized cells migrated into the retina, where they expressed various markers of retinal neurons. These observations indicate that adult human neural retina harbors a population of cells that express both Muller glial and stem cell markers and suggest that these cells may have potential use for cell-based therapies to restore retinal function. Disclosure of potential conflicts of interest is found at the end of this article.

Lee, B. C., et al. (2016). "PGE2 maintains self-renewal of human adult stem cells via EP2-mediated autocrine signaling and its production is regulated by cell-to-cell contact." Sci Rep **6**: 26298.

Mesenchymal stem cells (MSCs) possess unique immunomodulatory abilities. Many studies have elucidated the clinical efficacy and underlying mechanisms of MSCs in immune disorders. Although immunoregulatory factors, such as Prostaglandin E2 (PGE2), and their mechanisms of action on immune cells have been revealed, their effects on MSCs and regulation of their production by the culture environment are less clear. Therefore, we investigated the autocrine effect of PGE2 on human adult stem cells from cord blood or adipose tissue, and the regulation of its production by cell-to-cell contact, followed by the determination of its immunomodulatory properties. MSCs were treated with specific inhibitors to suppress PGE2 secretion, and proliferation was assessed. PGE2 exerted an autocrine regulatory function in MSCs by triggering E-Prostanoid (EP) 2 receptor. Inhibiting PGE2 production led to growth arrest, whereas addition of MSC-derived PGE2 restored proliferation. The level of PGE2 production from an equivalent number of MSCs was down-regulated via gap junctional intercellular communication. This cell contact-mediated decrease in PGE2 secretion down-regulated the suppressive effect of MSCs on immune cells. In conclusion, PGE2 produced by MSCs contributes to maintenance of self-renewal capacity through EP2 in an autocrine manner, and PGE2 secretion is down-regulated by cell-to-cell contact, attenuating its immunomodulatory potency.

Lee, J. and T. Tumbar (2017). "Linking chromatin dynamics, cell fate plasticity, and tissue homeostasis in adult mouse hair follicle stem cells." Mol Life **1**(1): 15-21.

Cellular plasticity for fate acquisition is associated with distinct chromatin states, which include histone modifications, dynamic association of chromatin factors with the DNA, and global chromatin compaction and nuclear organization. While embryonic stem cell (ESC) plasticity in vitro and its link with chromatin states have been characterized in depth, little is known about tissue stem cell plasticity in vivo, during adult tissue homeostasis. Recently, we reported a distinct globally low level of histone H3 K4/9/27me3 in mouse hair follicle stem cells (HFSCs) during quiescence. This occurred at the stage preceding fate acquisition, when HFSC fate plasticity must be at its highest. This hypomethylated state was required for proper skin homeostasis and timely hair cycle. Here, we show both in the live tissue and in cell culture that at quiescence HFSCs have higher exchange rates for core histone H2B when compared with proliferative or differentiated cells. This denoted a hyperdynamic chromatin state, which was previously associated with high cell fate plasticity in ESCs. Moreover, we find that quiescent HFSCs display a higher propensity for de-differentiation in response to Yamanaka's reprogramming factors in vivo. These results further support our recent model in which HFSCs render their chromatin into a specific state at quiescence, which is attuned to higher cell fate plasticity.

Lei, L., et al. (2007). "Biological character of human adipose-derived adult stem cells and influence of donor age on cell replication in culture." Sci China C Life Sci **50**(3): 320-328.

To investigate the biological character of human adipose-derived adult stem cells (hADAS cells) when cultured in vitro and the relationship between hADAS cell's replication activity and the donor's age factor, and to assess the stem cells as a new source for tissue engineering. hADAS cells are isolated from human adipose tissue of different age groups (from adolescents to olds: <20 years old, 21-40 years old, 41-60 years old and >61 years old groups). The protein markers (CD29, CD34, CD44, CD45, CD49d, HLA-DR, CD106) of hADAS cells were detected by flow cytometry (FCM) to identify the stem cell, and the cell cycle was examined for P20 hADAS cells to evaluate

the safety of the subculture in vitro. The generative activity of hADAS cells in different age groups was also examined by MTT method. The formula "TD = t x log2/logNt - logN0" was used to get the time doubling (TD) of the cells. The results showed that the cells kept heredity stabilization by chromosome analysis for at least 20 passages. The TD of these cells increased progressively by ageing, and the TD of the <20 years old group was lower than that of the >61 years old group (statistical analysis of variance (ANOVA), P=0.002, P<0.05). These findings suggested that a higher level of hADAS cells replication activity was found in the younger donators, and they represent novel and valuable seed cells for studies of tissue engineering.

Lermen, D., et al. (2010). "Neuro-muscular differentiation of adult porcine skin derived stem cell-like cells." PLoS One **5**(1): e8968.

BACKGROUND: Due to the genetic relationship to humans, porcine stem cells are a very important model system to investigate cell differentiation, associated cell signaling pathways, and cell fate. Porcine skin derived stem cells have been isolated from mid-gestation porcine fetus recently. To our knowledge, stem cells from the skin of the adult porcine organism have not been isolated until now. Hence, to our knowledge, we here describe the isolation, expansion, characterization and differentiation of multipotent porcine skin derived stem cell-like cells (pSSCs) from the adult porcine organism for the first time. METHODOLOGY/PRINCIPAL FINDINGS: pSSCs had a spindle shaped morphology similar to mesenchymal stem cells (MSCs). They could be maintained proliferatively active in vitro for more than 120 days and were able to form colonies from single cells. pSSCs expressed Sox2 and Oct3/4, both transcription factors essential to the pluripotent and self-renewing phenotypes of embryonic stem cells, which recently gained attention due to their function in inducing pluripotent stem cells. Furthermore, the expression of the progenitor marker nestin, the somatic stem cell markers Bcrp1/ABCG2, Bmi1, and Stat3 was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in undifferentiated pSSCs. Flow cytometry revealed the expression of the MSC related proteins CD9, CD29, CD44 and CD105, but not CD90. After neuronal differentiation cells with a characteristic morphology of neuronal and smooth muscle-like cells were present in the cultures. Subsequent immunochemistry and flow cytometry revealed the down-regulation of nestin and the up-regulation of the neuron specific protein beta-III-tubulin and the astrocyte marker GFAP. Also, alpha-SMA expressing cells increased during differentiation suggesting the neuro-muscular differentiation of these skin derived cells. pSSCs could also be induced to differentiate into adipocyte-like cells when cultured under specific conditions. CONCLUSIONS/SIGNIFICANCE: Adult porcine skin harbors a population of stem cell-like cells (pSSCs) that can be isolated via enzymatic digestion. These pSSCs show characteristic features of MSCs originated in other tissues and express the embryonic stem cell marker Oct3/4, Sox2, and Stat3. Furthermore, pSSCs have the potential to differentiate into cells from two different germ lines, the ectoderm (neurons, astrocytes) and the mesoderm (smooth muscle cells, adipocytes).

Levy, Y. S., et al. (2004). "Embryonic and adult stem cells as a source for cell therapy in Parkinson's disease." J Mol Neurosci **24**(3): 353-386.

The rationale behind the use of cells as therapeutic modalities for neurodegenerative diseases in general, and in Parkinson's disease (PD) in particular, is that they will improve patient's functioning by replacing the damaged cell population. It is reasoned that these cells will survive, grow neurites, establish functional synapses, integrate best and durably with the host tissue mainly in the striatum, renew the impaired wiring, and lead to meaningful clinical improvement. To increase the generation of dopamine, researchers have already transplanted non-neuronal cells, without any genetic manipulation or after introduction of genes such as tyrosine hydroxylase, in animal models of PD. Because these cells were not of neuronal origin, they developed without control, did not integrate well into the brain parenchyma, and their survival rates were low. Clinical experiments using cell transplantation as a therapy for PD have been conducted since the 1980s. Most of these experiments used fetal dopaminergic cells originating in the ventral mesencephalic tissue obtained from fetuses. Although it was shown that the transplanted cells survived and some patients benefited from this treatment, others suffered from severe dyskinesia, probably caused by the graft's excessive and uncontrolled production and release of dopamine. It is now recognized that cell-replacement strategy will be effective in PD only if the transplanted cells have the same abilities, such as dopamine synthesis and control release, reuptake, and metabolizing dopamine, as the original dopaminergic neurons. Recent studies on embryonic and adult stem cells have demonstrated that cells are able to both self-renew and produce differentiated tissues, including dopaminergic neurons. These new methods offer real hope for tissue replacement in a wide range of diseases, especially PD. In this review we summarize the evidence of dopaminergic neuron generation from embryonic and adult stem cells, and discuss their application for cell therapy in PD.

Li, J., et al. (2015). "Chondrogenic priming of human fetal synovium-derived stem cells in an adult stem cell matrix microenvironment." Genes Dis **2**(4): 337-346.

Cartilage defects are a challenge to treat clinically due to the avascular nature of cartilage. Low immunogenicity and extensive proliferation and multidifferentiation potential make fetal stem cells a promising source for regenerative medicine. In this study, we aimed to determine whether fetal synovium-derived stem cells (FSDSCs) exhibited replicative senescence and whether expansion on decellularized extracellular matrix (dECM) deposited by adult SDSCs (AECM) promoted FSDSCs' chondrogenic potential. FSDSCs from passage 2 and 9 were compared for chondrogenic potential, using Alcian blue staining for sulfated glycosaminoglycans (GAGs), biochemical analysis for DNA and GAG amounts, and real-time PCR for chondrogenic genes including ACAN and COL2A1. Passage 3 FSDSCs were expanded for one passage on plastic flasks (PL), AECM, or dECM deposited by fetal SDSCs (FECM). During expansion, cell proliferation was evaluated using flow cytometry for proliferation index, stem cell surface markers, and resistance to hydrogen peroxide. During chondrogenic induction, expanded FSDSCs were evaluated for tri-lineage differentiation capacity. We found that cell expansion enhanced FSDSCs' chondrogenic potential at least up to passage 9. Expansion on dECMs promoted FSDSCs' proliferative and survival capacity and adipogenic differentiation but not osteogenic capacity. AECM-primed FSDSCs exhibited an enhanced chondrogenic potential.

Li, X., et al. (2008). "Epigenetic regulation of the stem cell mitogen Fgf-2 by Mbd1 in adult neural stem/progenitor cells." J Biol Chem **283**(41): 27644-27652.

Whether and how mechanisms intrinsic to stem cells modulate their proliferation and differentiation are two central questions in stem cell biology. Although exogenous basic fibroblast growth factor 2 (FGF-2/Fgf-2) is commonly used to expand adult neural stem/progenitor cells (NSPCs) in vitro, we do not yet understand the functional significance or the molecular regulation of Fgf-2 expressed endogenously by adult NSPCs. We previously demonstrated that methylated CpG binding protein 1 (MBD1/Mbd1) is a transcriptional repressor of Fgf-2 and is enriched in adult brains. Mbd1 deficiency in mice selectively affected adult neurogenesis and the differentiation of NSPCs. Here we show that an Mbd1 and DNA methylation-mediated epigenetic mechanism regulated the expression of stem cell mitogen Fgf-2 in adult NSPCs. Mbd1 bound to the Fgf-2 promoter and regulates its expression in adult NSPCs. In the absence of functional Mbd1, the Fgf-2 promoter was hypomethylated, and treatment with a DNA methylation inhibitor resulted in increased Fgf-2 expression in adult NSPCs. We further demonstrated that both acute knockdown of Mbd1 or overexpression of Fgf-2 in adult NSPCs inhibited their neuronal differentiation, which could be responsible for the neurogenic deficits observed in Mbd1-deficient mice. These data indicate that intrinsic epigenetic mechanisms play critical roles in the regulation of adult NSPC functions.

Li, Y., et al. (2018). "Transcription factor TBX18 promotes adult rat bone mesenchymal stem cell differentiation to biological pacemaker cells." Int J Mol Med **41**(2): 845-851.

Bone mesenchymal stem cells (BMSCs) are currently considered the optimal stem cells for biological pacemaker cell transformation. The cardiacspecific transcription factor TBox protein 18 (TBX18) is essential for sinoatrial node (SAN) formation, particularly formation of the head region that generates the electrical impulses that induce heart contraction. The present study aimed to confirm the effects of TBX18 on biological pacemaker differentiation of rat BMSCs. Flow cytometry was used to identify the surface markers of BMSCs, in order to acquire pure mesenchymal stem cells. Subsequently, BMSCs were transduced with TBX18 or green fluorescent protein adenovirus vectors. The effects of TBX18 were evaluated using SANspecific makers including TBX18, alphaactin, cardiac troponin I, hyperpolarizationactivated cyclic nucleotidegated channel 4 and connexin 43 by reverse transcriptionquantitative polymerase chain reaction, western blotting and immunofluorescence. The findings demonstrated that direct conversion of BMSCs to biological pacemaker cells via TBX18 is a feasible method in the field of cardiology.

Lin, H. T., et al. (2006). "Characterization of pancreatic stem cells derived from adult human pancreas ducts by fluorescence activated cell sorting." World J Gastroenterol **12**(28): 4529-4535.

AIM: To isolate putative pancreatic stem cells (PSCs) from human adult tissues of pancreas duct using serum-free, conditioned medium. The characterization of surface phenotype of these PSCs was analyzed by flow cytometry. The potential for pancreatic lineage and the capability of beta-cell differentiation in these PSCs were evaluated as well. METHODS: By using serum-free medium supplemented with essential growth factors, we attempted to isolate the putative PSCs which has been reported to express nestin and pdx-1. The Matrigel(TM) was employed to evaluate the differential capacity of isolated cells. Dithizone staining, insulin content/secretion measurement, and immunohistochemistry staining were used to monitor the differentiation. Fluorescence activated cell sorting (FACS) was used to detect the phenotypic markers of putative PSCs. RESULTS: A monolayer of spindle-like cells was cultivated. The putative PSCs expressed pdx-

1 and nestin. They were also able to differentiate into insulin-, glucagon-, and somatostatin-positive cells. The spectrum of phenotypic markers in PSCs was investigated; a similarity was revealed when using human bone marrow-derived stem cells as the comparative experiment, such as CD29, CD44, CD49, CD50, CD51, CD62E, PDGFR-alpha, CD73 (SH2), CD81, CD105(SH3). CONCLUSION: In this study, we successfully isolated PSCs from adult human pancreatic duct by using serum-free medium. These PSCs not only expressed nestin and pdx-1 but also exhibited markers attributable to mesenchymal stem cells. Although work is needed to elucidate the role of these cells, the application of these PSCs might be therapeutic strategies for diabetes mellitus.

Liu, L., et al. (2009). "Stem cell regulatory gene expression in human adult dental pulp and periodontal ligament cells undergoing odontogenic/osteogenic differentiation." J Endod **35**(10): 1368-1376.

INTRODUCTION: During development and regeneration, odontogenesis and osteogenesis are initiated by a cascade of signals driven by several master regulatory genes. METHODS: In this study, we investigated the differential expression of 84 stem cell-related genes in dental pulp cells (DPCs) and periodontal ligament cells (PDLCs) undergoing odontogenic/osteogenic differentiation. RESULTS: Our results showed that, although there was considerable overlap, certain genes had more differential expression in PDLCs than in DPCs. CCND2, DLL1, and MME were the major upregulated genes in both PDLCs and DPCs, whereas KRT15 was the only gene significantly downregulated in PDLCs and DPCs in both odontogenic and osteogenic differentiation. Interestingly, a large number of regulatory genes in odontogenic and osteogenic differentiation interact or crosstalk via Notch, Wnt, transforming growth factor beta (TGF-beta)/bone morphogenic protein (BMP), and cadherin signaling pathways, such as the regulation of APC, DLL1, CCND2, BMP2, and CDH1. Using a rat dental pulp and periodontal defect model, the expression and distribution of both BMP2 and CDH1 have been verified for their spatial localization in dental pulp and periodontal tissue regeneration. CONCLUSIONS: This study has generated an overview of stem cell-related gene expression in DPCs and PDLCs during odontogenic/osteogenic differentiation and revealed that these genes may interact through the Notch, Wnt, TGF-beta/BMP, and cadherin signaling pathways to play a crucial role in determining the fate of dental derived cell and dental tissue regeneration. These findings provided a new insight into the molecular mechanisms of the dental tissue mineralization and regeneration.

Liu, L. N., et al. (2013). "Comparison of drug and cell-based delivery: engineered adult mesenchymal stem cells expressing soluble tumor necrosis factor receptor II prevent arthritis in mouse and rat animal models." Stem Cells Transl Med **2**(5): 362-375.

Rheumatoid arthritis (RA) is a systemic autoimmune disease with unknown etiology where tumor necrosis factor-alpha (TNFalpha) plays a critical role. Etanercept, a recombinant fusion protein of human soluble tumor necrosis factor receptor II (hsTNFR) linked to the Fc portion of human IgG1, is used to treat RA based on the rationale that sTNFR binds TNFalpha and blocks TNFalpha-mediated inflammation. We compared hsTNFR protein delivery from genetically engineered human mesenchymal stem cells (hMSCs) with etanercept. Blocking TNFalpha-dependent intercellular adhesion molecule-1 expression on transduced hMSCs and inhibition of nitric oxide production from TNFalpha-treated bovine chondrocytes by conditioned culture media from transduced hMSCs demonstrated the functionality of the hsTNFR construction. Implanted hsTNFR-transduced mesenchymal stem cells (MSCs) reduced mouse serum circulating TNFalpha generated from either implanted TNFalpha-expressing cells or lipopolysaccharide induction more effectively than etanercept (TNFalpha, 100%; interleukin [IL]-1alpha, 90%; and IL-6, 60% within 6 hours), suggesting faster clearance of the soluble tumor necrosis factor receptor (sTNFR)-TNFalpha complex from the animals. In vivo efficacy of sTNFR-transduced MSCs was illustrated in two (immune-deficient and immune-competent) arthritic rodent models. In the antibody-induced arthritis BalbC/SCID mouse model, intramuscular injection of hsTNFR-transduced hMSCs reduced joint inflammation by 90% compared with untransduced hMSCs; in the collagen-induced arthritis Fischer rat model, both sTNFR-transduced rat MSCs and etanercept inhibited joint inflammation by 30%. In vitro chondrogenesis assays showed the ability of TNFalpha and IL1alpha, but not interferon gamma, to inhibit hMSC differentiation to chondrocytes, illustrating an additional negative role for inflammatory cytokines in joint repair. The data support the utility of hMSCs as therapeutic gene delivery vehicles and their potential to be used in alleviating inflammation within the arthritic joint.

Liu, S., et al. (2014). "Effect of transplantation of human embryonic stem cell-derived neural progenitor cells on adult neurogenesis in aged hippocampus." Am J Stem Cells **3**(1): 21-26.

Adult neurogenesis occurs within the special microenvironment in the subgranular zone of the hippocampus and the subventricular zone of the lateral ventricle of the mammalian brain. The special microenvironment is known as neurogenic niches.

Multiple cell types, including endothelial cells, astroglia, ependymal cells, immature progeny of neural stem cells, and mature neurons, comprise the neurogenic niche. Differentiation of embryonic stem cells towards the neural lineage results in the generation of different neuronal subtypes and non-neuronal cells (mainly astrocytes). Therefore, it is reasonable to hypothesize that transplantation of human embryonic stem cell-derived neural progenitor cells can be used to modify neurogenic niches for facilitating adult neurogenesis. Furthermore, if generated new neurons are functionally integrated into the existing circuits of the aged hippocampus, synaptic plasticity in the hippocampus and learning/memory functions in aged mice should be enhanced. In this article, we provide a comprehensive review of the concepts in the regulation of adult neurogenesis by neurogenic niches and discuss the molecular mechanisms underlying the effect of stem cell transplantation on adult neurogenesis in aged hippocampus.

Long, X., et al. (2005). "Neural cell differentiation in vitro from adult human bone marrow mesenchymal stem cells." Stem Cells Dev **14**(1): 65-69.

Bone marrow (BM) mesenchymal stem cells (MSCs) are cells capable of expanding and differentiating in vitro into nonhematopoietic cells. Neurotrophic cytokines, such as human epidermal growth factor (hEGF) and bovine fibroblast growth factor (bFGF) can induce differentiation into neural cells (NCs). When BM MSCs were cultured with hEGF and bFGF, RNA expression of neuronal specific markers Nestin, MAP-2, and tyrosine hydroxylase (TH) were observed. We tested a new cytokine combination to generate mature NCs. The plastic-adherent cells were collected and then split when they were 90% confluent from an enriched mononuclear cell layer. At passage 3, MSCs were cultured in neural differentiation media (dbcAMP, IBMX, FGF-8, BDNF, hEGF, and bFGF in NEUROBASAL media plus B27). Cells were counted on day 6. Immunofluorescent staining and reverse transcriptase (RT)-PCR were performed to evaluate the expression of neural markers. On day 6, 66% of cells developed dendrites and presented typical neural cell morphology. Some cells were positive for early neural markers Nestin and beta-tubulin III. Cells expressing mature neuronal markers (NF, NeuN, Tau, Nurr1, GABA, oligodendryte GalC, and glial GFAP) were also seen. By adding hEGF, bFGF, dbcAMP, IBMX, BDNF, and bFGF-8 into NEUROBASAL media plus B27, BM MSCs were directed toward becoming early and mature NCs.

Luyckx, A., et al. (2011). "Oct4-negative multipotent adult progenitor cells and mesenchymal stem cells as regulators of T-cell alloreactivity in mice." Immunol Lett **137**(1-2): 78-81.

Multipotent adult progenitor cells (MAPC) are clinically being explored as an alternative to mesenchymal stem cells (MSC) for the immunomodulatory control of graft-versus-host disease (GvHD). Here, we performed an explorative study of the immunomodulatory potential of mouse MAPC (mMAPC), in comparison with that of MSC (mMSC) using experimental models of T-cell alloreactivity. Suppressive effects of Oct4-expressing mMAPC have been described previously; here, we studied mMAPC expressing low to no Oct4 ('mClone-3'), recently shown to be most representative for the human MAPC counterpart. mClone-3 and mMSC exhibited similar immunophenotype and in vitro immunogenic behavior. Allogeneic T-cell<-->dendritic cell-proliferation assays showed strong dose-dependent T-cell-suppressive effects of both mClone-3 and mMSC. In a popliteal lymph node assay, mClone-3 and mMSC equally suppressed in vivo alloreactive T-cell expansion. We conclude that mouse MAPC and MSC exhibit similar immunosuppressive behavior in in vitro and local in vivo GvHD assays.

Lynch, L., et al. (2007). "Cells with haematopoietic stem cell phenotype in adult human endometrium: relevance to infertility?" Hum Reprod **22**(4): 919-926.

BACKGROUND: Uterine lymphoid cell repertoires are specialized in order to meet the twin demands of successful pregnancy and local immunosurveillance. The possibility that some of these populations might differentiate locally from progenitor cells has been proposed. METHODS: Endometrial tissue from women with a history of infertility as well as fertile controls was examined for haematopoietic stem cells (HSCs) and lymphoid progenitors using three-colour flow cytometry. RESULTS: Significant populations of phenotypic HSCs (CD34+ CD45+) were detected in all samples, a high proportion of which co-expressed the differentiation marker CD45RA (45.7%), indicating ongoing differentiation. Almost 30% of uterine HSCs co-expressed CD56 and 44% co-expressed CD7, suggesting the presence of lymphoid progenitors. Small proportions expressed CD127 and CD122, receptors for interleukin (IL)-7 and IL-15, respectively. HSC numbers were similar in the endometrial samples from fertile and infertile women. However, the proportion co-expressing the natural killer (NK) antigen CD56 was significantly increased compared with HSCs found in the endometrium of fertile controls (P = 0.002). CONCLUSIONS: This is the first demonstration of cells with an HSC phenotype in the human endometrium, and increased proportions of NK progenitors in endometrium of women with infertility suggests a dysregulation of this pathway that may contribute to infertility.

Ma, D. K., et al. (2008). "G9a and Jhdm2a regulate embryonic stem cell fusion-induced reprogramming of adult neural stem cells." Stem Cells **26**(8): 2131-2141.

Somatic nuclei can be reprogrammed to pluripotency through fusion with embryonic stem cells (ESCs). The underlying mechanism is largely unknown, primarily because of a lack of effective approaches to monitor and quantitatively analyze transient, early reprogramming events. The transcription factor Oct4 is expressed specifically in pluripotent stem cells, and its reactivation from somatic cell genome constitutes a hallmark for effective reprogramming. Here we developed a double fluorescent reporter system using engineered ESCs and adult neural stem cells/progenitors (NSCs) to simultaneously and independently monitor cell fusion and reprogramming-induced reactivation of transgenic Oct4-enhanced green fluorescent protein (EGFP) expression. We demonstrate that knockdown of a histone methyltransferase, G9a, or overexpression of a histone demethylase, Jhdm2a, promotes ESC fusion-induced Oct4-EGFP reactivation from adult NSCs. In addition, coexpression of Nanog and Jhdm2a further enhances the ESC-induced Oct4-EGFP reactivation. Interestingly, knockdown of G9a alone in adult NSCs leads to demethylation of the Oct4 promoter and partial reactivation of the endogenous Oct4 expression from adult NSCs. Our results suggest that ESC-induced reprogramming of somatic cells occurs with coordinated actions between erasure of somatic epigenome and transcriptional resetting to restore pluripotency. These mechanistic findings may guide more efficient reprogramming for future therapeutic applications of stem cells. Disclosure of potential conflicts of interest is found at the end of this article.

Mademtzoglou, D., et al. (2018). "Cellular localization of the cell cycle inhibitor Cdkn1c controls growth arrest of adult skeletal muscle stem cells." Elife **7**.

Adult skeletal muscle maintenance and regeneration depend on efficient muscle stem cell (MuSC) functions. The mechanisms coordinating cell cycle with activation, renewal, and differentiation of MuSCs remain poorly understood. Here, we investigated how adult MuSCs are regulated by CDKN1c (p57(kip2)), a cyclin-dependent kinase inhibitor, using mouse molecular genetics. In the absence of CDKN1c, skeletal muscle repair is severely impaired after injury. We show that CDKN1c is not expressed in quiescent MuSCs, while being induced in activated and proliferating myoblasts and maintained in differentiating myogenic cells. In agreement, isolated Cdkn1c-deficient primary myoblasts display differentiation defects and increased proliferation. We further show that the subcellular localization of CDKN1c is dynamic; while CDKN1c is initially localized to the cytoplasm of activated/proliferating myoblasts, progressive nuclear translocation leads to growth arrest during differentiation. We propose that CDKN1c activity is restricted to differentiating myoblasts by regulated cyto-nuclear relocalization, coordinating the balance between proliferation and growth arrest.

Mahmoudifar, N. and P. M. Doran (2010). "Extent of cell differentiation and capacity for cartilage synthesis in human adult adipose-derived stem cells: comparison with fetal chondrocytes." Biotechnol Bioeng **107**(2): 393-401.

This study evaluated the extent of differentiation and cartilage biosynthetic capacity of human adult adipose-derived stem cells relative to human fetal chondrocytes. Both types of cell were seeded into nonwoven-mesh polyglycolic acid (PGA) scaffolds and cultured under dynamic conditions with and without addition of TGF-beta1 and insulin. Gene expression for aggrecan and collagen type II was upregulated in the stem cells in the presence of growth factors, and key components of articular cartilage such as glycosaminoglycan (GAG) and collagen type II were synthesized in cultured tissue constructs. However, on a per cell basis and in the presence of growth factors, accumulation of GAG and collagen type II were, respectively, 3.4- and 6.1-fold lower in the stem cell cultures than in the chondrocyte cultures. Although the stem cells synthesized significantly higher levels of total collagen than the chondrocytes, only about 2.4% of this collagen was collagen type II. Relative to cultures without added growth factors, treatment of the stem cells with TGF-beta1 and insulin resulted in a 59% increase in GAG synthesis, but there was no significant change in collagen production even though collagen type II gene expression was upregulated 530-fold. In contrast, in the chondrocyte cultures, synthesis of collagen type II and levels of collagen type II as a percentage of total collagen more than doubled after growth factors were applied. Although considerable progress has been achieved to develop differentiation strategies and scaffold-based culture techniques for adult mesenchymal stem cells, the extent of differentiation of human adipose-derived stem cells in this study and their capacity for cartilage synthesis fell considerably short of those of fetal chondrocytes.

Makoolati, Z., et al. (2017). "Embryonic stem cell derived germ cells induce spermatogenesis after transplantation into the testes of an adult mouse azoospermia model." Clin Sci (Lond) **131**(18): 2381-2395.

The present study aimed to: (i) identify the exogenous factors that allow in vitro differentiation of mouse spermatogonial stem cells (SSCs) from embryonic stem cells (ESCs); (ii) evaluate the effects of Sertoli cells in SSC enrichment; and (iii) assess the

success of transplantation using in vitro differentiated SSCs in a mouse busulfan-treated azoospermia model. A 1-day-old embryoid body (EB) received 5 ng/ml of bone morphogenetic protein 4 (BMP4) for 4 days, 3 microM retinoic acid (RA) in a SIM mouse embryo-derived thioguanine and ouabain resistant (STO) co-culture system for 7 days, and was subsequently co-cultured for 2 days with Sertoli cells in the presence or absence of a leukaemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF) and RA composition, and in the presence of these factors in simple culture medium. Higher viability, proliferation and germ cell gene expression were seen in the presence of the LIF, bFGF and RA composition, on top of Sertoli cells. Immunocytochemistry results showed higher CDH1 expression in this group. Sertoli co-culture had no effects on SSC proliferation. Eight weeks after transplantation, injected cells were observed at the base of the seminiferous tubules and in the recipient testes. The number of spermatogonia and the mass of the testes were higher in transplanted testes relative to the control group. It seems that transplantation of these cells can be useful in infertility treatment.

Maleki, M., et al. (2014). "Comparison of mesenchymal stem cell markers in multiple human adult stem cells." Int J Stem Cells **7**(2): 118-126.

OBJECTIVES: Mesenchymal stem cells (MSCs) are adult stem cells which identified by adherence to plastic, expression of cell surface markers including CD44, CD90, CD105, CD106, CD166, and Stro-1, lack of the expression of hematopoietic markers, no immunogenic effect and replacement of damaged tissues. These properties led to development of progressive methods to isolation and characterization of MSCs from various sources for therapeutic applications in regenerative medicine. METHODS: We isolated MSC-like cells from testis biopsies, ovary, hair follicle and umbilical cord Wharton's jelly and investigated the expression of specific cell surface antigens using flow cytometry in order to verify stemness properties of these cells. RESULTS: All four cell types adhered to plastic culture flask a few days after primary culture. All our cells positively expressed common MSC- specific cell surface markers. Moreover, our results revealed the expression of CD19and CD45 antigens in these cells. CONCLUSION: According to our results, high expression of CD44 in spermatogonial stem cells (SSCs), hair follicle stem cells (HFSCs),granulosa cells (GCs)and Wharton's jelly-MSCs (WJ-MSCs)may help them to maintain stemness properties. Furthermore, we suggest that CD105+SSCs, HFSCs and WJ-MSCs revealed the osteogenic potential of these cells. Moreover, high expression of CD90 in SSCs and HFSCs may associate to higher growth and differentiation potential of these cells. Further, the presence of CD19 on SSCs and GCs may help them to efficiency in response to trans-membrane signals. Thus, these four types of MSCs may be useful in clinical applications and cell therapy.

Manceur, A., et al. (2007). "Flow cytometric screening of cell-penetrating peptides for their uptake into embryonic and adult stem cells." Anal Biochem **364**(1): 51-59.

There is an increasing appreciation of the potential of cell-penetrating peptides (CPPs) as vectors to deliver peptides, proteins, and DNA into cells. However, the absolute and relative efficacy of various CPPs for applications targeting stem cells and primary cells is unclear. In this study, we have developed a two-step loading method and a flow cytometric assay to systematically compare the cellular uptake of five CPPs into embryonic stem cells, neurospheres (NSs), primary bone marrow hematopoietic progenitor (Sca-1(+)Lin(-)) cells, and hematopoietic cell lines (TF-1, K562, and FDCP Mix). The series of CPPs tested included three arginine-rich peptides; one was derived from HIV transactivator of transcription (TAT), one was derived from Antennapedia (Antp), and the third was a synthetic peptide known as protein transduction domain 4 (PTD4). Two hydrophobic peptides were also tested; one was derived from Kaposi fibroblast growth factor (K-FGF), and one was derived from PreS2 surface antigen of hepatitis B virus (PreS2-TLM). Our results indicate, for the first time, that arginine-rich CPPs can internalize into primary NSs and bone marrow Sca-1(+)Lin(-) cells. In addition, in all cell types examined, the uptake of arginine-rich CPPs is significantly greater than that of hydrophobic peptides.

Manceur, A. P. and J. Audet (2009). "Measurement of cell-penetrating peptide-mediated transduction of adult hematopoietic stem cells." Methods Mol Biol **482**: 43-54.

The ability of cell-penetrating peptides (CPPs) to cross cell membranes and transport cargo into cells makes them an attractive tool for the molecular engineering of stem cells. Even though the exact mechanism of transduction remains unclear, their potential has been demonstrated for diverse applications, including hematopoietic stem cell expansion and the generation of islets cells from embryonic stem cells. Several parameters can affect the intracellular delivery of CPP-based constructs. Those include the type of cells targeted, the type of CPP used, and the properties of the cargo. For this reason, it is important to have a means to quantitatively assess the transduction efficiency of specific constructs in the cell type of interest in order to select the best vector for a specific application. In this chapter, we describe a method to measure the uptake of HIV transactivator of transcription (TAT) and the homeobox protein Antennapedia (Antp) constructs in primary hematopoietic progenitor cells and hematopoietic cell

lines. This method is useful to compare, select, and optimize different strategies to deliver CPP-based constructs into a given cell type.

Manesia, J. K., et al. (2017). "Distinct Molecular Signature of Murine Fetal Liver and Adult Hematopoietic Stem Cells Identify Novel Regulators of Hematopoietic Stem Cell Function." Stem Cells Dev **26**(8): 573-584.

During ontogeny, fetal liver (FL) acts as a major site for hematopoietic stem cell (HSC) maturation and expansion, whereas HSCs in the adult bone marrow (ABM) are largely quiescent. HSCs in the FL possess faster repopulation capacity as compared with ABM HSCs. However, the molecular mechanism regulating the greater self-renewal potential of FL HSCs has not yet extensively been assessed. Recently, we published RNA sequencing-based gene expression analysis on FL HSCs from 14.5-day mouse embryo (E14.5) in comparison to the ABM HSCs. We reanalyzed these data to identify key transcriptional regulators that play important roles in the expansion of HSCs during development. The comparison of FL E14.5 with ABM HSCs identified more than 1,400 differentially expressed genes. More than 200 genes were shortlisted based on the gene ontology (GO) annotation term "transcription." By morpholino-based knockdown studies in zebrafish, we assessed the function of 18 of these regulators, previously not associated with HSC proliferation. Our studies identified a previously unknown role for tdg, uhrf1, uchl5, and ncoa1 in the emergence of definitive hematopoiesis in zebrafish. In conclusion, we demonstrate that identification of genes involved in transcriptional regulation differentially expressed between expanding FL HSCs and quiescent ABM HSCs, uncovers novel regulators of HSC function.

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

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

Margaryan, N. V., et al. (2017). "Targeting the Stem Cell Properties of Adult Breast Cancer Cells: Using Combinatorial Strategies to Overcome Drug Resistance." Curr Mol Biol Rep **3**(3): 159-164.

Purpose of review: Cancer is a major public health problem worldwide. In aggressive cancers, which are heterogeneous in nature, there exists a paucity of targetable molecules that can be used to predict outcome and response to therapy in patients, especially those in the high risk category with a propensity to relapse following chemotherapy. This review addresses the challenges pertinent to treating aggressive cancer cells with inherent stem cell properties, with a special focus on triple-negative breast cancer (TNBC). Recent findings: Plasticity underlies the cancer stem cell (CSC) phenotype in aggressive cancers like TNBC. Progenitors and CSCs implement similar signaling pathways to sustain growth, and the convergence of embryonic and tumorigenic signaling pathways has led to the discovery of novel oncofetal targets, rigorously regulated during normal development, but aberrantly reactivated in aggressive forms of cancer. Summary: Translational studies have shown that Nodal, an embryonic morphogen, is reactivated in aggressive cancers, but not in normal tissues, and underlies tumor growth, invasion, metastasis and drug resistance. Front-

line therapies do not inhibit Nodal, but when a combinatorial approach is used with an agent such as doxorubicin followed by anti-Nodal antibody therapy, significant decreases in cell growth and viability occur. These findings are of special interest in the development of new therapeutic interventions that target the stem cell properties of cancer cells to overcome drug resistance and metastasis.

Martin, J., et al. (2008). "Adult lung side population cells have mesenchymal stem cell potential." Cytotherapy **10**(2): 140-151.

BACKGROUND: The development of stem cell therapy for pulmonary diseases remains a challenge. Many diverse cell types reside within the lung and a common stem cell has not yet been identified. A basic understanding of lung stem cell fate during disease may prove important for drug intervention as well as autologous therapies. Niches for resident mesenchymal stem cells (MSC) have been identified in many adult tissues and more recently in the lung. We present data to confirm the observation that non-hematopoietic CD45(neg) lung side population (SP) cells contain MSC, single cells capable of multilineage differentiation. METHODS We carried these observations forward by analyzing the MSC potential of single-cell clones, as well as their chromosomal stability and telomerase activity. RESULTS: The expression of MSC markers was characterized in mouse CD45(neg) lung SP by flow cytometry on freshly isolated or cultured clonal populations. The karyotype of these cells was subsequently assayed by banding analysis, and telomerase activity was assessed using quantitative polymerase chain reaction. MSC differentiation potential was confirmed by the characteristic ability of single-cell clones to differentiate into cells of three mesenchymal lineages, chondrocytes, adipocytes and osteocytes. Differentiation was confirmed by histochemical analysis. All analyzed populations of CD45(neg) lung SP expressed mesenchymal markers (CD44, CD90, CD105, CD106, CD73 and Sca-I) and lacked hematopoietic markers (CD45, c-kit, CD11b, CD34 and CD14). The cultured and clonal CD45(neg) lung SP had normal chromosomal structures and expressed high levels of telomerase. After being expanded and cultured in differentiation medium, all populations of CD45(neg) lung SP demonstrated adipogenic, osteogenic and chrondrogenic potential. Adult CD45(neg) lung SP cells are a source of MSC. DISCUSSION: In defining this tissue-specific stem cell population in the lung, we are now better able to clarify a potential role for them in lung diseases.

Martinez-Herrero, S., et al. (2012). "Adrenomedullin as a growth and cell fate regulatory factor for adult neural stem cells." Stem Cells Int **2012**: 804717.

The use of stem cells as a strategy for tissue repair and regeneration is one of the biomedical research areas that has attracted more interest in the past few years. Despite the classic belief that the central nervous system (CNS) was immutable, now it is well known that cell turnover occurs in the mature CNS. Postnatal neurogenesis is subjected to tight regulation by many growth factors, cell signals, and transcription factors. An emerging molecule involved in this process is adrenomedullin (AM). AM, a 52-amino acid peptide which exerts a plethora of physiological functions, acts as a growth and cell fate regulatory factor for adult neural stem and progenitor cells. AM regulates the proliferation rate and the differentiation into neurons, astrocytes, and oligodendrocytes of stem/progenitor cells, probably through the PI3K/Akt pathway. The active peptides derived from the AM gene are able to regulate the cytoskeleton dynamics, which is extremely important for mature neural cell morphogenesis. In addition, a defective cytoskeleton may impair cell cycle and migration, so AM may contribute to neural stem cell growth regulation by allowing cells to pass through mitosis. Regulation of AM levels may contribute to program stem cells for their use in medical therapies.

Masaki, T., et al. (2013). "Reprogramming adult Schwann cells to stem cell-like cells by leprosy bacilli promotes dissemination of infection." Cell **152**(1-2): 51-67.

Differentiated cells possess a remarkable genomic plasticity that can be manipulated to reverse or change developmental commitments. Here, we show that the leprosy bacterium hijacks this property to reprogram adult Schwann cells, its preferred host niche, to a stage of progenitor/stem-like cells (pSLC) of mesenchymal trait by downregulating Schwann cell lineage/differentiation-associated genes and upregulating genes mostly of mesoderm development. Reprogramming accompanies epigenetic changes and renders infected cells highly plastic, migratory, and immunomodulatory. We provide evidence that acquisition of these properties by pSLC promotes bacterial spread by two distinct mechanisms: direct differentiation to mesenchymal tissues, including skeletal and smooth muscles, and formation of granuloma-like structures and subsequent release of bacteria-laden macrophages. These findings support a model of host cell reprogramming in which a bacterial pathogen uses the plasticity of its cellular niche for promoting dissemination of infection and provide an unexpected link between cellular reprogramming and host-pathogen interaction.

Matsu-Ura, T., et al. (2018). "WNT Takes Two to Tango: Molecular Links between the Circadian Clock and the Cell Cycle in Adult Stem Cells." J Biol Rhythms **33**(1): 5-14.

Like two dancers, the circadian clock and cell cycle are biological oscillators engaged in bidirectional communication, resulting in circadian clock-gated cell division cycles in species ranging from cyanobacteria to mammals. The identified mechanisms for this phenomenon have expanded beyond intracellular molecular coupling components to include intercellular connections. However, detailed molecular mechanisms, dynamics, and physiological functions of the circadian clock and cell cycle as coupled oscillators remain largely unknown. In this review, we discuss current understanding of this connection in light of recent findings that have uncovered intercellular coupling between the circadian clock in Paneth cells and the cell cycle in intestinal stem cells via WNT signaling. This extends the impact of circadian rhythms regulating the timing of cell divisions beyond the intracellular domain of homogenous cell populations into dynamic, multicellular systems. In-depth understanding of the molecular links and dynamics of these two oscillators will identify potential targets and temporal regimens for effective chronotherapy.

Maya-Espinosa, G., et al. (2015). "Mouse embryonic stem cell-derived cells reveal niches that support neuronal differentiation in the adult rat brain." Stem Cells **33**(2): 491-502.

A neurogenic niche can be identified by the proliferation and differentiation of its naturally residing neural stem cells. However, it remains unclear whether "silent" neurogenic niches or regions suitable for neural differentiation, other than the areas of active neurogenesis, exist in the adult brain. Embryoid body (EB) cells derived from embryonic stem cells (ESCs) are endowed with a high potential to respond to specification and neuralization signals of the embryo. Hence, to identify microenvironments in the postnatal and adult rat brain with the capacity to support neuronal differentiation, we transplanted dissociated EB cells to conventional neurogenic and non-neurogenic regions. Our results show a neuronal differentiation pattern of EB cells that was dependent on the host region. Efficient neuronal differentiation of EB cells occurred within an adjacent region to the rostral migratory stream. EB cell differentiation was initially patchy and progressed toward an even distribution along the graft by 15-21 days post-transplantation, giving rise mostly to GABAergic neurons. EB cells in the striatum displayed a lower level of neuronal differentiation and derived into a significant number of astrocytes. Remarkably, when EB cells were transplanted to the striatum of adult rats after a local ischemic stroke, increased number of neuroblasts and neurons were observed. Unexpectedly, we determined that the adult substantia nigra pars compacta, considered a non-neurogenic area, harbors a robust neurogenic environment. Therefore, neurally uncommitted cells derived from ESCs can detect regions that support neuronal differentiation within the adult brain, a fundamental step for the development of stem cell-based replacement therapies.

McQualter, J. L., et al. (2013). "TGF-beta signaling in stromal cells acts upstream of FGF-10 to regulate epithelial stem cell growth in the adult lung." Stem Cell Res **11**(3): 1222-1233.

Tissue resident mesenchymal stromal cells (MSCs) contribute to tissue regeneration through various mechanisms, including the secretion of trophic factors that act directly on epithelial stem cells to promote epithelialization. However, MSCs in tissues constitute a heterogeneous population of stromal cells and different subtypes may have different functions. In this study we show that CD166(neg) and CD166(pos) lung stromal cells have different proliferative and differentiative potential. CD166(neg) lung stromal cells exhibit high proliferative potential with the capacity to differentiate along the lipofibroblastic and myofibroblastic lineages, whereas CD166(pos) lung stromal cells have limited proliferative potential and are committed to the myofibroblastic lineage. Moreover, we show that CD166(pos) lung stromal cells do not share the same epithelial-supportive capacity as their CD166(neg) counterparts, which support the growth of lung epithelial stem cell (EpiSPC) colonies in vitro. In addition, ex vivo expansion of lung stromal cells also resulted in the loss of epithelial-supportive capacity, which could be reinstated by inhibition of the TGF-beta signaling pathway. We show that epithelial-supportive capacity correlated with the level of FGF-10 expression and the reactivation of several lung development-associated genes. In summary, these studies suggest that TGF-beta signaling in stromal cells acts upstream of FGF-10 to regulate epithelial stem cell growth in the adult lung.

Miszta-Lane, H., et al. (2006). "Stem cell sources for clinical islet transplantation in type 1 diabetes: embryonic and adult stem cells." Med Hypotheses **67**(4): 909-913.

Lifelong immunosuppressive therapy and inadequate sources of transplantable islets have led the islet transplantation benefits to less than 0.5% of type 1 diabetics. Whereas the potential risk of infection by animal endogenous viruses limits the uses of islet xeno-transplantation, deriving islets from stem cells seems to be able to overcome the current problems of islet shortages and immune compatibility. Both embryonic (derived from the inner cell mass of blastocysts) and adult stem cells (derived from adult tissues) have shown controversial results in secreting insulin in vitro and normalizing hyperglycemia in vivo. ESCs research is thought to have much greater developmental potential than adult stem cells; however it is still in the basic research phase. Existing ESC lines are not

believed to be identical or ideal for generating islets or beta-cells and additional ESC lines have to be established. Research with ESCs derived from humans is controversial because it requires the destruction of a human embryo and/or therapeutic cloning, which some believe is a slippery slope to reproductive cloning. On the other hand, adult stem cells are already in some degree specialized, recipients may receive their own stem cells. They are flexible but they have shown mixed degree of availability. Adult stem cells are not pluripotent. They may not exist for all organs. They are difficult to purify and they cannot be maintained well outside the body. In order to draw the future avenues in this field, existent discrepancies between the results need to be clarified. In this study, we will review the different aspects and challenges of using embryonic or adult stem cells in clinical islet transplantation for the treatment of type 1 diabetes.

Mizrak, S. C., et al. (2010). "Embryonic stem cell-like cells derived from adult human testis." Hum Reprod **25**(1): 158-167.

BACKGROUND: Given the significant drawbacks of using human embryonic stem (hES) cells for regenerative medicine, the search for alternative sources of multipotent cells is ongoing. Studies in mice have shown that multipotent ES-like cells can be derived from neonatal and adult testis. Here we report the derivation of ES-like cells from adult human testis. METHODS: Testis material was donated for research by four men undergoing bilateral castration as part of prostate cancer treatment. Testicular cells were cultured using StemPro medium. Colonies that appeared sharp edged and compact were collected and subcultured under hES-specific conditions. Molecular characterization of these colonies was performed using RT-PCR and immunohistochemistry. (Epi)genetic stability was tested using bisulphite sequencing and karyotype analysis. Directed differentiation protocols in vitro were performed to investigate the potency of these cells and the cells were injected into immunocompromised mice to investigate their tumorigenicity. RESULTS: In testicular cell cultures from all four men, sharp-edged and compact colonies appeared between 3 and 8 weeks. Subcultured cells from these colonies showed alkaline phosphatase activity and expressed hES cell-specific genes (Pou5f1, Sox2, Cripto1, Dnmt3b), proteins and carbohydrate antigens (POU5F1, NANOG, SOX2 and TRA-1-60, TRA-1-81, SSEA4). These ES-like cells were able to differentiate in vitro into derivatives of all three germ layers including neural, epithelial, osteogenic, myogenic, adipocyte and pancreatic lineages. The pancreatic beta cells were able to produce insulin in response to glucose and osteogenic-differentiated cells showed deposition of phosphate and calcium, demonstrating their functional capacity. Although we observed small areas with differentiated cell types of human origin, we never observed extensive teratomas upon injection of testis-derived ES-like cells into immunocompromised mice. CONCLUSIONS: Multipotent cells can be established from adult human testis. Their easy accessibility and ethical acceptability as well as their non-tumorigenic and autogenic nature make these cells an attractive alternative to human ES cells for future stem cell therapies.

Mo, X. (2007). "[Adult stem cells and stem cell disease]." Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi **21**(7): 759-762.

OBJECTIVE: To summarize the characteristics of adult stem cells and to introduce the definition and the features of stem cell disease. METHODS: Literature concerning adult stem cells and stem cell disease was extensively reviewed. RESULTS: Adult stem cells were localized in tissues and organs, and were able to generate function cells to replace cell loss during a lifetime of wear and tear. The stem cells had self-renewal to maintain themselves and undergo aging within the lifespan of an organism. The dysfunction of stem cells was capable to cause diseases, which could be defined as stem cell disease in human. The disorder of self renewal and differentiation in stem cells could increase the cellular proliferation, produce proliferative diseases such as tumors. The stem cells with self renewal defect, differentiation blockage, or aged stem cells could not supply enough function cells for tissue refreshment. The defect of tissue refreshment caused degenerative diseases. CONCLUSION: Studies on the stem cell self renew, differentiation, and aging can provide knowledge to understand the mechanism of stem cell diseases and develop technique to diagnose and treat these diseases.

Mold, J. E., et al. (2010). "Fetal and adult hematopoietic stem cells give rise to distinct T cell lineages in humans." Science **330**(6011): 1695-1699.

Although the mammalian immune system is generally thought to develop in a linear fashion, findings in avian and murine species argue instead for the developmentally ordered appearance (or "layering") of distinct hematopoietic stem cells (HSCs) that give rise to distinct lymphocyte lineages at different stages of development. Here we provide evidence of an analogous layered immune system in humans. Our results suggest that fetal and adult T cells are distinct populations that arise from different populations of HSCs that are present at different stages of development. We also provide evidence that the fetal T cell lineage is biased toward immune tolerance. These observations offer a mechanistic explanation for the tolerogenic properties of the developing fetus and for variable degrees of immune responsiveness at birth.

Moore, K. E., et al. (2006). "Alternative sources of adult stem cells: a possible solution to the embryonic stem cell debate." Gend Med **3**(3): 161-168.

The complex moral and ethical debate surrounding the definition of the origins of human life, together with conflicting current and proposed legislation on state and federal levels, is hindering the course of research into the therapeutic uses of human embryonic stem cells. However, newly identified sources of adult stem cells, free from many of the ethical and legal concerns attached to embryonic stem cell research, may offer great promise for the advancement of medicine. These alternative sources may alleviate the need to resolve the stem cell debate before further therapeutic benefits of stem cell research can be realized. While legislation and ethics evolve to address the legal and moral issues of embryonic stem cell research, innovative researchers will continue to search for and find real and present solutions for cell-based therapies using adult stem cells.

Moss, J. and N. Toni (2013). "A circuit-based gatekeeper for adult neural stem cell proliferation: Parvalbumin-expressing interneurons of the dentate gyrus control the activation and proliferation of quiescent adult neural stem cells." Bioessays **35**(1): 28-33.

Newborn neurons are generated in the adult hippocampus from a pool of self-renewing stem cells located in the subgranular zone (SGZ) of the dentate gyrus. Their activation, proliferation, and maturation depend on a host of environmental and cellular factors but, until recently, the contribution of local neuronal circuitry to this process was relatively unknown. In their recent publication, Song and colleagues have uncovered a novel circuit-based mechanism by which release of the neurotransmitter, gamma-aminobutyric acid (GABA), from parvalbumin-expressing (PV) interneurons, can hold radial glia-like (RGL) stem cells of the adult SGZ in a quiescent state. This tonic GABAergic signal, dependent upon the activation of gamma(2) subunit-containing GABA(A) receptors of RGL stem cells, can thus prevent their proliferation and subsequent maturation or return them to quiescence if previously activated. PV interneurons are thus capable of suppressing neurogenesis during periods of high network activity and facilitating neurogenesis when network activity is low.

Muller-Borer, B. J., et al. (2006). "Acquired cell-to-cell coupling and "cardiac-like" calcium oscillations in adult stem cells in a cardiomyocyte microenvironment." Conf Proc IEEE Eng Med Biol Soc **1**: 576-579.

Adult-derived stem cells have recently been found to respond in vivo to inductive signals from the microenvironment and to differentiate into a phenotype that is characteristic of cells in that microenvironment. We examined the differentiation potential of an adult liver stem cell line (WBF344) in a cardiac microenvironment in vitro. WBF344 cells were established from a single cloned non-parenchymal epithelial cell isolated from a normal male adult rat liver. Genetically modified, WBF344 cells that express beta-galactosidase, green fluorescent protein (GFP) or mitochondrial red fluorescent protein (DsRed) were co-cultured with rat neonatal cardiac cells. After 4-14 days, we identified WBF344-derived cardiomyocytes that were elongated, binucleated and expressed the cardiac specific proteins cardiac troponin T, cardiac troponin I and N cadherin. These WBF344-derived cardiomyocytes also exhibited myofibrils, sarcomeres, and a nascent sarcoplasmic reticulum. Furthermore, rhythmically beating WBF344-derived cardiomyocytes displayed "cardiac-like" calcium transients similar to the surrounding neonatal cardiomyocytes. Fluorescent recovery after photobleaching demonstrated that WBF344-derived cardiomyocytes were electrically coupled with adjacent neonatal cardiomyocytes through gap junctions (GJs). Collectively, these results support the conclusion that these adult-derived liver stem cells respond to signals generated in a cardiac microenvironment in vitro acquiring a cardiomyocyte phenotype and function. The identification of micro-environmental signals that appear to cross germ layer and species specificities should prove valuable in understanding the regulation of normal development and stem cell differentiation in vivo.

Munsie, M. J., et al. (2000). "Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei." Curr Biol **10**(16): 989-992.

Pluripotent human stem cells isolated from early embryos represent a potentially unlimited source of many different cell types for cell-based gene and tissue therapies [1-3]. Nevertheless, if the full potential of cell lines derived from donor embryos is to be realised, the problem of donor-recipient tissue matching needs to be overcome. One approach, which avoids the problem of transplant rejection, would be to establish stem cell lines from the patient's own cells through therapeutic cloning [3,4]. Recent studies have shown that it is possible to transfer the nucleus from an adult somatic cell to an unfertilised oocyte that is devoid of maternal chromosomes, and achieve embryonic development under the control of the transferred nucleus [5-7]. Stem cells isolated from such a cloned embryo would be genetically identical to the patient and pose no risk of immune rejection. Here, we report the isolation of pluripotent murine stem cells from reprogrammed adult somatic cell nuclei. Embryos were generated by direct injection of mechanically isolated cumulus cell nuclei into mature oocytes. Embryonic stem (ES) cells isolated from cumulus-cell-derived blastocysts displayed the characteristic

morphology and marker expression of conventional ES cells and underwent extensive differentiation into all three embryonic germ layers (endoderm, mesoderm and ectoderm) in tumours and in chimaeric foetuses and pups. The ES cells were also shown to differentiate readily into neurons and muscle in culture. This study shows that pluripotent stem cells can be derived from nuclei of terminally differentiated adult somatic cells and offers a model system for the development of therapies that rely on autologous, human pluripotent stem cells.

Muthna, D., et al. (2010). "Irradiation of adult human dental pulp stem cells provokes activation of p53, cell cycle arrest, and senescence but not apoptosis." Stem Cells Dev **19**(12): 1855-1862.

Adult human dental pulp contains stem cells (DPSCs) that are capable of differentiation into osteoblasts, odontoblasts, adipocytes, and neuronal-like cells. Because these cells have potential use in tissue regeneration, herein we characterized the response of DPSC lines to ionizing radiation (IR). These DPSC lines have been developed from the extracted molars of healthy donors. DPSCs were cultivated in a unique media supplemented with epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). Since tissue homeostasis depends on a precise balance among cell proliferation, senescence, and cell death, we explored the effects of IR (2-20 Gy) on the proliferative activity of DPSCs and the molecular pathways involved. Even the highest dose used (20 Gy) did not induce DPSC apoptosis. After irradiation with doses of 6 and 20 Gy, DPSCs accumulated in the G2 phase of the cell cycle. DPSCs responded to IR (20 Gy) with senescence detected as SA-beta-galactosidase positivity, beginning on the third day after irradiation. Twenty-four hours after irradiation, p53 and its serine 15 and 392 phosphorylated forms were detected. At this time, p21 (WAF1) was induced. Increases in protein p16 were observed from the third day following irradiation and continued till the end of the examination (Day 13). We conclude that DPSCs respond to IR-induced damage by permanent cell cycle arrest in the G2 phase and by stress-induced premature senescence.

Na, I. K., et al. (2013). "Rabbit antithymocyte globulin (thymoglobulin) impairs the thymic output of both conventional and regulatory CD4+ T cells after allogeneic hematopoietic stem cell transplantation in adult patients." Haematologica **98**(1): 23-30.

Rabbit antithymocyte globulin-Genzyme is used to prevent graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Common disadvantages of treatment are infectious complications. The effects of rabbit antithymocyte globulin-Genzyme on thymic function have not been well-studied. Multicolor flow cytometry was used to analyze the kinetics of conventional and regulatory T cells in adult patients treated (n=12) or not treated (n=8) with rabbit antithymocyte globulin-Genzyme during the first 6 months after allogeneic hematopoietic stem cell transplantation. Patients treated with rabbit antithymocyte globulin-Genzyme had almost undetectable levels of recent thymic emigrants (CD45RA(+)CD31(+)) of both conventional and regulatory CD4T cells throughout the 6 months after allogeneic hematopoietic stem cell transplantation whereas CD4(+)CD45RA-memory T cells were less affected, but their levels were also significantly lower than in patients not treated with rabbit antithymocyte globulin-Genzyme. In vitro, rabbit antithymocyte globulin-Genzyme induced apoptosis and cytolysis of human thymocytes, and its cytotoxic effects were greater than those of rabbit antithymocyte globulin-Fresenius. Rabbit antithymocyte globulin-Genzyme in combination with a conditioning regimen strongly impairs thymic recovery of both conventional and regulatory CD4(+) T cells. The sustained depletion of conventional and regulatory CD4(+)T cells carries a high risk of both infections and graft-versus-host disease. Our data indicate that patients treated with rabbit antithymocyte globulin-Genzyme could benefit from thymus-protective therapies and that trials comparing this product with other rabbit antithymocyte globulin preparations or lymphocyte-depleting compounds would be informative.

Na, X. D., et al. (2004). "[Analysis of the different effects of murine bone marrow endothelial cell conditioned medium on the growth of embryonic and adult hematopoietic stem/progenitor cells in vitro]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **12**(3): 255-260.

In the present study, the effects of murine bone marrow endothelial cell conditioned medium (mBMEC-CM) on the growth of yolk sac and bone marrow hematopoietic stem/progenitor cells (HSPC) were investigated. Nonadherent cells of yolk sac and bone marrow were collected for semisolid culture assay of CFU-GM and HPP-CFC after being cultured in DMEM with 10% FBS, 10% mBMEC-CM and/or FL (5 ng/ml), TPO (2 ng/ml) for 24 hours. The number of CFU-GM and HPP-CFC was counted by day 7 and 14 respectively. Atlas cDNA Expression Array was used for analysis of cytokine receptor expression of yolk sac and bone marrow HSPC. The results showed that mBMEC-CM could support the expansion of CFU-GM and HPP-CFC in liquid culture system. The expansion effects of mBMEC-CM were enhanced by combination with FL and TPO. mBMEC-CM was more effective on expansion of bone marrow CFU-GM and HPP-CFC than that of yolk sac CFU-GM and HPP-CFC. The differential expression of cytokine receptors were detected between yolk sac and bone marrow HSPC. PDGF-Rbeta, PDGF-Ralpha and corticotropin releasing factor receptor (CRFR) were only expressed

in yolk sac hematopoietic cells while IFN-gammaR, GM-CSFR, Dopamine D2R and follicle-stimulating hormone receptor were only expressed in bone marrow hematopoietic cells. In conclusion, mBMEC-CM could support the growth and proliferation of yolk sac and bone marrow HSPC, and this effect was further enhanced by addition of FL and TPO. mBMEC-CM was more effective on expansion of bone marrow HSPC than on expansion of yolk sac HSPC. The comparative study indicated that the different expressions of cytokine receptors existed between yolk sac and bone marrow hematopoietic cells, which might lead to the difference in expansion in vitro between embryonic and adult HSPC.

Nagai, Y., et al. (2015). "T memory stem cells are the hierarchical apex of adult T-cell leukemia." Blood **125**(23): 3527-3535.

Adult T-cell leukemia (ATL) is a peripheral CD4(+) T-cell neoplasm caused by human T-cell leukemia virus type 1 (HTLV-1). Despite several investigations using human specimens and mice models, the exact origin of ATL cells remains unclear. Here we provide a new insight into the hierarchical architecture of ATL cells. HTLV-1-infected cells and dominant ATL clones are successfully traced back to CD45RA(+) T memory stem (TSCM) cells, which were recently identified as a unique population with stemlike properties, despite the fact that the majority of ATL cells are CD45RA(-)CD45RO(+) conventional memory T cells. TSCM cells from ATL patients are capable of both sustaining themselves in less proliferative mode and differentiating into other memory T-cell populations in the rapidly propagating phase. In a xenograft model, a low number of TSCM cells efficiently repopulate identical ATL clones and replenish downstream CD45RO(+) memory T cells, whereas other populations have no such capacities. Taken together, these findings demonstrate the phenotypic and functional heterogeneity and the hierarchy of ATL cells. TSCM cells are identified as the hierarchical apex capable of reconstituting identical ATL clones. Thus, this is the first report to demonstrate the association of a T-cell malignancy with TSCM cells.

Nardi, N. B. (2005). "All the adult stem cells, where do they all come from? An external source for organ-specific stem cell pools." Med Hypotheses **64**(4): 811-817.

Stem cells can self-renew and maintain the ability to differentiate into mature lineages. Whereas the "stemness" of embryonic stem cells is not discussed, the primitiveness of a stem cell type within adult organisms is not well determined. Data presently available are either inconclusive or controversial regarding two main topics: maintenance or senescente of the adult stem cell pool; and pluripotentiality of the cells. While programmed senescence or apoptosis following uncorrected mutations represent no problem for mature cells, the maintenance of the stem cell pool itself must be assured. Two different mechanisms can be envisaged for that. In the first mechanism, which is generally accepted, stem cells originate during ontogeny along with the organ which they are responsible for, and remain there during all the lifespan of the organism. Several observations derived from recent reports allow the suggestion of a second mechanism. These observations include: organ-specific stem cells are senescent; adult stem cells circulate in the organism; stem cell niches are essential for the existence and function of stem cells; adult stem cells can present lineage markers; embryo-like, pluripotent stem cells are present in adult organisms, as shown by the development of teratomas, tumors composed of derivatives of the three germ layers; and the fact that the gonads may be a reservoir of embryo-like, pluripotent stem cells in adult organisms. The second mechanism for the maintenance of adult stem cells compartments implies a source external to the organ they belong, consisting of pluripotent, embryo-like cells of unrestricted life span, presenting efficient mechanisms for avoiding or correcting mutations and capable to circulate in the organism. According to this model, primitive stem cells exist in a specific organ in adult organisms. They undergo asymmetrical divisions, which originate one "true" stem cell and another one which enters the pool of adult stem cells, circulating through the entire organism. Upon signals liberated by organ-specific niches, this cell becomes activated to express lineage-specific genes, homes to that particular organ and repopulates its stem cell compartment, differentiating thus in what is seen as the organ-specific stem cell. The gonads are the natural candidates for homing the primitive stem cells in adult organisms. The model proposed in this work for the maintenance of organ-specific stem cell pools from an external source, represented by primitive, embryo-like germinal stem cells present in testes and ovaries, may contribute to the more complete understanding of this complex issue.

Navarro, M., et al. (2012). "The significance of the host inflammatory response on the therapeutic efficacy of cell therapies utilising human adult stem cells." Exp Cell Res **318**(4): 361-370.

Controlling the fate of implanted hMSCs is one of the major drawbacks to be overcome to realize tissue engineering strategies. In particular, the effect of the inflammatory environment on hMSCs behaviour is poorly understood. Studying and mimicking the inflammatory process in vitro is a very complex and challenging task that involves multiple variables. This research addressed the questions using in vitro co-cultures of primary derived hMSCs together with human peripheral blood mononucleated cells (PBMCs);

the latter are key agents in the inflammatory process. This work explored the in vitro phenotypic changes of hMSCs in co-culture direct contact with monocytes and lymphocytes isolated from blood using both basal and osteogenic medium. Our findings indicated that hMSCs maintained their undifferentiated phenotype and pluripotency despite the contact with PBMCs. Moreover, hMSCs demonstrated increased proliferation and were able to differentiate specifically down the osteogenic lineage pathway. Providing significant crucial evidence to support the hypothesis that inflammation and host defence mechanisms could be utilised rather than avoided and combated to provide for the successful therapeutic application of stem cell therapies.

Neal, A., et al. (2012). "The satellite cell in male and female, developing and adult mouse muscle: distinct stem cells for growth and regeneration." PLoS One **7**(5): e37950.

Satellite cells are myogenic cells found between the basal lamina and the sarcolemma of the muscle fibre. Satellite cells are the source of new myofibres; as such, satellite cell transplantation holds promise as a treatment for muscular dystrophies. We have investigated age and sex differences between mouse satellite cells in vitro and assessed the importance of these factors as mediators of donor cell engraftment in an in vivo model of satellite cell transplantation. We found that satellite cell numbers are increased in growing compared to adult and in male compared to female adult mice. We saw no difference in the expression of the myogenic regulatory factors between male and female mice, but distinct profiles were observed according to developmental stage. We show that, in contrast to adult mice, the majority of satellite cells from two week old mice are proliferating to facilitate myofibre growth; however a small proportion of these cells are quiescent and not contributing to this growth programme. Despite observed changes in satellite cell populations, there is no difference in engraftment efficiency either between satellite cells derived from adult or pre-weaned donor mice, male or female donor cells, or between male and female host muscle environments. We suggest there exist two distinct satellite cell populations: one for muscle growth and maintenance and one for muscle regeneration.

Neuss, S., et al. (2004). "Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing." Stem Cells **22**(3): 405-414.

Human mesenchymal stem cells (hMSC) are adult stem cells with multipotent capacities. The ability of mesenchymal stem cells to differentiate into many cell types, as well as their high ex vivo expansion potential, makes these cells an attractive therapeutic tool for cell transplantation and tissue engineering. hMSC are thought to contribute to tissue regeneration, but the signals governing their mobilization, diapedesis into the bloodstream, and migration into the target tissue are largely unknown. Here we report that hepatocyte growth factor (HGF) and the cognate receptor HGFR/c-met are expressed in hMSC, on both the RNA and the protein levels. The expression of HGF was downregulated by transforming growth factor beta. HGF stimulated chemotactic migration but not proliferation of hMSC. Therefore the HGF/c-met signaling system may have an important role in hMSC recruitment sites of tissue regeneration. The controlled regulation of HGF/c-met expression may be beneficial in tissue engineering and cell therapy employing hMSC.

Ng, C. P., et al. (2014). "Enhanced ex vivo expansion of adult mesenchymal stem cells by fetal mesenchymal stem cell ECM." Biomaterials **35**(13): 4046-4057.

Large-scale expansion of highly functional adult human mesenchymal stem cells (aMSCs) remains technologically challenging as aMSCs lose self renewal capacity and multipotency during traditional long-term culture and their quality/quantity declines with donor age and disease. Identification of culture conditions enabling prolonged expansion and rejuvenation would have dramatic impact in regenerative medicine. aMSC-derived decellularized extracellular matrix (ECM) has been shown to provide such microenvironment which promotes MSC self renewal and "stemness". Since previous studies have demonstrated superior proliferation and osteogenic potential of human fetal MSCs (fMSCs), we hypothesize that their ECM may promote expansion of clinically relevant aMSCs. We demonstrated that aMSCs were more proliferative (approximately 1.6 x) on fMSC-derived ECM than aMSC-derived ECMs and traditional tissue culture wares (TCPS). These aMSCs were smaller and more uniform in size (median +/- interquartile range: 15.5 +/- 4.1 mum versus 17.2 +/- 5.0 mum and 15.5 +/- 4.1 mum for aMSC ECM and TCPS respectively), exhibited the necessary biomarker signatures, and stained positive for osteogenic, adipogenic and chondrogenic expressions; indications that they maintained multipotency during culture. Furthermore, fMSC ECM improved the proliferation (approximately 2.2 x), size (19.6 +/- 11.9 mum vs 30.2 +/- 14.5 mum) and differentiation potential in late-passaged aMSCs compared to TCPS. In conclusion, we have established fMSC ECM as a promising cell culture platform for ex vivo expansion of aMSCs.

Nomura, T., et al. (2010). "EphB signaling controls lineage plasticity of adult neural stem cell niche cells." Cell Stem Cell **7**(6): 730-743.

Stem cells remain in specialized niches over the lifespan of the organism in many organs to ensure tissue homeostasis and enable regeneration. How the

niche is maintained is not understood, but is probably as important as intrinsic stem cell self-renewal capacity for tissue integrity. We here demonstrate a high degree of phenotypic plasticity of the two main niche cell types, ependymal cells and astrocytes, in the neurogenic lateral ventricle walls in the adult mouse brain. In response to a lesion, astrocytes give rise to ependymal cells and ependymal cells give rise to niche astrocytes. We identify EphB2 forward signaling as a key pathway regulating niche cell plasticity. EphB2 acts downstream of Notch and is required for the maintenance of ependymal cell characteristics, thereby inhibiting the transition from ependymal cell to astrocyte. Our results show that niche cell identity is actively maintained and that niche cells retain a high level of plasticity.

Ortega, F., et al. (2011). "Using an adherent cell culture of the mouse subependymal zone to study the behavior of adult neural stem cells on a single-cell level." Nat Protoc **6**(12): 1847-1859.

A comprehensive understanding of the cell biology of adult neural stem cells (aNSCs) requires direct observation of aNSC division and lineage progression in the absence of niche-dependent signals. Here we describe a culture preparation of the adult mouse subependymal zone (SEZ), which allows for continuous single-cell tracking of aNSC behavior. The protocol involves the isolation (approximately 3 h) and culture of cells from the adult SEZ at low density in the absence of mitogenic growth factors in chemically defined medium and subsequent live imaging using time-lapse video microscopy (5-7 d); these steps are followed by postimaging immunocytochemistry to identify progeny (approximately 7 h). This protocol enables the observation of the progression from slow-dividing aNSCs of radial/astroglial identity up to the neuroblast stage, involving asymmetric and symmetric cell divisions of distinct fast-dividing precursors. This culture provides an experimental system for studying instructive or permissive effects of signal molecules on aNSC modes of cell division and lineage progression.

P, M., et al. (2011). "Adult mesenchymal stem cells and cell surface characterization - a systematic review of the literature." Open Orthop J **5**(Suppl 2): 253-260.

Human adult mesenchymal stem cells (MSCs) were first identified by Friedenstein et al. when observing a group of cells that developed into fibroblastic colony forming cells (CFU-F). Ever since, the therapeutic uses and clinical applications of these cells have increased research and interest in this field. MSCs have the potential to be used in tissue engineering, gene therapy, transplants and tissue injuries. However, identifying these cells can be a challenge. Moreover, there are no articles bringing together and summarizing the cell surface markers of MSCs in adults. The purpose of this study is to summarize all the available information about the cell surface characterization of adult human MSCs by identifying and evaluating all the published literature in this field. We have found that the most commonly reported positive markers are CD105, CD90, CD44, CD73, CD29, CD13, CD34, CD146, CD106, CD54 and CD166. The most frequently reported negative markers are CD34, CD14, CD45, CD11b, CD49d, CD106, CD10 and CD31. A number of other cell surface markers including STRO-1, SH2, SH3, SH4, HLA-A, HLA-B, HLA-C, HLA-DR, HLA-I, DP, EMA, DQ (MHC Class II), CDIO5, Oct 4, Oct 4A, Nanog, Sox-2, TERT, Stat-3, fibroblast surface antigen, smooth muscle alpha-actin, vimentin, integrin subunits alpha4, alpha5, beta1, integrins alphavbeta3 and alphavbeta5 and ICAM-1 have also been reported. Nevertheless, there is great discrepancy and inconsistency concerning the information available on the cell surface profile of adult MSCs and we suggest that further research is needed in this field to overcome the problem.

Pallafacchina, G., et al. (2010). "An adult tissue-specific stem cell in its niche: a gene profiling analysis of in vivo quiescent and activated muscle satellite cells." Stem Cell Res **4**(2): 77-91.

The satellite cell of skeletal muscle provides a paradigm for quiescent and activated tissue stem cell states. We have carried out transcriptome analyses on satellite cells purified by flow cytometry from Pax3(GFP/+) mice. We compared samples from adult skeletal muscles where satellite cells are mainly quiescent, with samples from growing muscles or regenerating (mdx) muscles, where they are activated. Analysis of regulation that is shared by both activated states avoids other effects due to immature or pathological conditions. This in vivo profile differs from that of previously analyzed satellite cells activated after cell culture. It reveals how the satellite cell protects itself from damage and maintains quiescence, while being primed for activation on receipt of the appropriate signal. This is illustrated by manipulation of the corepressor Dach1, and by the demonstration that quiescent satellite cells are better protected from oxidative stress than those from mdx or 1-week-old muscles. The quiescent versus in vivo activated comparison also gives new insights into how the satellite cell controls its niche on the muscle fiber through cell adhesion and matrix remodeling. The latter also potentiates growth factor activity through proteoglycan modification. Dismantling the extracellular matrix is important for satellite cell activation when the expression of proteinases is up-regulated, whereas transcripts for their inhibitors are high in quiescent cells. In keeping with this, we demonstrate that metalloproteinase function is required for efficient regeneration in vivo.

Panula, S., et al. (2011). "Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells." Hum Mol Genet **20**(4): 752-762.

Historically, our understanding of molecular genetic aspects of human germ cell development has been limited, at least in part due to inaccessibility of early stages of human development to experimentation. However, the derivation of pluripotent stem cells may provide the necessary human genetic system to study germ cell development. In this study, we compared the potential of human induced pluripotent stem cells (iPSCs), derived from adult and fetal somatic cells to form primordial and meiotic germ cells, relative to human embryonic stem cells. We found that approximately 5% of human iPSCs differentiated to primordial germ cells (PGCs) following induction with bone morphogenetic proteins. Furthermore, we observed that PGCs expressed green fluorescent protein from a germ cell-specific reporter and were enriched for the expression of endogenous germ cell-specific proteins and mRNAs. In response to the overexpression of intrinsic regulators, we also observed that iPSCs formed meiotic cells with extensive synaptonemal complexes and post-meiotic haploid cells with a similar pattern of ACROSIN staining as observed in human spermatids. These results indicate that human iPSCs derived from reprogramming of adult somatic cells can form germline cells. This system may provide a useful model for molecular genetic studies of human germline formation and pathology and a novel platform for clinical studies and potential therapeutical applications.

Pare, J. and J. Sherley (2013). "Ex vivo Expansion of Human Adult Pancreatic Cells with Properties of Distributed Stem Cells by Suppression of Asymmetric Cell Kinetics." J Stem Cell Res Ther **3**(4): 149.

Transplantation therapy for type I diabetes (T1D) might be improved if pancreatic stem cells were readily available for investigation. Unlike macroscopic islets, pancreatic tissue stem cells could more easily access the retroperitoneal pancreatic environment and thereby might achieve more effective pancreatic regeneration. Unfortunately, whether the adult pancreas actually contains renewing stem cells continues as a controversial issue in diabetes research. We evaluated a new method developed in our lab for expanding renewing distributed stem cells (DSCs) from adult tissues as a means to provide more evidence for adult pancreatic stem cells, and potentially advance their availability for future clinical investigation. The new method was designed to switch DSCs from asymmetric self-renewal to symmetric self-renewal, which promotes their exponential expansion in culture with reduced production of differentiated cells. Called suppression of asymmetric cell kinetics (SACK), the method uses natural purine metabolites to accomplish the self-renewal pattern shift. The SACK purine metabolites xanthine, xanthosine, and hypoxanthine were evaluated for promoting expansion of DSCs from the pancreas of adult human postmortem donors. Xanthine and xanthosine were effective for deriving both pooled and clonal populations of cells with properties indicative of human pancreatic DSCs. The expanded human cell strains had signature SACK agent-suppressible asymmetric cell kinetics, produced Ngn3+ bipotent precursors for alpha-cells and beta-cells, and were non-tumorigenic in immunodeficient mice. Our findings support the existence of pancreatic DSCs in the adult human pancreas and indicate a potential path to increasing their availability for future clinical evaluation.

Park, K. H., et al. (2014). "Expression of polysialylated neural cell adhesion molecules on adult stem cells after neuronal differentiation of inner ear spiral ganglion neurons." Biochem Biophys Res Commun **453**(2): 282-287.

During brain development, polysialylated (polySia) neural cell adhesion molecules (polySia-NCAMs) modulate cell-cell adhesive interactions involved in synaptogenesis, neural plasticity, myelination, and neural stem cell (NSC) proliferation and differentiation. Our findings show that polySia-NCAM is expressed on NSC isolated from adult guinea pig spiral ganglion (GPSG), and in neurons and Schwann cells after differentiation of the NSC with epidermal, glia, fibroblast growth factors (GFs) and neurotrophins. These differentiated cells were immunoreactive with mAb's to polySia, NCAM, beta-III tubulin, nestin, S-100 and stained with BrdU. NSC could regenerate and be differentiated into neurons and Schwann cells. We conclude: (1) polySia is expressed on NSC isolated from adult GPSG and on neurons and Schwann cells differentiated from these NSC; (2) polySia is expressed on neurons primarily during the early stage of neuronal development and is expressed on Schwann cells at points of cell-cell contact; (3) polySia is a functional biomarker that modulates neuronal differentiation in inner ear stem cells. These new findings suggest that replacement of defective cells in the inner ear of hearing impaired patients using adult spiral ganglion neurons may offer potential hope to improve the quality of life for patients with auditory dysfunction and impaired hearing disorders.

Pekovic, V. and C. J. Hutchison (2008). "Adult stem cell maintenance and tissue regeneration in the ageing context: the role for A-type lamins as intrinsic modulators of ageing in adult stem cells and their niches." J Anat **213**(1): 5-25.

Adult stem cells have been identified in most mammalian tissues of the adult body and are known to support the continuous repair and regeneration of

tissues. A generalized decline in tissue regenerative responses associated with age is believed to result from a depletion and/or a loss of function of adult stem cells, which itself may be a driving cause of many age-related disease pathologies. Here we review the striking similarities between tissue phenotypes seen in many degenerative conditions associated with old age and those reported in age-related nuclear envelope disorders caused by mutations in the LMNA gene. The concept is beginning to emerge that nuclear filament proteins, A-type lamins, may act as signalling receptors in the nucleus required for receiving and/or transducing upstream cytosolic signals in a number of pathways central to adult stem cell maintenance as well as adaptive responses to stress. We propose that during ageing and in diseases caused by lamin A mutations, dysfunction of the A-type lamin stress-resistant signalling network in adult stem cells, their progenitors and/or stem cell niches leads to a loss of protection against growth-related stress. This in turn triggers an inappropriate activation or a complete failure of self-renewal pathways with the consequent initiation of stress-induced senescence. As such, A-type lamins should be regarded as intrinsic modulators of ageing within adult stem cells and their niches that are essential for survival to old age.

Pelacho, B., et al. (2011). "Adult stem cells: from new cell sources to changes in methodology." J Cardiovasc Transl Res **4**(2): 154-160.

Cardiovascular diseases constitute the first cause of mortality and morbidity worldwide. Alternative treatments like transplantation of (stem) cell populations derived from several adult tissue sources, like the bone marrow, skeletal muscle, or even adipose tissue, have been already employed in diverse clinical trials. Results from these studies and previous animal studies have reached to the conclusion that stem cells induce a benefit in the treated hearts, which is exerted mainly through paracrine mechanisms and not through direct differentiation as it was initially expected. However, a strong technical limitation for the stem cell therapy, which is the low level of cell survival and engraftment, diminishes their potential. Thus, new strategies like combination of the cells with bioengineering techniques have been developed and are being subject of intense research, suggesting that new strategies may improve the efficacy of these therapies. In this review, we will discuss the different therapeutic approaches, drawbacks, and future expectations of new regenerative therapies for cardiovascular diseases.

Peyrard, T., et al. (2011). "Banking of pluripotent adult stem cells as an unlimited source for red blood cell production: potential applications for alloimmunized patients and rare blood challenges." Transfus Med Rev **25**(3): 206-216.

The transfusion of red blood cells (RBCs) is now considered a well-settled and essential therapy. However, some difficulties and constraints still occur, such as long-term blood product shortage, blood donor population aging, known and yet unknown transfusion-transmitted infectious agents, growing cost of the transfusion supply chain management, and the inescapable blood group polymorphism barrier. Red blood cells can be now cultured in vitro from human hematopoietic, human embryonic, or human-induced pluripotent stem cells (hiPSCs). The highly promising hiPSC technology represents a potentially unlimited source of RBCs and opens the door to the revolutionary development of a new generation of allogeneic transfusion products. Assuming that in vitro large-scale cultured RBC production efficiently operates in the near future, we draw here some futuristic but realistic scenarios regarding potential applications for alloimmunized patients and those with a rare blood group. We retrospectively studied a cohort of 16,486 consecutive alloimmunized patients (10-year period), showing 1 to 7 alloantibodies with 361 different antibody combinations. We showed that only 3 hiPSC clones would be sufficient to match more than 99% of the 16,486 patients in need of RBC transfusions. The study of the French National Registry of People with a Rare Blood Phenotype/Genotype (10-year period) shows that 15 hiPSC clones would cover 100% of the needs in patients of white ancestry. In addition, one single hiPSC clone would meet 73% of the needs in alloimmunized patients with sickle cell disease for whom rare cryopreserved RBC units were required. As a result, we consider that a very limited number of RBC clones would be able to not only provide for the need for most alloimmunized patients and those with a rare blood group but also efficiently allow for a policy for alloimmunization prevention in multiply transfused patients.

Pfefferle, L. W. and G. A. Wray (2013). "Insights from a chimpanzee adipose stromal cell population: opportunities for adult stem cells to expand primate functional genomics." Genome Biol Evol **5**(10): 1995-2005.

Comparisons between humans and chimpanzees are essential for understanding traits unique to each species. However, linking important phenotypic differences to underlying molecular changes is often challenging. The ability to generate, differentiate, and profile adult stem cells provides a powerful but underutilized opportunity to investigate the molecular basis for trait differences between species within specific cell types and in a controlled environment. Here, we characterize adipose stromal cells (ASCs) from Clint, the chimpanzee whose genome was first sequenced. Using imaging and RNA-Seq, we compare the chimpanzee ASCs with three

comparable human cell lines. Consistent with previous studies on ASCs in humans, the chimpanzee cells have fibroblast-like morphology and express genes encoding components of the extracellular matrix at high levels. Differentially expressed genes are enriched for distinct functional classes between species: immunity and protein processing are higher in chimpanzees, whereas cell cycle and DNA processing are higher in humans. Although hesitant to draw definitive conclusions from these data given the limited sample size, we wish to stress the opportunities that adult stem cells offer for studying primate evolution. In particular, adult stem cells provide a powerful means to investigate the profound disease susceptibilities unique to humans and a promising tool for conservation efforts with nonhuman primates. By allowing for experimental perturbations in relevant cell types, adult stem cells promise to complement classic comparative primate genomics based on in vivo sampling.

Phinney, D. G., et al. (2006). "Murine mesenchymal stem cells transplanted to the central nervous system of neonatal versus adult mice exhibit distinct engraftment kinetics and express receptors that guide neuronal cell migration." Stem Cells Dev **15**(3): 437-447.

Mesenchymal stem cells (MSCs) have demonstrated efficacy as cellular vectors for treating a variety of nervous system disorders. Nevertheless, few studies have quantified MSC engraftment levels or explored the mechanisms that promote their survival and migration in nervous tissue. In this study, we compared the engraftment kinetics and anatomical distribution of murine, male MSCs injected intracranially into neonatal versus adult female mice using a real-time PCR assay that targets the mouse SRY gene. These analyses revealed that MSCs exhibited low but equivalent engraftment levels in the central nervous system (CNS) of neonatal and adult transplant recipients at 12 days post-injection. However, MSC engraftment levels were significantly greater at 60 and 150 days post-transplantation in neonates as compared to adults. Despite these differences, engrafted MSCs were widely distributed along the neuraxis of the CNS in both transplant groups. Collectively, these data indicate that proliferation, but not engraftment and migration, of MSCs in brain are regulated by the host microenvironment. Using a genomics approach, we also identified MSC subpopulations that express neural adhesion proteins and receptors that regulate neuronal cell migration in brain, including cadherin 2, neurexin 1, ninjurin 1, neogenin 1, neuropilin 2, and roundabout homolog 1 and 4. Functional studies indicate these proteins confer cell adhesion and migration of MSCs in response to the appropriate chemoattractant. On the basis of these findings, we conclude that the unique molecular composition of MSC subpopulations imparts to them an inherent capacity to engraft and migrate in brain. These subpopulations may represent more potent cellular vectors for treating CNS disorders.

Pisati, F., et al. (2007). "Induction of neurotrophin expression via human adult mesenchymal stem cells: implication for cell therapy in neurodegenerative diseases." Cell Transplant **16**(1): 41-55.

In animal models of neurological disorders for cerebral ischemia, Parkinson's disease, and spinal cord lesions, transplantation of mesenchymal stem cells (MSCs) has been reported to improve functional outcome. Three mechanisms have been suggested for the effects of the MSCs: transdifferentiation of the grafted cells with replacement of degenerating neural cells, cell fusion, and neuroprotection of the dying cells. Here we demonstrate that a restricted number of cells with differentiated astroglial features can be obtained from human adult MSCs (hMSCs) both in vitro using different induction protocols and in vivo after transplantation into the developing mouse brain. We then examined the in vitro differentiation capacity of the hMSCs in coculture with slices of neonatal brain cortex. In this condition the hMSCs did not show any neuronal transdifferentiation but expressed neurotrophin low-affinity (NGFR(p75)) and high-affinity (trkC) receptors and released nerve growth factor (NGF) and neurotrophin-3 (NT-3). The same neurotrophin's expression was demonstrated 45 days after the intracerebral transplantation of hMSCs into nude mice with surviving astroglial cells. These data further confirm the limited capability of adult hMSC to differentiate into neurons whereas they differentiated in astroglial cells. Moreover, the secretion of neurotrophic factors combined with activation of the specific receptors of transplanted hMSCs demonstrated an alternative mechanism for neuroprotection of degenerating neurons. hMSCs are further defined in their transplantation potential for treating neurological disorders.

Pochampally, R. R., et al. (2004). "Rat adult stem cells (marrow stromal cells) engraft and differentiate in chick embryos without evidence of cell fusion." Proc Natl Acad Sci U S A **101**(25): 9282-9285.

Cell fusion was recently reported to account for the plasticity of adult stem cells in vivo. Adult stem cells, referred to as mesenchymal stem cells or marrow stromal cells, from rat marrow, were infused into 1.5- to 2-day-old chick embryos. After 4 days, the rat cells had expanded 1.3- to 33-fold in one-third of surviving embryos. The cells engrafted into many tissues, and no multinuclear cells were detected. The most common site of engraftment was the heart, apparently because the cells were infused just above the dorsal aorta. Some of the cells in the heart expressed cardiotin, and alpha-heavy-chain myosin. GFP(+) cells reisolated from the embryos had a rat karyotype. Therefore, the cells

engrafted and partially differentiated without evidence of cell fusion.

Porlan, E., et al. (2016). "Stable and Efficient Genetic Modification of Cells in the Adult Mouse V-SVZ for the Analysis of Neural Stem Cell Autonomous and Non-autonomous Effects." J Vis Exp(108): 53282.

Relatively quiescent somatic stem cells support life-long cell renewal in most adult tissues. Neural stem cells in the adult mammalian brain are restricted to two specific neurogenic niches: the subgranular zone of the dentate gyrus in the hippocampus and the ventricular-subventricular zone (V-SVZ; also called subependymal zone or SEZ) in the walls of the lateral ventricles. The development of in vivo gene transfer strategies for adult stem cell populations (i.e. those of the mammalian brain) resulting in long-term expression of desired transgenes in the stem cells and their derived progeny is a crucial tool in current biomedical and biotechnological research. Here, a direct in vivo method is presented for the stable genetic modification of adult mouse V-SVZ cells that takes advantage of the cell cycle-independent infection by LVs and the highly specialized cytoarchitecture of the V-SVZ niche. Specifically, the current protocol involves the injection of empty LVs (control) or LVs encoding specific transgene expression cassettes into either the V-SVZ itself, for the in vivo targeting of all types of cells in the niche, or into the lateral ventricle lumen, for the targeting of ependymal cells only. Expression cassettes are then integrated into the genome of the transduced cells and fluorescent proteins, also encoded by the LVs, allow the detection of the transduced cells for the analysis of cell autonomous and non-autonomous, niche-dependent effects in the labeled cells and their progeny.

Prindull, G. (2005). "Hypothesis: cell plasticity, linking embryonal stem cells to adult stem cell reservoirs and metastatic cancer cells?" Exp Hematol **33**(7): 738-746.

Embryonal stem (ES) cells are the earliest ontogenetically identifiable stem cells of the embryo proper for all subsequent mesenchymal stem cells and for highly specialized differentiated cells. This review characterizes, in a working hypothesis, the role of reversible EMT/MET (epithelialmesenchymal transition) as a manifestation of cell plasticity 1) in the development of ES cells to adult stem cells (hematopoietic stem cells) and 2) in metastasizing cancer cells. Animal studies support the concept that EMT/MET is a key manifestation of cell plasticity in the development of ES cells to adult stem cells, and in conversion of localized to metastasizing cancer cells. In fact, ES cells may persist to postnatal life, in cytologically verifiable form and/or within the frame of EMT/MET, as ultimate reservoir for adult stem cells. Furthermore, EMT could possibly serve as a conceptional link between physiologic and pathologic signaling pathways. Clonal confirmation in humans is necessary.

Prockop, D. J., et al. (2003). "One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues." Proc Natl Acad Sci U S A **100 Suppl 1**: 11917-11923.

Most recent evidence suggests that the process of tissue repair is driven by stem-like cells that reside in multiple tissues but are replenished by precursor cells from bone marrow. Among the candidates for the reparative cells are the adult stem cells from bone marrow referred to as either mesenchymal stem cells or marrow stromal cells (MSCs). We recently found that after MSCs were replated at very low densities to generate single-cell-derived colonies, they did not exit a prolonged lag period until they synthesized and secreted considerable quantities of Dickkopf-1, an inhibitor of the canonical Wnt signaling pathway. We also found that when the cells were cocultured with heat-shocked pulmonary epithelial cells, they differentiated into epithelial cells. Most of the MSCs differentiated without evidence of cell fusion but up to one-quarter underwent cell fusion with the epithelial cells. A few also underwent nuclear fusion. The results are consistent with the interesting possibility that MSCs and similar cells repair tissue injury by three different mechanisms: creation of a milieu that enhances regeneration of endogenous cells, transdifferentiation, and perhaps cell fusion.

Raasch, K., et al. (2015). "Identification of Nucleoside Analogs as Inducers of Neuronal Differentiation in a Human Reporter Cell Line and Adult Stem Cells." Chem Biol Drug Des **86**(2): 129-143.

Nucleoside analogs (NSAs) were among the first chemotherapeutic agents and could also be useful for the manipulation of cell fate. To investigate the potential of NSAs for the induction of neuronal differentiation, we developed a novel phenotypic assay based on a human neuron-committed teratocarcinoma cell line (NT2) as a model for neuronal progenitors and constructed a NT2-based reporter cell line that expressed eGFP under the control of a neuron-specific promoter. We tested 38 structurally related NSAs and determined their activity to induce neuronal differentiation by immunocytochemistry of neuronal marker proteins, live cell imaging, fluorometric detection and immunoblot analysis. We identified twelve NSAs, which induced neuronal differentiation to different extents. NSAs with highest activity carried a halogen substituent at their pyrimidine nucleobase and an unmodified or 2'-O-methyl substituted 2-deoxy-beta-D-ribofuranosyl residue as glyconic moiety. Cladribine, a purine nucleoside with similar structural features and in use to treat leukemia and multiple sclerosis, induced also differentiation of adult human neural crest-derived stem cells. Our results suggest that

NSAs could be useful for the manipulation of neuronal cell fate in cell replacement therapy or treatment of neurodegenerative disorders. The data on the structure and function relationship will help to design compounds with increased activity and low toxicity.

Rabin, D. M., et al. (2013). "Chronic oxidative stress upregulates Drusen-related protein expression in adult human RPE stem cell-derived RPE cells: a novel culture model for dry AMD." Aging (Albany NY) **5**(1): 51-66.

PURPOSE: The goal of this study was to examine changes in the expression of transcripts and proteins associated with drusen in Age-related Macular Degeneration (AMD) after exposing human retinal pigment epithelium (hRPE) cells to chronic oxidative stress. METHODS: Primary adult human RPE cells were isolated from cadaveric donor eyes. The subpopulation of RPE stem cells (RPESCs) was activated, expanded, and then differentiated into RPE progeny. Confluent cultures of RPESC-derived hRPE and ARPE-19 cells were exposed to a regimen of tert-butylhydroperoxide (TBHP) for 1-5 days. After treatment, gene expression was measured by quantitative PCR (qPCR), protein expression was assessed by immunocytochemistry and transepithelial resistance and cell toxicity were measured. RESULTS: hRPE cells exposed to a regimen of TBHP for 5 days upregulate expression of several molecules identified in drusen, including molecular chaperones and pro-angiogenic factors. 5-day TBHP treatment was significantly more effective than 1-day treatment at eliciting these effects. The extent of hRPE response to 5-day treatment varied significantly between individual donors, nevertheless, 6 transcripts were reliably significantly upregulated. ARPE-19 cells treated with the same 5-day stress regime did not show the same pattern of response and did not upregulate this group of transcripts. CONCLUSIONS: RPESC-derived hRPE cells change significantly when exposed to repeated oxidative stress conditions, upregulating expression of several drusen-related proteins and transcripts. This is consistent with the hypothesis that hRPE cells are competent to be a source of proteins found in drusen deposits. Our results suggest that donor-specific genetic and environmental factors influence the RPE stress response. ARPE-19 cells appear to be less representative of AMD-like changes than RPESC-derived hRPE. This adult stem cell-based system using chronic TBHP treatment of hRPE represents a novel in vitro model useful for the study of drusen formation and dry AMD pathophysiology.

Radtke, S., et al. (2016). "The frequency of multipotent CD133(+)CD45RA(-)CD34(+) hematopoietic stem cells is not increased in fetal liver compared with adult stem cell sources." Exp Hematol **44**(6): 502-507.

The cell surface marker CD133 has been used to describe a revised model of adult human hematopoiesis, with hematopoietic stem cells and multipotent progenitors (HSCs/MPPs: CD133(+)CD45RA(-)CD34(+)) giving rise to lymphomyeloid-primed progenitors (LMPPs: CD133(+)CD45RA(+)CD34(+)) and erythromyeloid progenitors (EMPs: CD133(low)CD45RA(-)CD34(+)). Because adult and fetal hematopoietic stem and progenitor cells (HSPCs) differ in their gene expression profile, differentiation capabilities, and cell surface marker expression, we were interested in whether the reported segregation of lineage potentials in adult human hematopoiesis would also apply to human fetal liver. CD133 expression was easily detected in human fetal liver cells, and the defined hematopoietic subpopulations were similar to those found for adult HSPCs. Fetal HSPCs were enriched for EMPs and HSCs/MPPs, which were primed toward erythromyeloid differentiation. However, the frequency of multipotent CD133(+)CD45RA(-)CD34(+) HSPCs was much lower than previously reported and comparable to that of umbilical cord blood. We noted that engraftment in NSG (NOD scid gamma [NOD.Cg-Prkdc(scid) Il2rg(tm1Wjl)/SzJ]) mice was driven mostly by LMPPs, confirming recent findings that repopulation in mice is not a unique feature of multipotent HSCs/MPPs. Thus, our data challenge the general assumption that human fetal liver contains a greater percentage of multipotent HSCs/MPPs than any adult HSC source, and the mouse model may have to be re-evaluated with respect to the type of readout it provides.

Reinshagen, H., et al. (2011). "Corneal surface reconstruction using adult mesenchymal stem cells in experimental limbal stem cell deficiency in rabbits." Acta Ophthalmol **89**(8): 741-748.

PURPOSE: To investigate the ability of mesenchymal stem cells (MSC) to transdifferentiate to corneal epithelial cells in experimental limbal stem cell deficiency in rabbits. METHODS: Total limbal stem cell deficiency was produced in 21 right eyes of 21 New Zealand rabbits; 6 eyes served as controls (group 1, G1). After removal of the conjunctival overgrowth, five eyes received amniotic membrane transplantation (AMT; G2). In four eyes, autologous limbal stem cell transplantation from the healthy eye was performed with AMT (G3). In another six eyes, enriched autologous MSC were injected under the amniotic membrane (AM) (G4). Within 280 days, corneoscleral discs were analysed for goblet cells, cytokeratin (CK) 3/12, connexin 43, beta(1) -integrin, CK 19, alpha-enolase, p63 and ATP-binding cassette transporter subtype G-2 (ABCG-2) distribution patterns. RESULTS: Cultivated MSC were positive for CK 3/12 and alpha-enolase, but negative for ABCG-2, p63 and

connexin 43. On rabbit corneas, CK 3/12 was expressed in all corneal regions in all groups, but with significantly different intensities. Among all other parameters, expression levels of ABCG-2, beta(1) -integrin and connexin 43 were significantly different between the transplanted groups and the control group. After a mean follow-up time of 172 (47-280) days, goblet cells were rarely present in the central cornea (G1-4). CONCLUSION: CK 3/12 is not highly specific for differentiated corneal epithelium. Further, goblet cells are not a reliable marker for conjunctivalization in rabbits. Expression of ABCG-2, beta(1)-integrin and connexin 43 after mesenchymal stem cell transplantation may indicate their ability to maintain their stem cell character or to transdifferentiate to epithelial progenitor cells.

Ren, Y., et al. (2014). "Analysis of the stem cell characteristics of adult stem cells from Arbas white Cashmere goat." Biochem Biophys Res Commun **448**(2): 121-128.

Studies have shown that multipotent adult stem cells possess differentiation characteristics similar to embryonic stem cells and pluripotent stem cells. We aimed to explore these similarities further by examining the expression of the pluripotency and stemness biomarkers, AKP, IL-6, Nanog, Oct-4, Rex-1, Sox-2 and TERT, as well as the triploblastic biomarkers, Sox-1, Myod1 and Gata-6 in adipose-derived stem cells (ADSCs), bone marrow stem cells (BMSCs) and muscle-derived satellite cells (MDSCs). These were isolated from adult Arbas white Cashmere goats and cultured in vitro. Immunocytochemistry, reverse transcription quantitative PCR and Western blotting were used to analyze the protein and mRNA expression of the markers. To investigate the ability of ADSCs, BMSCs and MDSCs to differentiate and cause tumors in vivo they were injected into immunodeficient mice (NOD-SCID). All results were compared to those for mouse embryonic stem cells (mESCs). Immunocytochemistry showed that AKP, IL-6, Nanog, Oct-4, Rex-1 and TERT were expressed in ADSCs, BMSCs and MDSCs, whereas Sox-2 was not. In ADSCs, the expression of IL-6 mRNA was relatively high, followed by Nanog and Oct-4, while Rex-1 and TERT expression were the lowest ($P<0.01$). In BMSCs, the expression of Rex-1 was relatively high, followed by IL-6, while Oct-4, Nanog and TERT were comparatively low ($P<0.01$). In MDSCs, the expression of IL-6, Nanog and Oct-4 were relatively high, while TERT was comparatively low ($P<0.01$). However, no expression of Sox-2 mRNA was detected in any of the three cell lines. The expression of Sox-1, Myod1 and Gata-6 was observed to different degrees in all three cell lines ($P<0.01$); the expression pattern in MDSCs was different from that in ADSCs and BMSCs. Western blotting indicated that no expression of Sox-2 and Rex-1 protein occurred in ADSCs, BMSCs and MDSCs, while the other five proteins were all expressed to different degrees ($P<0.01$); the expression pattern was consistent with the mRNA results. In contrast to the mESCs, no teratoma tissue or triploblastic differentiation appendages were formed in the immunodeficient mice after injection of ADSCs, BMSCs and MDSCs. Our results suggest that the three adult goat stem cell types are non-oncogenic and have stemness characteristics similar to embryonic stem cells. Of these, MDSCs were found to exhibit the most ESC-like properties and would make the best candidates for clinical application.

Resende, R. R., et al. (2010). "Influence of spontaneous calcium events on cell-cycle progression in embryonal carcinoma and adult stem cells." Biochim Biophys Acta **1803**(2): 246-260.

Spontaneous Ca(2+) events have been observed in diverse stem cell lines, including carcinoma and mesenchymal stem cells. Interestingly, during cell cycle progression, cells exhibit Ca(2+) transients during the G(1) to S transition, suggesting that these oscillations may play a role in cell cycle progression. We aimed to study the influence of promoting and blocking calcium oscillations in cell proliferation and cell cycle progression, both in neural progenitor and undifferentiated cells. We also identified which calcium stores are required for maintaining these oscillations. Both in neural progenitor and undifferentiated cells calcium oscillations were restricted to the G1/S transition, suggesting a role for these events in progression of the cell cycle. Maintenance of the oscillations required calcium influx only through inositol 1,4,5-triphosphate receptors (IP(3)Rs) and L-type channels in undifferentiated cells, while neural progenitor cells also utilized ryanodine-sensitive stores. Interestingly, promoting calcium oscillations through IP(3)R agonists increased both proliferation and levels of cell cycle regulators such as cyclins A and E. Conversely, blocking calcium events with IP(3)R antagonists had the opposite effect in both undifferentiated and neural progenitor cells. This suggests that calcium events created by IP(3)Rs may be involved in cell cycle progression and proliferation, possibly due to regulation of cyclin levels, both in undifferentiated cells and in neural progenitor cells.

Rim, J. S., et al. (2005). "Mesenchymal stem cells from the outer ear: a novel adult stem cell model system for the study of adipogenesis." FASEB J **19**(9): 1205-1207.

Adipocytes arise from multipotent stem cells of mesodermal origin, which also give rise to the muscle, bone, and cartilage lineages. However, signals and early molecular events that commit multipotent stem cells into the adipocyte lineage are not well established mainly due to lack of an adequate model

system. We have identified a novel source of adult stem cells from the external murine ears referred to here as an ear mesenchymal stem cells (EMSC). EMSC have been isolated from several standard and mutant strains of mice. They are self-renewing, clonogenic, and multipotent, since they give rise to osteocytes, chondrocytes, and adipocytes. The in vitro characterization of EMSC indicates very facile adipogenic differentiation. Morphological, histochemical, and molecular analysis after the induction of differentiation showed that EMSC maintain adipogenic potentials up to fifth passage. A comparison of EMSC to the stromal-vascular (S-V) fraction of fat depots, under identical culture conditions (isobutyl-methylxanthine, dexamethasone, and insulin), revealed much more robust and consistent adipogenesis in EMSC than in the S-V fraction. In summary, we show that EMSC can provide a novel, easily obtainable, primary culture model for the study of adipogenesis.

Rios, M. and D. A. Williams (1990). "Systematic analysis of the ability of stromal cell lines derived from different murine adult tissues to support maintenance of hematopoietic stem cells in vitro." J Cell Physiol **145**(3): 434-443.

Hematopoietic stem cells interact with a complex microenvironment both in vivo and in vitro. In association with this microenvironment, murine stem cells are maintained in vitro for several months. Fibroblast-like stromal cells appear to be important components of the microenvironment, since several laboratories have demonstrated that cloned stromal cell lines support hematopoiesis in vitro. The importance of the tissue of origin of such cell lines remains unknown, since systematic generation of stromal cell lines from adult tissues has never been accomplished. In addition, the capacity of stromal cell lines to support reconstituting stem cell has not been examined. We have previously described an efficient and rapid method for the immortalization of primary bone marrow stromal cell lines (Williams et al., Mol. Cell. Biol. 8:3864-3871, 1988) which can be used to systematically derive cell lines from multiple tissues of the adult mouse. Here we report the immortalization of primary murine lung, kidney, skin, and bone marrow stromal cells using a recombinant retrovirus vector (U19-5) containing the simian virus large T antigen (SV40 LT) and the neophosphotransferase gene. The interaction of these stromal cells with factor-dependent cells Patterson-Mix (FDCP-Mix), colony forming units-spleen (CFU-S), and reconstituting hematopoietic stem cells was studied in order to analyze the ability of such lines to support multipotent stem cells in vitro. These studies revealed that stromal cell lines from these diverse tissues were morphologically and phenotypically similar and that they quantitatively bound CFU-S and FDCP-Mix cells equally well. However, only those cell lines derived from bone marrow-supported maintenance of day 12 CFU-S in vitro. One lung-derived stromal cell line, ULU-3, supported the survival of day 8 CFU-S, but not the more primitive CFU-S12. A bone marrow-derived stromal cell line, U2, supported the survival of long-term reconstituting stem cells for up to 3 weeks in vitro as assayed by reconstitution 1 year post-transplant. These studies suggest that adherence of HSC to stromal cells is necessary but not sufficient for maintenance of these stem cell populations and that bone marrow provides specific signals relating to hematopoietic stem cell survival and proliferation.

Riz, I. and R. G. Hawley (2017). "Increased expression of the tight junction protein TJP1/ZO-1 is associated with upregulation of TAZ-TEAD activity and an adult tissue stem cell signature in carfilzomib-resistant multiple myeloma cells and high-risk multiple myeloma patients." Oncoscience **4**(7-8): 79-94.

Tight junction protein 1 (TJP1) has recently been proposed as a biomarker to identify multiple myeloma (MM) patients most likely to respond to bortezomib- and carfilzomib-based proteasome inhibitor regimens. Herein we report increased expression of TJP1 during the adaptive response mediating carfilzomib resistance in the LP-1/Cfz MM cell line. Moreover, increased TJP1 expression delineated a subset of relapsed/refractory MM patients on bortezomib-based therapy sharing an LP-1/Cfz-like phenotype characterized by activation of interacting transcriptional effectors of the Hippo signaling cascade (TAZ and TEAD1) and an adult tissue stem cell signature. siRNA-mediated knockdown of TJP1 or TAZ/TEAD1 partially sensitized LP-1/Cfz cells to carfilzomib. Connectivity Map analysis identified translation inhibitors as candidate therapeutic agents targeting this molecular phenotype. We confirmed this prediction by showing that homoharringtonine (omacetaxine mepesuccinate) - the first translation inhibitor to be approved by the U.S. Food and Drug Administration - displayed potent cytotoxic activity on LP-1/Cfz cells. Homoharringtonine treatment reduced the levels of TAZ and TEAD1 as well as the MM-protective proteins Nrf2 and MCL1. Thus, our data suggest the importance of further studies evaluating translation inhibitors in relapsed/refractory MM. On the other hand, use of TJP1 as a MM biomarker for proteasome inhibitor sensitivity requires careful consideration.

Robertson, M. J., et al. (2008). "Neural stem cell engineering: directed differentiation of adult and embryonic stem cells into neurons." Front Biosci **13**: 21-50.

Both adult neural stem cells and embryonic stem cells have shown the capacity to differentiation into multiple cell types of the adult nervous system.

They will therefore serve as valuable systems for basic investigations of cell fate choice mechanisms, as well as play important future roles in applications ranging from regenerative medicine to drug screening. However, there are significant challenges remaining, including the identification of signaling factors that specify cell fate in the stem cell niche, the analysis of intracellular targets and mechanisms of these extracellular signals, and the development of ex vivo culture systems that can exert efficient control over cell function. This review will discuss progress in the identification of signaling mechanisms and culture systems that regulate neural differentiation, neuronal differentiation, and neuronal subtype specification.

Rocheteau, P., et al. (2012). "A subpopulation of adult skeletal muscle stem cells retains all template DNA strands after cell division." Cell **148**(1-2): 112-125.

Satellite cells are adult skeletal muscle stem cells that are quiescent and constitute a poorly defined heterogeneous population. Using transgenic Tg:Pax7-nGFP mice, we show that Pax7-nGFP(Hi) cells are less primed for commitment and have a lower metabolic status and delayed first mitosis compared to Pax7-nGFP(Lo) cells. Pax7-nGFP(Hi) can give rise to Pax7-nGFP(Lo) cells after serial transplantations. Proliferating Pax7-nGFP(Hi) cells exhibit lower metabolic activity, and the majority performs asymmetric DNA segregation during cell division, wherein daughter cells retaining template DNA strands express stem cell markers. Using chromosome orientation-fluorescence in situ hybridization, we demonstrate that all chromatids segregate asymmetrically, whereas Pax7-nGFP(Lo) cells perform random DNA segregation. Therefore, quiescent Pax7-nGFP(Hi) cells represent a reversible dormant stem cell state, and during muscle regeneration, Pax7-nGFP(Hi) cells generate distinct daughter cell fates by asymmetrically segregating template DNA strands to the stem cell. These findings provide major insights into the biology of stem cells that segregate DNA asymmetrically.

Roose, H., et al. (2017). "Major depletion of SOX2(+) stem cells in the adult pituitary is not restored which does not affect hormonal cell homeostasis and remodelling." Sci Rep **7**(1): 16940.

The pituitary gland contains SOX2-expressing stem cells. However, their functional significance remains largely unmapped. We investigated their importance by depleting SOX2(+) cells through diphtheria toxin (DT)-mediated ablation. DT treatment of adult Sox2(CreERT2/+);R26(iDTR/+) mice (after tamoxifen-induced expression of DT receptor in SOX2(+) cells) resulted in 80% obliteration of SOX2(+) cells in the endocrine pituitary, coinciding with reduced pituisphere-forming activity. Counterintuitively for a stem cell population, the SOX2(+) cell compartment did not repopulate. Considering the more active phenotype of the stem cells during early-postnatal pituitary maturation, SOX2(+) cell ablation was also performed in 4- and 1-week-old animals. Ablation grade diminished with decreasing age and was accompanied by a proliferative reaction of the SOX2(+) cells, suggesting a rescue attempt. Despite this activation, SOX2(+) cells did also not recover. Finally, the major SOX2(+) cell depletion in adult mice did not affect the homeostatic maintenance of pituitary hormonal cell populations, nor the corticotrope remodelling response to adrenalectomy challenge. Taken together, our study shows that pituitary SOX2(+) fail to regenerate after major depletion which does not affect adult endocrine cell homeostasis and remodelling. Thus, pituitary SOX2(+) cells may constitute a copious stem cell reserve or may have other critical role(s) still to be clearly defined.

Sallustio, F., et al. (2017). "Inhibin-A and Decorin Secreted by Human Adult Renal Stem/Progenitor Cells Through the TLR2 Engagement Induce Renal Tubular Cell Regeneration." Sci Rep **7**(1): 8225.

Acute kidney injury (AKI) is a public health problem worldwide. Several therapeutic strategies have been made to accelerate recovery and improve renal survival. Recent studies have shown that human adult renal progenitor cells (ARPCs) participate in kidney repair processes, and may be used as a possible treatment to promote regeneration in acute kidney injury. Here, we show that human tubular ARPCs (tARPCs) protect physically injured or chemically damaged renal proximal tubular epithelial cells (RPTECs) by preventing cisplatin-induced apoptosis and enhancing proliferation of survived cells. tARPCs without toll-like receptor 2 (TLR2) expression or TLR2 blocking completely abrogated this regenerative effect. Only tARPCs, and not glomerular ARPCs, were able to induce tubular cell regeneration process and it occurred only after damage detection. Moreover, we have found that ARPCs secreted inhibin-A and decorin following the RPTEC damage and that these secreted factors were directly involved in cell regeneration process. Polysaccharide synthetic vesicles containing these molecules were constructed and co-cultured with cisplatin damaged RPTECs. These synthetic vesicles were not only incorporated into the cells, but they were also able to induce a substantial increase in cell number and viability. The findings of this study increase the knowledge of renal repair processes and may be the first step in the development of new specific therapeutic strategies for renal repair.

Sart, S., et al. (2009). "Ear mesenchymal stem cells: an efficient adult multipotent cell population fit for rapid and scalable expansion." J Biotechnol **139**(4): 291-299.

Bone marrow mesenchymal stem cells (BM-MSCs) have the potential to be used for tissue

engineering. Nevertheless, they exhibit a low growth rate that limits their availability. In this work we use an alternative model of MSCs from the outer ear (ear mesenchymal stem cells, E-MSCs). These cells bear the characteristics of progenitor cells because of their ability to be differentiated into the three lineages of chondrocytes, osteocytes and adipocytes. This model cell population had a threefold higher cell growth rate compared to BM-MSCs. This allowed rapid testing of the scalability in microcarrier culture using bead-to-bead transfer and also enabled their expansion in a 1-l bioreactor. The cells were able to maintain their potential for differentiation into the above three lineages. Therefore, E-MSCs appear to be an attractive model for assessing a number of bioengineering parameters that may affect the behavior of adult stem cells in culture.

Schnerch, A., et al. (2013). "Human embryonic stem cell-derived hematopoietic cells maintain core epigenetic machinery of the polycomb group/Trithorax Group complexes distinctly from functional adult hematopoietic stem cells." Stem Cells Dev **22**(1): 73-89.

Hematopoietic cells derived from human embryonic stem cells (hESCs) have a number of potential utilities, including the modeling of hematological disorders in vitro, whereas the use for cell replacement therapies has proved to be a loftier goal. This is due to the failure of differentiated hematopoietic cells, derived from human pluripotent stem cells (hPSCs), to functionally recapitulate the in vivo properties of bona fide adult hematopoietic stem/progenitor cells (HSPCs). To better understand the limitations of differentiation programming at the molecular level, we have utilized differential gene expression analysis of highly purified cells that are enriched for hematopoietic repopulating activity across embryonic, fetal, and adult human samples, including in vivo explants of human HSPCs 8-weeks post-transplantation. We reveal that hESC-derived hematopoietic progenitor cells (eHPCs) fail to express critical transcription factors which are known to govern self-renewal and myeloid/lymphoid development and instead retain the expression of Polycomb Group (PcG) and Trithorax Group (TrxG) factors which are more prevalent in embryonic cell types that include EZH1 and ASH1L, respectively. These molecular profiles indicate that the differential expression of the core epigenetic machinery comprising PcGs/TrxGs in eHPCs may serve as previously unexplored molecular targets that direct hematopoietic differentiation of PSCs toward functional HSPCs in humans.

Scopelliti, A., et al. (2014). "Local control of intestinal stem cell homeostasis by enteroendocrine cells in the adult Drosophila midgut." Curr Biol **24**(11): 1199-1211.

BACKGROUND: Enteroendocrine cells populate gastrointestinal tissues and are known to translate local cues into systemic responses through the release of hormones into the bloodstream. RESULTS: Here we report a novel function of enteroendocrine cells acting as local regulators of intestinal stem cell (ISC) proliferation through modulation of the mesenchymal stem cell niche in the Drosophila midgut. This paracrine signaling acts to constrain ISC proliferation within the epithelial compartment. Mechanistically, midgut enteroendocrine cells secrete the neuroendocrine hormone Bursicon, which acts-beyond its known roles in development-as a paracrine factor on the visceral muscle (VM). Bursicon binding to its receptor, DLGR2, the ortholog of mammalian leucine-rich repeat-containing G protein-coupled receptors (LGR4-6), represses the production of the VM-derived EGF-like growth factor Vein through activation of cAMP. CONCLUSIONS: We therefore identify a novel paradigm in the regulation of ISC quiescence involving the conserved ligand/receptor Bursicon/DLGR2 and a previously unrecognized tissue-intrinsic role of enteroendocrine cells.

Sequiera, G. L., et al. (2017). "Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells as an Individual-Specific and Renewable Source of Adult Stem Cells." Methods Mol Biol **1553**: 183-190.

This chapter deals with the employment of human-induced pluripotent stem cells (hiPSCs) as a candidate to differentiate into mesenchymal stem cells (MSCs). This would enable to help establish a regular source of human MSCs with the aim of avoiding the problems associated with procuring the MSCs either from different healthy individuals or patients, limited extraction potentials, batch-to-batch variations or from diverse sources such as bone marrow or adipose tissue. The procedures described herein allow for a guided and ensured approach for the regular maintenance of hiPSCs and their subsequent differentiation into MSCs using the prescribed medium. Subsequently, an easy protocol for the successive isolation and purification of the hiPSC-differentiated MSCs is outlined, which is carried out through passaging and can be further sorted through flow cytometry. Further, the maintenance and expansion of the resultant hiPSC-differentiated MSCs using appropriate characterization techniques, i.e., Reverse-transcription PCR and immunostaining is also elaborated. The course of action has been deliberated keeping in mind the awareness and the requisites available to even beginner researchers who mostly have access to regular consumables and medium components found in the general laboratory.

Shafiee, A., et al. (2016). "Evaluation and comparison of the in vitro characteristics and chondrogenic capacity of four adult stem/progenitor cells for

cartilage cell-based repair." J Biomed Mater Res A **104**(3): 600-610.

Cell-based therapy is being considered as a promising approach to regenerate damaged cartilage. Though, autologous chondrocyte implantation is the most effective strategy currently in use, but is hampered by some drawbacks seeking comprehensive research to surmount existing limitations or introducing alternative cell sources. In this study, we aimed to evaluate and compare the in vitro characteristics and chondrogenic capacity of some easily available adult cell sources for use in cartilage repair which includes: bone marrow-derived mesenchymal stem cells (MSC), adipose tissue-derived MSC, articular chondrocyte progenitors, and nasal septum-derived progenitors. Human stem/progenitor cells were isolated and expanded. Cell's immunophenotype, biosafety, and cell cycle status were evaluated. Also, cells were seeded onto aligned electrospun poly (l-lactic acid)/poly (epsilon-caprolactone) nanofibrous scaffolds and their proliferation rate as well as chondrogenic potential were assessed. Cells were almost phenotypically alike as they showed similar cell surface marker expression pattern. The aligned nanofibrous hybrid scaffolds could support the proliferation and chondrogenic differentiation of all cell types. However, nasal cartilage progenitors showed a higher proliferation potential and a higher chondrogenic capacity. Though, mostly similar in the majority of the studied features, nasal septum progenitors demonstrated a higher chondrogenic potential that in combination with their higher proliferation rate and easier access to the source tissue, introduces it as a promising cell source for cartilage tissue engineering and regenerative medicine. (c) 2015 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 104A: 600-610, 2016.

Shen, Q., et al. (2008). "Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions." Cell Stem Cell **3**(3): 289-300.

There is an emerging understanding of the importance of the vascular system within stem cell niches. Here, we examine whether neural stem cells (NSCs) in the adult subventricular zone (SVZ) lie close to blood vessels, using three-dimensional whole mounts, confocal microscopy, and automated computer-based image quantification. We found that the SVZ contains a rich plexus of blood vessels that snake along and within neuroblast chains. Cells expressing stem cell markers, including GFAP, and proliferation markers are closely apposed to the laminin-containing extracellular matrix (ECM) surrounding vascular endothelial cells. Apical GFAP+ cells are admixed within the ependymal layer and some span between the ventricle and blood vessels, occupying a specialized microenvironment. Adult SVZ progenitor cells express the laminin receptor alpha6beta1 integrin, and blocking this inhibits their adhesion to endothelial cells, altering their position and proliferation in vivo, indicating that it plays a functional role in binding SVZ stem cells within the vascular niche.

Sherley, J. L. (2002). "Asymmetric cell kinetics genes: the key to expansion of adult stem cells in culture." ScientificWorldJournal **2**: 1906-1921.

A singular challenge in stem cell research today is the expansion and propagation of functional adult stem cells. Unlike embryonic stem cells, which are immortal in culture, adult stem cells are notorious for the difficulty encountered when attempts are made to expand them in culture. One overlooked reason for this difficulty may be the inherent asymmetric cell kinetics of stem cells in postnatal somatic tissues. Senescence is the expected fate of a culture whose growth depends on adult stem cells that divide with asymmetric cell kinetics. Therefore, the bioengineering of strategies to expand adult stem cells in culture requires knowledge of cellular mechanisms that control asymmetric cell kinetics. The properties of several genes recently implicated to function in a cellular pathway(s) that regulates asymmetric cell kinetics are discussed. Understanding the function of these genes in asymmetric cell kinetics mechanisms may be the key that unlocks the adult stem cell expansion problem.

Sherley, J. L. (2002). "Asymmetric cell kinetics genes: the key to expansion of adult stem cells in culture." Stem Cells **20**(6): 561-572.

A singular challenge in stem cell research today is the expansion and propagation of functional adult stem cells. Unlike embryonic stem cells, which are immortal in culture, adult stem cells are notorious for the difficulty encountered when attempts are made to expand them in culture. One overlooked reason for this difficulty may be the inherent asymmetric cell kinetics of stem cells in postnatal somatic tissues. Senescence is the expected fate of a culture whose growth depends on adult stem cells that divide with asymmetric cell kinetics. Therefore, the bioengineering of strategies to expand adult stem cells in culture requires knowledge of cellular mechanisms that control asymmetric cell kinetics. The properties of several genes recently implicated to function in a cellular pathway(s) that regulates asymmetric cell kinetics are discussed. Understanding the function of these genes in asymmetric cell kinetics mechanisms may be the key that unlocks the adult stem cell expansion problem.

Shimizu, Y., et al. (2002). "Characterization of 'adult-type' mast cells derived from human bone marrow CD34(+) cells cultured in the presence of stem cell factor and interleukin-6. Interleukin-4 is not required for constitutive expression of CD54, Fc epsilon RI alpha and chymase, and CD13 expression is reduced

during differentiation." Clin Exp Allergy **32**(6): 872-880.

BACKGROUND: In vitro-derived human mast cells exhibit different properties, depending in part on the source of progenitor cells. Most investigations have used fetal liver, cord blood or peripheral blood. Few have used adult bone marrow. OBJECTIVE: Human mast cells derived in vitro from the CD34(+) progenitors in bone marrow and cord blood that had been cultured with recombinant human stem cell factor (rhSCF) and recombinant human interleukin-6 (rhIL-6) were compared. METHODS AND RESULTS: After 12 weeks of culture, nearly all of the cells were mast cells, and nearly all of these had cytoplasmic granules containing both tryptase and chymase (MCTC type), stained metachromatically with acidic toluidine blue, and expressed CD117 on the cell surface. Both tryptase protein and mRNA were detected by two weeks of culture. Chymase mRNA and protein were detected at 4 weeks but not at 2 weeks of culture. By 12 weeks, chymase content per cell, measured by ELISA, was significantly higher ($P < 0.05$) in human bone marrow-derived mast cells (HBMMC) (5.6 +/- 0.9 pg) than in cord blood-derived mast cells (CBMC) (2.4 +/- 0.9 pg), whereas histamine and tryptase levels were not significantly different. Of the cluster designations tested, CD29, CD49d, CD51 and CD61 were strongly expressed on HBMMC. CD54 and Fc epsilon RI alpha also were expressed constitutively. Approximately half of CD34-sorted cells at day 0 were CD13(+) and this diminished as mast cell maturation occurred. Electron microscopy revealed that 12-week-old HBMMC had many secretory granules that contained spherical electron dense cores surrounded by electron lucent space, consistent with previous reports of immature MCTC cells developing in vivo. CONCLUSIONS: CD34(+) progenitors of human bone marrow are a rich source of mast cell progenitors capable of expressing granule and surface markers of mature mast cells in the presence of rhSCF and rhIL-6.

Silberstein, L., et al. (2005). "Transgenic analysis of the stem cell leukemia +19 stem cell enhancer in adult and embryonic hematopoietic and endothelial cells." Stem Cells **23**(9): 1378-1388.

Appropriate transcriptional regulation is critical for the biological functions of many key regulatory genes, including the stem cell leukemia (SCL) gene. As part of a systematic dissection of SCL transcriptional regulation, we have previously identified a 5,245-bp SCL +18/19 enhancer that targeted embryonic endothelium together with embryonic and adult hematopoietic progenitors and stem cells (HSCs). This enhancer is proving to be a powerful tool for manipulating hematopoietic progenitors and stem cells, but the design and interpretation of such transgenic studies require a detailed understanding of enhancer activity in vivo. In this study, we demonstrate that the +18/19 enhancer is active in mast cells, megakaryocytes, and adult endothelium. A 644-bp +19 core enhancer exhibited similar temporal and spatial activity to the 5,245-bp +18/19 fragment both during development and in adult mice. Unlike the +18/19 enhancer, the +19 core enhancer was only active in adult mice when linked to the eukaryotic reporter gene human placental alkaline phosphatase. Activity of a single core enhancer in HSCs, endothelium, mast cells, and megakaryocytes suggests possible overlaps in their respective transcriptional programs. Moreover, activity in a proportion of thymocytes and other SCL-negative cell types suggests the existence of a silencer elsewhere in the SCL locus.

Sinanan, A. C., et al. (2004). "Human adult craniofacial muscle-derived cells: neural-cell adhesion-molecule (NCAM; CD56)-expressing cells appear to contain multipotential stem cells." Biotechnol Appl Biochem **40**(Pt 1): 25-34.

Skeletal muscle has been well characterized as a reservoir of myogenic precursors or satellite cells with the potential to participate in cellular repopulation therapies for muscle dysfunction. Recent evidence, however, suggests that the postnatal muscle compartment can be considered an alternative to bone marrow as a source of multipotent cells or muscle-derived stem cells (MDSCs). MDSCs, when primed with appropriate environmental cues, can differentiate into a variety of non-muscle cells. The present study describes the application of a new technique for the isolation of adult human myoblasts and putative MDSCs, based on microbead-immunomagnetic selection of CD56+ cells, derived from craniofacial skeletal muscle, and details changes in morphological/molecular phenotype of the purified cells when maintained in either a myogenic or a non-myogenic milieu. Multiple immunofluorescence microscopy and two-colour flow-cytometric analysis of proliferating CD56+ cultures revealed positive staining for myogenic markers (CD56, desmin and M-cadherin) as well as putative stem-cell markers [the antigens CD34, CD90 and CD106, and Flk-1 (fetal liver kinase-1)/VEGFR-2 (vascular-endothelial-growth-factor receptor)]. Confluent cultures subjected to cycles of adipogenic or osteogenic induction contained either adipocytes or osteoblasts and myotubes. In conclusion, the CD56+ subpopulation within adult human skeletal muscle is heterogeneous and is composed of both lineage-committed myogenic cells and multipotent cells (the candidate MDSCs), which are able to form non-muscle tissue such as fat and bone.

Singh, A., et al. (2013). "Protein Kinase C epsilon , Which Is Linked to Ultraviolet Radiation-Induced Development of Squamous Cell Carcinomas,

Stimulates Rapid Turnover of Adult Hair Follicle Stem Cells." J Skin Cancer **2013**: 452425.

To find clues about the mechanism by which kinase C epsilon (PKC epsilon) may impart susceptibility to ultraviolet radiation (UVR)-induced development of cutaneous squamous cell carcinomas (SCC), we compared PKC epsilon transgenic (TG) mice and their wild-type (WT) littermates for (1) the effects of UVR exposures on percent of putative hair follicle stem cells (HSCs) and (2) HSCs proliferation. The percent of double HSCs (CD34+ and alpha 6-integrin or CD34+/CD49f+) in the isolated keratinocytes were determined by flow cytometric analysis. Both single and chronic UVR treatments (1.8 kJ/m(2)) resulted in an increase in the frequency of double positive HSCs in PKC epsilon TG mice as compared to their WT littermates. To determine the rate of proliferation of bulge region stem cells, a 5-bromo-2'-deoxyuridine labeling (BrdU) experiment was performed. In the WT mice, the percent of double positive HSCs retaining BrdU label was 28.4 +/- 0.6% compared to 4.0 +/- 0.06% for the TG mice, an approximately 7-fold decrease. A comparison of gene expression profiles of FACS sorted double positive HSCs showed increased expression of Pes1, Rad21, Tfdp1 and Cks1b genes in TG mice compared to WT mice. Also, PKC epsilon over expression in mice increased the clonogenicity of isolated keratinocytes, a property commonly ascribed to stem cells.

Spadaccio, C., et al. (2010). "Predifferentiated adult stem cells and matrices for cardiac cell therapy." Asian Cardiovasc Thorac Ann **18**(1): 79-87.

Stem cell therapy is a major field of research worldwide, with increasing clinical application, especially in cardiovascular pathology. However, the best stem cell source and type with optimal safety for functional engraftment remains unclear. An intermediate cardiac precommitted phenotype expressing some of the key proteins of a mature cardiomyocyte would permit better integration into the cardiac environment. The predifferentiated cells would receive signals from the environment, thus achieving gradual and complete differentiation. In cell transplantation, survival and engraftment within the environment of the ischemic myocardium represents a challenge for all types of cells, regardless of their state of differentiation. An alternative strategy is to embed cells in a 3-dimensional structure replicating the extracellular matrix, which is crucial for full tissue restoration and prevention of ventricular remodeling. The clinical translation of cell therapy requires avoidance of potentially harmful drugs and cytokines, and rapid off-the-shelf availability of cells. The combination of predifferentiated cells with a functionalized scaffold, locally releasing molecules tailored to promote in-situ completion of differentiation and improve homing, survival, and function, could be an exciting approach that might circumvent the potential undesired effects of growth factor administration and improve tissue restoration.

Spees, J. L., et al. (2003). "Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma." Proc Natl Acad Sci U S A **100**(5): 2397-2402.

To investigate stem cell differentiation in response to tissue injury, human mesenchymal stem cells (hMSCs) were cocultured with heat-shocked small airway epithelial cells. A subset of the hMSCs rapidly differentiated into epithelium-like cells, and they restored the epithelial monolayer. Immunocytochemistry and microarray analyses demonstrated that the cells expressed many genes characteristic of normal small airway epithelial cells. Some hMSCs differentiated directly after incorporation into the epithelial monolayer but other hMSCs fused with epithelial cells. Surprisingly, cell fusion was a frequent rather than rare event, in that up to 1% of the hMSCs added to the coculture system were recovered as binucleated cells expressing an epithelial surface epitope. Some of the fused cells also underwent nuclear fusion.

Staber, P. B., et al. (2013). "Sustained PU.1 levels balance cell-cycle regulators to prevent exhaustion of adult hematopoietic stem cells." Mol Cell **49**(5): 934-946.

To provide a lifelong supply of blood cells, hematopoietic stem cells (HSCs) need to carefully balance both self-renewing cell divisions and quiescence. Although several regulators that control this mechanism have been identified, we demonstrate that the transcription factor PU.1 acts upstream of these regulators. So far, attempts to uncover PU.1's role in HSC biology have failed because of the technical limitations of complete loss-of-function models. With the use of hypomorphic mice with decreased PU.1 levels specifically in phenotypic HSCs, we found reduced HSC long-term repopulation potential that could be rescued completely by restoring PU.1 levels. PU.1 prevented excessive HSC division and exhaustion by controlling the transcription of multiple cell-cycle regulators. Levels of PU.1 were sustained through autoregulatory PU.1 binding to an upstream enhancer that formed an active looped chromosome architecture in HSCs. These results establish that PU.1 mediates chromosome looping and functions as a master regulator of HSC proliferation.

Staszkiewicz, J., et al. (2010). "Cell growth characteristics, differentiation frequency, and immunophenotype of adult ear mesenchymal stem cells." Stem Cells Dev **19**(1): 83-92.

Ear mesenchymal stem cells (EMSCs) represent a readily accessible population of stem-like

cells that are adherent, clonogenic, and have the ability to self-renew. Previously, we have demonstrated that they can be induced to differentiate into adipocyte, osteocyte, chondrocyte, and myocyte lineages. The purpose of the current study was to characterize the growth kinetics of the cells and to determine their ability to form colonies of fibroblasts, adipocytes, osteocytes, and chondrocytes. In addition, the immunophenotypes of freshly isolated and culture-expanded cells were evaluated. From 1 g of tissue, we were able to isolate an average of 7.8 x 10(6) cells exhibiting a cell cycle length of approximately 2-3 days. Colony-forming unit (CFU) assays indicated high proliferation potential, and confirmed previously observed multipotentiality of the cells. Fluorescence-activated cell sorting (FACS) showed that EMSCs were negative for hematopoietic markers (CD4, CD45), proving that they did not derive from circulating hematopoietic cells. The FACS analyses also showed high expression of stem cell antigen-1 (Sca-1) with only a minor population of cells expressing CD117, thus identifying Sca-1 as the more robust stem cell biomarker.

Stockl, S., et al. (2013). "Sox9 modulates cell survival and adipogenic differentiation of multipotent adult rat mesenchymal stem cells." J Cell Sci **126**(Pt 13): 2890-2902.

Sox9 is a key transcription factor in early chondrogenesis with distinct roles in differentiation processes and during embryonic development. Here, we report that Sox9 modulates cell survival and contributes to the commitment of mesenchymal stem cells (MSC) to adipogenic or osteogenic differentiation lineages. We found that the Sox9 activity level affects the expression of the key transcription factor in adipogenic differentiation, C/EBPbeta, and that cyclin D1 mediates the expression of the osteogenic marker osteocalcin in undifferentiated adult bone-marrow-derived rat MSC. Introducing a stable Sox9 knockdown into undifferentiated rat MSC resulted in a marked decrease in proliferation rate and an increase in apoptotic activity. This was linked to a profound upregulation of p21 and cyclin D1 gene and protein expression accompanied by an induction of caspase 3/7 activity and an inhibition of Bcl-2. We observed that Sox9 silencing provoked a delayed S-phase progression and an increased nuclear localization of p21. The protein stability of cyclin D1 was induced in the absence of Sox9 presumably as a function of altered p38 signalling. In addition, the major transcription factor for adipogenic differentiation, C/EBPbeta, was repressed after silencing Sox9. The nearly complete absence of C/EBPbeta protein as a result of increased destabilization of the C/EBPbeta mRNA and the impact on osteocalcin gene expression and protein synthesis, suggests that a delicate balance of Sox9 level is not only imperative for proper chondrogenic differentiation of progenitor cells, but also affects the adipogenic and probably osteogenic differentiation pathways of MSC.

Stripp, B. R. (2008). "Hierarchical organization of lung progenitor cells: is there an adult lung tissue stem cell?" Proc Am Thorac Soc **5**(6): 695-698.

Dynamic changes to the developing lung endoderm during the process of lung development result in the establishment of functionally distinct epithelial compartments that vary both in their cellular composition and mechanisms contributing to their maintenance in adulthood. This focused review compares the hierarchical organization of cells within slowly and rapidly renewing tissues as a basis to better understand cellular and molecular mechanisms regulating epithelial maintenance and repair in the lung.

Sung, L. Y., et al. (2006). "Differentiated cells are more efficient than adult stem cells for cloning by somatic cell nuclear transfer." Nat Genet **38**(11): 1323-1328.

Since the creation of Dolly via somatic cell nuclear transfer (SCNT), more than a dozen species of mammals have been cloned using this technology. One hypothesis for the limited success of cloning via SCNT (1%-5%) is that the clones are likely to be derived from adult stem cells. Support for this hypothesis comes from the findings that the reproductive cloning efficiency for embryonic stem cells is five to ten times higher than that for somatic cells as donors and that cloned pups cannot be produced directly from cloned embryos derived from differentiated B and T cells or neuronal cells. The question remains as to whether SCNT-derived animal clones can be derived from truly differentiated somatic cells. We tested this hypothesis with mouse hematopoietic cells at different differentiation stages: hematopoietic stem cells, progenitor cells and granulocytes. We found that cloning efficiency increases over the differentiation hierarchy, and terminally differentiated postmitotic granulocytes yield cloned pups with the greatest cloning efficiency.

Tai, M. H., et al. (2005). "Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis." Carcinogenesis **26**(2): 495-502.

The Oct3/4 gene, a POU family transcription factor, has been noted as being specifically expressed in embryonic stem cells and in tumor cells but not in cells of differentiated tissues. With the ability to isolate adult human stem cells it became possible to test for the expression of Oct3/4 gene in adult stem cells and to test the stem cell theory of carcinogenesis. Using antibodies and PCR primers we tested human breast, liver, pancreas, kidney, mesenchyme and gastric stem

cells, the cancer cell lines HeLa and MCF-7 and human, dog and rat tumors for Oct4 expression. The results indicate that adult human stem cells, immortalized non-tumorigenic cells and tumor cells and cell lines, but not differentiated cells, express Oct4. Oct4 is expressed in a few cells found in the basal layer of human skin epidermis. The data demonstrate that adult stem cells maintain expression of Oct4, consistent with the stem cell hypothesis of carcinogenesis.

Tamai, Y., et al. (2013). "Potential contribution of a novel Tax epitope-specific CD4+ T cells to graft-versus-Tax effect in adult T cell leukemia patients after allogeneic hematopoietic stem cell transplantation." J Immunol **190**(8): 4382-4392.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for adult T cell leukemia/lymphoma (ATL) caused by human T cell leukemia virus type 1 (HTLV-1). We previously reported that Tax-specific CD8(+) cytotoxic T lymphocyte (CTL) contributed to graft-versus-ATL effects in ATL patients after allo-HSCT. However, the role of HTLV-1-specific CD4(+) T cells in the effects remains unclear. In this study, we showed that Tax-specific CD4(+) as well as CD8(+) T cell responses were induced in some ATL patients following allo-HSCT. To further analyze HTLV-1-specific CD4(+) T cell responses, we identified a novel HLA-DRB1*0101-restricted epitope, Tax155-167, recognized by HTLV-1-specific CD4(+) Th1-like cells, a major population of HTLV-1-specific CD4(+) T cell line, which was established from an ATL patient at 180 d after allo-HSCT from an unrelated seronegative donor by in vitro stimulation with HTLV-1-infected cells from the same patient. Costimulation of PBMCs with both the identified epitope (Tax155-167) and known CTL epitope peptides markedly enhanced the expansion of Tax-specific CD8(+) T cells in PBMCs compared with stimulation with CTL epitope peptide alone in all three HLA-DRB1*0101(+) patients post-allo-HSCT tested. In addition, direct detection using newly generated HLA-DRB1*0101/Tax155-167 tetramers revealed that Tax155-167-specific CD4(+) T cells were present in all HTLV-1-infected individuals tested, regardless of HSCT. These results suggest that Tax155-167 may be the dominant epitope recognized by HTLV-1-specific CD4(+) T cells in HLA-DRB1*0101(+)-infected individuals and that Tax-specific CD4(+) T cells may augment the graft-versus-Tax effects via efficient induction of Tax-specific CD8(+) T cell responses.

Tang, X., et al. (2016). "Conversion of adult human peripheral blood mononuclear cells into induced neural stem cell by using episomal vectors." Stem Cell Res **16**(2): 236-242.

Human neural stem cells (NSCs) hold great promise for research and therapy in neural diseases. Many studies have shown direct induction of NSCs from human fibroblasts, which require an invasive skin biopsy and a prolonged period of expansion in cell culture prior to use. Peripheral blood (PB) is routinely used in medical diagnoses, and represents a noninvasive and easily accessible source of cells. Here we show direct derivation of NSCs from adult human PB mononuclear cells (PB-MNCs) by employing episomal vectors for transgene delivery. These induced NSCs (iNSCs) can expand more than 60 passages, can exhibit NSC morphology, gene expression, differentiation potential, and self-renewing capability and can give rise to multiple functional neural subtypes and glial cells in vitro. Furthermore, the iNSCs carry a specific regional identity and have electrophysiological activity upon differentiation. Our findings provide an easily accessible approach for generating human iNSCs which will facilitate disease modeling, drug screening, and possibly regenerative medicine.

Tauc, H. M., et al. (2014). "Isolating intestinal stem cells from adult Drosophila midguts by FACS to study stem cell behavior during aging." J Vis Exp(94).

Aging tissue is characterized by a continuous decline in functional ability. Adult stem cells are crucial in maintaining tissue homeostasis particularly in tissues that have a high turnover rate such as the intestinal epithelium. However, adult stem cells are also subject to aging processes and the concomitant decline in function. The Drosophila midgut has emerged as an ideal model system to study molecular mechanisms that interfere with the intestinal stem cells' (ISCs) ability to function in tissue homeostasis. Although adult ISCs can be easily identified and isolated from midguts of young flies, it has been a major challenge to study endogenous molecular changes of ISCs during aging. This is due to the lack of a combination of molecular markers suitable to isolate ISCs from aged intestines. Here we propose a method that allows for successful dissociation of midgut tissue into living cells that can subsequently be separated into distinct populations by FACS. By using dissociated cells from the esg-Gal4, UAS-GFP fly line, in which both ISCs and the enteroblast (EB) progenitor cells express GFP, two populations of cells are distinguished based on different GFP intensities. These differences in GFP expression correlate with differences in cell size and granularity and represent enriched populations of ISCs and EBs. Intriguingly, the two GFP-positive cell populations remain distinctly separated during aging, presenting a novel technique for identifying and isolating cell populations enriched for either ISCs or EBs at any time point during aging.

Tecirlioglu, R. T., et al. (2010). "Derivation and maintenance of human embryonic stem cell line on human adult skin fibroblast feeder cells in serum

replacement medium." In Vitro Cell Dev Biol Anim **46**(3-4): 231-235.

Human embryonic stem (hES) cells were originally isolated and maintained on mouse embryonic fibroblast (MEF) feeder layers in the presence of fetal bovine serum (FBS). However, if the hES cells are to be used for therapeutic applications, it is preferable to regulatory authorities that they be derived and cultured in animal-free conditions to prevent mouse antigen contamination that would exacerbate an immune response to foreign proteins, and the potential risk of transmission of retroviral and other zoonotic pathogens to humans. As a step towards this goal, we derived a new hES cell line (MISCES-01) on human adult skin fibroblasts as feeder cells using serum replacement (SR) medium. The MISCES-01 cells have a normal diploid karyotype (46XX), express markers of pluripotency (OCT4, GCTM-2, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4, and alkaline phosphatase) and following in vitro and in vivo differentiation, give rise to derivatives of the three primary germ layers. This cell line can be obtained for research purposes from the Australian Stem Cell Centre (http://www.stemcellcentre.edu.au).

Thomas, T., et al. (2006). "Expression of endoderm stem cell markers: evidence for the presence of adult stem cells in human thyroid glands." Thyroid **16**(6): 537-544.

OBJECTIVE: Adult stem cells have been detected in several human tissues. The object of this study was to investigate whether they also occur in the human thyroid gland. DESIGN: The expression of the stem cell marker Oct- 4 and the early endodermal markers GATA-4 and HNF4alpha was analyzed in histologic slides and cultured cells derived from goiters, in the FRTL5 cell line, and the HTh74, HTC, C643, and XTC133 thyroid carcinoma cell lines. MAIN OUTCOME: Stem cell markers were detectable in all primary cultures whereas in the differentiated FRTL5 cell line no expression was observed. Expression of stem cell marker mRNA was not affected by thyrotropin (TSH) stimulation and did not decrease when cells underwent several passages. Immunostaining of cultured cells and of histologic slides of goitrous tissues showed only single cells that were immunoreactive for Oct-4, GATA-4, and HNF4a. Expression of Oct-4 but not of endodermal marker GATA-4 was also detectable in some thyroid carcinoma cell lines. Fluorescence-activated cell sorter (FACS) analysis demonstrated cell populations that were positive for either Oct-4, GATA-4, or HNF4alpha but negative for thyroglobulin. When these putative, FACS-sorted stem cell populations were further analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR), expression of all stem cell markers and of Pax8 but not of thyroglobulin mRNA was detectable.

Tiede, S., et al. (2010). "Endocrine controls of primary adult human stem cell biology: thyroid hormones stimulate keratin 15 expression, apoptosis, and differentiation in human hair follicle epithelial stem cells in situ and in vitro." Eur J Cell Biol **89**(10): 769-777.

Here we demonstrate that physiological concentrations of the thyroid hormones T3 and T4 enhance the KERATIN 15 promoter activity and expression in epithelial stem cells of adult human scalp hair follicles in situ and in vitro. Additionally, T3 and T4 stimulate expression of the immuno-inhibitory surface molecule CD200. Subsequently, T3 and T4 induce apoptosis and differentiation and inhibit clonal growth of these progenitor cells in vitro. These data suggest that human hair follicle bulge-derived epithelial stem cells underlie profound, previously unknown hormonal regulation by thyroid hormones, and show that primary human keratin 15-GFP+ progenitor cells can be exploited to further elucidate fundamental endocrine controls of human epithelial stem cells.

Toda, H., et al. (2003). "Stem cell-derived neural stem/progenitor cell supporting factor is an autocrine/paracrine survival factor for adult neural stem/progenitor cells." J Biol Chem **278**(37): 35491-35500.

Recent evidence suggests that adult neural stem/progenitor cells (ANSCs) secrete autocrine/paracrine factors and that these intrinsic factors are involved in the maintenance of adult neurogenesis. We identified a novel secretory molecule, stem cell-derived neural stem/progenitor cell supporting factor (SDNSF), from adult hippocampal neural stem/progenitor cells by using the signal sequence trap method. The expression of SDNSF in adult central nervous system was localized to hippocampus including dentate gyrus, where the neurogenesis persists throughout life. In induced neurogenesis status seen in ischemically treated hippocampus, the expression of SDNSF was up-regulated. As functional aspects, SDNSF protein provided a dose-dependent survival effect for ANSC following basic fibroblast growth factor 2 (FGF-2) withdrawal. ANSCs treated by SDNSF also retain self-renewal potential and multipotency in the absence of FGF-2. However, SDNSF did not have mitogenic activity, nor was it a cofactor that promoted the mitogenic effects of FGF-2. These data suggested an important role of SDNSF as an autocrine/paracrine factor in maintaining stem cell potential and lifelong neurogenesis in adult central nervous system.

Torsvik, A., et al. (2012). "Comment to: "Spontaneous transformation of adult mesenchymal stem cells from cynomolgus macaques in vitro" by Z. Ren et al. Exp. Cell Res. 317 (2011) 2950-2957: spontaneous

transformation of mesenchymal stem cells in culture: facts or fiction?" Exp Cell Res **318**(5): 441-443.

There is at present a controversy in the literature whether MSCs are susceptible to spontaneous in vitro transformation or not. Several groups have reported spontaneous transformation of MSCs from various species. However, some of these reports were not true transformations and later proven to be due to cross-contaminating cancer cells. To date there is no solid evidence that MSCs can undergo spontaneous transformation in culture. Only two groups used DNA fingerprinting to authenticate their transformed cells, and both groups later showed cross-contamination of cancer cells in their cultures. In this commentary, we address the paper "Spontaneous transformation of adult mesenchymal stem cells from cynomolgus macaques in vitro" by Z. Ren et al. Exp. Cell Res. 317 (2011) 2950-2957. In this article the authors characterize the transformed mesenchymal cells (TMCs) and claim to have verified their origin. We question the authentication of the TMCs made by the authors and we also believe it is in the interest of the scientific community, that a highly controversial finding, such as spontaneous transformation of MSCs, should be properly verified by stringent methods, preferably DNA fingerprinting, in order to validate if an actual transformation event has occurred.

Trapecar, M., et al. (2014). "Myogenic progenitors and imaging single-cell flow analysis: a model to study commitment of adult muscle stem cells." J Muscle Res Cell Motil **35**(5-6): 249-257.

Research on skeletal muscles suffers from a lack of appropriate human models to study muscle formation and regeneration on the regulatory level of single cells. This hampers both basic understanding and the development of new therapeutic approaches. The use of imaging multicolour flow cytometry and myogenic stem cells can help fill this void by allowing researchers to visualize and quantify the reaction of individual cultured cells to bioactives or other physiological impulses. As proof of concept, we subjected human CD56+ satellite cells to reference bioactives follistatin and Malva sylvestris extracts and then used imaging multicolor flow cytometry to visualize the stepwise activation of myogenic factors MyoD and myogenin in individual cells. This approach enabled us to evaluate the potency of these bioactives to stimulate muscle commitment.

Trosko, J. E. (2006). "From adult stem cells to cancer stem cells: Oct-4 Gene, cell-cell communication, and hormones during tumor promotion." Ann N Y Acad Sci **1089**: 36-58.

Carcinogenesis is characterized by "initiation," "promotion," and "progression" phases. The "stem cell theory" and "de-differentiation" theories are used to explain the origin of cancer. Growth control for stem cells, which lack functional gap junctional intercellular communication (GJIC), involves negative soluble or niche factors, while for progenitor cells, it involves GJIC. Tumor promoters, hormones, and growth factors inhibit GJIC reversibly. Oncogenes stably inhibit GJIC. Cancer cells, which lack growth control and the ability to terminally differentiate and to apoptose, lack GJIC. The Oct3/4 gene, a POU (Pit-Oct-Unc) family of transcription factors was thought to be expressed only in embryonic stem cells and in tumor cells. With the availability of normal adult human stem cells, tests for the expression of Oct3/4 gene and the stem cell theory in human carcinogenesis became possible. Human breast, liver, pancreas, kidney, mesenchyme, and gastric stem cells, HeLa and MCF-7 cells, and canine tumors were tested with antibodies and polymerase chain reaction (PCR) primers for Oct3/4. Adult human breast stem cells, immortalized nontumorigenic and tumor cell lines, but not the normal differentiated cells, expressed Oct3/4. Adult human differentiated cells lose their Oct-4 expression. Oct3/4 is expressed in a few cells found in the basal layer of human skin epidermis. The data demonstrate that normal adult stem cells and cancer stem cells maintain expression of Oct3/4, consistent with the stem cell hypothesis of carcinogenesis. These Oct-4 positive cells might represent the "cancer stem cells." A strategy to target "cancer stem cells" is to suppress the Oct-4 gene in order to cause the cells to differentiate.

Trosko, J. E., et al. (2005). "The role of human adult stem cells and cell-cell communication in cancer chemoprevention and chemotherapy strategies." Mutat Res **591**(1-2): 187-197.

Since carcinogenesis is a multi-stage, multi-mechanism process, involving mutagenic, cell death and epigenetic mechanisms, during the "initiation/promotion/and progression" phases, chemoprevention must be based on understanding the underlying mechanism(s) of each phase, In principle, prevention of each of these phases could reduce the risk to cancer. However, because reducing the mutagenic/initiation phase to a zero level is impossible, the most efficacious intervention would be at the promotion phase that requires a sustained exposure to promoting conditions/agents. In addition, assuming the "target" cells for carcinogenesis are the pluri-potent stem cells and their early progenitor or transit cells, chemoprevention strategies for inhibiting the promotion of these two types of pre-malignant "initiated" cells will require different kinds of agents. A hypothesis will be proposed that involves adult stem cells, which express Oct-4 gene and lack gap junctional intercellular communication (GJIC-) or the early progenitor cells which express GJIC+ and are partially-differentiated, if initiated, will be promoted by agents that either inhibit secreted negative growth regulators

or by inhibitors of GJIC. Consequently, anti-tumor promoting chemopreventing agents to each of these two types of initiated cells must have different mechanisms of action and work on different target cells. Assuming stem cells are target cells for carcinogenesis, an alternative method of chemoprevention would be to reduce the stem cell pool. Many classes of anti-tumor promoter chemopreventive agents, such as green tea components, resveratrol, caffeic acid phenethylene ester, either up-regulate GJIC in stem cells or prevent the down regulation of GJIC by tumor promoters in early progenitor cells.

Trosko, J. E. and M. H. Tai (2006). "Adult stem cell theory of the multi-stage, multi-mechanism theory of carcinogenesis: role of inflammation on the promotion of initiated stem cells." Contrib Microbiol **13**: 45-65.

Inflammation, induced by microbial agents, radiation, endogenous or exogenous chemicals, has been associated with chronic diseases, including cancer. Since carcinogenesis has been characterized as consisting of the 'initiation', 'promotion' and 'progression' phases, the inflammatory process could affect any or all three phases. The stem cell theory of carcinogenesis has been given a revival, in that isolated human adult stem cells have been isolated and shown to be 'targets' for neoplastic transformation. Oct4, a transcription factor, has been associated with adult stem cells, as well as their immortalized and tumorigenic derivatives, but not with the normal differentiated daughters. These data are consistent with the stem cell theory of carcinogenesis. In addition, Gap Junctional Intercellular Communication (GJIC) seems to play a major role in cell growth. Inhibition of GJIC by non-genotoxic chemicals or various oncogenes seems to be the mechanism for the tumor promotion and progression phases of carcinogenesis.

Wakayama, T. (2003). "Cloned mice and embryonic stem cell lines generated from adult somatic cells by nuclear transfer." Oncol Res **13**(6-10): 309-314.

Mice can now be cloned from cultured and noncultured adult-, fetus-, male-, or female-derived cells. Using the mouse as a model, research is moving towards a comprehensive description of clones generated by somatic cell nuclear transfer. In addition, embryonic stem (ES) cell lines can be generated from adult somatic cells via nuclear transfer (ntES cells). ntES cells contribute to an extensive variety of cell types including neurons in vitro and germ cells in vivo. Recent advances in mouse cloning are reported to illustrate its strengths and promise in the study of mammalian biology and biomedicine.

Wakayama, T. (2006). "Establishment of nuclear transfer embryonic stem cell lines from adult somatic cells by nuclear transfer and its application." Ernst Schering Res Found Workshop(60): 111-123.

Nuclear transfer can be used to generate embryonic stem cell (ntESC) lines from a patient's own somatic cells. We have shown that ntESCs can be generated relatively easily from a variety of mouse genotypes and cell types of both sexes, even though it may be more difficult to generate clones directly. Several reports have already demonstrated that ntESCs can be used in regenerative medicine in order to rescue immunodeficient or infertile phenotypes. However, it is unclear whether ntES cells are identical to fertilized embryonic stem cells (ESCs). This review seeks to describe the phenotype and possible abnormalities of ntESC lines.

Wakayama, T., et al. (2001). "Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer." Science **292**(5517): 740-743.

Embryonic stem (ES) cells are fully pluripotent in that they can differentiate into all cell types, including gametes. We have derived 35 ES cell lines via nuclear transfer (ntES cell lines) from adult mouse somatic cells of inbred, hybrid, and mutant strains. ntES cells contributed to an extensive variety of cell types, including dopaminergic and serotonergic neurons in vitro and germ cells in vivo. Cloning by transfer of ntES cell nuclei could result in normal development of fertile adults. These studies demonstrate the full pluripotency of ntES cells.

Wang, H., et al. (2013). "Adult renal mesenchymal stem cell-like cells contribute to juxtaglomerular cell recruitment." J Am Soc Nephrol **24**(8): 1263-1273.

The renin-angiotensin-aldosterone system (RAAS) regulates BP and salt-volume homeostasis. Juxtaglomerular (JG) cells synthesize and release renin, which is the first and rate-limiting step in the RAAS. Intense pathologic stresses cause a dramatic increase in the number of renin-producing cells in the kidney, termed JG cell recruitment, but how this occurs is not fully understood. Here, we isolated renal CD44(+) mesenchymal stem cell (MSC)-like cells and found that they differentiated into JG-like renin-expressing cells both in vitro and in vivo. Sodium depletion and captopril led to activation and differentiation of these cells into renin-expressing cells in the adult kidney. In summary, CD44(+) MSC-like cells exist in the adult kidney and can differentiate into JG-like renin-producing cells under conditions that promote JG cell recruitment.

Wang, J. and K. Liu (2003). "[Method of differentiation of adult human bone marrow mesenchymal stem cell into Schwann-like cells in vitro]." Beijing Da Xue Xue Bao Yi Xue Ban **35**(2): 202-206.

OBJECTIVE: To establish a method of committedly induced adult bone marrow mesenchymal stem cells differentiating into schwann' s-like cells in vitro. METHODS: Adult human bone marrow

mononuclear cells were separated by gradient centrifugation on Percoll(density 1.073 g.ml-1). The cells were incubated in low-glucose DMEM with 10% fetal bovine serum. Cell surface antigenic features were analyzed by flow cytometry technique. Passages 2, 3, 5 and 8 of MSCs were committedly induced to differentiate into Schwann-like cells. S-100, GFAP was detected by immunohistochemistry staining. RESULTS: The mesenchymal stem cells separated by percoll were expanded for more than 16 passages, increased about 6 x 10(7) folds. Flow cytometry results showed that: the CD29+ CD44+ CD34- CD45- cells separated from percoll immediately were 32.47% +/- 3.49%; The CD29+ CD44+ CD34- CD45- cells incubated in DMEM-LG with 10% fetal bovine serum were around 94.38% +/- 1.50%. Immunohistochemistry staining results indicated that induced with beta-ME + bFGF S-100 positive cells were 90% +/- 4%, while about 21% +/- 5% cells expressed GFAP. CONCLUSION: MSCs in human adult bone marrow are capable of being committedly induced to differentiate into Schwann-like cells in vitro.

Wang, Y., et al. (2010). "Paracrine signals from mesenchymal cell populations govern the expansion and differentiation of human hepatic stem cells to adult liver fates." Hepatology **52**(4): 1443-1454.

UNLABELLED: The differentiation of embryonic or determined stem cell populations into adult liver fates under known conditions yields cells with some adult-specific genes but not others, aberrant regulation of one or more genes, and variations in the results from experiment to experiment. We tested the hypothesis that sets of signals produced by freshly isolated, lineage-dependent mesenchymal cell populations would yield greater efficiency and reproducibility in driving the differentiation of human hepatic stem cells (hHpSCs) into adult liver fates. The subpopulations of liver-derived mesenchymal cells, purified by immunoselection technologies, included (1) angioblasts, (2) mature endothelia, (3) hepatic stellate cell precursors, (4) mature stellate cells (pericytes), and (5) myofibroblasts. Freshly immunoselected cells of each of these subpopulations were established in primary cultures under wholly defined (serum-free) conditions that we developed for short-term cultures and were used as feeders with hHpSCs. Feeders of angioblasts yielded self-replication, stellate cell precursors caused lineage restriction to hepatoblasts, mature endothelia produced differentiation into hepatocytes, and mature stellate cells and/or myofibroblasts resulted in differentiation into cholangiocytes. Paracrine signals produced by the different feeders were identified by biochemical, immunohistochemical, and quantitative reverse-transcription polymerase chain reaction analyses, and then those signals were used to replace the feeders in monolayer and three-dimensional cultures to elicit the desired biological responses from hHpSCs. The defined paracrine signals were proved to be able to yield reproducible responses from hHpSCs and to permit differentiation into fully mature and functional parenchymal cells. CONCLUSION: Paracrine signals from defined mesenchymal cell populations are important for the regulation of stem cell populations into specific adult fates; this finding is important for basic and clinical research as well as industrial investigations.

Wang, Z., et al. (2009). "Conditional deletion of STAT5 in adult mouse hematopoietic stem cells causes loss of quiescence and permits efficient nonablative stem cell replacement." Blood **113**(20): 4856-4865.

Currently, there is a major need in hematopoietic stem cell (HSC) transplantation to develop reduced-intensity regimens that do not cause DNA damage and associated toxicities and that allow a wider range of patients to receive therapy. Cytokine receptor signals through c-Kit and c-Mpl can modulate HSC quiescence and engraftment, but the intracellular signals and transcription factors that mediate these effects during transplantation have not been defined. Here we show that loss of one allele of signal transducer and activator of transcription 5 (STAT5) in nonablated adult mutant mice permitted engraftment with wild-type HSC. Conditional deletion of STAT5 using Mx1-Cre caused maximal reduction in STAT5 mRNA (> 97%) and rapidly decreased quiescence-associated c-Mpl downstream targets (Tie-2, p57), increased HSC cycling, and gradually reduced survival and depleted the long-term HSC pool. Host deletion of STAT5 was persistent and permitted efficient donor long-term HSC engraftment in primary and secondary hosts in the absence of ablative conditioning. Overall, these studies establish proof of principle for targeting of STAT5 as novel transplantation conditioning and demonstrate, for the first time, that STAT5, a mitogenic factor in most cell types, including hematopoietic progenitors, is a key transcriptional regulator that maintains quiescence of HSC during steady-state hematopoiesis.

Watanabe, J., et al. (2001). "Autologous stem cell transplantations for recurrent adult T cell leukaemia/lymphoma using highly purified CD34+ cells derived from cryopreserved peripheral blood stem cells." Leuk Lymphoma **42**(5): 1115-1117.

We performed double autologous PBSCT in one fulminantly relapsed ATL patient. Cryopreserved PBSCs containing tumour cells were thawed, and CD34+ cells were selected for by immunomagnetic beads, with the aim of decreasing the number of re-infused tumour cells. The patient received 0.72x10(6) and 0.90x10(6) CD34+ cells/kg. The graft contained less than 0.1% T cells. The patient had a very good

recovery, and maintained a good quality of life, until she died on Day 339 after her first PBSCT in third relapse. We conclude that autologous PBSCT for ATL patient has benefit in some instances, and cryopreserved PBSCs can be used for CD34+ selection.
West, M. D., et al. (2016). "Adult Versus Pluripotent Stem Cell-Derived Mesenchymal Stem Cells: The Need for More Precise Nomenclature." Curr Stem Cell Rep **2**: 299-303.

The complexity of human pluripotent stem cell (hPSC) fate represents both opportunity and challenge. In theory, all somatic cell types can be differentiated from hPSCs, opening the door to many opportunities in transplant medicine. However, such clinical applications require high standards of purity and identity, that challenge many existing protocols. This underscores the need for increasing precision in the description of cell identity during hPSC differentiation. We highlight one salient example, namely, the numerous published reports of hPSC-derived mesenchymal stem cells (MSCs). We suggest that many of these reports likely represent an improper use of certain cluster of differentiation (CD) antigens in defining bone marrow-derived MSCs. Instead, most such hPSC-derived mesenchymal cells are likely a complex mixture of embryonic anlagen, primarily of diverse mesodermal and neural crest origins, making precise identification, reproducible manufacture, and uniform differentiation difficult to achieve. We describe a potential path forward that may provide more precision in nomenclature, and cells with higher purity and identity for potential therapeutic use.
Wirths, S., et al. (2013). "Shared cell surface marker expression in mesenchymal stem cells and adult sarcomas." Stem Cells Transl Med **2**(1): 53-60.

Advanced adult soft-tissue sarcomas (STSs) are rare tumors with a dismal prognosis and limited systemic treatment options. STSs may originate from mesenchymal stem cells (MSCs); the latter have mainly been isolated from adult bone marrow as plastic-adherent cells with differentiation capacity into mesenchymal tissues. Recently, a panel of antibodies has been established that allows for the prospective isolation of primary MSCs with high selectivity. Similar to cancer stem cells in other malignancies, sarcoma stem cells may bear immunophenotypic similarity with the corresponding precursor, that is, MSCs. We therefore set out to establish the expression pattern of MSC markers in sarcoma cell lines and primary tumor samples by flow cytometry. In addition, fibroblasts from different sources were examined. The results document a significant amount of MSC markers shared by sarcoma cells. The expression pattern includes uniformly expressed markers, as well as MSC markers that only stained subpopulations of sarcoma cells. Expression of W5C5, W8B2 (tissue nonspecific alkaline phosphatase [TNAP]), CD344 (frizzled-4), and CD271 marked subpopulations displaying increased proliferation potential. Moreover, CD271+ cells displayed in vitro doxorubicin resistance and an increased capacity to form spheres under serum-free conditions. Interestingly, another set of antigens, including the bona fide progenitor cell markers CD117 and CD133, were not expressed. Comparative expression patterns of novel MSC markers in sarcoma cells, as well as fibroblasts and MSCs, are presented. Our data suggest a hierarchical cytoarchitecture of the most common adult type sarcomas and introduce W5C5, TNAP, CD344, and CD271 as potential sarcoma progenitor cell markers.
Wong, C. E., et al. (2006). "Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin." J Cell Biol **175**(6): 1005-1015.

Given their accessibility, multipotent skin-derived cells might be useful for future cell replacement therapies. We describe the isolation of multipotent stem cell-like cells from the adult trunk skin of mice and humans that express the neural crest stem cell markers p75 and Sox10 and display extensive self-renewal capacity in sphere cultures. To determine the origin of these cells, we genetically mapped the fate of neural crest cells in face and trunk skin of mouse. In whisker follicles of the face, many mesenchymal structures are neural crest derived and appear to contain cells with sphere-forming potential. In the trunk skin, however, sphere-forming neural crest-derived cells are restricted to the glial and melanocyte lineages. Thus, self-renewing cells in the adult skin can be obtained from several neural crest derivatives, and these are of distinct nature in face and trunk skin. These findings are relevant for the design of therapeutic strategies because the potential of stem and progenitor cells in vivo likely depends on their nature and origin.
Woo, D. H., et al. (2016). "Comparison of adult stem cells derived from multiple stem cell niches." Biotechnol Lett **38**(5): 751-759.

OBJECTIVES: Adult stem cells (ASCs) have great potential for tissue regeneration; however, comparative studies of ASCs from different niches are required to understand the characteristics of each population for their potential therapeutic uses. RESULTS: We compared the proliferation, stem cell marker expression, and differentiation potential of ASCs from bone marrow, skin dermis, and adipose tissue. ASCs from bone marrow and skin dermis showed 50-100 % increased proliferation in comparison to the ASCs from adipose tissues. Furthermore, ASCs from each stem cell niche showed differential expression of stem cell marker genes, and preferentially differentiated into cell types of their tissue of origin. CONCLUSION: Different characters

of each ASC might be major factors for their effective use for therapeutics and tissue regeneration.

Yamasaki, R., et al. (2007). "Small number of HTLV-1-positive cells frequently remains during complete remission after allogeneic hematopoietic stem cell transplantation that are heterogeneous in origin among cases with adult T-cell leukemia/lymphoma." Leukemia **21**(6): 1212-1217.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can provide long-term remission for patients with adult T-cell leukemia/lymphoma (ATLL) caused by human retrovirus, human T-lymphocyte virus (HTLV-1). To understand how HTLV-1-positive cells including ATLL cells were suppressed by allo-HSCT, we examined HTLV-1 provirus load and residual ATLL cells in peripheral blood of transplant recipients using PCR-based tests. We found that the copy number of HTLV-1 genome, called provirus, became very small in number after allo-HSCT; however, in most cases, provirus did not disappear even among long-term survivors. Tumor-specific PCR tests demonstrated that most of HTLV-1-positive cells that remained long after transplantation were not primary ATLL cells but donor-derived HTLV-1-positive cells. We also found a case having very low amount of residual disease in peripheral blood even long after transplantation. There was only one recipient in whom we failed to show the presence of HTLV-1 genome and antibody against HTLV-1 even with an extensive search, which strongly suggested the elimination of HTLV-1 after allo-HSCT. These results demonstrated that after allo-HSCT the small amount of residual HTLV-1-positive cells were heterogeneous in origin and that long-term disease control for ATLL could be obtained without the complete elimination of HTLV-1.

Yamazaki, J., et al. (2009). "Identification of cancer stem cells in a Tax-transgenic (Tax-Tg) mouse model of adult T-cell leukemia/lymphoma." Blood **114**(13): 2709-2720.

Adult T-cell leukemia/lymphoma (ATL) is a malignant lymphoproliferative disorder caused by HTLV-I infection. In ATL, chemotherapeutic responses are generally poor, which has suggested the existence of chemotherapy-resistant cancer stem cells (CSCs). To identify CSC candidates in ATL, we have focused on a Tax transgenic mouse (Tax-Tg) model, which reproduces ATL-like disease both in Tax-Tg animals and also after transfer of Tax-Tg splenic lymphomatous cells (SLCs) to nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Using a limiting dilution transplantation, it was estimated that one CSC existed per 10(4) SLCs (0.01%). In agreement with this, we have successfully identified candidate CSCs in a side population (0.06%), which overlapped with a minor population of CD38(-)/CD71(-)/CD117(+) cells (0.03%). Whereas lymphoma did not develop after transplantation of 10(2) SLCs, 10(2) CSCs could consistently regenerate the original lymphoma. In addition, lymphoma and CSCs could also be demonstrated in the bone marrow and CD117(+) CSCs were observed in both osteoblastic and vascular niches. In the CSCs, Tax, Notch1, and Bmi1 expression was down-regulated, suggesting that the CSCs were derived from Pro-T cells or early hematopoietic progenitor cells. Taken together, our data demonstrate that CSCs certainly exist and have the potential to regenerate lymphoma in our mouse model.

Yan, R., et al. (2012). "A new finding concerning adenoviral-mediated gene transfer: a high-level, cell-specific transgene expression in the neural stem cells of adult mice." J Virol Methods **186**(1-2): 1-6.

Adenoviruses are highly efficient vectors for gene transfer into brain cells. Restricting transgene expression to specific cell types and maintaining long-term expression are major goals for adenoviral-mediated gene transfer in the central nervous system. Human adenovirus type 5 (Ad5) mediated transgene expression is described under the control of the murine cytomegalovirus (mCMV) immediate-early promoter. It was found that the neural stem cells in the dentate subgranular zone were transduced preferentially, minimal neurons were transduced in the granule cell layer of the dentate gyrus, no EGFP was detected in the pyramidal cell layers of CAl to CA3 area and EGFP activity can be detected for 2 months after infection. Therefore, the mCMV-adenoviral vectors can be used both for studying the function of various genes in the differentiation of neural stem cells and, ultimately, for gene therapy or to modulate specific gene expression.

Yang, F., et al. (2009). "Gene delivery to human adult and embryonic cell-derived stem cells using biodegradable nanoparticulate polymeric vectors." Gene Ther **16**(4): 533-546.

Gene delivery to stem cells holds great potential for tissue regeneration and delivery of therapeutic proteins. The major barrier is the lack of safe and efficient delivery methods. Here, we report enhanced gene delivery systems for human stem cells using biodegradable polymeric vectors. A library of poly (beta-amino esters) end-modified derivatives was developed and optimized for high transfection efficiency and low cytotoxicity for three human stem cell lines including human mesenchymal stem cells (hMSCs), human adipose-derived stem cells (hADSCs) and human embryonic stem cell-derived cells (hESCds). In the presence of 10% serum, leading end-modified C32 polymeric vectors exhibited significantly high transfection efficiency in hMSCs (27+/-2%), hADSCs (24+/-3%) and hESCds (56+/-11%), with high cell viability (87-97%) achieved in all cell types. Our

results show that poly(beta-amino esters) as a class, and end-modified versions of C32 in particular, are efficient polymeric vectors for gene delivery to both adult and embryonic-derived stem cells.

Yang, J., et al. (2009). "Low connexin channel-dependent intercellular communication in human adult hematopoietic progenitor/stem cells: probing mechanisms of autologous stem cell therapy." Cell Commun Adhes **16**(5-6): 138-145.

Human bone marrow is a clinical source of autologous progenitor stem cells showing promise for cardiac repair following ischemic insult. Functional improvements following delivery of adult bone marrow CD34(+) cells into heart tissue may require metabolic/electrical communication between participating cells. Since connexin43 (Cx43) channels are implicated in cardiogenesis and provide intercellular connectivity in the heart, the authors analyzed the expression of 20 connexins (Cx) in CD34(+) cells and in monocytes and granulocytes in bone marrow and spinal cord. Reverse transcriptase-polymerase chain reaction (RT-PCR) detected only low expression of Cx43 and Cx37. Very low level dye coupling was detected by flow cytometry between CD34(+) cells and other Cx43 expressing cells, including HL-1 cardiac cells, and was not inhibited by specific gap junction inhibitors. The results indicate that CD34(+) cells are unlikely to communicate via gap junctions and the authors conclude that use of CD34(+) cells to repair damaged hearts is unlikely to involve gap junctions. The results concur with the hypothesis that bone marrow cells elicit improved cardiac function through release of undefined paracrine mediators.

Yasin, M. (2013). "Removing the cells from adult bone marrow derived stem cell therapy does not eliminate cardioprotection." Eur J Cardiothorac Surg **43**(4): 840-848.

OBJECTIVES: The debate as to whether adult stem cell therapy is regenerative or not continues. The non-regenerative benefits of adult bone marrow-derived stem cell therapy were investigated by testing whether the supernatant derived from unfractionated bone marrow mononuclear cells might be cardioprotective in an animal model of myocardial ischaemia-reperfusion injury. METHODS: Regional myocardial reperfusion injury was acquired by 25 min reversible left anterior descending coronary artery (LAD) occlusion followed by 2 h reperfusion, in anaesthetized Wistar male rats. Unfractionated bone marrow mononuclear cells (BMMNC) isolated from sibling Wistar male rat whole bone marrow were phenotyped by fluorescence activated cell sorting flowcytometry for the haematopoietic stem cell surface markers c-kit, CD34, CD45 and CD133. Animals subjected to regional myocardial reperfusion injury received either 10 million BMMNC or BMMNC supernatant (BMS); both were collected in 0.5 ml phosphate-buffered saline and delivered by intravenous bolus at the onset of reperfusion. The left ventricular region distal to the LAD occlusion point was excised for measurement of myocardial infarct size and proteomic analysis, which was used to identify whether there were any differences in myocardial proteins associated with intravenous injection of either BMMNC or BMS. RESULTS: BMMNC were phenotyped to be c-kit(+) (7 +/- 1%), CD34(+) (7 +/- 1%), CD45(+) (54 +/- 6%), CD133(+) (15 +/- 1%). The supernatant reduced myocardial infarct size (BMS 34 +/- 2%, n = 15 vs control 57 +/- 2%, n = 7, P < 0.0001), which was comparable to the reduction in infarct size afforded by the injection of cells (BMMNC 33 +/- 3% vs control 57 +/- 2%, n = 10, P < 0.0001). Proteomics of hearts treated with either BMS or BMMNC demonstrated higher expression of (i) anti-apoptotic signal transduction protein: 14-3-3-epsilon (1.5-fold); (ii) anti-oxidants: peroxiredoxin-6 (2.1-fold); (iii) heat shock proteins: alpha B-crystallin (1.7-fold), heat shock protein 72 (2.8-fold), tumour necrosis factor receptor-1 associated protein (2.3-fold), ischaemia responsive protein-94 (1.6-fold); (iv) glycolytic protein: glyceraldehyde-3-phosphate dehydrogenase (2.3-fold); (v) mitochondrial respiratory proteins: mitochondrial aconitase (4.7-fold), voltage-dependent anion-selective channel protein-1 (VDAC-1) (2.7-fold). CONCLUSIONS: Regional myocardial reperfusion injury can be attenuated by intravenous administration of either BMMNC or BMS at the onset of reperfusion, which suggests adult stem cells mediate non-regenerative cardioprotection.

Ye, L., et al. (2017). "Insights into the Development of the Adult Leydig Cell Lineage from Stem Leydig Cells." Front Physiol **8**: 430.

Adult Leydig cells (ALCs) are the steroidogenic cells in the testes that produce testosterone. ALCs develop postnatally from a pool of stem cells, referred to as stem Leydig cells (SLCs). SLCs are spindle-shaped cells that lack steroidogenic cell markers, including luteinizing hormone (LH) receptor and 3beta-hydroxysteroid dehydrogenase. The commitment of SLCs into the progenitor Leydig cells (PLCs), the first stage in the lineage, requires growth factors, including Dessert Hedgehog (DHH) and platelet-derived growth factor-AA. PLCs are still spindle-shaped, but become steroidogenic and produce mainly androsterone. The next transition in the lineage is from PLC to the immature Leydig cell (ILC). This transition requires LH, DHH, and androgen. ILCs are ovoid cells that are competent for producing a different form of androgen, androstanediol. The final stage in the developmental lineage is ALC. The transition to ALC involves the reduced expression of 5alpha-reductase 1,

a step that is necessary to make the cells to produce testosterone as the final product. The transitions along the Leydig cell lineage are associated with the progressive down-regulation of the proliferative activity, and the up-regulation of steroidogenic capacity, with each step requiring unique regulatory signaling.

Yeh, C. Y., et al. (2018). "Mossy Cells Control Adult Neural Stem Cell Quiescence and Maintenance through a Dynamic Balance between Direct and Indirect Pathways." Neuron **99**(3): 493-510 e494.

Mossy cells (MCs) represent a major population of excitatory neurons in the adult dentate gyrus, a brain region where new neurons are generated from radial neural stem cells (rNSCs) throughout life. Little is known about the role of MCs in regulating rNSCs. Here we demonstrate that MC commissural projections structurally and functionally interact with rNSCs through both the direct glutamatergic MC-rNSC pathway and the indirect GABAergic MC-local interneuron-rNSC pathway. Specifically, moderate MC activation increases rNSC quiescence through the dominant indirect pathway, while high MC activation increases rNSC activation through the dominant direct pathway. In contrast, MC inhibition or ablation leads to a transient increase of rNSC activation, but rNSC depletion only occurs after chronic ablation of MCs. Together, our study identifies MCs as a critical stem cell niche component that dynamically controls adult NSC quiescence and maintenance under various MC activity states through a balance of direct glutamatergic and indirect GABAergic signaling onto rNSCs.

Yeo, B. K., et al. (2016). "Valosin-containing protein is a key mediator between autophagic cell death and apoptosis in adult hippocampal neural stem cells following insulin withdrawal." Mol Brain **9**: 31.

BACKGROUND: Programmed cell death (PCD) plays essential roles in the regulation of survival and function of neural stem cells (NSCs). Abnormal regulation of this process is associated with developmental and degenerative neuronal disorders. However, the mechanisms underlying the PCD of NSCs remain largely unknown. Understanding the mechanisms of PCD in NSCs is crucial for exploring therapeutic strategies for the treatment of neurodegenerative diseases. RESULT: We have previously reported that adult rat hippocampal neural stem (HCN) cells undergo autophagic cell death (ACD) following insulin withdrawal without apoptotic signs despite their normal apoptotic capabilities. It is unknown how interconnection between ACD and apoptosis is mediated in HCN cells. Valosin-containing protein (VCP) is known to be essential for autophagosome maturation in mammalian cells. VCP is abundantly expressed in HCN cells compared to hippocampal tissue and neurons. Pharmacological and genetic inhibition of VCP at basal state in the presence of insulin modestly impaired autophagic flux, consistent with its known role in autophagosome maturation. Of note, VCP inaction in insulin-deprived HCN cells significantly decreased ACD and down-regulated autophagy initiation signals with robust induction of apoptosis. Overall autophagy level was also substantially reduced, suggesting the novel roles of VCP at initial step of autophagy. CONCLUSION: Taken together, these data demonstrate that VCP may play an essential role in the initiation of autophagy and mediation of crosstalk between ACD and apoptosis in HCN cells when autophagy level is high upon insulin withdrawal. This is the first report on the role of VCP in regulation of NSC cell death. Elucidating the mechanism by which VCP regulates the crosstalk of ACD and apoptosis will contribute to understanding the molecular mechanism of PCD in NSCs.

Yoon, J., et al. (2005). "Transdifferentiation of mesenchymal stem cells into cardiomyocytes by direct cell-to-cell contact with neonatal cardiomyocyte but not adult cardiomyocytes." Ann Hematol **84**(11): 715-721.

Recent studies have demonstrated that direct cell-to-cell interaction is one of the microenvironment factors for transdifferentiation of adult stem cells into cardiomyocytes. We investigated whether transdifferentiation of mesenchymal stem cells (MSCs) into cardiomyocytes was dependent on developmental stages of cocultured cardiomyocytes, and direct cell-to-cell interaction was essential for transdifferentiation. MSCs were isolated from adult rat and cocultured in four different ways: (1) with neonatal cardiomyocytes, (2) with adult cardiomyocytes, (3) with neonatal cardiomyocytes on the cell culture inserts, and (4) with the conditioned medium from neonatal cardiomyocytes. After 5 days of coculture with neonatal cardiomyocytes, 9.40+/-1.15% of 1,1'-dioctadecyl-1-3,3,3',3'-tetramethylindocarbocyanine perchlorate labeled MSCs expressed sarcomeric-alpha-actinin. Immunocytochemistry showed that only these MSCs expressed the cardiac markers and were not observed with other coculture condition as well as conditioned medium. Calcein-AM labeling of cardiomyocytes showed gap junctional communication between 56.1+/-2.0% of MSCs (24 h after labeling, n=5) and neonatal cardiomyocytes. These findings suggest that MSCs are capable of differentiating into cardiomyocytes when directly cocultured with neonatal cardiomyocytes by cell-to-cell interaction, but not with adult cardiomyocytes or conditioned medium.

Yu, K., et al. (2016). "Beta Cell Regeneration in Adult Mice: Controversy Over the Involvement of Stem Cells." Curr Stem Cell Res Ther **11**(7): 542-546.

Islet transplantation is an effective therapy for severe diabetes. Nevertheless, the short supply of donor

pancreases constitutes a formidable obstacle to its extensive clinical application. This shortage heightens the need for alternative sources of insulin-producing beta cells. Since mature beta cells have a very slow proliferation rate, which further declines with age, great efforts have been made to identify beta cell progenitors in the adult pancreas. However, the question whether facultative beta cell progenitors indeed exist in the adult pancreas remains largely unresolved. In the current review, we discuss the problems in past studies and review the milestone studies and recent publications.

Yuzwa, S. A., et al. (2017). "Developmental Emergence of Adult Neural Stem Cells as Revealed by Single-Cell Transcriptional Profiling." Cell Rep **21**(13): 3970-3986.

Adult neural stem cells (NSCs) derive from embryonic precursors, but little is known about how or when this occurs. We have addressed this issue using single-cell RNA sequencing at multiple developmental time points to analyze the embryonic murine cortex, one source of adult forebrain NSCs. We computationally identify all major cortical cell types, including the embryonic radial precursors (RPs) that generate adult NSCs. We define the initial emergence of RPs from neuroepithelial stem cells at E11.5. We show that, by E13.5, RPs express a transcriptional identity that is maintained and reinforced throughout their transition to a non-proliferative state between E15.5 and E17.5. These slowly proliferating late embryonic RPs share a core transcriptional phenotype with quiescent adult forebrain NSCs. Together, these findings support a model wherein cortical RPs maintain a core transcriptional identity from embryogenesis through to adulthood and wherein the transition to a quiescent adult NSC occurs during late neurogenesis.

Zangrossi, S., et al. (2007). "Oct-4 expression in adult human differentiated cells challenges its role as a pure stem cell marker." Stem Cells **25**(7): 1675-1680.

The Oct-4 transcription factor, a member of the POU family that is also known as Oct-3 and Oct3/4, is expressed in totipotent embryonic stem cells (ES) and germ cells, and it has a unique role in development and in the determination of pluripotency. ES may have their postnatal counterpart in the adult stem cells, recently described in various mammalian tissues, and Oct-4 expression in putative stem cells purified from adult tissues has been considered a real marker of stemness. In this context, normal mature adult cells would not be expected to show Oct-4 expression. On the contrary, we demonstrated, using reverse transcription-polymerase chain reaction (PCR) (total RNA, Poly A+), real-time PCR, immunoprecipitation, Western blotting, band shift, and immunofluorescence, that human peripheral blood mononuclear cells, genetically stable and mainly terminally differentiated cells with well defined functions and a limited lifespan, express Oct-4. These observations raise the question as to whether the role of Oct-4 as a marker of pluripotency should be challenged. Our findings suggest that the presence of Oct-4 is not sufficient to define a cell as pluripotent, and that additional measures should be used to avoid misleading results in the case of an embryonic-specific gene with a large number of pseudogenes that may contribute to false identification of Oct-4 in adult stem cells. These unexpected findings may provide new insights into the role of Oct-4 in fully differentiated cells. Disclosure of potential conflicts of interest is found at the end of this article.

Zhang, H., et al. (2014). "Life-long in vivo cell-lineage tracing shows that no oogenesis originates from putative germline stem cells in adult mice." Proc Natl Acad Sci U S A **111**(50): 17983-17988.

Whether or not oocyte regeneration occurs in adult life has been the subject of much debate. In this study, we have traced germ-cell lineages over the life spans of three genetically modified mouse models and provide direct evidence that oogenesis does not originate from any germline stem cells (GSCs) in adult mice. By selective ablation of all existing oocytes in a Gdf9-Cre;iDTR mouse model, we have demonstrated that no new germ cells were ever regenerated under pathological conditions. By in vivo tracing of oocytes and follicles in the Sohlh1-CreER(T2);R26R and Foxl2-CreER(T2);mT/mG mouse models, respectively, we have shown that the initial pool of oocytes is the only source of germ cells throughout the life span of the mice and that no adult oogenesis ever occurs under physiological conditions. Our findings clearly show that there are no GSCs that contribute to adult oogenesis in mice and that the initial pool of oocytes formed in early life is the only source of germ cells throughout the entire reproductive life span.

Zhang, L., et al. (2013). "NGF induces adult stem Leydig cells to proliferate and differentiate during Leydig cell regeneration." Biochem Biophys Res Commun **436**(2): 300-305.

Nerve growth factor (NGF) has been reported to be involved in male reproductive physiology. However, few reports have described the activity of NGF during Leydig cell development. The objective of the present study was to examine the role of NGF during stem-Leydig-cell (SLC) regeneration. We investigated the effects of NGF on Leydig-cell (LC) regeneration by measuring mRNA levels in the adult rat testis after ethane dimethanesulfonate (EDS) treatment. Furthermore, we used the established organ culture model of rat seminiferous tubules to examine the regulation of NGF during SLC proliferation and differentiation using EdU staining, real-time PCR and western blotting. Progenitor Leydig cells (PLCs) and

immature Leydig cells (ILCs) were also used to investigate the effects of NGF on LCs at different developmental stages. NGF mRNA levels changed significantly during Leydig-cell regeneration in vivo. In vitro, NGF significantly promoted the proliferation of stem Leydig cells and also induced steroidogenic enzyme gene expression and 3beta-HSD protein expression. The data from PLCs and ILCs showed that NGF could increase Cyclin D1 and Hsd 17b3 mRNA levels in PLCs and Cyclin D1 mRNA levels in ILCs. These results indicate that NGF may play an important role during LC regeneration by regulating the proliferation and differentiation of LCs at different developmental stages, from SLCs to PLCs and from PLCs to ILCs. The discovery of this effect of NGF on Leydig cells will provide useful information for developing new potential therapies for PADAM (Partial Androgen Deficiency in the Aging Male).

Zhang, P. Z., et al. (2013). "Up-regulation of stromal cell-derived factor-1 enhances migration of transplanted neural stem cells to injury region following degeneration of spiral ganglion neurons in the adult rat inner ear." Neurosci Lett **534**: 101-106.

Neural stem cell (NSC) transplantation into the cochlea is widely used for the treatment of spiral ganglion neuron (SGN) degenerative disease and injury in the animal models, but the migration of the transplanted NSCs to the injury region is difficult and the mechanism is still unclear. In this study, we aimed to validate whether the SGN-degenerated cochlear microenvironment plays a role in the NSC migration and investigated whether stromal cell-derived factor-1 (SDF-1) was involved in the NSCs migration. Using a rat SGN degeneration model, we demonstrated that the transplanted NSCs are more likely to migrate to the injury region during the early post-injury (EPI) than the late post-injury (LPI) stage and the control cochlea. We found that the expressions of SDF-1 increased transiently after SGN degeneration. Additionally, we showed that the NSCs express CXCR4, a receptor for SDF-1. We observed that the region to which the transplanted NSC localized coincides with the region where the SDF-1 is highly expressed following the degeneration of SGNs. Finally, we observed that the increased SDF-1 is derived from the Schwann cells in the SGN-degenerated model. These results suggest that SDF-1, which is derived from cochlear Schwann cells and up-regulated in the early injury microenvironment, plays a beneficial role in the NSC migration to the injury region. Optimizing SDF-1 expression in the host microenvironment or increasing the CXCR4 expression of the donor stem cells may improve the migration efficiency of transplanted cells toward the injury region in the cochlea.

Zhang, Y., et al. (2015). "sb203580 preconditioning recharges matrix-expanded human adult stem cells for chondrogenesis in an inflammatory environment - A feasible approach for autologous stem cell based osteoarthritic cartilage repair." Biomaterials **64**: 88-97.

Autologous stem cells are a promising cell source for cartilage regeneration; however, cell replicative senescence and joint posttraumatic inflammation provide challenges in bringing this treatment modality to fruition. In this study, we hypothesized that preconditioning with p38 MAPK inhibitor (sb203580) would recharge decellularized extracellular matrix (dECM) expanded human synovium-derived stem cell (hSDSC) chondrogenesis in an inflammatory environment. We found that preconditioning with sb203580 greatly enhanced dECM expanded hSDSC proliferation and chondrogenic potential while supplementation with sb203580 in an induction medium dramatically retarded hSDSC chondrogenic differentiation, even for dECM expanded cells. We also found that sb203580 preconditioning enhanced matrix-expanded hSDSC chondrogenic capacity even in an interleukin-1 (IL-1) induced inflammatory environment. Non-detectable expression of HLA-DR in the hSDSCs grown on allogeneic dECM indicates the feasibility of commercial preparation of these dECMs from healthy, young donors for patients who need autologous transplantation. Our study indicated that p38 MAPK inhibitor has a distinctive priming effect on dECM mediated stem cell cartilage regeneration. Combined rejuvenation with sb203580 and dECM expansion can precondition hSDSCs' resurfacing capacity for osteoarthritic patients with cartilage defects.

Zhao, M., et al. (2007). "Evidence for the presence of stem cell-like progenitor cells in human adult pancreas." J Endocrinol **195**(3): 407-414.

The origin of cells replacing ageing beta-cells in adult life is unknown. This study assessed the expression of classic stem cell markers: Oct4, Sox2 and CD34 in islet-enriched fractions versus exocrine cell-enriched fractions from 25 adult human pancreases following human islet isolation. Expression of Oct4, Sox2 and CD34 mRNAs was found in all cell samples, with no significant differences between endocrine and exocrine cell fractions. Immunohistochemical staining for Oct4, Sox2, CD133, CD34, CK19, insulin and nestin on human pancreas sections showed that the majority of Oct4(+ve) cells were found in the walls of small ducts. Similar localisations were observed for Sox2(+ve) cells. The majority of Sox2(+ve) cells were found to co-express Oct4 proteins, but not vice versa. Cells positive for Oct4 and Sox2 appeared to be a unique cell population in the adult human pancreases without co-expression for CK19, CD34, CD133, insulin and nestin proteins. The numbers of Oct4(+ve) and Sox2(+ve) cells varied among donors and were

approximately 1-200 and 1-30 per 100 000 pancreatic cells respectively.

Ziebell, F., et al. (2014). "Mathematical modelling of adult hippocampal neurogenesis: effects of altered stem cell dynamics on cell counts and bromodeoxyuridine-labelled cells." J R Soc Interface **11**(94): 20140144.

In the adult hippocampus, neurogenesis-the process of generating mature granule cells from adult neural stem cells-occurs throughout the entire lifetime. In order to investigate the involved regulatory mechanisms, knockout (KO) experiments, which modify the dynamic behaviour of this process, were conducted in the past. Evaluating these KOs is a non-trivial task owing to the complicated nature of the hippocampal neurogenic niche. In this study, we model neurogenesis as a multicompartmental system of ordinary differential equations based on experimental data. To analyse the results of KO experiments, we investigate how changes of cell properties, reflected by model parameters, influence the dynamics of cell counts and of the experimentally observed counts of cells labelled by the cell division marker bromodeoxyuridine (BrdU). We find that changing cell proliferation rates or the fraction of self-renewal, reflecting the balance between symmetric and asymmetric cell divisions, may result in multiple time phases in the response of the system, such as an initial increase in cell counts followed by a decrease. Furthermore, these phases may be qualitatively different in cells at different differentiation stages and even between mitotically labelled cells and all cells existing in the system.

Zuk, P. A. (2009). "The intracellular distribution of the ES cell totipotent markers OCT4 and Sox2 in adult stem cells differs dramatically according to commercial antibody used." J Cell Biochem **106**(5): 867-877.

To characterize ES cells, researchers have at their disposal a list of pluripotent markers, such as OCT4. In their quest to determine if adult stem cell populations, such as MSCs and ASCs, are pluripotent, several groups have begun to report the expression of these markers in these cells. Consistent with this, human ASCs (hASCs) are shown in this study to express a plethora of ES pluripotent markers at the gene and protein level, including OCT4, Sox2, and Nanog. When intracellular distribution is examined in hASCs, both OCT4 and Sox2 are expressed within the nuclei of hASCs, consistent with their expression patterns in ES cells. However, a significant amount of expression can be noted within the hASC cytoplasm and a complete absence of nuclear expression is observed for Nanog. Recent descriptions of OCT4 transcript variants may explain the cytoplasmic expression of OCT4 in hASCs and consistent with this, hASCs do express both the OCT4A and 4B transcript variants at the gene level. However, discrepancies arise when these three pluripotent markers are studied at the protein level. Specifically, distinct differences in intracellular expression patterns were noted for OCT4, Sox2, and Nanog from commercial antibody to commercial antibody. These antibody discrepancies persisted when hMSCs and rat ASCs and MSCs were examined. Therefore, confirming the expression of OCT4, Sox2, and Nanog in adult stem cells with today's commercial antibodies must be carefully considered before the designation of pluripotent can be granted.

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3/22/2022