Stem Cell Multipotent Literatures

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

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into many different types of cells in life bodies, that are exciting to scientists because of their potential to develop into many different cells, tissues and organs. Stem cells can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003). Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situuition or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a tipical and important topic of life science.

2. Definition of Stem Cells

The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell" (Stedman's Medical Dictionary, 2002).

3. Characterization of Stem Cell

Stem cell is totipotent, that means it holds all the genetic information of the living body and it can develop into a mature cell. Stem cell is a single cell that can give rise to progeny that differentiate into any of the specialized cells of embryonic or adult tissue. The ultimate stem cells (fertilized egg) divide to branches of cells that form various differentiated tissues or organs. During these early decisions, each progeny cell retains totipotency. Through divisions and differentiations the embryonic stem cells lose totipotency and gain differentiated function. During normal tissue renewal in adult organs, tissue stem cells give rise to progeny that differentiate into mature functioning cells of that tissue. Stem cells losing totipotentiality are progenitor cells. Except for germinal cells, which retain totipotency, most stem cells in adult tissues have reduced potential to produce different cells.

4. Sources of Stem Cells

Aristotle (384-322 BC) deduced that the embryo was derived from mother's menstrual blood, which was based on the concept that living animals arose from slime or decaying matter. This concept was accepted in western world for over 2000 years, and it controlled western philosophy for over 2000 years either. In 1855, Virchow supposed that all cells in an organism are derived from preexisting cells. Now we know that all the human cells arise from a preexisting stem cell – the fertilized egg, that come from the mating of a man and a woman naturally but now can be produced in the laboratory tube. The counter hypothesis of spontaneous generation was accepted until 1864, when the French scientist Louis Pasteur demonstrated that there would be no microorganisms' growing after sterilizing and sealing.

The animal body has an unlimited source of stem cells, almost. However, the problem is not in locating these stem cells, but in isolating them from their tissue source.

Five key stem cells have been isolated from human: (1) Blastocysts; (2) Early embryos; (3) Fetal tissue; (4) Mature tissue; (5) Mature cells that can be grown into stem cells.

Up to today, only stem cells taken from adults or children (known generically as "adult stem cells") have been used extensively and effectively in the treatment of degenerative diseases.

5. Embryonic Stem Cell

Embryonic stem cells hold great promise for treating degenerative diseases, including diabetes, Parkinson's, Alzheimer's, neural degeneration, and

cardiomyopathies (Bavister, 2005). Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Embryonic stem cells can replicate indefinitely. This makes it feasible to culture the cells on a large scaled for cell transplantation therapy in clinical application. Embryonic stem cells are pluripotent and have the potential to differentiate into all three germ layers of the mammalian body including the germ cells.

6. Somatic Stem Cell

Normally to say that somatic stem cells differentiate only into specific tissue cells wherein they reside. However, somatic stem cells can differentiate into cells other than those of their tissue of origin. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart and peripheral blood possess stem or progenitor cells with the capacity to transdifferentiate. Due to this developmental plasticity, somatic stem cells may have potential in autologous regenerative medicine, circumventing problems like rejection and the ethically challenged use of embryocyte stem cells.

7. Isolation and Characterisation of Stem Cells

As the example, the following is describing the isolation and characterization of the putative prostatic stem cell, which was done by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in 2003. The detail methods have been described by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in the article "Novel method for the isolation and characterisation of the putative prostatic stem cell" in the journal Cytometry A in 2003 (Bhatt, 2003).

7.1 Prostatic tissue collection and culture

When using human tissue, formal consent by the donator must be obtained before tissue collection. Tissue sections are obtained under sterile conditions. Each individual tissue section is bisected with half being sent for histological analysis for diagnostic evaluation and the remainder used for tissue culture. After then, tissue sections are chopped and placed in collagenase type I at 200 U/ml in RPMI 1640 medium with 2% v/v FCS overnight on a shaking platform at 37°C. The digest is then broken down further by shaking in 0.1% trypsin in PBS with 1% BSA and 1 mM ethylenediaminetetraacetic acid (EDTA) for 15-20 min. The cell suspension is then washed three times in PBS with 1% BSA and 1 mM EDTA before resuspending in RPMI 10% v/v FCS. Prostate epithelial cells are separated from fibroblasts by differential centrifugation (360 g, 1 min without braking). This process produced a supernatant enriched for fibroblasts and a pellet enriched for epithelia. The epithelial cell suspension is then spun on a metrizamide gradient (1.079 g/ml), and the cells are isolated from the interface (Bhatt, 2003).

7.2 Ber-EP4/α_2/CD45 labelling of cells

Isolated epithelial cells are labeled at ambient temperature with either anti-human integrin α_2 monoclonal antibody or Ber-EP4 antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Samples are then dual labelled with CD45-FITC (1 μg/ml in 1% BSA/PBS) for 30 min (Bhatt, 2003).

7.3 Ber-EP4/α_2 and Hoechst labelling for flow cytometry

Isolated epithelial cells are labelled at ambient temperature with anti-human integrin α_2 monoclonal antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Hoechst staining could be performed by using the protocol for HSC as described by Rupesh, et al (Bhatt, 2003). Briefly, epithelial cells are resuspended in Hoechst buffer (Hanks' balanced salts solution, 10% FCS, 1% D-glucose, and 20 mM HEPES) and warmed to 37°C. Hoechst 33342 is then added to give a final concentration of 2 μM and the cells incubated at 37°C for 2 h. Fifteen min before the end of incubation, the cells are labelled with monoclonal anti-human Ber-EP4 directly conjugated to FITC (8 μg/ml). The cells are then washed in ice-cold Hoechst buffer before resuspending in ice-cold Hoechst buffer containing propidium iodide (PI) at 20 ng/ml (Bhatt, 2003).

7.4 Flow cytometry isolation of the SP fraction

Flow cytometry is carried out using a Becton Dickinson FACS Vantage SE flow cytometer. Hoechst 33342 is excited with an argon ion, ultraviolet-enhanced laser at 350 nm, and its fluorescence is measured with a 424/44 BP filter (Hoechst BLUE) and a 675DF20 BP optical filter (Hoechst RED; Omega Optical, Brattleboro VT). A 640 LP dichroic mirror is used to separate the emission wavelengths. PI fluorescence is also measured through the 675DF20 BP (having been excited at 350 nm). A second argon ion laser is used to excite the additional fluorochrome PE-Cy7 at 488 nM. PE-Cy7 is measured using a 787RDF40 (Omega Optical) filter (Bhatt, 2003).

7.5 Cell cycle characterisation of SP fraction

Epithelial cells are isolated and all fractions are resuspended in Hoechst buffer and warmed to 37°C. Hoechst 33342 is then added to give a concentration of 2 μM and incubated at 37°C for 45 min. Pyronin Y (250 ng/μl) is added to each tube, and the samples are incubated for 45 min. Monoclonal anti-human Ber-EP4 FITC (8 μg/ml) is added as appropriate 15 min

before the end. After this, ice-cold Hoechst buffer is added immediately and the samples are washed then resuspended in ice-cold Hoechst buffer. The samples are analysed immediately by flow cytometry. Flow cytometry is performed using a modification of the method described above. Cells under study are selected by positive labelling for Ber-EP4 FITC before being analysed for Hoechst and Pyronin Y staining. These cells are then analysed by plotting the Hoechst profile on the x-axis and Pyronin Y along the y-axis in a linear scale (Bhatt, 2003).

7.6 Cytokeratin phenotype studies

Samples are processed as above, divided into two fractions, and labelled with either cytokeratin 8 or 14 indirectly conjugated to PE-Cy5. Samples are then dual labelled with Ber-EP4 FITC and integrin α_2 PE-CY7. Flow cytometry is performed as described and analysed on forward (FSC) and side (SSC) scatter (Bhatt, 2003).

8 Application of Stem Cells in Clinical Medicine

There are over four thousand registered diseases specifically linked to genetic abnormalities. Although stem cells are unlikely to provide powerful treatment for these diseases, they are unique in their potential application to these diseases.

Indeed, in many research projects, scientists have demonstrated that stem cells can be used to replenish or rejuvenate damaged cells within the immune system of the human body and that damaged stem cells can repair themselves and their neighbors. For example, in what is regarded as the first documented case of successful gene-therapy "surgery", scientists at the Necker Hospital for Sick Children in Paris of French succeeded in treating two infants diagnosed with Severe Combined Immunodeficiency Disease, a life-threatening degenerative disease caused by defects on the male (X) chromosome. With the identification of stem cell plasticity several years ago, multiple reports raised hopes that tissue repair by stem cell transplantation could be within reach in the near future (Kashofer, 2005). In cardiovascular medicine, the possibility to cure heart failure with newly generated cardiomyocytes has created the interest of many researchers (Condorelli, 2005). Gene clone techniques can be widely used in the stem cell researches and applications (Ma, 2004).

9 Debates on Stem Cell Research

There are a lot of debates on the stem cell research. Stem cell research is a high-tech question and the people involved in this rebates should have certain scientific knowledge on the stem cell. It is OK for the politicians or religionists to show their opinions on any topic they are interested in, but not suitable for them to make decisions (or make laws) that will significantly influence the scientific research as this field the politicians or religionists are not specialized. Such as, it is not suitable for the American President George W. Bush to show the power in the stem cell research. It is scientists' job. When politics and science collide, science should do scientific way, rather political way. Major ethical and scientific debates surround the potential of stem cells to radically alter therapies in health care (Williams, 2005).

10. Literatures

Abkowitz, J. L., P. J. Fialkow, et al. (1984). "Pancytopenia as a clonal disorder of a multipotent hematopoietic stem cell." J Clin Invest **73**(1): 258-61.

Hematopoiesis was investigated in a 14-yr-old girl who had a 2-yr history of stable asymptomatic pancytopenia and who was also heterozygous at the structural locus for glucose-6-phosphate dehydrogenase (G-6-PD). There was no morphologic or cytogenetic evidence for preleukemia and no suggestion of Fanconi anemia. In the skin and sheep erythrocytes-rosetted T lymphocytes, the ratio of G-6-PD A/B activities was 1:1. However, only type B activity was found in peripheral blood erythrocytes, granulocytes, and platelets. Most erythroid bursts and all granulocyte/macrophage colonies formed in methylcellulose culture were derived from the abnormal clone. These findings demonstrate that (a) some cases of pancytopenia are stem cell diseases that apparently develop clonally; (b) circulating differentiated cells originate from this clone; (c) despite a hypoproliferative anemia, the in vivo expression of presumably normal (nonclonal) progenitors is suppressed. In this patient, the relationship between clonal dominance and possible malignancy may be assessed prospectively.

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): 193-7.

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.

Anzai, H., M. Nagayoshi, et al. (1999). "Self-renewal and differentiation of a basic fibroblast growth factor-dependent multipotent hematopoietic cell line derived from embryonic stem cells." Dev Growth Differ **41**(1): 51-8.

Despite the accumulation of informat on on the origin of hematopoietic stem cells, it is still unclear how these cells are generated in ontogeny. Isolation of cell lines equivalent to early embryonic hematopoietic progenitor cells can be helpful. A multipotent hematopoietic progenitor cell line, A-6, was isolated from H-1 embryonic stem (ES) cells. The self-renewal of A-6 cells was supported by basic-fibroblast growth factor (b-FGF) and their differentiation into definitive erythroid cells, granulocytes and macrophages was induced after co-culture with ST-2 stromal cells. A-6 cells were positive for the surface markers of hematopoietic stem cell, c-kit, CD31, CD34, Flt3/Flk2, PgP-1, and HSA, but were negative for that of the differentiated cells. Reverse transcription-polymerase chain reaction analysis showed that A-6 cells produced mRNA from SCL/tal-1 and GATA-2 genes. Among various cytokines examined, on y stem cell factor (SCF) and Flt3/Flk2 ligand (FL) supported the proliferation of A-6 cells instead of b-FGF. The FL, as well as b-FGF, supported the self-renewal of A-6 cells, whereas SCF induced differentiation into myeloid cells. A-6 cells will be useful for the characterization of hematopoietic progenitor cells derived from ES cells and provide a model system to realize the control mechanisms between self-renewal and different ation of hematopoietic stem cells.

Asami, M., M. Owhashi, et al. (1991). "Susceptibility of multipotent haemopoietic stem cell deficient W/Wv mice to Plasmodium berghei-infection." Immunol Cell Biol **69 (Pt 5)**: 355-60.

The susceptibility of haemopoietic stem cell deficient W/Wv mice to infection with Plasmodium berghei was examined. The mean survival time of W/Wv mice after the infection was shorter than that of the +/+ mice. Splenomegaly, a characteristic pathological change of the host after infection with malaria parasites was not observed in W/Wv mice. When haemopoietic activity of the infected mice was examined, a substantial increase in number of multipotent haemopoietic stem cells (CFU-S) and the committed stem cells for granulocytes and macrophages (CFU-GM) or for erythrocytes (CFU-E) was observed in the bone marrow and spleen of +/+ but not of W/Wv mice. CFU-S were not detected in W/Wv mice before or after infection. The number of CFU-GM and CFU-E in bone marrow and spleen of W/Wv mice decreased after infection. Bone marrow grafting from +/+ to W/Wv mice 8 weeks before infection prolonged the mean survival time of the mice and effectively restored the number of CFU-S in the spleen of W/Wv mice. These results indicate that multi-potent haemopoietic stem cells play an important role in the host's defence mechanisms against P. berghei-infection.

Berking, S. (1979). "Control of nerve cell formation from multipotent stem cells in Hydra." J Cell Sci **40**: 193-205.

Feeding of starved animals provides a very short signal which determines stem cells to differentiate into nerve cells after the next mitosis. Only those stem cells become determined which are just in the middle of their S-phase at the time of feeding. Stem cells of any other stage of the cycle do not become determined. Nerve cell determination is suppressed by very low concentrations of an endogenous inhibitor. The inhibitor exerts its effect only during the first half of the S-phase, not before and not after this period. Based on these finding it is proposed that stem cells are susceptible to 2 different signals during the first half of their S-phase; one signal allows the development into nerve cells, the other prevents this development. Within this period the decision whether to become a nerve cell or not is reversible. It becomes fixed at the end of this period.

Cai, J., Y. Wu, et al. (2002). "Properties of a fetal multipotent neural stem cell (NEP cell)." Dev Biol **251**(2): 221-40.

Multipotent neural stem cells (NSCs) present in the developing neural tube (E10.5, neuroepithelial cells; NEP) were examined for the expression of candidate stem cell markers, and the expression of these markers was compared with later appearing precursor cells (E14.5) that can be distinguished by the expression of embryonic neural cell adhesion

molecule (E-NCAM) and A2B5. NEP cells possess gap junctions, express connexins, and appear to lack long cilia. Most candidate markers, including Nestin, Presenilin, Notch, and Numb, were expressed by both NEP cells as well as other cell populations. Fibroblast growth factor receptor 4 (FGFR4), Frizzled 9 (Fz9), and SRY box-containing gene 2 (Sox2) as assessed by immunocytochemistry and in situ hybridization are markers that appear to distinguish NSCs from other precursor cells. Neither Hoechst 33342 nor rhodamine-123 staining, telomerase (Tert) expression, telomerase activity, or breakpoint cluster region protein 1 (Bcrp1) transporter expression could be used to distinguish NEP stem cells from other dividing cells. NEP cells, however, lacked expression of several lineage markers that are expressed by later appearing cells. These included absence of expression of CD44, E-NCAM, A2B5, epidermal growth factor receptor (EGFR), and platelet-derived growth factor receptor-alpha (PDGFR alpha), suggesting that negative selection using cell surface epitopes could be used to isolate stem cell populations from mixed cultures of cells. Using mixed cultures of cells isolated from E14.5 stage embryos, we show that NEP cells can be enriched by depleting differentiating cells that express E-NCAM or A2B5 immunoreactivity. Overall, our results show that a spectrum of markers used in combination can reliably distinguish multipotent NSCs from other precursor cells as well as differentiated cells present in the CNS.

Cairns, L., M. Ciro, et al. (2003). "Induction of globin mRNA expression by interleukin-3 in a stem cell factor-dependent SV-40 T-antigen-immortalized multipotent hematopoietic cell line." J Cell Physiol **195**(1): 38-49.

Erythropoiesis requires the stepwise action on immature progenitors of several growth factors, including stem cell factor (SCF), interleukin 3 (IL-3), and erythropoietin (Epo). Epo is required to sustain proliferation and survival of committed progenitors and might further modulate the level of expression of several erythroid genes, including globin genes. Here we report a new SCF-dependent immortalized mouse progenitor cell line (GATA-1 ts SCF) that can also grow in either Epo or IL-3 as the sole growth factor. When grown in SCF, these cells show an "open" chromatin structure of the beta-globin LCR, but do not significantly express globin. However, Epo or IL-3 induce globin expression and are required for its maintainance. This effect of IL-3 is unexpected as IL-3 was previously reported either to be unable to induce hemoglobinization, or even to antagonize it. This suggests that GATA-1 ts SCF cells may have progressed to a stage in which globin genes are already poised for expression and only require signal(s) that can be elicited by either Epo or IL-3. Through the use of inhibitors, we suggest that p38 may be one of the molecules modulating induction and maintenance of globin expression.

Cuneo, A., S. Kerim, et al. (1989). "Translocation t(6;9) occurring in acute myelofibrosis, myelodysplastic syndrome, and acute nonlymphocytic leukemia suggests multipotent stem cell involvement." Cancer Genet Cytogenet **42**(2): 209-19.

The cytological and cytogenetic features of six patients with myeloid neoplasia and t(6;9)(p23;q34) including a case of acute myelofibrosis (AMF), a refractory anemia with excess of blasts (RAEB), and four cases of acute nonlymphocytic leukemia (ANLL) are described. Two patients in this series, both affected by ANLL type M2, presented an increase of bone marrow basophils, suggesting that this cytological-cytogenetic association is not absolute and that it may be more frequently observed in ANLL with maturation. All patients with de novo ANLL showed associated myelodysplastic features, and one patient presented a dysmyelopoietic syndrome, later evolving into ANLL. The presence of the t(6;9) in a range of myeloid neoplasias, with either concurrent myelodysplastic features or a preleukemic phase in cases of ANLL, provide evidence that this chromosome aberration may always involve a multipotent myeloid stem cell. Data on toxic exposure of the patients suggests that myeloproliferative disorders with the t(6;9) may frequently represent environmentally induced neoplasias.

Cuneo, A., C. Mecucci, et al. (1989). "Multipotent stem cell involvement in megakaryoblastic leukemia: cytologic and cytogenetic evidence in 15 patients." Blood **74**(5): 1781-90.

Cytologic and cytogenetic results obtained from patients fulfilling the FAB criteria for the diagnosis of acute nonlymphocytic leukemia (ANLL) of megakaryocytic lineage (ANLL-M7) are reported. Eleven cases were de novo ANLL-M7, of whom three presented with acute myelofibrosis. Four cases were megakaryoblastic transformations of chronic myelogenous leukemia (two cases), refractory anemia with excess of blasts (one case), and polycythemia vera (one case). Four patients showed a minority of granular blasts, with occasional Auer rods in one. Positive myeloperoxidase and/or sudan black-B stainings and CD13 positivity in these cases were consistent with the presence of a myeloid involvement. Morphologic evidence of associated myelodysplastic features was detected in all evaluable patients with de novo ANLL-M7. These cytologic findings indicate that ANLL-M7 may frequently

represent a multilineage proliferation. Cytogenetic studies revealed -7/7q- and +8, alone or in combination with additional aberrations, in three cases each. Rearrangements involving bands 3q21 or 3q26 were seen in two patients and +21, as an additional aberration, in one. Other structural rearrangements all observed in a single patient were inv(16)(p13q22) at megakaryoblastic relapse with bone marrow eosinophilia, t(13;20)(q13 or 14;q11), del(20)(q11), and der(7)t(7;17)(p14;q22). Most breakpoints of these aberrations are located at bands frequently rearranged in malignant myeloid stem cell disorders. A review of 31 cases of the literature showed a frequent occurrence of -7/7q- and -5/5q- in ANLL-M7. Many of the chromosome aberrations so far described in ANLL-M7 appear to be shared by a spectrum of myeloid neoplasias and may be related to mechanisms conferring proliferative advantage to undifferentiated stem cells.

De Filippis, L., G. Lamorte, et al. (2007). "A novel, immortal, and multipotent human neural stem cell line generating functional neurons and oligodendrocytes." Stem Cells **25**(9): 2312-21.

The discovery and study of neural stem cells have revolutionized our understanding of the neurogenetic process, and their inherent ability to adopt expansive growth behavior in vitro is of paramount importance for the development of novel therapeutics based on neural cell replacement. Recent advances in high-throughput assays for drug development and gene discovery dictate the need for rapid, reproducible, long-term expansion of human neural stem cells (hNSCs). In this view, the complement of wild-type cell lines currently available is insufficient. Here we report the establishment of a stable human neural stem cell line (immortalized human NSCs [IhNSCs]) by v-myc-mediated immortalization of previously derived wild-type hNSCs. These cells demonstrate three- to fourfold faster proliferation than wild-type cells in response to growth factors but retain rather similar properties, including multipotentiality. By molecular biology, biochemistry, immunocytochemistry, fluorescence microscopy, and electrophysiology, we show that upon growth factor removal, IhNSCs completely downregulate v-myc expression, cease proliferation, and differentiate terminally into three major neural lineages: astrocytes, oligodendrocytes, and neurons. The latter are functional, mature cells displaying clear-cut morphological and physiological features of terminally differentiated neurons, encompassing mostly the GABAergic, glutamatergic, and cholinergic phenotypes. Finally, IhNSCs produce bona fide oligodendrocytes in fractions up to 20% of total cell number. This is in contrast to the negligible propensity of hNSCs to generate oligodendroglia reported so far. Thus, we describe an immortalized hNSC line endowed with the properties of normal hNSCs and suitable for developing the novel, reliable assays and reproducible high-throughput gene and drug screening that are essential in both diagnostics and cell therapy studies.

Fialkow, P. J., G. B. Faguet, et al. (1981). "Evidence that essential thrombocythemia is a clonal disorder with origin in a multipotent stem cell." Blood **58**(5): 916-9.

Essential thrombocythemia is characterized by proliferation of hematopoietic tissue predominantly involving megakaryocytes and resulting in marked thrombocytosis. The disorder has some clinical and laboratory features that resemble those seen in the clonal multipotent stem cell disorders chronic myelogenous leukemia, polycythemia vera, and agnogenic myeloid metaplasia. It has been argued that essential thrombocythemia should be classified together with those disorders as a myeloproliferative syndrome. However, without knowledge of the numbers and types of cells that are involved in essential thrombocythemia, this suggestion remains speculative. Three patients with thrombocytosis were studied. The diagnosis of essential thrombocythemia was considered to be firm in two patients and probable in the third one. The X-linked glucose-6-phosphate dehydrogenase locus was used as a cell marker. Whereas both A and B types of glucose-6-phosphate dehydrogenase were found in nonhematopoietic tissues, only a single-enzyme type was found in the granulocytes, red cells, and platelets from each patient. These data indicate that the disorders in these three patients are clonal and involve multipotent stem cells.

Fialkow, P. J., R. J. Jacobson, et al. (1980). "Philadelphia chromosome (Ph1)-negative chronic myelogenous leukemia (CML): a clonal disease with origin in a multipotent stem cell." Blood **56**(1): 70-3.

It has been shown with glucose 6-phosphate dehydrogenase (G-6-PD) mosaicism that Ph1-positive chronic myelogenous leukemia (CML) is a clonal disease that involves multipotent hematopoietic stem cells. We now report G-6-PD studies of a 79-yr-old woman with Ph1-negative CML. Equal amounts of B and A-type activities were found in nonhematopoietic tissues, indicating that the patient was heterozygous for G-6-PD. In contrast, only A-type G-6-PD was found in marrow cells, blood erythrocytes, leukocytes, and platelets and in granulocyte-monocyte and eosinophil colonies grown from blood mononuclear cells. Unlike most cases of PH1-positive CML, colony growth in this patient increased during blastic

transformation and the colonies contained only immature monocytic cells. The data indicate that in this patient, Ph1-negative CML is similar to the Ph1-positive form of the disease in involvement of multipotent stem cells and probable clonal origin, but the two disorders differ in the rapidity with which they enter blastic transformation and in the pattern of granulocyte-monocyte colony growth at that time.

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

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

Fujita, S. (2003). "The discovery of the matrix cell, the identification of the multipotent neural stem cell and the development of the central nervous system." Cell Struct Funct **28**(4): 205-28.

In the early 1960s I applied 3H-thymidine autoradiography to the study of the cells constituting the neural tube, and found that its wall was composed solely of one kind of single-layered epithelial cell, which perform an elevator movement between the mitotic and DNA-synthetic zones in the wall in accord with the cell cycle. They were identified as multipotent stem cells of the central nervous sytem (CNS) to which I gave the name of matrix cells. (3)H-thymidine autoradiography also revealed the chronology of development of these matrix cells: At first they proliferate only to expand the population (stage I), then switch to differentiate specific neuroblasts in given sequences (stage II), and finally change themselves into ependymoglioblasts, common progenitors of ependymal cells and neuroglia (stage III). Based on these findings, I proposed a monophyletic view of cytogenesis of the central nervous sytem. This matrix cell theory claiming the existence of multipotent stem cells has long been the target of severe criticism and not been accepted among neuro-embryologists for a long time. Recent findings by experimental and clinical neuroscientists on the importance of stem cells have renewed interest in the nature and biology of the multipotent neural stem cells. The present paper describes how the concept of the matrix cell (multipotent neural stem cells in vivo) emerged and what has come out from this view over the last 45 years, and how the basic concept of the matrix cell theory has recently been reconfirmed after a long period of controversy and neglect.

Gritti, A., P. Frolichsthal-Schoeller, et al. (1999). "Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain." J Neurosci **19**(9): 3287-97.

The subventricular zone (SVZ) of the adult mammalian forebrain contains kinetically distinct precursor populations that contribute new neurons to the olfactory bulb. Because among forebrain precursors there are stem-like cells that can be cultured in the presence of mitogens such as epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2), we asked whether distinct subsets of stem-like cells coexist within the SVZ or whether the proliferation of a single type of SVZ stem-like cell is controlled by several GFs. We show that the latter is the case. Thus cells isolated from the SVZ coexpress the EGF and FGF receptors; by quantitative analysis, the number of stem-like cells isolated from the SVZ by either FGF2 or EGF is the same, whereas no additive effect occurs when these factors are used together. Furthermore, short-term administration of high-dose [3H]thymidine in vivo depletes both the EGF- and FGF2-responsive stem-like cell populations equally, showing they possess closely similar proliferation kinetics and likely belong to the constitutively proliferating SVZ compartment. By subcloning and population analysis, we demonstrate that responsiveness to more than one GF endows SVZ cells with an essential stem cell feature, the ability to vary self-renewal, that was until now undocumented in CNS stem-like cells. The multipotent stem cell-like population that expands slowly in the presence of FGF2 in culture switches to a faster growth mode when exposed to EGF alone and expands even faster when exposed to both GFs together. Analogous responses are observed when the GFs are used in the reverse order, and furthermore, these growth rate modifications are fully reversible.

Han, X. D., S. W. Chung, et al. (1995). "Identification of a unique membrane-bound molecule on a hemopoietic stem cell line and on multipotent progenitor cells." Proc Natl Acad Sci U S A **92**(24): 11014-8.

Hemopoietic stem cells are a distinct population of cells that can differentiate into multilineages of hemopoietic cells and have long-term repopulation capability. A few membrane-bound molecules have been found to be preferentially, but not uniquely, present on the surface of these primitive cells. We report here the identification of a unique 105-kDa glycoprotein on the surface of hemopoietic stem cell line BL3. This molecule, recognized by the absorbed antiserum, is not present on the surface of myeloid progenitors 32D and FDC-P1 cells, EL4 T cells, and NIH 3T3 fibroblasts. This antiserum can also be used to block the proliferation of BL3 cells even in the presence of mitogen-stimulated spleen cell conditioned medium, which is known to have a stimulating activity on BL3 cells. It can also inhibit development of in vitro, fetal liver cell-derived multilineage colonies, but not other types of colonies, and of in vivo bone marrow cell-derived colony-forming unit spleen foci. These data suggest that gp105 plays an important role in hemopoietic stem cell differentiation.

Holstein, T. W. and C. N. David (1990). "Putative intermediates in the nerve cell differentiation pathway in hydra have properties of multipotent stem cells." Dev Biol **142**(2): 401-5.

We have investigated the properties of nerve cell precursors in hydra by analyzing the differentiation and proliferation capacity of interstitial cells in the peduncle of Hydra oligactis, which is a region of active nerve cell differentiation. Our results indicate that about 50% of the interstitial cells in the peduncle can grow rapidly and also give rise to nematocyte precursors when transplanted into a gastric environment. If these cells were committed nerve cell precursors, one would not expect them to differentiate into nematocytes nor to proliferate apparently without limit. Therefore we conclude that cycling interstitial cells in peduncles are not intermediates in the nerve cell differentiation pathway but are stem cells. The remaining interstitial cells in the peduncle are in G1 and have the properties of committed nerve cell precursors (Holstein and David, 1986). Thus, the interstitial cell population in the peduncle contains both stem cells and noncycling nerve precursors. The presence of stem cells in this region makes it likely that these cells are the immediate targets of signals which give rise to nerve cells.

Izadyar, F., F. Pau, et al. (2008). "Generation of multipotent cell lines from a distinct population of male germ line stem cells." Reproduction **135**(6): 771-84.

Spermatogonial stem cells (SSCs) maintain spermatogenesis by self-renewal and generation of spermatogonia committed to differentiation. Under certain in vitro conditions, SSCs from both neonatal and adult mouse testis can reportedly generate multipotent germ cell (mGC) lines that have characteristics and differentiation potential similar to embryonic stem (ES) cells. However, mGCs generated in different laboratories showed different germ cell characteristics, i.e., some retain their SSC properties and some have lost them completely. This raises an important question: whether mGC lines have been generated from different subpopulations in the mouse testes. To unambiguously identify and track germ line stem cells, we utilized a transgenic mouse model expressing green fluorescence protein under the control of a germ cell-specific Pou5f1 (Oct4) promoter. We found two distinct populations among the germ line stem cells with regard to their expression of transcription factor Pou5f1 and c-Kit receptor. Only the POU5F1+/c-Kit+ subset of mouse germ line stem cells, when isolated from either neonatal or adult testes and cultured in a complex mixture of growth factors, generates cell lines that express pluripotent ES markers, i.e., Pou5f1, Nanog, Sox2, Rex1, Dppa5, SSEA-1, and alkaline phosphatase, exhibit high telomerase activity, and differentiate into multiple lineages, including beating cardiomyocytes, neural cells, and chondrocytes. These data clearly show the existence of two distinct populations within germ line stem cells: one destined to become SSC and the other with the ability to generate multipotent cell lines with some pluripotent characteristics. These findings raise interesting questions about the relativity of pluripotency and the plasticity of germ line stem cells.

Janssen, J. W., M. Buschle, et al. (1989). "Clonal analysis of myelodysplastic syndromes: evidence of multipotent stem cell origin." Blood **73**(1): 248-54.

Restriction fragment length polymorphisms (RFLPs) of the X-chromosome genes hypoxanthine phosphoribosyl transferase (HPRT) and phosphoglycerate kinase (PGK) were studied in 34 female patients with primary myelodysplastic syndromes (MDS). Twelve patients (35%) were heterozygous at the HPRT or PGK loci for BamHI or BglI RFLPs, respectively. In eight patients showing PGK polymorphisms, clonality was determined by X-chromosome inactivation analysis. These included patients from different morphologic subtypes: four with refractory anemia (RA), two with RA and ring

sideroblasts (RARS), one patient with RA with excess of blasts (RAEB), and one with chronic myelomonocytic leukemia (CMML). A monoclonal pattern of X-chromosome inactivation was observed in seven cases. In a further case characterized by bone marrow hypoplasia, peripheral blood (PB) leukocytes were polyclonal in origin. Following low-dose cytarabine therapy, reversion to polyclonal hematopoiesis was observed in a case of RAEB indicating the presence of residual normal hematopoietic stem cells with the capacity for marrow reconstitution. The clonal relation of lymphoid and granulocyte/monocyte lineages was studied directly in two cases of CMML exhibiting somatic mutations of N-ras or Ki-ras oncogenes. By selective oligonucleotide hybridization to ras gene sequences amplified in vitro by the polymerase chain reaction, a mutated ras allele was demonstrated in PB granulocytes, monocytes, and B and T lymphocytes of both patients. We conclude that MDS arise from a multipotent hematopoietic stem cell with the potential for myeloid and lymphoid differentiation.

Jensen, K. B., C. A. Collins, et al. (2009). "Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis." Cell Stem Cell **4**(5): 427-39.

Lrig1 is a marker of human interfollicular epidermal stem cells and helps maintain stem cell quiescence. We show that, in mouse epidermis, Lrig1 defines the hair follicle junctional zone adjacent to the sebaceous glands and infundibulum. Lrig1 is a Myc target gene; loss of Lrig1 increases the proliferative capacity of stem cells in culture and results in epidermal hyperproliferation in vivo. Lrig1-expressing cells can give rise to all of the adult epidermal lineages in skin reconstitution assays. However, during homeostasis and on retinoic acid stimulation, they are bipotent, contributing to the sebaceous gland and interfollicular epidermis. beta-catenin activation increases the size of the junctional zone compartment, and loss of Lrig1 causes a selective increase in beta-catenin-induced ectopic hair follicle formation in the interfollicular epidermis. Our results suggest that Lrig1-positive cells constitute a previously unidentified reservoir of adult mouse interfollicular epidermal stem cells.

Kawase, Y., Y. Yanagi, 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.

Kim, S. W., H. Han, et al. (2006). "Successful stem cell therapy using umbilical cord blood-derived multipotent stem cells for Buerger's disease and ischemic limb disease animal model." Stem Cells **24**(6): 1620-6.

Buerger's disease, also known as thromboangiitis obliterans, is a nonatherosclerotic, inflammatory, vasoocclusive disease. It is characterized pathologically as a panangiitis of medium and small blood vessels, including both arteries and adjacent veins, especially the distal extremities (the feet and the hands). There is no curative medication or surgery for this disease. In the present study, we transplanted human leukocyte antigen-matched human umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) into four men with Buerger's disease who had already received medical treatment and surgical therapies. After the stem cell transplantation, ischemic rest pain suddenly disappeared from their affected extremities. The necrotic skin lesions were healed within 4 weeks. In the follow-up angiography, digital capillaries were increased in number and size. In addition, vascular resistance in the affected extremities, compared with the preoperative examination, was markedly decreased due to improvement of the peripheral circulation. Because an animal model of Buerger's disease is absent and also to understand human results, we transplanted human UCB-derived MSCs to athymic nude mice with hind limb ischemia by femoral artery ligation. Up to 60% of the hind limbs were salvaged in the femoral artery-ligated animals. By in situ hybridization, the human UCB-derived MSCs were detected in the arterial walls of the ischemic hind limb in the treated group. Therefore, it is suggested that human UCB-derived MSC transplantation may be a

new and useful therapeutic armament for Buerger's disease and similar ischemic diseases.

Laywell, E. D., P. Rakic, et al. (2000). "Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain." Proc Natl Acad Sci U S A **97**(25): 13883-8.

The mammalian brain contains a population of neural stem cells (NSC) that can both self-renew and generate progeny along the three lineage pathways of the central nervous system (CNS), but the in vivo identification and localization of NSC in the postnatal CNS has proved elusive. Recently, separate studies have implicated ciliated ependymal (CE) cells, and special subependymal zone (SEZ) astrocytes as candidates for NSC in the adult brain. In the present study, we have examined the potential of these two NSC candidates to form multipotent spherical clones-neurospheres-in vitro. We conclude that CE cells are unipotent and give rise only to cells within the glia cell lineage, although they are capable of forming spherical clones when cultured in isolation. In contrast, astrocyte monolayers from the cerebral cortex, cerebellum, spinal cord, and SEZ can form neurospheres that give rise both to neurons and glia. However, the ability to form neurospheres is restricted to astrocyte monolayers derived during the first 2 postnatal wk, except for SEZ astrocytes, which retain this capacity in the mature forebrain. We conclude that environmental factors, simulated by certain in vitro conditions, transiently confer NSC-like attributes on astrocytes during a critical period in CNS development.

Matulka, L. A., A. A. Triplett, et al. (2007). "Parity-induced mammary epithelial cells are multipotent and express cell surface markers associated with stem cells." Dev Biol **303**(1): 29-44.

Parity-induced mammary epithelial cells (PI-MECs) are defined as a pregnancy hormone-responsive cell population that activates the promoter of late milk protein genes during the second half of pregnancy and lactation. However, unlike their terminally differentiated counterparts, these cells do not undergo programmed cell death during post-lactational remodeling of the gland. We previously demonstrated that upon transplantation into an epithelial-free mammary fat pad, PI-MECs exhibited two important features of multipotent mammary epithelial progenitors: a) self-renewal, and b) contribution to ductal and alveolar morphogenesis. In this new report, we introduce a new method to viably label PI-MECs. Using this methodology, we analyzed the requirement of ovarian hormones for the maintenance of this epithelial subtype in the involuted mammary gland. Furthermore, we examined the expression of putative stem cell markers and found that a portion of GFP-labeled PI-MECs were part of the CD24(+)/CD49f(high) mammary epithelial subtype, which has recently been suggested to contain multipotent stem cells. Subsequently, we demonstrated that isolated PI-MECs were able to form mammospheres in culture, and upon transplantation, these purified epithelial cells were capable of establishing a fully functional mammary gland. These observations suggest that PI-MECs contain multipotent progenitors that are able to self renew and generate diverse epithelial lineages present in the murine mammary gland.

Motohashi, T., H. Aoki, et al. (2007). "Multipotent cell fate of neural crest-like cells derived from embryonic stem cells." Stem Cells **25**(2): 402-10.

Neural crest cells migrate throughout the embryo and differentiate into diverse derivatives: the peripheral neurons, cranial mesenchymal cells, and melanocytes. Because the neural crest cells have critical roles in organogenesis, detailed elucidation of neural crest cell differentiation is important in developmental biology. We recently reported that melanocytes could be induced from mouse ESCs. Here, we improved the culture system and showed the existence of neural crest-like precursors. The addition of retinoic acid to the culture medium reduced the hematopoiesis and promoted the expression of the neural crest marker genes. The colonies formed contained neural crest cell derivatives: neurons and glial cells, together with melanocytes. This suggested that neural crest-like cells assuming multiple cell fates had been generated in these present cultures. To isolate the neural crest-like cells, we analyzed the expression of c-Kit, a cell-surface protein expressed in the early stage of neural crest cells in vivo. The c-Kit-positive (c-Kit(+)) cells appeared as early as day 9 of the culture period and expressed the transcriptional factors Sox10 and Snail, which are expressed in neural crest cells. When the c-Kit(+) cells were separated from the cultures and recultured, they frequently formed colonies containing neurons, glial cells, and melanocytes. Even a single c-Kit(+) cell formed colonies that contained these three cell types, confirming their multipotential cell fate. The c-Kit(+) cells were also capable of migrating along neural crest migratory pathways in vivo. These results indicate that the c-Kit(+) cells isolated from melanocyte-differentiating cultures of ESCs are closely related to neural crest cells.

Muller, I., S. Kordowich, et al. (2008). "Application of multipotent mesenchymal stromal cells in pediatric patients following allogeneic stem cell transplantation." Blood Cells Mol Dis **40**(1): 25-32.

Multipotent mesenchymal stromal cells (MSC) have immunomodulatory effects. The aim of this study was to demonstrate safety and feasibility of MSC transfusion in pediatric patients who had undergone allogeneic stem cell transplantation from MMFD, MUD, MMUD and MSD. Patients with posttransplant complications based on deregulated immune effector cells who may benefit from an immunomodulatory effect of MSC had been selected. MSC were isolated from the hematopoietic stem cell donors in five cases and from a third party parental donor in two cases. We transfused ex vivo-expanded MSC in 11 doses into seven pediatric patients. Cell doses were escalated based on availability from 0.4x10(6) to 3.0x10(6) per kg bodyweight No adverse effects were detected with a maximum follow-up of 29 months. One out of three patients showed slight improvement of chronic GVHD. Two patients with severe acute GvHD did not progress to cGvHD. One patient received MSC to stabilize graft function after secondary haploidentical transplantation. One patient recovered from trilineage failure due to severe hemophagocytosis. This is the first case of a pediatric patient treated with MSC for trilineage failure after haploidentical stem cell transplantation from her father. We report the first series of 11 transfusions of expanded MSC in pediatric patients with immunological complications after allogeneic transplantation. Transfusion of MSC was safe and encouraging improvements in some patients were observed.

Nilsson, T., L. Nilsson, et al. (2004). "MDS/AML-associated cytogenetic abnormalities in multiple myeloma and monoclonal gammopathy of undetermined significance: evidence for frequent de novo occurrence and multipotent stem cell involvement of del(20q)." Genes Chromosomes Cancer **41**(3): 223-31.

Multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) are characterized cytogenetically by 14q32 rearrangements, -13/13q-, and various trisomies. Occasionally, karyotypic patterns characteristic of myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) occur in MM, often signifying therapy-related (t)-MDS/t-AML. Comparison of cytogenetic features in all published MMs (n = 993) and t-MDS/t-AML post-MM (n = 117) revealed significant differences in complexity and ploidy levels and in most genomic changes. Thus, these features often can be used to distinguish between MM and t-MDS/t-AML. Rarely, myeloid-associated aberrations are detected in MM without any signs of MDS/AML. To characterize such abnormalities in MM/MGUS, we ascertained all 122 MM and 26 MGUS/smoldering MM (SMM) cases analyzed in our department. Sixty-six (54%) MMs and 8 (31%) MGUS/SMMs were karyotypically abnormal, of which 6 (9%) MMs and 3 (38%) MGUS/SMMs displayed myeloid abnormalities, that is, +8 (1 case) and 20q- (8 cases) as the sole anomalies, without any evidence of MDS/AML. One patient developed AML, whereas no MDS/AML occurred in the remaining 8 patients. In one MGUS with del(20q), fluorescence in situ hybridization analyses revealed its presence in CD34+CD38- (hematopoietic stem cells), CD34+CD38+ (progenitors), CD19+ (B cells), and CD15+ (myeloid cells). The present data indicate that 20q- occurs in 10% of karyotypically abnormal MM/MGUS cases and that it might arise at a multipotent progenitor/stem cell level.

Ohlstein, B. and A. Spradling (2007). "Multipotent Drosophila intestinal stem cells specify daughter cell fates by differential notch signaling." Science **315**(5814): 988-92.

The adult Drosophila midgut contains multipotent intestinal stem cells (ISCs) scattered along its basement membrane that have been shown by lineage analysis to generate both enterocytes and enteroendocrine cells. ISCs containing high levels of cytoplasmic Delta-rich vesicles activate the canonical Notch pathway and down-regulate Delta within their daughters, a process that programs these daughters to become enterocytes. ISCs that express little vesiculate Delta, or are genetically impaired in Notch signaling, specify their daughters to become enteroendocrine cells. Thus, ISCs control daughter cell fate by modulating Notch signaling over time. Our studies suggest that ISCs actively coordinate cell production with local tissue requirements by this mechanism.

Pierce, A., E. Spooncer, et al. (2002). "BCR-ABL alters the proliferation and differentiation response of multipotent hematopoietic cells to stem cell factor." Oncogene **21**(19): 3068-75.

Chronic myeloid leukaemia (CML), a hematopoietic stem cell disorder is characterized by the expression of BCR-ABL. To investigate the effects of BCR-ABL on multipotent hematopoietic cells, a temperature sensitive BCR-ABL tyrosine kinase was expressed in the cell line, FDCP-Mix. BCR-ABL mediated an increase in c-kit expression that correlated with an enhanced mitogenic response to SCF. This was not observed in the absence of Bcr-Abl kinase activity or presence of the BCR-ABL inhibitor STI571, which also inhibits c-kit. When cultured in a combination of SCF plus G-CSF the FDCP-Mix cells undergo neutrophilic differentiation over a 7-10 day period. When BCR-ABL was active there was a marked inhibition of cell maturation

compared to control cells in which BCR-ABL was either inactive or not present. However, BCR-ABL did not block differentiation as the cells eventually undergo terminal maturation. These data argue that BCR-ABL is directly responsible for the enhanced response to SCF reported in CML progenitor cells. Furthermore, although the primary effect of STI571 is via direct inhibition of BCR-ABL, STI571 additionally reduces the enhanced response to SCF. Thus there are two sites of STI571 action of potential importance in Bcr-Abl expressing cells.

Rajasingh, J., E. Lambers, et al. (2008). "Cell-free embryonic stem cell extract-mediated derivation of multipotent stem cells from NIH3T3 fibroblasts for functional and anatomical ischemic tissue repair." Circ Res **102**(11): e107-17.

The oocyte-independent source for the generation of pluripotent stem cells is among the ultimate goals in regenerative medicine. We report that on exposure to mouse embryonic stem cell (mESC) extracts, reversibly permeabilized NIH3T3 cells undergo dedifferentiation followed by stimulus-induced redifferentiation into multiple lineage cell types. Genome-wide expression profiling revealed significant differences between NIH3T3 control and ESC extract-treated NIH3T3 cells including the reactivation of ESC-specific transcripts. Epigenetically, ESC extracts induced CpG demethylation of Oct4 promoter, hyperacetylation of histones 3 and 4, and decreased lysine 9 (K-9) dimethylation of histone 3. In mouse models of surgically induced hindlimb ischemia or acute myocardial infarction transplantation of reprogrammed NIH3T3 cells significantly improved postinjury physiological functions and showed anatomic evidence of engraftment and transdifferentiation into skeletal muscle, endothelial cell, and cardiomyocytes. These data provide evidence for the generation of functional multipotent stem-like cells from terminally differentiated somatic cells without the introduction of retroviral mediated transgenes or ESC fusion.

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

Communication within the hematopoietic-neuroendocrine-immune axis is partly mediated by neurotransmitters (e.g. substance P, SP) and cytokines. SP mediates neuromodulation partly through the stimulation of bone marrow (BM) progenitors. This study shows that SP, through the neurokinin-1 receptor, stimulates the proliferation of primitive hematopoietic progenitors: cobblestone-forming cells (CAFC, CD34+). This effect is optimal when macrophage is included within the fibroblast support. Indirect induction of IL-1 could be important in the proliferation of CAFC colonies by SP. Phenotypic and functional studies suggest that SP might directly interact with the CD34+/CD45(dim) population. These studies indicate that SP can initiate a cascade of biological responses in the BM stroma and stem cells to stimulate hematopoiesis.

Sart, S., Y. J. Schneider, et al. (2009). "Ear mesenchymal stem cells: an efficient adult multipotent cell population fit for rapid and scalable expansion." J Biotechnol **139**(4): 291-9.

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.

Satoh, M., H. Sugino, et al. (2000). "Activin promotes astrocytic differentiation of a multipotent neural stem cell line and an astrocyte progenitor cell line from murine central nervous system." Neurosci Lett **284**(3): 143-6.

The effects of activin A were investigated on the development of a multipotent neural stem cell line (MEB5) and an astrocyte progenitor cell line (AP-16) that were established from murine central nervous system (CNS). Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis demonstrated that each cell line expresses both type I and type II activin receptors and signaling molecules for activin, Smad2, Smad3, and Smad4. Activin A did not affect the proliferation of MEB5 and AP-16 cells. When each cell line was treated alone with activin A, glial fibrillary acidic protein (GFAP), a marker for astrocytes, was induced in AP-16 cells, but not in MEB5 cells. However, activin A accelerated the leukemia inhibitory factor (LIF)-induced astroglial

differentiation of MEB5 cells. These results suggest that activin promotes astrocyte differentiation of CNS neural progenitors, and the competence to activin is different between multipotent stem cells and unipotent astrocyte progenitor cells.

Shinohara, T., N. Takuwa, et al. (1983). "Chronic myelomonocytic leukemia with a chromosome abnormality (46,XY,20q-) in all dividing myeloid cells: evidence for clonal origin in a multipotent stem cell common to granulocyte, monocyte, erythrocyte, and thrombocyte." Am J Hematol **15**(3): 289-93.

In a typical case of chronic myelomonocytic leukemia (CMML), a chromosome abnormality, 46,XY,20q-, was observed in all the dividing cells including up to 16-ploid cells in the bone marrow and the blood. As the mitotic figures could be easily seen not only in myelomonocytoid cells but also in erythroblasts in the bone marrow smear, it was concluded that all the cell lineages except lymphocytes had the abnormality. The present case will support the view that the leukemic process in CMML affects a multipotent stem cell rather than a granulocyte-monocyte committed stem cell.

Sletvold, O. and O. D. Laerum (1988). "Multipotent stem cell (CFU-S) numbers and circadian variations in aging mice." Eur J Haematol **41**(3): 230-6.

The multipotent stem cell (CFU-S) numbers were studied in aging female C3H mice (16, 21 and 26 months old, respectively) versus young controls (3 months old). Using the spleen colony technique, the d-8 CFU-S numbers were measured every 3 h during the 24-h period at three different times of the year. Prominent circadian variations were found in young mice. The peak and trough values were significantly different also in aging mice, although the peak-trough differences were declining. When comparing young and old mice at different times of the 24-h period, the CFU-S numbers were sometimes significantly different, but often not. The 24-h mean values were consistently declining during aging. Young mice had different circadian variation patterns and 24-h mean values when examined at different times of the year. It is concluded that the d-8 CFU-S numbers decline in aging mice. Conflicting reports may partly be due to neglect of physiological variations.

Taghon, T., K. Thys, et al. (2003). "Homeobox gene expression profile in human hematopoietic multipotent stem cells and T-cell progenitors: implications for human T-cell development." Leukemia **17**(6): 1157-63.

Class I homeobox (HOX) genes comprise a large family of transcription factors that have been implicated in normal and malignant hematopoiesis. However, data on their expression or function during T-cell development is limited. Using degenerated RT-PCR and Affymetrix microarray analysis, we analyzed the expression pattern of this gene family in human multipotent stem cells from fetal liver (FL) and adult bone marrow (ABM), and in T-cell progenitors from child thymus. We show that FL and ABM stem cells are similar in terms of HOX gene expression, but significant differences were observed between these two cell types and child thymocytes. As the most immature thymocytes are derived from immigrated FL and ABM stem cells, this indicates a drastic change in HOX gene expression upon entry into the thymus. Further analysis of HOX-A7, HOX-A9, HOX-A10, and HOX-A11 expression with specific RT-PCR in all thymocyte differentiation stages showed a sequential loss of 3' region HOX-A cluster genes during intrathymic T-cell development and an unexpected expression of HOX-A11, previously not recognized to play a role in hematopoiesis. Also HOX-B3 and HOX-C4 were expressed throughout thymocyte development. Overall, these data provide novel evidence for an important role of certain HOX genes in human T-cell development.

Tait, L., P. Dawson, et al. (1996). "Multipotent human breast stem cell line MCF1OAT." Int J Oncol **9**(2): 263-7.

Human breast epithelial MCF10AT cells injected with Matrigel into the subcutis of nude/beige mice form simple ducts which, with time, resemble human proliferative breast disease and sporadically progress to carcinoma. Basement membrane was visualized with a silver stain and myoepithelial cells detected by their reactivity with antibody against smooth muscle actin. The ducts formed in situ are bilayered, composed of both myoepithelial and luminal epithelial layers circumscribed by a distinct basement membrane. The human origins of both luminal and myoepithelial layers were confirmed with fluorescent in situ hybridization with a probe for human chromosome 9. Electron microscopic studies reveal actin bundles and the formation of hemidesmosomes bordering the basement membrane, consistent with myoepithelium, as well as the formation of desmosomes between luminal and myoepithelial cells.

Tavian, M., C. Robin, et al. (2001). "The human embryo, but not its yolk sac, generates lympho-myeloid stem cells: mapping multipotent hematopoietic cell fate in intraembryonic mesoderm." Immunity **15**(3): 487-95.

We have traced emerging hematopoietic cells along human early ontogeny by culturing embryonic tissue rudiments in the presence of stromal cells that

promote myeloid and B cell differentiation, and by assaying T cell potential in the NOD-SCID mouse thymus. Hematogenous potential was present inside the embryo as early as day 19 of development in the absence of detectable CD34+ hematopoietic cells, and spanned both lymphoid and myeloid lineages from day 24 in the splanchnopleural mesoderm and derived aorta where CD34+ progenitors appear at day 27. By contrast, hematopoietic cells arising in the third week yolk sac, as well as their progeny at later stages, were restricted to myelopoiesis and therefore are unlikely to contribute to definitive hematopoiesis in man.

Tomita, Y., K. Matsumura, et al. (2005). "Cardiac neural crest cells contribute to the dormant multipotent stem cell in the mammalian heart." J Cell Biol **170**(7): 1135-46.

Arodent cardiac side population cell fraction formed clonal spheroids in serum-free medium, which expressed nestin, Musashi-1, and multi-drug resistance transporter gene 1, markers of undifferentiated neural precursor cells. These markers were lost following differentiation, and were replaced by the expression of neuron-, glial-, smooth muscle cell-, or cardiomyocyte-specific proteins. Cardiosphere-derived cells transplanted into chick embryos migrated to the truncus arteriosus and cardiac outflow tract and contributed to dorsal root ganglia, spinal nerves, and aortic smooth muscle cells. Lineage studies using double transgenic mice encoding protein 0-Cre/Floxed-EGFP revealed undifferentiated and differentiated neural crest-derived cells in the fetal myocardium. Undifferentiated cells expressed GATA-binding protein 4 and nestin, but not actinin, whereas the differentiated cells were identified as cardiomyocytes. These results suggest that cardiac neural crest-derived cells migrate into the heart, remain there as dormant multipotent stem cells-and under the right conditions-differentiate into cardiomyocytes and typical neural crest-derived cells, including neurons, glia, and smooth muscle.

van de Ven, C., D. Collins, et al. (2007). "The potential of umbilical cord blood multipotent stem cells for nonhematopoietic tissue and cell regeneration." Exp Hematol **35**(12): 1753-65.

Stem cells have been isolated from human embryos, fetal tissue, umbilical cord blood (UCB), and also from "adult" sources. Adult stem cells are found in many tissues of the body and are capable of maintaining, generating, and replacing terminally differentiated cells. A source of pluripotent stem cells has been recently identified in UCB that can also differentiate across tissue lineage boundaries into neural, cardiac, epithelial, hepatocytic, and dermal tissue. Thus, UCB may provide a future source of stem cells for tissue repair and regeneration. Its widespread availability makes UCB an attractive source for tissue regeneration. UCB-derived stem cells offer multiple advantages over adult stem cells, including their immaturity, which may play a significant role in reduced rejection after transplantation into a mismatched host and their ability to produce larger quantities of homogenous tissue or cells. While research with embryonic stem cells continues to generate considerable controversy, human umbilical stem cells provide an alternative cell source that has been more ethically acceptable and appears to have widespread public support. This review will summarize the in vitro and in vivo studies examining UCB stem cells and their potential use for therapeutic application for nonhematopoietic tissue and cell regeneration.

Voog, J., C. D'Alterio, et al. (2008). "Multipotent somatic stem cells contribute to the stem cell niche in the Drosophila testis." Nature **454**(7208): 1132-6.

Adult stem cells reside in specialized microenvironments, or niches, that have an important role in regulating stem cell behaviour. Therefore, tight control of niche number, size and function is necessary to ensure the proper balance between stem cells and progenitor cells available for tissue homeostasis and wound repair. The stem cell niche in the Drosophila male gonad is located at the tip of the testis where germline and somatic stem cells surround the apical hub, a cluster of approximately 10-15 somatic cells that is required for stem cell self-renewal and maintenance. Here we show that somatic stem cells in the Drosophila testis contribute to both the apical hub and the somatic cyst cell lineage. The Drosophila orthologue of epithelial cadherin (DE-cadherin) is required for somatic stem cell maintenance and, consequently, the apical hub. Furthermore, our data indicate that the transcriptional repressor escargot regulates the ability of somatic cells to assume and/or maintain hub cell identity. These data highlight the dynamic relationship between stem cells and the niche and provide insight into genetic programmes that regulate niche size and function to support normal tissue homeostasis and organ regeneration throughout life.

Zhao, L., G. Li, et al. (2009). "Comparison of multipotent differentiation potentials of murine primary bone marrow stromal cells and mesenchymal stem cell line C3H10T1/2." Calcif Tissue Int **84**(1): 56-64.

Murine C3H10T1/2 cells have many features of mesenchymal stem cells (MSCs). Whether or not the multipotent differentiation capability of C3H10T1/2 cells is comparable to that of primary

bone marrow-derived MSCs (BM-MSCs) was investigated in this study. For in vitro osteogenic differentiation, both BM-MSCs and C3H10T1/2 cells differentiated to osteoblastic cell lineage and showed positive staining for alkaline phosphatase (ALP) and increased mRNA expression of Runx2, Col1alphaI, and osteocalcin. C3H10T1/2 cells and BM-MSCs induced similar amounts of bone formation in the biomaterials. Under chondrogenic induction in the presence of TGF-beta1, cell pellets of both BM-MSCs and C3H10T1/2 cells formed cartilage-like tissues with cartilage matrix components including proteoglycan, type II collagen, and aggrecan. However, C3H10T1/2 cells presented lower adipogenic differentiation potential, with only about 10% C3H10T1/2 cells (but about 70% of BM-MSCs) being committed to adipogenesis. In this study we confirmed that C3H10T1/2 cells coimplanted with osteoconductive scaffolds can form bone spontaneously in vivo and that C3H10T1/2 cells have a basal level of osteocalcin expression, suggesting that they may be a good alternative source of primary BM-MSCs for investigating osteogenic and chondrogenic differentiation in bone or cartilage tissue engineering studies. Caution is needed when using C3H10T1/2 cells for adipogenic studies as they appear to have lower adipogenic potential than BM-MSCs.

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