Isolation and Characterization of Stem Cells 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 isolation and characterization of stem cell.
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Key words: stem cell; life; gene; DNA; protein; isolation; characterization

Introduction

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).

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).

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 labeled with CD45-FITC (1 μg/ml in 1% BSA/PBS) for 30 min (Bhatt, 2003).

3. Ber-EP4/ α_2 and Hoechst labelling for flow cytometry

Isolated epithelial cells are labeled 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 labeled 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).

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).

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 analyzed 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 analyzed for Hoechst and Pyronin Y staining. These cells are then analyzed 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 labeled with either cytokeratin 8 or 14 indirectly conjugated to PE-Cy5. Samples are then dual labeled with Ber-EP4 FITC and integrin α_2 PE-CY7. Flow cytometry is performed as described and analyzed on forward (FSC) and side (SSC) scatter (Bhatt, 2003).

8. Literatures:

Alessandri, G., S. Pagano, et al. (2004). "Isolation and culture of human muscle-derived stem cells able to differentiate into myogenic and neurogenic cell lineages." Lancet **364**(9448): 1872-83.

BACKGROUND: Skeletal-muscle-derived stem cells seem to be a distinct population of immature progenitors of satellite cells, but their functional properties remain unclear, especially in human adult tissue. We investigated their differentiation in samples of skeletal muscle obtained from adults undergoing cardiovascular surgery. METHODS: Samples were obtained from the brachioradialis muscle of 12 patients in whom the radial artery was the conduit for myocardial revascularisation. The stem cells were isolated by a procedure similar to that used for rat gastrocnemius and cultured in medium optimised for growth of neural stem cells. Cytometry was used for phenotypic characterisation and immunocytochemistry and RT-PCR to assess differentiation. Immunohistochemistry was used to examine engraftment of skeletal-muscle-derived stem cells into injured rat spinal cord. FINDINGS: The skeletal-muscle stem cells consisted of two distinct types: one with the typical spindle morphology of satellite cells, the other of rounded cells. Some cultures could be maintained for longer than 6 months. The cells were mainly positive for desmin and to a lesser extent CD105, vimentin, and AC133/CD133, but negative for FLK-1/KDR, CD34, CD31, CD45, von Willebrand factor, Ve-cadherins, and BCL2. After in-vitro differentiation, the cells were able to organise skeletal-muscle fibres and stained positively for striated-muscle actin, smooth-muscle actin, and desmin. Moreover, they differentiated into astrocytes and neurons, as confirmed by positive staining for characteristic proteins. INTERPRETATION: Adult human skeletal muscle includes a population of progenitor stem cells that can generate cells of the same lineage and cells with neurogenic properties. Muscle may therefore be a tissue source for the isolation of pluripotent stem cells for development of cell-based therapies for human myogenic and neurogenic diseases.

Basch, R. S., T. Panagiotatos, et al. (1981). "Demonstration of a hematopoietic stem cell antigen (SC-1) on a murine lymphoma and isolation of variants lacking the antigen." J Cell Physiol **107**(3): 379-84.

Murine multipotential hematopoietic stem cells (CFU-s) bear an antigen (SC-1) which is recognized by heterologous antisera to mouse brain. We have found that cloned Thy-1 negative variants of the T-cell lymphoma RL male 1 are sensitive to complement-mediated cytolysis by anti-brain serum and can absorb the anti-stem cell activity from the antiserum. We have isolated several subclones derived from a primary Thy-1 negative variant which are not susceptible to anti-brain serum. The surface of the resistant lines has little or no antigen capable of binding anti-mouse brain antibodies as measured by either immunofluorescence or a radioimmunoassay. These lines are also unable to absorb the antibodies responsible for the cytotoxic effect of rabbit anti-mouse brain serum against CFU-s. We conclude that

the predominant antigen, serologically detectable on Thy-1 negative variants of RL male 1, is SC-1.

Battula, V. L., S. Treml, et al. (2009). "Isolation of functionally distinct mesenchymal stem cell subsets using antibodies against CD56, CD271, and mesenchymal stem cell antigen-1." Haematologica **94**(2): 173-84.

BACKGROUND: Conventionally, mesenchymal stem cells are functionally isolated from primary tissue based on their capacity to adhere to a plastic surface. This isolation procedure is hampered by the unpredictable influence of co-cultured hematopoietic and/or other unrelated cells and/or by the elimination of a late adhering mesenchymal stem cells subset during removal of undesired cells. To circumvent these limitations, several antibodies have been developed to facilitate the prospective isolation of mesenchymal stem cells. Recently, we described a panel of monoclonal antibodies with superior selectivity for mesenchymal stem cells, including the monoclonal antibodies W8B2 against human mesenchymal stem cell antigen-1 (MSCA-1) and 39D5 against a CD56 epitope, which is not expressed on natural killer cells. DESIGN AND METHODS: Bone marrow derived mesenchymal stem cells from healthy donors were analyzed and isolated by flow cytometry using a large panel of antibodies against surface antigens including CD271, MSCA-1, and CD56. The growth of mesenchymal stem cells was monitored by colony formation unit fibroblast (CFU-F) assays. The differentiation of mesenchymal stem cells into defined lineages was induced by culture in appropriate media and verified by immunostaining. RESULTS: Multicolor cell sorting and CFU-F assays showed that mesenchymal stem cells were approximately 90-fold enriched in the MSCA-1(+)CD56(-) fraction and approximately 180-fold in the MSCA-1(+)CD56(+) fraction. Phenotype analysis revealed that the expression of CD10, CD26, CD106, and CD146 was restricted to the MSCA-1(+)CD56(-) mesenchymal stem cells subset and CD166 to MSCA-1(+)CD56(+/-) mesenchymal stem cells. Further differentiation of these subsets showed that chondrocytes and pancreatic-like islets were predominantly derived from MSCA-1(+)CD56(+/-) cells whereas adipocytes emerged exclusively from MSCA-1(+)CD56(-) cells. The culture of single sorted MSCA-1(+)CD56(+) cells resulted in the appearance of phenotypically heterogeneous clones with distinct proliferation and differentiation capacities. CONCLUSIONS: Novel mesenchymal stem cells subsets with distinct phenotypic and functional properties were identified. Our data suggest that the MSCA-1(+)CD56(+) subset is an attractive starting population for autologous chondrocyte transplantation.

Baum, C. M., I. L. Weissman, et al. (1992). "Isolation of a candidate human hematopoietic stem-cell population." Proc Natl Acad Sci U S A **89**(7): 2804-8.

We have identified a rare (0.05-0.1%) subset of human fetal bone marrow cells that contains multipotent hematopoietic precursors. The population of human precursor cells that express Thy-1 and CD34 but no known lineage markers is enriched for clonogenic activity that establishes long-term, multilineage (myelomonocytic and B lymphoid) cultures on mouse marrow stromal lines. Further, the Thy-1+CD34+ subset that takes up little of the fluorescent mitochondrial dye rhodamine 123 contains virtually all the cells that establish long-term cultures. In human fetal thymus transplanted into SCID (severe combined immunodeficiency) mice, Thy-1+CD34+ fetal bone marrow cells differentiate into T lymphocytes. In two of nine cases, allogeneic Thy-1+CD34+ cells could engraft intact human fetal bone marrow grown in SCID mice, resulting in donor-derived myeloid and B cells. By extrapolation, the rare human Thy-1+Lin-CD34+ cell population contains pluripotent hematopoietic progenitors; we propose that it is highly enriched for candidate hematopoietic stem cells.

Baumert, B., K. Grymula, et al. (2008). "An optimization of hematopoietic stem and progenitor cell isolation for scientific and clinical purposes by the application of a new parameter determining the hematopoietic graft efficacy." Folia Histochem Cytobiol **46**(3): 299-305.

The transplantation of hematopoietic stem and progenitor cells (HSPC) is an established lifesaving therapy. Bone marrow (BM), harvested from heparinized cadaveric organ donors, peripheral blood (PB) and cord blood (CB), are important sources of hematopoietic stem cells. HSPCs, which are used for transplantation purposes, are routinely evaluated in terms of number of mononuclear cells (MNCs), CD34+ MNCs count and viability. The efficacy of grafting is determined additionally in clonogenic tests in vitro. These tests deliver important information about the number of HSPCs and their proliferative potential. Unfortunately, they do not give a possibility to evaluate the functional HSPC chemotactic reactivity in the SDF-1 gradient, which is probably the key phenomenon for HSPC homing after transplantation procedure. Thus, the aim of our study was to optimize HSPC isolation according to their chemotactic reactivity in SDF-1 gradient. Using multiparameter cell sorter (FACS Aria, BD) we examined the HSPCs attracted by SDF-1 on a single

cell level. The population of cells which participated in the chemotactic process was highly enriched in CXCR4+lin-AC133+CD45+ cells (referred as hematopoietic stem cells) and to our surprise in CXCR4+lin-AC133+CD45- cells (referred as pluripotent stem cells) in quantitative amounts. Since reactivity of HSPCs may depend on various factors involved in the protocol of their isolation and short-term storage, we tested the most commonly used anticoagulants (ACD, CPDA-1, EDTA and Heparin) and culture media (DME, IMDM, RPMI). HSPCs, harvested from CB, PB and BM, were subsequently investigated for clonogenic growth of CFU-GM in methylcellulose cultures and for the level of apoptosis by employing annexin V staining. Evaluating clonogenic potential, ability of chemotactic reactivity in SDF-1 gradient and intensification of apoptosis of HSPC as the most safe anticoagulant and medium were selected. This study has proved that chemotactic reactivity of HSPCs is a new but very important parameter which should be included in the procedure of their isolation.

Bhatt, R. I., M. D. Brown, et al. (2003). "Novel method for the isolation and characterisation of the putative prostatic stem cell." Cytometry A **54**(2): 89-99.

BACKGROUND: Prostate stem cells, responsible for the development, maturation, and function of the prostate, have been implicated in the aetiology of both benign prostate hyperplasia (BPH) and prostate cancer (CaP). However, research has been hampered by the lack of a definitive stem cell marker. We have adapted the protocol for differential Hoechst 33342 uptake by hemopoietic stem cells to enable isolation of putative stem cells from the prostate. METHODS: Prostate epithelial cells isolated from prostate tissue obtained from patients with BPH after transurethral resection of the prostate were stained with Hoechst 33342. The Hoechst 33342 Red/Blue flow cytometry profile was then determined. Hoechst 33342 and Pyronin Y staining was used to determined the cell cycle status. RESULTS: A verapamil-sensitive side population (SP) can be isolated from primary prostate tissue accounting for 1.38% +/- 0.07% of prostate epithelial cells. Cell cycle analysis of this SP population revealed that the majority of SP cells are in either G0 (12.38 +/- 0.31%) or G1 (63.19 +/- 2.13%). CONCLUSIONS: The Hoechst 33342 dye efflux protocol can be adapted for the isolation of a SP from primary prostate tissue.

Boquest, A. C., A. Shahdadfar, et al. (2005). "Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after in vitro cell culture." Mol Biol Cell **16**(3): 1131-41.

Stromal stem cells proliferate in vitro and may be differentiated along several lineages. Freshly isolated, these cells have been too few or insufficiently pure to be thoroughly characterized. Here, we have isolated two populations of CD45-CD34+CD105+ cells from human adipose tissue which could be separated based on expression of CD31. Compared with CD31+ cells, CD31- cells overexpressed transcripts associated with cell cycle quiescence and stemness, and transcripts involved in the biology of cartilage, bone, fat, muscle, and neural tissues. In contrast, CD31+ cells overexpressed transcripts associated with endothelium and the major histocompatibility complex class II complex. Clones of CD31- cells could be expanded in vitro and differentiated into cells with characteristics of bone, fat, and neural-like tissue. On culture, transcripts associated with cell cycle quiescence, stemness, certain cytokines and organ specific genes were down-regulated, whereas transcripts associated with signal transduction, cell adhesion, and cytoskeletal +CD105+CD31- cells from human adipose tissue have stromal stem cell properties which may make them useful for tissue engineering.

Brunt, K. R., S. R. Hall, et al. (2007). "Endothelial progenitor cell and mesenchymal stem cell isolation, characterization, viral transduction." Methods Mol Med **139**: 197-210.

Endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) have emerged as potentially useful substrates for neovascularization and tissue repair and bioengineering. EPCs are a heterogeneous group of endothelial cell precursors originating in the hematopoietic compartment of the bone marrow. MSCs are a rare population of fibroblast-like cells derived from the bone marrow stroma, constituting approximately 0.001-0.01% of the nucleated cells in the marrow. Both cells types have been isolated from the bone marrow. In addition, EPC can be isolated from peripheral blood as well as the spleen, and MSC has also been isolated from peripheral adipose tissue. Several approaches have been used for the isolation of EPC and MSC, including density centrifugation and magnetic bead selection. Phenotypic characterization of both cell types is carried out using immunohistochemical detection and fluorescence-activated cell sorting analysis of cell-surface molecule expression. However, the lack of specific markers for each cell type renders their characterization difficult and ambiguous. In this chapter, we describe the methods that we use routinely for isolation, characterization, and genetic modification of EPC and MSC from

human, rabbit, and mouse peripheral blood and bone marrow.

Busch, C., P. M. Bareiss, et al. (2009). "Isolation of three stem cell lines from human sacrococcygeal teratomas." J Pathol **217**(4): 589-96.

Sacrococcygeal teratomas (SCTs) are benign tumours of the newborn with absolute indication for surgery directly after birth. We recently described the presence of stem cells positive for the stem cell markers nanog and Oct4 in SCTs. Here we report the isolation of three stem cell lines from three different SCTs. Cells were propagated in mesenchymal or in embryonic stem cell medium. Non-clonal homogeneous stem cell lines were obtained after two to three passages and characterized in vitro by immunocytochemistry, RT-PCR, western blot, FACS analysis, and metaphase spreads. The differentiation potential was tested in vitro and in vivo. The isolated cell lines, which we refer to as human sacrococcygeal teratoma stem cells (hSctSCs), express nanog, Oct4 and stella, and are negative for malignancy markers alpha-fetoprotein and carcinoembryonic antigen. They can be induced in vitro to express neuronal, osteogenic, and chondrogenic traits. After grafting in vivo, spontaneous integration into the neural crest of the chick embryo and teratoma formation in the nude mouse were obtained. Our results indicate that SCTs are derived from remnants of the epiblast-derived primitive streak, which in the human embryo normally regresses but forms teratomas in children affected with SCT. The hSctSCs therefore may be comparable to mouse epiblast-derived stem cells (EpiSCs) and share characteristic features with human embryonic stem (hES) cells. Thus, SCT tissue obtained after surgery appears to be a novel source for the generation of human stem cells without the ethical implications associated with hES cells.

Cantoni, N., M. Weisser, et al. (2009). "Infection prevention strategies in a stem cell transplant unit: impact of change of care in isolation practice and routine use of high dose intravenous immunoglobulins on infectious complications and transplant related mortality." Eur J Haematol **83**(2): 130-8.

OBJECTIVE: Nursing in 'live islands' and routine high dose intravenous immunoglobulins after allogeneic hematopoietic stem cell transplantation were abandoned by many teams in view of limited evidence and high costs. METHODS: This retrospective single-center study examines the impact of change from nursing in 'live islands' to care in single rooms (SR) and from high dose to targeted intravenous immunoglobulins (IVIG) on mortality and infection rate of adult patients receiving an allogeneic stem cell or bone marrow transplantation in two steps and three time cohorts (1993-1997, 1997-2000, 2000-2003). RESULTS: Two hundred forty-eight allogeneic hematopoetic stem cell transplantations were performed in 227 patients. Patient characteristics were comparable in the three cohorts for gender, median age, underlying disease, and disease stage, prophylaxis for graft versus host disease (GvHD) and cytomegalovirus constellation. The incidence of infections (78.4%) and infection rates remained stable (rates/1000 days of neutropenia for sepsis 17.61, for pneumonia 6.76). Cumulative incidence of GvHD and transplant-related mortality did not change over time. CONCLUSIONS: Change from nursing in 'live islands' to SR and reduction of high dose to targeted IVIG did not result in increased infection rates or mortality despite an increase in patient age. These results support the current practice.

Cao, S., F. Wang, et al. (2009). "Isolation and culture of primary bovine embryonic stem cell colonies by a novel method." J Exp Zool A Ecol Genet Physiol **311**(5): 368-76.

Authentic bovine embryonic stem (ES) cell lines have not been established despite progress made for more than two decades. Isolation and culture of primary ES cell colonies are the first critical step towards establishment of stable ES cell lines. Here we report a novel method designated as "Separate and Seed" that contributes remarkably to efficient derivation of bovine primary ES-like cell colonies from blastocysts. These primary cultured bovine ES-like cells exhibit morphology typical of ES cells and express pluripotent molecular markers including Oct4, Nanog and alkaline phosphatase. Interestingly, bovine primary ES-like cell colonies distinctively express both stage-specific embryonic antigens 1 and 4 (SSEA1 and SSEA4), unlike mouse and human ES cells. These pluripotent markers may be used for characterization of authentic bovine ES cell lines in later studies. In contrast, whole embryos or inner cell mass (ICM) used for primary culture by conventional methods fails to produce primary bovine ES cell colonies that express all pluripotent stem cell markers shown above. Furthermore, bFGF improves growth and maintained undifferentiated state of bovine ES-like cells for several passages, whereas LIF and ERK inhibitor PD98059 known to promote pluripotency of mouse ES cells are unable to sustain bovine ES-like cells. Although continued efforts are required for improving long-term culture of bovine ES cells, this novel "Separate and Seed" method provides an initial effective step that may eventually lead to derivation of authentic bovine ES cell lines.

Chakrabarti, S., K. E. Collingham, et al. (2000). "Isolation of viruses from stools in stem cell transplant

recipients: a prospective surveillance study." Bone Marrow Transplant **25**(3): 277-82.

We prospectively examined stool specimens for enteric viruses in 75 stem cell transplant recipients (autologous 48, allogeneic 27) to determine the frequency and significance of these infections. Only six patients (8%) had a positive isolate. Five of these were allograft recipients (18%) compared to one autograft recipient (2%) (P = 0.02). Unrelated donor BMT recipients were at the highest risk for a viral isolate (OR = 10.5). Adenovirus was the commonest isolate (four patients). One patient each had an echovirus, enterovirus and small round structured virus identified. No correlation was found between the severity of gastro-intestinal symptoms and detection of a viral pathogen. There was no correlation with GVHD or CMV status. The only risk factor identified for isolation of an enterovirus was allogeneic BMT from an unrelated donor. There was a negative correlation with PBSC grafts. All the patients infected with an enteric virus had concomitant infection with other pathogens, compared to only 18% of uninfected patients (P = 0.001). The non-relapse mortality of the infected patients was 50% and only 7% in the uninfected patients (P = 0.01, OR = 12.5), although the isolated virus was the direct cause of death in one patient only. This study indicates a low rate of enteric virus isolation in recipients of PBSC grafts, both autologous and allogeneic. However, unrelated donor BMT is associated with a higher risk of enteric virus infection and an adverse outcome. Bone Marrow Transplantation (2000) 25, 277-282.

Cocola, C., P. Anastasi, et al. (2009). "Isolation of canine mammary cells with stem cell properties and tumour-initiating potential." Reprod Domest Anim **44 Suppl 2**: 214-7.

Recent data suggest that mammary carcinogenesis may be driven by cancer stem cells (CSCs) derived from mutated adult stem cells, which have acquired aberrant cell self-renewal or by progenitor cells that have acquired the capacity for cell self-renewal. Spontaneous mammary cancers in cats and dogs are important models for the understanding of human breast cancer and may represent alternative species model systems that can significantly contribute to the study of human oncogenesis. With the goal of identifying markers for isolating human breast CSCs, we have generated a canine model system to isolate and characterize normal and CSCs from dog mammary gland. Insight into the hierarchical organization of canine tumours may contribute to the development of universal concepts in oncogenesis by CSCs. Cells with stem cell properties were isolated from normal and tumoural canine breast tissue and propagated as mammospheres and tumourspheres in long-term non-adherent culture conditions. We showed that cells obtained from spheres that display self-renewing properties, have multi-lineage differentiation potential, could generate complex branched tubular structures in vitro and form tumours in NOD/SCID mice. We analysed these cells for the expression of human stem and CSC markers and are currently investigating the tumour-initiating properties of these cells and the hierarchical organization of normal and neoplastic canine mammary tissue.

Conigliaro, A., M. Colletti, et al. (2008). "Isolation and characterization of a murine resident liver stem cell." Cell Death Differ **15**(1): 123-33.

Increasing evidence provides support that mammalian liver contains stem/progenitor cells, but their molecular phenotype, embryological derivation, biology and their role in liver cell turnover and regeneration remain to be further clarified. In this study, we report the isolation, characterization and reproducible establishment in line of a resident liver stem cell (RLSC) with immunophenotype and differentiative potentiality distinct from other previously described liver precursor/stem cells. RLSCs, derived from fetal and neonatal murine livers as well as from immortalized hepatocytic MMH lines and established in lines, are Sca+, CD34-, CD45-, alpha-fetoprotein+ and albumin-. This molecular phenotype suggests a non-hematopoietic origin. RLSC transcriptional profile, defined by microArray technology, highlighted the expression of a broad spectrum of 'plasticity-related genes' and 'developmental genes' suggesting a multi-differentiative potentiality. Indeed, RLSCs spontaneously differentiate into hepatocytes and cholangiocytes and, when cultured in appropriate conditions, into mesenchymal and neuro-ectodermal cell lineages such as osteoblasts/osteocytes, chondrocytes, astrocytes and neural cells. RLSC capability to spontaneously differentiate into hepatocytes, the lack of albumin expression and the broad differentiative potentiality locate them in a pre-hepatoblast/liver precursor cells hierarchical position. In conclusion, RLSCs may provide a useful tool to improve liver stem cell knowledge and to assess new therapeutic approaches for liver diseases.

Cortes, J. L., L. Sanchez, et al. (2008). "Whole-blastocyst culture followed by laser drilling technology enhances the efficiency of inner cell mass isolation and embryonic stem cell derivation from good- and poor-quality mouse embryos: new insights for derivation of human embryonic stem cell lines." Stem Cells Dev **17**(2): 255-67.

The optimization of human embryonic stem (hES) cell line derivation methods is challenging because many worldwide laboratories have neither access to spare human embryos nor ethical approval for using supernumerary human embryos for hES cell derivation purposes. Additionally, studies performed directly on human embryos imply a waste of precious human biological material. In this study, we developed a new strategy based on the combination of whole-blastocyst culture followed by laser drilling destruction of the trophoectoderm for improving the efficiency of inner cell mass (ICM) isolation and ES cell derivation using murine embryos. Embryos were divided into good- and poor-quality embryos. We demonstrate that the efficiency of both ICM isolation and ES cell derivation using this strategy is significantly superior to whole-blastocyst culture or laser drilling technology itself. Regardless of the ICM isolation method, the ES cell establishment depends on a feeder cell growth surface. Importantly, this combined methodology can be successfully applied to poor-quality blastocysts that otherwise would not be suitable for laser drilling itself nor immunosurgery in an attempt to derive ES cell lines due to the inability to distinguish the ICM. The ES cell lines derived by this combined method were characterized and shown to maintain a typical morphology, undifferentiated phenotype, and in vitro and in vivo three germ layer differentiation potential. Finally, all ES cell lines established using either technology acquired an aneuploid karyotype after extended culture periods, suggesting that the method used for ES cell derivation does not seem to influence the karyotype of the ES cells after extended culture. This methodology may open up new avenues for further improvements for the derivation of hES cells, the majority of which are derived from frozen, poor-quality human embryos.

Dadd, G., P. McMinn, et al. (2003). "Protective isolation in hemopoietic stem cell transplants: a review of the literature and single institution experience." J Pediatr Oncol Nurs **20**(6): 293-300.

Princess Margaret Hospital for Children, Perth, Western Australia, is a pediatric bone marrow transplant center. This center has both laminar flow and HEPA- (high-efficiency particulate air-)filtered rooms for children undergoing allogeneic and autologous transplantation. HEPA-filtered rooms on negative pressure are used to nurse oncology children with infectious diseases. Over the winter months of 2001, there was an increased demand for single rooms for children with infectious diseases. Over the same period, a number of transplants were planned. Consequently, to guide practice decisions, a review of the literature and a survey of nursing practice in Australian and North American pediatric oncology units were undertaken. Findings showed that protective isolation measures such as positive-pressure single rooms, low microbial diets, and strict hand washing should be used only for children requiring allogeneic transplants. Use of other isolation measures were found to be of no added value for transplantation. As autologous transplants are increasingly performed in outpatient clinics, these children should not require the same level of protective isolation.

De Coppi, P., G. Bartsch, Jr., et al. (2007). "Isolation of amniotic stem cell lines with potential for therapy." Nat Biotechnol **25**(1): 100-6.

Stem cells capable of differentiating to multiple lineages may be valuable for therapy. We report the isolation of human and rodent amniotic fluid-derived stem (AFS) cells that express embryonic and adult stem cell markers. Undifferentiated AFS cells expand extensively without feeders, double in 36 h and are not tumorigenic. Lines maintained for over 250 population doublings retained long telomeres and a normal karyotype. AFS cells are broadly multipotent. Clonal human lines verified by retroviral marking were induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages. Examples of differentiated cells derived from human AFS cells and displaying specialized functions include neuronal lineage cells secreting the neurotransmitter L-glutamate or expressing G-protein-gated inwardly rectifying potassium channels, hepatic lineage cells producing urea, and osteogenic lineage cells forming tissue-engineered bone.

Dekaney, C. M., J. M. Rodriguez, et al. (2005). "Isolation and characterization of a putative intestinal stem cell fraction from mouse jejunum." Gastroenterology **129**(5): 1567-80.

BACKGROUND & AIMS: Although there have been many recent advances regarding the biology of intestinal stem cells, the field has been hampered significantly by the lack of a method to isolate these cells. Therefore, the aim of this study was to explore the hypothesis that viable intestinal stem cells can be isolated as a side population (SP) by fluorescence-activated cell sorting after staining with the DNA-binding dye Hoechst 33342. METHODS: Preparations of individual cells from either whole mucosa or epithelium of mouse jejunum were stained with Hoechst 33342 and propidium iodide and then sorted using fluorescence-activated cell sorting. Cells were characterized using fluorochrome-labeled antibodies to surface markers, intracellular markers, and annexin V to detect early apoptosis. Total RNA

was isolated from sorted fractions and used for quantitative real-time reverse-transcription polymerase chain reaction to evaluate the expression of cell lineage markers and the intestinal stem-cell marker, Musashi-1. RESULTS: Adult and neonatal jejunum contain a viable population of cells that shows the SP phenotype and is sensitive to verapamil. This population of cells (from both mucosal and epithelial preparations) includes a CD45-negative fraction corresponding to nonhematopoietic cells, which shows minimal expression of surface markers typically found on stem cells from other tissues and of intracellular markers found in mesenchymal cells. Additionally, these cells were enriched for Musashi-1 and beta1-integrin, were cytokeratin positive, and survived in culture for up to 14 days. CONCLUSIONS: The CD45-negative SP fraction, although not pure, represents the successful isolation of a viable population significantly enriched in small intestinal epithelial stem cells.

Dou, J., M. Pan, et al. (2007). "Isolation and identification of cancer stem-like cells from murine melanoma cell lines." Cell Mol Immunol **4**(6): 467-72.

In current study, cancer stem-like cells in the murine melanoma B16F10 cells were investigated. CD phenotypes of the B16F10 cells were analyzed by flow cytometry, and the specific CD phenotype cells from the B16F10 cells were isolated by MACS. Then we used colony formation assay in soft agar media, the cell growth assay in serum-free culture media as well as the tumorigenicity investigation of the specific CD phenotype cells in C57BL/6 mice, respectively, to identify cancer stem-like cells in the B16F10 cells. The results showed that the B16F10 cells could form spherical clones in serum-free culture media, and the rate of clonegenesis of CD133+, CD44+ and CD44+CD133+ cells was higher than that of CD133-, CD44- and CD44+CD133- cells in soft agar media, respectively. The tumorigenic potential of CD133+, CD44+, CD44+CD133+ cells and CD44+CD133+CD24+ cells was stronger than that of CD133-, CD44-, CD44+CD133- cells and CD44+CD133+CD24- cells in mice, respectively. In conclusion, the CD44+CD133+CD24+ cells have some biological properties of cancer stem-like cells or are highly similar to the characteristics of cancer stem cells (CSC). These results provide an important method for identifying cancer stem-like cells in B16F10 cells and for further cancer target therapy.

Dvorakova, J., A. Hruba, et al. (2008). "Isolation and characterization of mesenchymal stem cell population entrapped in bone marrow collection sets." Cell Biol Int **32**(9): 1116-25.

Bone marrow is an important source of mesenchymal stem cells (MSCs), and a promising tool for cytotherapy. MSC utilization is limited by low cell yields obtained under standard isolation protocols. Herein, used bone marrow collection sets were evaluated as a valuable source of MSCs. Adherent cells washed from the collection sets were examined for widely accepted criteria defining MSCs. Significant numbers of cells (median 9million per set in passage 1) with colony-forming activity and high proliferative potential at low seeding densities were obtained. These cells were positive for essential MSC surface molecules (CD90, CD105, CD166, CD44, CD29) and negative for most haematopoietic and endothelial cell markers (CD45, CD34, CD11a, CD235a, HLA-DR, CD144). The cells were capable of differentiation along adipogenic, osteogenic and chondrogenic pathways. Washing out bone marrow collection sets may constitute a highly ethical source of MSCs for research purposes and may be utilized also in clinical applications.

Feng, J., M. van der Zwaag, et al. (2009). "Isolation and characterization of human salivary gland cells for stem cell transplantation to reduce radiation-induced hyposalivation." Radiother Oncol **92**(3): 466-71.

BACKGROUND: Recently, we showed that transplantation of 100-300 c-Kit(+) stem cells isolated from cultured salispheres ameliorates radiation-damage in murine salivary glands. The aim of this study is to optimize and translate these findings from mice to man. METHODS: Mouse and human non-malignant parotid and submandibular salivary gland tissue was collected and enzymatically digested. The remaining cell suspension was cultured according to our salisphere culture method optimized for murine salispheres. Salisphere cells were tested using 3D matrix culturing for their in vitro stem cell characteristics such as the potential to differentiate into tissue specific cell types. Several potential mouse and human salivary gland stem cells were selected using FACS. RESULTS: In human salivary gland, c-Kit(+) cells were only detected in excretory ducts as shown previously in mice. From both human parotid and submandibular gland cell suspensions salispheres could be grown, which when placed in 3D culture developed ductal structures and mucin-expressing acinar-like cells. Moreover, cells dispersed from primary salispheres were able to form secondary spheres in matrigel, a procedure that could be repeated for at least seven passages. Approximately 3000 c-Kit+ cells could be isolated from primary human salispheres per biopsy. CONCLUSION: Human salivary glands contain a similar 'putative' stem cell population as rodents, expressing c-kit and capable of in vitro differentiation and self-renewal. In the future,

these cells may have the potential to reduce radiotherapy-induced salivary gland dysfunction in patients.

Forsyth, N. R. and J. McWhir (2008). "Human embryonic stem cell telomere length impacts directly on clonal progenitor isolation frequency." Rejuvenation Res **11**(1): 5-17.

The pluripotentiality of human embryonic stem cells is expected to yield an abundance of clinically useful cell types. Using physiologic oxygen culture systems, we show that it is possible to isolate highly proliferative clonal progenitor cells from partially differentiated human embryonic stem cells. These progenitors have similar, though not identical, immunophenotypes with a resemblance to bone marrow-derived adherent stem cells. Through telomere length analysis of multiple early senescing clones, we were able to show that the starting telomere length of a human embryonic stem cell line impacts on the proliferative potential of clonally isolated partially differentiated mortal progeny. Proliferative clones undergo growth arrest with telomere lengths consistent with telomere-driven replicative senescence. To bypass this phenomenon, we transduced progenitor cells with ectopic hTERT (the limiting catalytic component of telomerase). This enabled telomerase immortalization without affecting differentiation potential or immunophenotype. In summary we describe the derivation of clonal progenitor cells from human embryonic stem cells and the relevance of parental cell telomere length to the frequency of highly proliferative clone isolation.

Fujimori, Y., K. Izumi, et al. (2009). "Isolation of small-sized human epidermal progenitor/stem cells by Gravity Assisted Cell Sorting (GACS)." J Dermatol Sci **56**(3): 181-7.

BACKGROUND: Small diameter characterizes epidermal progenitor/stem cells. We have developed Gravity Assisted Cell Sorting (GACS) to simply enrich small-sized epidermal progenitor/stem cells. OBJECTIVE: The cells sorted by GACS were characterized by fluorescence-activated cell sorting analysis, and cultured for up to 7 weeks. The cultured cells were then used for reconstruction of skin equivalent. METHODS: GACS was performed on primary cultures (primary cell) and passage 6-7 cultures (cultured cell) of keratinocytes. A keratinocyte suspension was sized into two groups: cells trapped by a 20 microm filter (trapped cells), and cells flowing through both a 20 and 11 microm filter (non-trapped cells). RESULTS: In the primary cell groups, viability of the trapped cells was 62.5+/-7.2% compared to 77.0+/-3.7% for the non-trapped cells. In the cultured cell groups, viability of the trapped cells was 64.3+/-14.9%, compared to the non-trapped cells (93.1+/-2.0%). Flow cytometric analysis showed better discrimination by cell size between trapped and non-trapped cells in culture than in the primary cell suspension. Non-trapped cells contained a larger number of cells with high levels of alpha6 integrin and low levels of CD71 (alpha6 integrin(bri)CD71(dim)), indicating an enriched progenitor/stem cell population. The difference in these markers between the non-trapped and trapped cells was seen in both the primary and cultured cell groups although this difference was more distinct in cultured cells. Culture of both groups showed that cultures originating from the trapped cells senesced after approximately 15 days while the non-trapped keratinocytes grew for up to 40 days. Manufacture of an epidermis/dermal device (artificial skin) showed that non-trapped cells formed a significantly thicker epithelial layer than the trapped cells, demonstrating the enhanced regenerative capability of the smaller diameter, alpha6 integrin(bri)CD71(dim) cells separated by GACS. CONCLUSION: These results indicate that GACS is simple and useful technique to enrich for epidermal progenitor/stem cell populations, and is more efficient when used on cells in culture.

Gallacher, L., B. Murdoch, et al. (2000). "Isolation and characterization of human CD34(-)Lin(-) and CD34(+)Lin(-) hematopoietic stem cells using cell surface markers AC133 and CD7." Blood **95**(9): 2813-20.

Recent evidence indicates that human hematopoietic stem cell properties can be found among cells lacking CD34 and lineage commitment markers (CD34(-)Lin(-)). A major barrier in the further characterization of human CD34(-) stem cells is the inability to detect this population using in vitro assays because these cells only demonstrate hematopoietic activity in vivo. Using cell surface markers AC133 and CD7, subfractions were isolated within CD34(-)CD38(-)Lin(-) and CD34(+)CD38(-)Lin(-) cells derived from human cord blood. Although the majority of CD34(-)CD38(-)Lin(-) cells lack AC133 and express CD7, an extremely rare population of AC133(+)CD7(-) cells was identified at a frequency of 0.2%. Surprisingly, these AC133(+)CD7(-) cells were highly enriched for progenitor activity at a frequency equivalent to purified fractions of CD34(+) stem cells, and they were the only subset among the CD34(-)CD38(-)Lin(-) population capable of giving rise to CD34(+) cells in defined liquid cultures. Human cells were detected in the bone marrow of non-obese/severe combined immunodeficiency (NOD/SCID) mice 8 weeks after transplantation of ex vivo-cultured AC133(+)CD7(-) cells isolated from the CD34(-)CD38(-)Lin(-)

population, whereas 400-fold greater numbers of the AC133(-)CD7(-) subset had no engraftment ability. These studies provide novel insights into the hierarchical relationship of the human stem cell compartment by identifying a rare population of primitive human CD34(-) cells that are detectable after transplantation in vivo, enriched for in vitro clonogenic capacity, and capable of differentiation into CD34(+) cells. (Blood. 2000;95:2813-2820)

Gendall, A. R., A. R. Dunn, et al. (1997). "Isolation and characterization of a leukemia inhibitory factor-independent embryonic stem cell line." Int J Biochem Cell Biol **29**(5): 829-40.

Leukemia inhibitory factor (LIF) is a mammalian cytokine that has a wide range of physiological activities, including the inhibition of differentiation of embryonic stem (ES) cells. We have used insertional mutagenesis in an attempt to isolate molecules that participate in LIF signal transduction via the LIF receptor. Using a robust screen for undifferentiated cells, we have isolated one ES cell line, Poly 27, that does not require exogenous LIF to remain undifferentiated in vitro. We present evidence that Poly 27 is not irreversibly committed to an undifferentiated phenotype, but can differentiate in vitro if cultured in the presence of chemical differentiating agents, while in syngeneic mice Poly 27 cells form tumours which are composed largely of undifferentiated cells. We have characterized the mechanism of factor independence in Poly 27, and shown it to be a result of autocrine LIF production. This LIF production is potentially the result of a mutation in a gene critically involved in regulating LIF production in ES cells.

Gertow, K., S. Przyborski, et al. (2007). "Isolation of human embryonic stem cell-derived teratomas for the assessment of pluripotency." Curr Protoc Stem Cell Biol **Chapter 1**: Unit1B 4.

This unit describes protocols on how to assess the developmental potency of human embryonic stem cells (hESCs) by performing xenografting into immunodeficient mice to induce teratoma formation. hESCs can be injected under the testis capsule, or alternatively into the kidney or subcutaneously. Teratomas that develop from grafted hESCs are surgically removed, fixed in formaldehyde, and paraffin embedded. The tissues in the teratoma are analyzed histologically to determine whether the hESCs are pluripotent and form tissues derived from of all three embryonic germ layers (ectoderm, mesoderm, and endoderm). Teratomas can also be fixed in Bouin's or cryosectioned for analysis, and they can be analyzed by immunohistochemistry for tissue markers. Methods for these procedures are included in this unit.

Gharaibeh, B., A. Lu, et al. (2008). "Isolation of a slowly adhering cell fraction containing stem cells from murine skeletal muscle by the preplate technique." Nat Protoc **3**(9): 1501-9.

This protocol details a procedure, known as the modified preplate technique, which is currently used in our laboratory to isolate muscle cells on the basis of selective adhesion to collagen-coated tissue culture plates. By employing this technique to murine skeletal muscle, we have been able to isolate a rapidly adhering cell (RAC) fraction within the earlier stages of the process, whereas a slowly adhering cell (SAC) fraction containing muscle-derived stem cells is obtained from the later stages of the process. This protocol outlines the methods and materials needed to isolate RAC and SAC populations from murine skeletal muscle. The procedure involves mechanical and enzymatic digestion of skeletal muscle tissue with collagenase XI, dispase and trypsin followed by plating the resultant muscle slurry on collagen type I-coated flasks where the cells adhere at different rates. The entire preplate technique requires 5 d to obtain the final preplate SAC population. Two to three additional days are usually required before this population is properly established. We also detail additional methodologies designed to further enrich the resultant cell population by continuing the modified preplating process on the SAC population. This process is known as replating and requires further time.

Goldschneider, I., D. Metcalf, et al. (1980). "Analysis of rat hemopoietic cells on the fluorescence-activated cell sorter. I. Isolation of pluripotent hemopoietic stem cells and granulocyte-macrophage progenitor cells." J Exp Med **152**(2): 419-37.

A scheme is presented whereby pluripotent hemopoietic stem cells (PHSC) from rat bone marrow can be enriched 320-fold with the aid of the fluorescence- activated cell sorter. This scheme is based on the observations that PHSC are strongly positive for Thy-1 antigen (upper 10th percentile); have light- scattering properties (size distribution) between those of bone marrow lymphocytes and myeloid progenitor cells; and are relatively resistant to cortisone. It is estimated that PHSC may constitute 80 percent of the cells isolated according to these parameters. Candidate PHSC are described at the light and electron microscopic levels. At least two populations of accessory cells appear to influence the number and/or the nature of the hemopoietic colonies that form in the in vivo spleen colony-forming unit assay. Putative amplifier cells are strongly Thy-1(+) and cortisone sensitive; putative suppressor cells are

weakly Thy-1(+) and cortisone resistant. Three subsets of granulocyte (G) -macrophage (M) progenitor cells (in vitro colony-forming cells [CFC]) are identified on the basis of relative fluorescence intensity for Thy-1 antigen: G-CFC are strongly Thy-l(+); M-CFC are weakly Thy-l(+); and cells that produce mixed G and M CFC have intermediate levels of Thy-1. GM-cluster-forming cells and mature G and M are Thy-1(-). The results suggest that G-CFC are bipotential cells that give rise to G and M-CFC; and that the latter produce mature M through a cluster-forming cell intermediate. Thy-1 antigen is also demonstrated on members of the eosinophil, megakaryocyte, erythrocyte, and lymphocyte cell series in rat bone marrow. In each instance, the relative concentration of Thy-1 antigen is inversely related to the state of cellular differentiation.

Grabel, L. B. (1984). "Isolation of a putative cell adhesion mediating lectin from teratocarcinoma stem cells and its possible role in differentiation." Cell Differ **15**(2-4): 121-4.

We have identified a cell surface teratocarcinoma stem cell lectin with a fucan/mannan specificity by the use of an erythrocyte rosetting assay as well as hemagglutination assay. We have also described experiments that suggest that the lectin is involved in mediating divalent cation-independent adhesion of the stem cells. This molecule has been purified from stem cell conditioned medium and identified as a polypeptide of 56 000 apparent molecular weight. An antibody has been raised to this 56K polypeptide (using material eluted from an SDS-polyacrylamide gel as the immunogen) and its specificity determined by protein blot analysis. In addition, we have recently observed that only carbohydrates recognized by the lectin interfere with in vitro embryoid body formation by the stem cells, suggesting that the lectin may be involved in differentiation.

Grisanti, L., I. Falciatori, et al. (2009). "Identification of spermatogonial stem cell subsets by morphological analysis and prospective isolation." Stem Cells **27**(12): 3043-52.

Spermatogenesis is maintained by a pool of spermatogonial stem cells (SSCs). Analyses of the molecular profile of SSCs have revealed the existence of subsets, indicating that the stem cell population is more heterogeneous than previously believed. However, SSC subsets are poorly characterized. In rodents, the first steps in spermatogenesis have been extensively investigated, both under physiological conditions and during the regenerative phase that follows germ cell damage. In the widely accepted model, the SSCs are type Asingle (As) spermatogonia. Here, we tested the hypothesis that As spermatogonia are phenotypically heterogeneous by analyzing glial cell line-derived neurotrophic factor (GDNF) family receptor alpha1 (GFRA1) expression in whole-mounted seminiferous tubules, via cytofluorimetric analysis and in vivo colonogenic assays. GFRA1 is a coreceptor for GDNF, a Sertoli cell-derived factor essential for SSC self-renewal and proliferation. Morphometric analysis demonstrated that 10% of As spermatogonia did not express GFRA1 but were colonogenic, as shown by germ cell transplantation assay. In contrast, cells selected for GFRA1 expression were not colonogenic in vivo. In human testes, GFRA1 was also heterogeneously expressed in Adark and in Apale spermatogonia, the earliest spermatogonia. In vivo 5-bromo-2'-deoxyuridine administration showed that both GFRA1(+) and GFRA1(-) As spermatogonia were engaged in the cell cycle, a finding supported by the lack of long-term label-retaining As spermatogonia. GFRA1 expression was asymmetric in 5% of paired cells, suggesting that As subsets may be generated by asymmetric cell division. Our data support the hypothesis of the existence of SSC subsets and reveal a previously unrecognized heterogeneity in the expression profile of As spermatogonia in vivo.

Gudjonsson, T., R. Villadsen, et al. (2002). "Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties." Genes Dev **16**(6): 693-706.

The epithelial compartment of the human breast comprises two distinct lineages: the luminal epithelial and the myoepithelial lineage. We have shown previously that a subset of the luminal epithelial cells could convert to myoepithelial cells in culture signifying the possible existence of a progenitor cell. We therefore set out to identify and isolate the putative precursor in the luminal epithelial compartment. Using cell surface markers and immunomagnetic sorting, we isolated two luminal epithelial cell populations from primary cultures of reduction mammoplasties. The major population coexpresses sialomucin (MUC(+)) and epithelial-specific antigen (ESA(+)) whereas the minor population has a suprabasal position and expresses epithelial specific antigen but no sialomucin (MUC(-)/ESA(+)). Two cell lines were further established by transduction of the E6/E7 genes from human papilloma virus type 16. Both cell lines maintained a luminal epithelial phenotype as evidenced by expression of the tight junction proteins, claudin-1 and occludin, and by generation of a high transepithelial electrical resistance on semipermeable filters. Whereas in clonal cultures, the MUC(+)/ESA(+) epithelial cell line was luminal epithelial restricted in

its differentiation repertoire, the suprabasal-derived MUC(-)/ESA(+) epithelial cell line was able to generate itself as well as MUC(+)/ESA(+) epithelial cells and Thy-1(+)/alpha-smooth muscle actin(+) (ASMA(+)) myoepithelial cells. The MUC(-)/ESA(+) epithelial cell line further differed from the MUC(+)/ESA(+) epithelial cell line by the expression of keratin K19, a feature of a subpopulation of epithelial cells in terminal duct lobular units in vivo. Within a reconstituted basement membrane, the MUC(+)/ESA(+) epithelial cell line formed acinus-like spheres. In contrast, the MUC(-)/ESA(+) epithelial cell line formed elaborate branching structures resembling uncultured terminal duct lobular units both by morphology and marker expression. Similar structures were obtained by inoculating the extracellular matrix-embedded cells subcutaneously in nude mice. Thus, MUC(-)/ESA(+) epithelial cells within the luminal epithelial lineage may function as precursor cells of terminal duct lobular units in the human breast.

Hamanoue, M., Y. Matsuzaki, et al. (2009). "Cell surface N-glycans mediated isolation of mouse neural stem cells." J Neurochem **110**(5): 1575-84.

The isolation of neural stem cells (NSCs) from the brain has been hampered by the lack of valid cell surface markers and the requirement for long-term in vitro cultivation that may lead to phenotype deterioration. However, few suitable specific cell surface antigens are available on NSCs that could be used for their prospective isolation. The present study demonstrated that the expression of complex type asparagine-linked oligosaccharide (N-glycans) was detected on brain cells dissociated from embryonic and adult brain using Phaseolus vulgaris erythroagglutinating lectin (E-PHA) which binds to biantennary complex type N-glycans, and demonstrated that E-PHA bound preferentially to purified NSCs, but not to neurons, microglia, or oligodendrocyte precursor cells. The labeling of dissociated mouse embryonic brain cells or adult brain cells with E-PHA enabled the enrichment of NSCs by 25-fold or 9-fold of the number of neurosphere-forming cells in comparison to that of unsorted cells, respectively. Furthermore, a lectin blot analysis revealed the presence of several glycoproteins which were recognized by E-PHA in the membrane fraction of the proliferating NSCs, but not in the differentiated cells. These results indicate that complex type N-glycans is a valuable cell surface marker for living mouse NSCs from both the embryonic and adult brain.

Hayashi, T., M. Asami, et al. (2006). "Isolation of planarian X-ray-sensitive stem cells by fluorescence-activated cell sorting." Dev Growth Differ **48**(6): 371-80.

The remarkable capability of planarian regeneration is mediated by a group of adult stem cells referred to as neoblasts. Although these cells possess many unique cytological characteristics (e.g. they are X-ray sensitive and contain chromatoid bodies), it has been difficult to isolate them after cell dissociation. This is one of the major reasons why planarian regenerative mechanisms have remained elusive for a long time. Here, we describe a new method to isolate the planarian adult stem cells as X-ray-sensitive cell populations by fluorescence-activated cell sorting (FACS). Dissociated cells from whole planarians were labeled with fluorescent dyes prior to fractionation by FACS. We compared the FACS profiles from X-ray-irradiated and non-irradiated planarians, and thereby found two cell fractions which contained X-ray-sensitive cells. These fractions, designated X1 and X2, were subjected to electron microscopic morphological analysis. We concluded that X-ray-sensitive cells in both fractions possessed typical stem cell morphology: an ovoid shape with a large nucleus and scant cytoplasm, and chromatoid bodies in the cytoplasm. This method of isolating X-ray-sensitive cells using FACS may provide a key tool for advancing our understanding of the stem cell system in planarians.

Herrera, M. B., S. Bruno, et al. (2006). "Isolation and characterization of a stem cell population from adult human liver." Stem Cells **24**(12): 2840-50.

Several studies suggested the presence of stem cells in the adult normal human liver; however, a population with stem cell properties has not yet been isolated. The purpose of the present study was to identify and characterize progenitor cells in normal adult human liver. By stringent conditions of liver cell cultures, we isolated and characterized a population of human liver stem cells (HLSCs). HLSCs expressed the mesenchymal stem cell markers CD29, CD73, CD44, and CD90 but not the hematopoietic stem cell markers CD34, CD45, CD117, and CD133. HLSCs were also positive for vimentin and nestin, a stem cell marker. The absence of staining for cytokeratin-19, CD117, and CD34 indicated that HLSCs were not oval stem cells. In addition, HLSCs expressed albumin, alpha-fetoprotein, and in a small percentage of cells, cytokeratin-8 and cytokeratin-18, indicating a partial commitment to hepatic cells. HLSCs differentiated in mature hepatocytes when cultured in the presence of hepatocyte growth factor and fibroblast growth factor 4, as indicated by the expression of functional cytochrome P450, albumin, and urea production. Under this condition, HLSCs downregulated alpha-fetoprotein and expressed cytokeratin-8 and cytokeratin-18. HLSCs were also

able to undergo osteogenic and endothelial differentiation when cultured in the appropriated differentiation media, but they did not undergo lipogenic differentiation. Moreover, HLSCs differentiated in insulin-producing islet-like structures. In vivo, HLSCs contributed to regeneration of the liver parenchyma in severe-combined immunodeficient mice. In conclusion, we here identified a pluripotent progenitor population in adult human liver that could provide a basis for cell therapy strategies.

Hisha, H., H. Yamada, et al. (1997). "Isolation and identification of hematopoietic stem cell-stimulating substances from Kampo (Japanese herbal) medicine, Juzen-taiho-to." Blood **90**(3): 1022-30.

We have previously found that TJ-48 has the capacity to accelerate recovery from hematopoietic injury induced by radiation and the anti-cancer drug mitomycin C (MMC). The effects are found to be due to its stimulation of spleen colony-forming unit (CFU-S) counts on day 14. In the present study, we attempt to isolate and purify the active components in TJ-48 extracts using a new in vitro hematopoietic stem cell (HSC) assay method. n-Hexane extract from TJ-48 shows a significant stimulatory activity. The extract is further fractionated by silica gel chromatography and HPLC in order to identify its active components. 1H-NMR and GC-EI-MS indicate that the active fraction is composed of free fatty acids (oleic acid and linolenic acid). When 27 kinds of free fatty acids (commercially available) are tested using the HSC proliferating assay, oleic acid, elaidic acid, and linolenic acid are found to have potent activity. The administration of oleic acid to MMC-treated mice enhances CFU-S counts on days 8 and 14 to twice the control group. These findings strongly suggest that fatty acids contained in TJ-48 actively promote the proliferation of HSCs. Although many mechanisms seem to be involved in the stimulation of HSC proliferation, we speculate that at least one of the signals is mediated by stromal cells, rather than any direct interaction with the HSCs.

Hsu, Y. R., W. C. Chang, et al. (1998). "Selective deamidation of recombinant human stem cell factor during in vitro aging: isolation and characterization of the aspartyl and isoaspartyl homodimers and heterodimers." Biochemistry **37**(8): 2251-62.

During in vitro aging, deamidation of recombinant human stem cell factor produced in Escherichia. coli was detected by HPLC analysis and by the release of soluble ammonia. The deamidation rate is very slow in buffers at low pH or at low temperatures; however, the rate is significantly accelerated in alkaline buffers such as sodium bicarbonate in combination with elevated temperatures. HPLC isolation of various deamidated forms followed by peptide mapping and mass spectrometric analyses revealed that the deamidation involves Asn10 in the sequence -T9NNV- near the N-terminus of the protein. Following peptide mapping analysis, significant amounts of aspartyl and isoaspartyl peptides were identified, indicating the conversion of asparagine into both aspartate and isoaspartate residues. As a result of spontaneous association-dissociation of stem cell factor dimer, a total of five deamidated forms, including two homodimers and three heterodimers, were detected and isolated. Cell proliferation assays showed that two rhSCF heterodimeric species, derived from dimerization between isoaspartyl and other stem cell factor monomers, retain only approximately half of the biological activity. The homodimer with isoaspartic acid in place of Asn10 is 50-fold less potent, while the aspartyl homodimer, either isolated during deamidation experiments or recombinantly prepared by site-directed mutagenesis (e.g., N10D and N10D/N11D variants), exhibits higher activity than the standard molecule. In comparison, synthetic N10A and N10E variants, though missing the deamidation site, are significantly less active. All these variants lacking the Asn10 deamidation site are relatively more stable than those containing the asparagine residue. The results indicate that the biological function and chemical stability of stem cell factor are influenced by the nature of the residue at position 10.

Huang, A. H., Y. K. Chen, et al. (2009). "Isolation and characterization of normal hamster buccal pouch stem/stromal cells--a potential oral cancer stem/stem-like cell model." Oral Oncol **45**(11): e189-95.

The hamster buccal pouch (HBP) is an appropriate experimental model for buccal squamous cell carcinoma (SCC). Our objective was to isolate and characterize the stem/stromal cells from normal HBP. HBP stem/stromal cells were successfully derived from three of five normal pouch tissues, which differentiated into adipogenic, chondrogenic, and osteogenic lineages, and also expressed stem cell and differentiation markers, indicating their stem cell origin and differentiation capability. These cells showed high expression of CD29, CD90, and CD105, markers specific for bone marrow stem cells, and exhibited very low expression of CD14, CD34, and CD45, markers specific for hematopoietic cells. Of the HBP stem/stromal cells isolated, 90% stained positively for cytoplasmic keratin, whereas 10% stained positively for vimentin. In conclusion, normal HBP stem/stromal cells provide a potential avenue for future experimental trials of cancer stem/stem-like cells for treatment of buccal SCC. In vitro, we may

detect the sequential changes of normal HBP stem/stromal cells during multistep oral carcinogenesis or the alternations of these cells upon irradiation treatment and/or chemotherapy.

Huang, D., Q. Gao, et al. (2009). "Isolation and identification of cancer stem-like cells in esophageal carcinoma cell lines." Stem Cells Dev **18**(3): 465-73.

Side population (SP) cells may play an important role in tumorigenesis and cancer therapy. We isolate and identify the cancer stem-like cells in human esophageal carcinoma (EC) cell lines, EC9706 and EC109 cells labeled with Hoechst 33342. Both the cell lines contained SP cells, and the cells that had the strongest dye efflux activity ("Tip"-SP cell) in EC9706 had higher clone formation efficiency than non-SP cells. When transplanted into nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, "Tip"-SP cells showed at least 50 times higher tumorigenicity than non-SP cells. Microarray analysis discriminated a differential gene expression profile between "Tip"-SP and non-SP cells, which is further tested using quantitative real-time RT-PCR. It is ascertained that several important stem cell-related genes (including OCT-4, SOX-2, BMI-1, and ZFX), two ATP-binding cassette (ABC) transporter genes (ABCG2 and ABCA5), and three Wnt and two Notch signal pathway-related genes (such as FZD10, PTGS2, KLF5, TTK, and RBM15) were upregulated in "Tip"-SP cells. Western blot showed a higher expression of beta-catenin protein in "Tip"-SP cells. All these indicated that the minority population described as "Tip"-SP cells possessed cancer stem cell character. Further understanding of tumor stem cell-specific traits will offer insights on the early stages of tumorigenesis for prevention and enhanced selectivity of antitumor therapeutics.

Husain, S. M., Y. Shou, et al. (2006). "Isolation, molecular cloning and in vitro expression of rhesus monkey (Macaca mulatta) prominin-1.s1 complementary DNA encoding a potential hematopoietic stem cell antigen." Tissue Antigens **68**(4): 317-24.

Human prominin-1 (CD133 or AC133) is an important cell surface marker used to isolate primitive hematopoietic stem cells. The commercially available antibody to human prominin-1 does not recognize rhesus prominin-1. Therefore, we isolated, cloned and characterized the complementary DNA (cDNA) of rhesus prominin-1 gene and determined its coding potential. Following the nomenclature of prominin family of genes, we named this cDNA as rhesus prominin-1.s1. The amino acid sequence data of the putative rhesus prominin-1.s1 could be used in designing antigenic peptides to raise antibodies for use in isolation of pure populations of rhesus prominin-1(+) hematopoietic cells. To the best of our knowledge, there has been no previously published report about the isolation of a prominin-1 cDNA from rhesus monkey (Macaca mulatta).

Jankowski, R. J., C. Haluszczak, et al. (2001). "Flow cytometric characterization of myogenic cell populations obtained via the preplate technique: potential for rapid isolation of muscle-derived stem cells." Hum Gene Ther **12**(6): 619-28.

Myoblast transplantation has been investigated as a therapy for muscle-related diseases and as a gene delivery vehicle for therapeutic recombinant proteins. Clinical successes involving muscle cell transplantation have been limited, in part because of poor donor cell survival, and the heterogeneous nature of myogenic donor cells has largely been ignored. We have previously reported an isolation technique, preplating, that results in purified myogenic cells that are capable of significantly higher rates of donor cell survival leading to enhanced gene transfer to skeletal muscle. Characterization of these purified cells revealed that they display markers common to stem cells and are capable of multilineage differentiation. This study was performed to phenotypically characterize, by flow cytometry, muscle-derived cell populations obtained by the preplate technique for the purpose of eventually developing a method to quickly identify and isolate viable muscle cells best suited for transplantation. Muscle cell cultures were analyzed for expression of the surface proteins Sca-1, c-Kit, and CD34. We found that the preplate technique purifies distinct myogenic cell subpopulations expressing CD34 alone (Sca-1 negative) and Sca-1 alone (CD34 negative), but that this expression is subject to change with time in culture. Isolation and transplantation of phenotypically pure Sca-1-positive myogenic cells, obtained by magnetic cell sorting, demonstrates the ability to quickly select viable myogenic cells capable of regenerating skeletal muscle and restoring dystrophin expression within dystrophic host skeletal muscle. Flow cytometric described phenotypes will aid in the rapid isolation of specific donor cell populations for muscle cell transplants and muscle cell-mediated gene therapies, thereby enhancing their future success.

Kato, K. and A. Radbruch (1993). "Isolation and characterization of CD34+ hematopoietic stem cells from human peripheral blood by high-gradient magnetic cell sorting." Cytometry **14**(4): 384-92.

CD34+ cells, present at a frequency of 0.18 +/- 0.052% among leukocytes from peripheral blood (PB), can be rapidly and efficiently (recovery of 39.0-74.0%) enriched to a frequency of 38.6-87.1% (54.4

+/- 12.3%) by high-gradient magnetic cell separation (MACS) for immunophenotyping, characterization in colony-forming cell (CFU) assays, and further purification to homogeneity (> 98%) by multiparameter fluorescence-activated cell sorting (FACS). Enriching PB-CD34+ cells for immunophenotyping allows the detection of small subpopulations, expressing the B-cell antigens CD10, CD19, and CD20, the T-cell antigens CD45RA and CD7, and a small subpopulation expressing high levels of CD34 (1.20 +/- 0.12%), which mostly coexpress CD19 (91.9 +/- 9.05%), CD20 (64.8 +/- 14.4%), and CD38 (84.5 +/- 10.3%). All PB-CD34+ cells express elevated levels of CD71 (transferrin receptor), with a subpopulation of high expressing cells, and CD38. Some cells express CD33. MACS-enriched PB-CD34+ cells show "normal" hematopoietic colony formation in vitro. The ease and efficiency of purification of large numbers of CD34+ cells from PB by MACS is not only relevant for the characterization of migrating stem cells but also opens new possibilities for stem cell transplantation and genetic manipulation of the hematopoietic system.

Kerkis, I., A. Kerkis, et al. (2006). "Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers." Cells Tissues Organs **184**(3-4): 105-16.

We report the isolation of a population of immature dental pulp stem cells (IDPSC), which express embryonic stem cell markers Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81 as well as several other mesenchymal stem cell markers during at least 25 passages while maintaining the normal karyotype and the rate of expansion characteristic of stem cells. The expression of these markers was maintained in subclones obtained from these cells. Moreover, in vitrothese cells can be induced to undergo uniform differentiation into smooth and skeletal muscles, neurons, cartilage, and bone under chemically defined culture conditions. After in vivo transplantation of these cells into immunocompromised mice, they showed dense engraftment in various tissues. The relative ease of recovery and the expression profiles of various markers justify further exploration of IDPSC for clinical therapy.

Kilty, I. C., R. Barraclough, et al. (1999). "Isolation of a potential neural stem cell line from the internal capsule of an adult transgenic rat brain." J Neurochem **73**(5): 1859-70.

A thermosensitive mutation of simian virus 40 large T antigen (LTA) gene, the tsA58 gene, was cloned downstream of the 6-kbp neurofilament light chain promoter in pPOLYIII and injected into the pronucleus of fertilised oocytes of Sprague-Dawley rats to develop a strain harbouring six copies of the transgene. Immunocytochemical staining of hemizygous adult tissues with antibodies to the C-terminus of LTA showed that the inactive form of LTA was expressed only in the fibres of the internal capsule and in the choroid plexus of the brain. Culturing the former region at 33 degrees C, the permissive temperature for LTA, yielded a cell line, NF2C, which produced active LTA and grew at 33 degrees C but which produced only inactive LTA and eventually died at the non-permissive temperature of 39 degrees C. This clonal cell line was heterogeneous at 33 degrees C, producing the precursor neuronal cell marker nestin and the glial-specific markers glial fibrillary acidic protein, vimentin and S100A1, as well as weakly producing the neuronal cell markers 68-kDa neurofilament protein (NF68) and microtubule-associated protein 2 (MAP2) in different subpopulations of cells. However, at 39 degrees C, the cells produced dendritic, neuronal-like processes and elevated levels of NF68 and MAP2, as well as the neuronal markers synaptophysin, neurone-specific enolase, and low levels of tau, all determined by western blotting and immunofluorescent staining. Basic fibroblast growth factor enhanced the growth of the cells at 33 degrees C but also enhanced the formation of dendritic neuronal-like processes at 39 degrees C. It is suggested that NF2C represents a potential stem cell line from adult brain that expresses precursor and glial cell markers at 33 degrees C but undergoes partial differentiation to a neuronal cell phenotype at 39 degrees C.

Kim, Y., V. Selvaraj, et al. (2006). "Recipient preparation and mixed germ cell isolation for spermatogonial stem cell transplantation in domestic cats." J Androl **27**(2): 248-56.

The loss of genetic diversity poses a serious threat to the conservation of endangered species, including wild felids. We are attempting to develop spermatogonial stem cell transplantation in the cat as a tool to preserve and propagate male germ-plasm from genetically valuable animals, be they threatened wild species or lines of cats used as models for inherited diseases. In this study, we investigated the use of local external beam radiation treatment to deplete the endogenous germ cells of male domestic cats, a step necessary to prepare them for use as recipients for transplantation. Testes of 5-month-old domestic cats were irradiated with a fractionated dose of 3 Gy per fraction for 3 consecutive days. These cats were castrated at 2, 4, 8, 16, and 32 weeks posttreatment, and progress of spermatogenesis was evaluated histologically and compared against age-matched

controls. Even at the latest time points, less than 10% of tubules contained germ cells at any stage of meiosis, showing the efficacy of this protocol. In addition, male germ cells were isolated from the testes of domestic cats using a 2-step enzymatic dissociation to establish a protocol for the preparation of donor cells. The presence and viability of spermatogonia within this population were demonstrated by successful transplantation into, and colonization of, mouse seminiferous tubules. The success of these protocols provides a foundation to perform spermatogonial stem cell transplantation in the domestic cat.

Kitani, H., N. Takagi, et al. (1996). "Isolation of a germline-transmissible embryonic stem (ES) cell line from C3H/He mice." Zoolog Sci **13**(6): 865-71.

We have isolated three embryonic stem (ES) cell lines from C3H/He mice using mouse STO cells as a feeder layer. One ES cell line (H-1) was male, and two (H-2 and H-3) were female, as determined by polymerase chain reaction, in situ hybridization, and karyotype analyses. All were immunocytochemically reactive with a C3H strain-specific antibody. Injection of cells from the female ES H-3 line into C57BL/6 blastocysts yielded four chimeras with slight coat color chimerism. All chimeras were male, and as expected, no germline-transmission was observed. By contrast, when male ES H-1 cells were injected into the perivitelline space of 8-cell C57BL/6 embryos, one male mouse with overt coat color chimerism was recovered, and it produced ES H-1-derived offspring exclusively. This germline-transmissible C3H/He cell line represents a novel addition to those ES lines currently employed for gene manipulation studies of development.

Kossack, N., J. Meneses, et al. (2009). "Isolation and characterization of pluripotent human spermatogonial stem cell-derived cells." Stem Cells **27**(1): 138-49.

Several reports have documented the derivation of pluripotent cells (multipotent germline stem cells) from spermatogonial stem cells obtained from the adult mouse testis. These spermatogonia-derived stem cells express embryonic stem cell markers and differentiate to the three primary germ layers, as well as the germline. Data indicate that derivation may involve reprogramming of endogenous spermatogonia in culture. Here, we report the derivation of human multipotent germline stem cells (hMGSCs) from a testis biopsy. The cells express distinct markers of pluripotency, form embryoid bodies that contain derivatives of all three germ layers, maintain a normal XY karyotype, are hypomethylated at the H19 locus, and express high levels of telomerase. Teratoma assays indicate the presence of human cells 8 weeks post-transplantation but limited teratoma formation. Thus, these data suggest the potential to derive pluripotent cells from human testis biopsies but indicate a need for novel strategies to optimize hMGSC culture conditions and reprogramming.

Lanzoni, G., F. Alviano, et al. (2009). "Isolation of stem cell populations with trophic and immunoregulatory functions from human intestinal tissues: potential for cell therapy in inflammatory bowel disease." Cytotherapy **11**(8): 1020-31.

BACKGROUND AIMS: Bone marrow (BM)- and adipose tissue (AT)-derived mesenchymal stromal cells (MSC) are currently under evaluation in phase III clinical trials for inflammatory bowel disease and other intestinal disease manifestations. The therapeutic efficacy of these treatments may derive from a combination of the differentiation, trophic and immunomodulatory abilities of the transplanted cells. We investigated intestinal tissues as sources of MSC: such cells may support tissue-specific functions and hold advantages for engraftment and contribution in the gastrointestinal environment. METHODS: Intestinal specimens were collected, and the mucosa and submucosa mechanically separated and enzymatically digested. Mesenchymal stromal populations were isolated, expanded and characterized under conditions commonly used for MSC. The differentiation potential, trophic effect and immunomodulatory ability were investigated. Results We successfully isolated and extensively expanded populations showing the typical MSC profile: CD29+, CD44+, CD73+, CD105+ and CD166+, and CD14(-), CD34(-) and CD45(-). Intestinal mucosal (IM) MSC were also CD117+, while submucosal cultures (ISM MSC) showed CD34+ subsets. The cells differentiated toward osteogenic, adipogenic and angiogenic commitments. Intestinal-derived MSC were able to induce differentiation and organization of intestinal epithelial cells (Caco-2) in three-dimensional collagen cultures. Immunomodulatory activity was evidenced in co-cultures with normal heterologous phytohemagglutinin-stimulated peripheral blood mononuclear cells. Conclusions Multipotent MSC can be isolated from intestinal mucosal and submucosal tissues. IM MSC and ISM MSC are able to perform trophic and immunomodulatory functions. These findings could open a pathway for novel approaches to intestinal disease treatment.

Lee, D. R., K. S. Kim, et al. (2006). "Isolation of male germ stem cell-like cells from testicular tissue of non-obstructive azoospermic patients and differentiation into haploid male germ cells in vitro." Hum Reprod **21**(2): 471-6.

BACKGROUND: The purpose of this study was to establish the culture conditions required to isolate, identify and expand male germ stem cell-like cells (GSC-LC) from the testicular tissue of patients with non-obstructive azoospermia (NOA). METHODS AND RESULTS: Testicular tissues obtained from patients (two with maturation arrest (MA, n = 2) and Sertoli cell-only syndrome (SCOS, n = 11) were dissociated and plated into gelatin-coated dishes. After 2-4 weeks, cultures from both MA patients (100%) and four SCOS patients (36.3%) exhibited multicellular colonies, which proliferated successfully until passage 10. GSC-LC in the colonies displayed alkaline phosphatase activity, as well as Oct-4 and integrin b1 expression after every passage. After the fifth passage, GSC-LC were differentiated by encapsulation in calcium alginate and further cultivation. At 2 and 6 weeks, cells expressed c-Kit, Scp3, testis-specific histone protein 2B (TH2B), and transition protein (TP)-1. Fluorescence in situ hybridization additionally disclosed a few tetraploid and haploid cells at 6 weeks. Human oocytes were activated in the absence of artificial activation and cleaved after the injection of presumptive spermatids. CONCLUSIONS: Our novel culture system may be useful for diagnosing the existence of germ cells and facilitating the treatment of NOA patients.

Lemmer, E. R., E. G. Shepard, et al. (1998). "Isolation from human fetal liver of cells co-expressing CD34 haematopoietic stem cell and CAM 5.2 pancytokeratin markers." J Hepatol **29**(3): 450-4.

BACKGROUND/AIMS: Ductal plate and bile duct cells in developing human liver express haematopoietic stem cell markers, such as c-kit and CD34, in association with cytokeratin markers CAM 5.2 and CK 18. The identification of such ductal plate cells as likely progenitors for both bile duct epithelial cells and hepatocytes and their possible reappearance as oval cells in the regenerating liver have generated much interest in their pluripotential capacities. This study aimed to isolate cells from human fetal liver that co-express haematopoietic stem cell and epithelial cell markers. METHODS: Human fetal liver was harvested following legal termination of pregnancy at week 14-22. CD34+ mononuclear cells were isolated from liver cell suspensions with immunomagnetic beads. Immunofluorescent staining, using anticytokeratin CAM 5.2 against CK 8 and 18, was performed on permeabilised CD34+ cells for flow cytometry and fluorescent microscopy. CD34+ cells were also stained for other stem cell markers (HLA-DR, c-kit) and committed haematopoietic cell markers (CD33, CD38). RESULTS: Approximately 0.9% (range 0.07-4.0%) of the mononuclear cells isolated were CD34+ cells. The number of mononuclear cells isolated correlated with fetal liver weight (r=0.508). About 3-8% of these CD34+ cells stained positively for CAM 5.2. In addition, CD34+ cells were positive for HLA-DR, but only a small percentage was positive for c-kit. Staining for the committed haematological markers, CD33 and CD38, was consistently negative. CONCLUSIONS: This study describes an immunoaffinity method for the enrichment from human fetal liver of cells that co-express haematopoietic stem cell and epithelial cell markers. Such cellular subsets may correspond to pluripotential ductal plate and bile duct cells.

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

Adult bone marrow stroma contains a source of mesenchymal stem cells (MSC) that have the capacity to self-renew and differentiate into multiple stromal lineages. These rare cells can be visualised indirectly by the formation of heterogeneous colonies, containing stem cells and their differentiated progeny in long-term culture. If MSC and their associated progenitor and precursor populations are to reach their full therapeutic potential, markers will be required to identify and characterize specific bone marrow stromal subsets. We sought to use phage display to generate antibodies against bone marrow mononuclear cells (BMMNC) enriched for colony forming cells. Initially, we identified our target cell population by comparing the colony forming efficiency (CFE) of CD49a-positive, STRO-1-positive and CD45-negative BMMNC subpopulations with unseparated BMMNC. Selection with anti-CD49a gave the greatest enrichment (19-fold) of colony forming cells and in light of these findings, we generated phage antibodies against CD49a-positive BMMNC by simultaneous positive/negative selection. A dominant clone (C15), generated after 3 rounds of selection, has been isolated and sequenced, then characterized for cell and tissue specificity. Sequence analysis showed that the V(H) and V(L) gene segments of C15 aligned most closely to the VH26/DP-47 and IGLV3S1/DPL16 germline V segments found in the synthetic repertoire. C15 bound to 4% of freshly isolated BMMNC and localized to osteoblastic cells and proximal marrow cells in areas of active bone formation in sections of osteophyte. C15 binding was upregulated in cultured bone marrow stromal cells (BMSC) and was also detected on bone-derived cell lines. This report demonstrates that phage display is a powerful tool for the isolation of antibodies against rare cell populations, and provides a platform for the future application of this technology

in the search for antigens on MSC and other rare cell populations.

Li, A., P. J. Simmons, et al. (1998). "Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype." Proc Natl Acad Sci U S A **95**(7): 3902-7.

Despite the central role of human epidermal stem cells in tissue homeostasis, wound repair, and neoplasia, remarkably little is known about these cells, largely due to the absence of molecular markers that distinguish them from other proliferative cells within the germinative/basal layer. Epidermal stem cells can be distinguished from other cells in the basal layer by their quiescent nature in vivo and their greater overall proliferative capacity. In this study, we demonstrate enrichment and isolation of a subpopulation of basal epidermal cells from neonatal human foreskin based on cell surface phenotype, which satisfy these criteria. These putative stem cells are distinguished from other basal cells by their characteristic expression of high levels of the adhesion molecule alpha6, a member of the integrin family (alpha6bri), and low levels of a proliferation-associated cell surface marker recognized by recently described mAb 10G7 (10G7(dim)). We conclude that cells with the phenotype alpha6bri10G7(dim) represent the epidermal stem cell population based on the demonstration that these cells (i) exhibit the greatest regenerative capacity of any basal cells, (ii) represent a minor subpopulation (approximately 10%) of immature epidermal cells, which (iii) are quiescent at the time of isolation from the epidermis, as determined by cell cycle analysis.

Littefield, C. L. (1991). "Cell lineages in Hydra: isolation and characterization of an interstitial stem cell restricted to egg production in Hydra oligactis." Dev Biol **143**(2): 378-88.

In an attempt to isolate unipotent stem cells (progenitors to the nerve cells, nematocytes, gland cells, and gametes) from Hydra oligactis females, animals were treated with a drug (hydroxyurea, HU) that preferentially lowers or eliminates the interstitial stem cells, leaving the epithelial tissue intact. In this epithelial environment, interstitial cells remaining after treatment will proliferate and differentiate, permitting a long-term analysis of their developmental capabilities. Following treatment of females with HU, animals were isolated that contained interstitial cells that gave rise to eggs only. Two clones of animals containing these cells were propagated for several years and the growth and differentiation behavior of the interstitial cells examined in their asexually produced offspring. During this time, the cells displayed an extensive proliferative capacity (classifying them as stem cells) and remained restricted to egg differentiation. It is proposed that both the sperm- and the egg-restricted stem cells arise from a multipotent stem cell, which also gives rise to the somatic cells (see above), and that, in hydra, sex is ultimately determined by interactions between cells of the two germ cell lineages.

Lodge, P., J. McWhir, et al. (2005). "Increased gp130 signaling in combination with inhibition of the MEK/ERK pathway facilitates embryonic stem cell isolation from normally refractory murine CBA blastocysts." Cloning Stem Cells **7**(1): 2-7.

The standard method for isolation of ES cells from strain 129 mice does not give rise to ES lines of CBA origin. We investigated the effect of inhibition of MEK/ERK signaling in combination with increased stimulation of gp130 signaling on derivation of ES cells from CBA blastocysts. Inhibition of MEKI and MEKII using the drug U0126 and stimulation of gp130 signaling by elevating the level of LIF present gave rise to ES-like lines in 22.6% of explants. No lines arose when MEK was inhibited in the absence of additional LIF stimulation, nor when additional LIF stimulation occurred in the absence of MEK inhibition. Typically for ES cell lines, CBA-derived cells contributed to chimeric mice and differentiated broadly in vitro. Increased levels of gp130 signaling led to similar levels of STAT3 activation in strain 129 and CBA ES cells. We conclude that CBA ES cells have a requirement for additional STAT3 activation.

Lu, H. S., M. D. Jones, et al. (1996). "Isolation and characterization of a disulfide-linked human stem cell factor dimer. Biochemical, biophysical, and biological comparison to the noncovalently held dimer." J Biol Chem **271**(19): 11309-16.

Distinct from the noncovalently linked recombinant human stem call factor (rhSCF) dimer, we report here the isolation and identification of an SDS-nondissociable dimer produced during folding/oxidation of rhSCF. Experimental evidence using various cleavage strategies and analyses shows that the isolated dimer is composed of two rhSCF monomers covalently linked by four disulfide bonds. The cysteines are paired as in the noncovalently associated dimer except that all pairings are intermolecular rather than intramolecular. Other structural models, involving intertwining of intramolecular disulfide loops, are ruled out. The molecule behaves similarly to the noncovalently associated dimer during ion-exchange or gel permeation chromatography. However, the disulfide-linked dimer exhibits increased hydrophobicity in reverse-phase columns and in the native state does not undergo spontaneous dimer dissociation-association as

seen for the noncovalent dimer. Spectroscopic analyses indicate that the disulfide-linked and noncovalently associated rhSCF dimers have grossly similar secondary and tertiary structures. In vitro, the disulfide-linked dimer exhibits approximately 3-fold higher biological activity in supporting growth of a hematopoietic cell line and stimulating hematopoietic cell colony formation from enriched human CD34+ cells. The molecule binds to the rhSCF receptor, Kit, with an efficiency only half that of the noncovalently associated dimer. Formation of intermolecular disulfides in the disulfide-linked dimer with retention of biological activity has implications for the three-dimensional structure of noncovalently held dimer and disulfide-linked dimer.

Marone, M., D. De Ritis, et al. (2002). "Cell cycle regulation in human hematopoietic stem cells: from isolation to activation." Leuk Lymphoma **43**(3): 493-501.

Hematopoietic stem cells (HSCs) reside mostly in the bone marrow and are defined by their ability to self-renew and to give rise by proliferation and differentiation to all blood lineages. Despite this strict definition HSCs cannot be unequivocally identified in the hematopoietic cell pool. Despite innumerable studies over the years, which focused on the search of the ideal phenotypic marker to selectively isolate stem cells, most of the known markers still define heterogeneous populations in different stages of commitment. Functional features attributed to stem cells have also been investigated, and among these the use of fluorescent markers which allow tracking of the cell division record of each cell. A second issue, after the initial isolation process, is the expansion ex vivo in order to obtain production of large numbers of homogeneous cell populations for both biological studies and clinical applications. Expansion ex vivo is difficult to modulate and normally occurs only along with commitment and consequent loss of multipotentiality. Moreover expansion obtained ex vivo is significantly reduced to that achievable in vivo. One of the key features of HSCs is a very slow proliferation rate, but when the appropriate stimuli are delivered, the proliferation rate can drastically increase. In normal physiological conditions a strict balance is maintained between the number of cells that maintain the original pool and those that proliferate and differentiate. Numerous data in recent years are providing some clue to elucidate the key steps in this tightly controlled process, but the dynamics that regulate which and how many cells self-renew to maintain the pool, and which proliferate and become committed to give rise to the mature blood elements, are still unclear.

Martin, G. R. (1981). "Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells." Proc Natl Acad Sci U S A **78**(12): 7634-8.

This report describes the establishment directly from normal preimplantation mouse embryos of a cell line that forms teratocarcinomas when injected into mice. The pluripotency of these embryonic stem cells was demonstrated conclusively by the observation that subclonal cultures, derived from isolated single cells, can differentiate into a wide variety of cell types. Such embryonic stem cells were isolated from inner cell masses of late blastocysts cultured in medium conditioned by an established teratocarcinoma stem cell line. This suggests that such conditioned medium might contain a growth factor that stimulates the proliferation or inhibits the differentiation of normal pluripotent embryonic cells, or both. This method of obtaining embryonic stem cells makes feasible the isolation of pluripotent cells lines from various types of noninbred embryo, including those carrying mutant genes. The availability of such cell lines should made possible new approaches to the study of early mammalian development.

McWhir, J., A. E. Schnieke, et al. (1996). "Selective ablation of differentiated cells permits isolation of embryonic stem cell lines from murine embryos with a non-permissive genetic background." Nat Genet **14**(2): 223-6.

Embryonic stem (ES) cells enable the engineering of precise modifications to the mouse genome by gene targeting. Although there are reports of cultured cell contributions to chimaeras in golden hamster, rat and pig, definitive ES cell lines which contribute to the germline have not been demonstrated in any species but mouse. Among mouse strains, genetic background strongly affects the efficiency of ES isolation, and almost all ES lines in use are derived from strain 129 (refs 1,4,5) or, less commonly, C57BL/6 (refs 6-8). The CBA strain is refractory to ES isolation and there are no published reports of CBA-derived ES lines. Hence, CBA mice may provide a convenient model of ES isolation in other species. In ES derivation it is critical that the primary explant be cultured for a sufficient time to allow multiplication of ES cell progenitors, yet without allowing extensive differentiation. Thus, differences in ES derivation between mouse strains may reflect differences in the control of ES progenitor cells by other lineages within the embryo. Here we describe a strategy to continuously remove differentiated cells by drug selection, which generates germline competent ES lines from genotypes that are non-permissive in the absence of selection.

Medlock, E. S., C. Mineo, et al. (1992). "Isolation of rat bone marrow mast lineage cells using Thy 1.1 and rat stem cell factor." J Cell Physiol **153**(3): 498-506.

Recent reports have shown that various marrow-derived cell populations respond vigorously to recombinant rat stem cell factor (rrSCF164), one form of the kit-ligand. In the present study, we isolated cell populations from rat bone marrow using the Thy 1.1 antigen (an antigen that in the rat is differentially expressed on primitive hemopoietic progenitor cells) and fluorescently conjugated rrSCF164 (rrSCF164-PE). We show that rrSCF164 only stimulates cells that are enriched in the brightest Thy 1.1 populations (Thy 1.1bright). Numerous cell lines were generated by serial passage in rrSCF164 containing medium, and the prototypic cell lines have been designated SRT002 and SRT003. Each cell line retains the Thy 1.1bright phenotype and does not respond to interleukins (IL) 1-8, IL-10, granulocyte (G) colony-stimulating factor (CSF), granulocyte macrophage (GM) CSF, M-CSF, or crude preparations of mitogen-stimulated T-cell supernatants. The Thy 1.1bright population of rat marrow was subdivided into a subset that binds rrSCF164-PE (Thy 1.1bright, rrSCF164+). The majority of these cells possess certain characteristics in common with marrow-derived mast cells and the Thy 1.1bright, rrSCF164 responsive cell lines, having similar granule morphology, being metachromatic, and reacting positively with alcian blue. Moreover, rats treated with rrSCF164 displayed significant increases in Thy 1.1bright, rrSCF164+ cells in the bone marrow. These studies show that the combination of Thy 1.1 and rrSCF164 makes possible the isolation of a unique subset of rat bone marrow cells that differentially express the Thy 1.1 antigen and the cell surface receptor c-kit, the majority of which are morphologically similar to marrow-derived mast cells.

Meindl, S., U. Schmidt, et al. (2006). "Characterization, isolation, and differentiation of murine skin cells expressing hematopoietic stem cell markers." J Leukoc Biol **80**(4): 816-26.

As the phenotype of adult dermal stem cells is still elusive, and the hematopoietic stem cell is one of the best-characterized stem cells in the body, we tested dermal cell suspensions, sections, and wholemounts in newborn and adult mice for hematopoietic stem cell marker expression. Phenotypic analysis revealed that a small population of CD45(+) cells and a large population of CD45(-) cells expressed CD34, CD117, and stem cell antigen-1 molecules. When cultivated in selected media supplemented with hematopoietic cytokines, total dermal cells, lineage(-), and/or highly enriched phenotypically defined cell subsets produced hematopoietic and nonhematopoietic colonies. When injected into lethally irradiated recipient mice, a small percentage of newborn dermal cells was able to migrate into hematopoietic tissues and the skin and survived through the 11-month monitoring period. Our ability to isolate a candidate autologous stem cell pool will make these cells ideal vehicles for genetic manipulation and gene therapy.

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

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

Mio, H., N. Kagami, et al. (1998). "Isolation and characterization of a cDNA for human mouse, and rat full-length stem cell growth factor, a new member of C-type lectin superfamily." Biochem Biophys Res Commun **249**(1): 124-30.

cDNA encoding stem cell growth factor (SCGF; 245 aa), a novel human growth factor for primitive hematopoietic progenitor cells, has been previously reported (Hiraoka, A., Sugimura, A., Seki, T., Nagasawa, T., Ohta, N., Shimonishi, M., Hagiya, M. and Shimizu, S. Proc. Natl. Acad. Sci. USA 94, 7577-7582, 1997). Here we report the cloning and characterization of a full-length SCGF cDNA. This protein consists of 323, 328 and 328 aa in the human, murine and rat forms, the latter two of which share

85.1% and 83.3% aa identity, and 90.4% and 90.4% aa similarity to the human protein, respectively. Because the newly identified human clone encodes the protein longer by 78 aa than that previously identified, we term the longer clone as hSCGF-alpha and the shorter one as hSCGF-beta. The computer-assisted homology search reveals that SCGF is a new member of the C-type lectin superfamily, and that SCGF shows the greatest homology to tetranectin among the members of the family (27.2-33.7% aa identity and 46.0-53.6% aa similarity). SCGF transcripts are detected in spleen, thymus, appendix, bone marrow and fetal liver. Fluorescent in situ hybridization mapping indicates that the SCGF gene is located on chromosome 19 at position q13.3 for human form and on chromosome 7 at position B3-B5 for murine form, which are close to flk-2/flt3 ligand and interleukin-11 genes of both human and murine species.

Miraglia, S., W. Godfrey, et al. (1997). "A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning." Blood **90**(12): 5013-21.

Phenotypic analysis of hematopoietic stem and progenitor cells (HSCs) has been an invaluable tool in defining the biology of stem cell populations. We have recently described the production of AC133, a monoclonal antibody (MoAb) that binds to a novel cell surface antigen present on a CD34(bright) subset of human HSCs. This antigen is a glycosylated protein with a molecular weight of 120 kD. Here, we report the molecular cloning of a cDNA encoding this antigen and show that it does not share homology with any previously described hematopoietic or other cell surface antigen(s). The AC133 polypeptide has a predicted size of 97 kD and contains five-transmembrane (5-TM) domains with an extracellular N-terminus and a cytoplasmic C-terminus. Whereas the expression of tetraspan (4-TM) and 7-TM molecules is well documented on mature and immature hematopoietic cells and leukocytes, this 5-TM type of structure containing two large (255-amino acid [aa] and 290-aa) extracellular loops is unique and does not share sequence homology with any known multi-TM family members. Expression of this protein appears limited to bone marrow in normal tissue by immunohistochemical staining; however, Northern analysis suggests that the mRNA transcript is present in a variety of tissues such as the kidney, pancreas, placenta, and fetal liver. The AC133 antigen is also expressed on subsets of CD34+ leukemias, suggesting that it may be an important early marker for HSCs, as well as the first described member of a new class of TM receptors.

Mitalipov, S., H. C. Kuo, et al. (2006). "Isolation and characterization of novel rhesus monkey embryonic stem cell lines." Stem Cells **24**(10): 2177-86.

ESCs are important as research subjects since the mechanisms underlying cellular differentiation, expansion, and self-renewal can be studied along with differentiated tissue development and regeneration in vitro. Furthermore, human ESCs hold promise for cell and tissue replacement approaches to treating human diseases. The rhesus monkey is a clinically relevant primate model that will likely be required to bring these clinical applications to fruition. Monkey ESCs share a number of properties with human ESCs, and their derivation and use are not affected by bioethical concerns. Here, we summarize our experience in the establishment of 18 ESC lines from rhesus monkey preimplantation embryos generated by the application of the assisted reproductive technologies. The newly derived monkey ESC lines were maintained in vitro without losing their chromosomal integrity, and they expressed markers previously reported present in human and monkey ESCs. We also describe initial efforts to compare the pluripotency of ESC lines by expression profiling, chimeric embryo formation, and in vitro-directed differentiation into endodermal, mesodermal, and ectodermal lineages.

Miyazaki, M., M. Hardjo, et al. (2007). "Isolation of a bone marrow-derived stem cell line with high proliferation potential and its application for preventing acute fatal liver failure." Stem Cells **25**(11): 2855-63.

Transplantation of hepatocytes or hepatocyte-like cells of extrahepatic origin is a promising strategy for treatment of acute and chronic liver failure. We examined possible utility of hepatocyte-like cells induced from bone marrow cells for such a purpose. Clonal cell lines were established from the bone marrow of two different rat strains. One of these cell lines, rBM25/S3 cells, grew rapidly (doubling time, approximately 24 hours) without any appreciable changes in cell properties for at least 300 population doubling levels over a period of 300 days, keeping normal diploid karyotype. The cells expressed CD29, CD44, CD49b, CD90, vimentin, and fibronectin but not CD45, indicating that they are of mesenchymal cell origin. When plated on Matrigel with hepatocyte growth factor and fibroblast growth factor-4, the cells efficiently differentiated into hepatocyte-like cells that expressed albumin, cytochrome P450 (CYP) 1A1, CYP1A2, glucose 6-phosphatase, tryptophane-2,3-dioxygenase, tyrosine aminotransferase, hepatocyte nuclear factor (HNF)1 alpha, and HNF4alpha. Intrasplenic transplantation of the differentiated cells prevented fatal liver failure in 90%-hepatectomized rats. In conclusion, a clonal stem cell line derived

from adult rat bone marrow could differentiate into hepatocyte-like cells, and transplantation of the differentiated cells could prevent fatal liver failure in 90%-hepatectomized rats. The present results indicate a promising strategy for treating human fatal liver diseases.

Muller-Sieburg, C. E., C. A. Whitlock, et al. (1986). "Isolation of two early B lymphocyte progenitors from mouse marrow: a committed pre-pre-B cell and a clonogenic Thy-1-lo hematopoietic stem cell." Cell **44**(4): 653-62.

Two novel early B lymphocyte precursor populations have been identified by their capacity to differentiate in Whitlock-Witte bone marrow cultures. Cells expressing neither the B lineage antigen B220 nor Thy-1 contain committed B cell precursors which differentiate in short-term culture into pre-B and B cells. The other population expresses low levels of Thy-1, and lacks B220 as well as the T cell markers L3T4 and Lyt-2. The Thy-1+ cells which initiate long-term B cell cultures contain clonogenic B cell precursors at a frequency of 1 in 11, a 100-fold enrichment over unseparated bone marrow. Thy-1+ cells are also highly enriched for myeloid-erythroid precursors (CFU-S). Thy-1+ cells allow long-term survival of lethally irradiated mice and fully reconstitute the hematopoietic system, including T and B lymphocyte compartments. These results indicate that this population (approximately 0.1% of bone marrow) may contain the pluripotent hematopoietic stem cell.

Munsie, M. J., A. E. Michalska, et al. (2000). "Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei." Curr Biol **10**(16): 989-92.

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.

Nilsson, L., I. Astrand-Grundstrom, et al. (2000). "Isolation and characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes: evidence for involvement at the hematopoietic stem cell level." Blood **96**(6): 2012-21.

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders characterized by ineffective hematopoiesis and frequent progression to acute myeloid leukemia. Within MDS, 5q-syndrome constitutes a distinct clinical entity characterized by an isolated deletion of the long arm of chromosome 5 (5q-), a relatively good prognosis, and infrequent transformation to acute leukemia. The cell of origin in 5q- syndrome as well as in other 5q-deleted MDS patients has not been established, but evidence for involvement of multiple myeloid (but not lymphoid) lineages has suggested that a myeloid-restricted progenitor rather than a pluripotent (lympho-myeloid) stem cell might be the primary target in most patients. Although in 9 patients no evidence of peripheral blood T-cell and only 1 case of B-cell involvement was found, the data herein support that 5q deletions occur in hematopoietic stem cells (HSCs) with a combined lympho-myeloid potential. First, in all investigated patients a minimum of 94% of cells in the minor CD34(+)CD38(-) HSC compartment were 5q deleted as determined by fluorescence in situ hybridization. Second, in 3 of 5 patients 5q aberrations were detected in a large fraction (25% to 90%) of purified CD34(+)CD19(+) pro-B cells. Furthermore, extensive functional characterization with regard to responsiveness to early-acting cytokines, long-term culture-initiating cells, and nonobese diabetic/severe combined immunodeficiency repopulating cells supported that MDS HSCs in 5q-deleted patients are CD34(+)CD38(-), but inefficient at reconstituting hematopoiesis.

Nishimune, Y., Y. Nishina, et al. (1989). "Isolation of mutants showing temperature-sensitive cell growth

from embryonal carcinoma cells: control of stem cell differentiation by incubation temperatures." Biochem Biophys Res Commun **165**(1): 65-72.

Embryonal carcinoma(EC) cells, the undifferentiated stem cells of teratocarcinomas, have many properties in common with pluripotent embryonic cells, and thus provide an excellent system for studying the early events involved in embryonic development and stem cell differentiation. We have isolated three novel mutants with temperature-sensitive(ts) cell growth that were able to differentiate at a non-permissive temperature for cell growth. These mutations affect the progression of the cell cycle, leading to the transient accumulation of cells in a specific phase, the S phase, of the cell cycle, which is likely to be the primary cause of stem cell differentiation of EC cells at non-permissive temperature. Isolation of these mutants strongly supports the notion that there is a close association between the inhibition of DNA synthesis and EC cell differentiation.

Ohyama, M. (2007). "William J. Cunliffe Scientific Awards. Advances in the study of stem-cell-enriched hair follicle bulge cells: a review featuring characterization and isolation of human bulge cells." Dermatology **214**(4): 342-51.

Hair follicles repeatedly regress and reconstitute themselves, suggesting the presence of intrinsic tissue stem cells. Using label-retaining cell technique to detect slow-cycling stem cells, hair follicle stem cells were detected in the bulge region of the outer root sheath, which provides the insertion point for the arrector pili muscle and marks the bottom of the permanent portion of hair follicles. Later studies elucidated important stem cell characteristics of the bulge cells, including high proliferative capacity and multipotency to regenerate the pilosebaceous unit as well as epidermis. Isolation of living bulge cells is now feasible. In addition, microarray analyses revealed the global gene expression profile of the bulge cells. However, most of those studies were performed in mouse hair follicles and our understanding of human bulge cells has been limited. Recently, remarkable progress was made in human bulge cell biology. The morphologically ill-defined human bulge boundary was precisely determined by the distribution of label-retaining cells. Laser capture microdissection enabled accurate isolation of human bulge cells and control cell populations. Microarray comparison analyses between isolated bulge and nonbulge cells elucidated the molecular signature of human bulge cells and identified cell surface markers for living bulge cell isolation. Importantly, isolated living human bulge cells demonstrated stem cell characteristics in vitro. In this review, recent advances in hair follicle bulge cell research are summarized, especially focusing on the characterization and isolation of human bulge cells.

Ohyama, M., A. Terunuma, et al. (2006). "Characterization and isolation of stem cell-enriched human hair follicle bulge cells." J Clin Invest **116**(1): 249-60.

The human hair follicle bulge is an important niche for keratinocyte stem cells (KSCs). Elucidation of human bulge cell biology could be facilitated by analysis of global gene expression profiles and identification of unique cell-surface markers. The lack of distinctive bulge morphology in human hair follicles has hampered studies of bulge cells and KSCs. In this study, we determined the distribution of label-retaining cells to define the human anagen bulge. Using navigated laser capture microdissection, bulge cells and outer root sheath cells from other follicle regions were obtained and analyzed with cDNA microarrays. Gene transcripts encoding inhibitors of WNT and activin/bone morphogenic protein signaling were overrepresented in the bulge, while genes responsible for cell proliferation were underrepresented, consistent with the existence of quiescent noncycling KSCs in anagen follicles. Positive markers for bulge cells included CD200, PHLDA1, follistatin, and frizzled homolog 1, while CD24, CD34, CD71, and CD146 were preferentially expressed by non-bulge keratinocytes. Importantly, CD200+ cells (CD200hiCD24loCD34loCD71loCD146lo) obtained from hair follicle suspensions demonstrated high colony-forming efficiency in clonogenic assays, indicating successful enrichment of living human bulge stem cells. The stem cell behavior of enriched bulge cells and their utility for gene therapy and hair regeneration will need to be assessed in in vivo assays.

Orlic, D., S. L. Laprise, et al. (1999). "Isolation of stem cell-specific cDNAs from hematopoietic stem cell populations." Ann N Y Acad Sci **872**: 243-54; discussion 254-5.

We have begun to isolate gene sequences that are specifically expressed in hematopoietic stem cells (HSCs). There are at least three fundamental requirements for the isolation of HSC-specific transcripts. First, highly enriched populations of HSCs, and an HSC-depleted cell population for comparison must be isolated. Secondly, the gene isolation procedures must be adapted to accommodate the small amounts of RNA obtained from purified HSCs. Finally, a defined screening strategy must be developed to focus on sequences to be examined in more detail. In this report, we describe the characterization of populations of HSCs that are

highly enriched (Lin- c-kitHI) or depleted (Lin- c-kitNEG) of HSCs. We compared two methods for gene isolation, differential display polymerase chain reaction (DD-PCR) and subtractive hybridization (SH), and found that the latter was more powerful and efficient in our hands. Lastly we describe the strategy that we have developed to screen clones for further study.

Ploemacher, R. E. and N. H. Brons (1988). "Isolation of hemopoietic stem cell subsets from murine bone marrow: I. Radioprotective ability of purified cell suspensions differing in the proportion of day-7 and day-12 CFU-S." Exp Hematol **16**(1): 21-6.

We have studied the ability of bone marrow cell suspensions greatly differing in the relative proportion of day-7 and day-12 spleen colony-forming units (CFU-S) to rescue mice from radiation-inflicted death, and to repopulate the irradiated bone marrow and spleen with nucleated cells. Counterflow centrifugal elutriation in combination with removal of adherent cells and fluorescence-activated cell sorting on differences in wheat germ agglutinin (WGA)-fluorescein isothiocyanate (FITC) affinity and light scatter properties were used consecutively to enrich large numbers of hemopoietic stem cells from mouse bone marrow. Enrichments of 50- to 200-fold have been achieved for day-12 CFU-S and radioprotective ability (RPA), permitting 50% of lethally irradiated mice to survive over a period of 30 days with as few as 50-80 donor cells. The ratio of day-7 and day-12 CFU-S in the various suspensions could be significantly modulated on the basis of their WGA binding and perpendicular light scatter characteristics. This finding enabled us to investigate the properties of day-7 and day-12 CFU-S with respect to their RPA. We found a highly significant log/log relationship between enrichment factors for (1) RPA, (2) the number of day-12 CFU-S, and (3) spleen cellularity as measured on day 13. In addition, similar numbers of sorted and unfractionated day-12 CFU-S were required to obtain the same level of protection. Enrichment for RPA was significantly less related to either the number of day-7 CFU-S injected, or the bone marrow cellularity of the irradiated mice on day 13.

Ploemacher, R. E. and N. H. Brons (1988). "Isolation of hemopoietic stem cell subsets from murine bone marrow: II. Evidence for an early precursor of day-12 CFU-S and cells associated with radioprotective ability." Exp Hematol **16**(1): 27-32.

Counterflow centrifugal elutriation (CCE) in combination with plastic adherence and fluorescence-activated cell sorting were used consecutively to enrich functionally different subpopulations of pluripotent hemopoietic stem cells (HSC) from mouse bone marrow. The nonadherent CCE fractions were labeled with wheat germ agglutinin (WGA)-fluorescein isothiocyanate (FITC) and sorted according to differences in fluorescence within various windows on the basis of forward (FLS) and perpendicular (PLS) light scatter. The sorted cells were then assayed for their (1) in vivo colony-forming ability (day-7 and day-12 spleen colony-forming units [CFU-S]), (2) radioprotective ability (RPA; 30-day survival), and (3) their ability to repopulate the bone marrow or spleen over a 13-day period with day-12 CFU-S, granulocyte-macrophage colony-forming units (CFU-GM), nucleated cells, or cells associated with RPA. The highest incidence of day-12 CFU-S and cells with RPA was obtained by sorting the most WGA-positive cells with relatively high PLS (enrichment, 50- to 200-fold), lowering the effective dose (ED 50/30) to an average of 80 cells. The separative procedure enabled hemopoietic stem cells that repopulate both bone marrow and spleen with secondary RPA cells, CFU-S-12, and CFU-GM to be enriched and separated from part of the RPA cells, CFU-S-12, and cells that reconstitute the cellularity of bone marrow and spleen. These data suggest that cells generating both day-12 CFU-S and RPA cells differ from day-12 CFU-S and RPA cells themselves on the basis of PLS characteristics and affinity for WGA. Such early stem cells have also been detected in sorted fractions meeting the FLS/PLS characteristics of lymphocytes.

Ponti, D., A. Costa, et al. (2005). "Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties." Cancer Res **65**(13): 5506-11.

Breast cancer-initiating cells have been recently identified in breast carcinoma as CD44+/CD24(-/low) cells, which exclusively retain tumorigenic activity and display stem cell-like properties. However, at present, direct evidence that breast cancer-initiating cells can be propagated in vitro is still lacking. We report here the isolation and in vitro propagation of breast cancer-initiating cells from three breast cancer lesions and from an established breast carcinoma cell line. Our breast carcinoma-derived cultures encompassed undifferentiated cells capable of self-renewal, extensive proliferation as clonal nonadherent spherical clusters, and differentiation along different mammary epithelial lineages (ductal and myoepithelial). Interestingly, cultured cells were CD44+/CD24- and Cx43-, overexpressed neoangiogenic and cytoprotective factors, expressed the putative stem cell marker Oct-4, and gave rise to new tumors when as few as 10(3) cells were injected into the mammary fat

pad of SCID mice. Long-term cultures of breast tumorigenic cells with stem/progenitor cell properties represent a suitable in vitro model to study breast cancer-initiating cells and to develop therapeutic strategies aimed at eradicating the tumorigenic subpopulation within breast cancer.

Pountos, I., D. Corscadden, et al. (2007). "Mesenchymal stem cell tissue engineering: techniques for isolation, expansion and application." Injury **38 Suppl 4**: S23-33.

Mesenchymal stem cells (MSCs) are undifferentiated multipotent cells which reside in various human tissues and have the potential to differentiate into osteoblasts, chondrocytes, adipocytes, fibroblasts and other tissues of mesenchymal origin. In the human body they could be regarded as readily available reservoirs of reparative cells capable to mobilize, proliferate and differentiate to the appropriate cell type in response to certain signals. These properties have triggered a variety of MSC-based therapies for pathologies including nonunions, osteogenesis imperfecta, cartilage damage and myocardial infarction. The outcome of these approaches is influenced by the methodologies and materials used during the cycle from the isolation of MSCs to their re-implantation. This review article focuses on the pathways that are followed from the isolation of MSCs, expansion and implantation.

Qiang, L., Y. Yang, et al. (2009). "Isolation and characterization of cancer stem like cells in human glioblastoma cell lines." Cancer Lett **279**(1): 13-21.

To identify and compare the features of stem like cells in human glioblastoma cell lines U251, U87MG, A172 with primary cultured glioblastoma stem cells, the ratio of CD133+ cells, the ability of tumor sphere formation, and self-renewing capacity of U251, U87MG, A172 cells in serum free medium plus EGF, bFGF and B27 supplement were detected. The results suggested that there might be more cancer stem like cells in U251 cells compared with others. CD133+ cells enriched in SP cells and in U251 cells cultured with the serum free medium. They expressed the neural stem cell markers CD133 and Nestin, but lacked of neuronal and astrocyte marker MAP2, beta-III tubulin and GFAP. They could apparently generate both neurons and glial cells after serum retrieved in vitro. Gli1, Bmi1, Notch2 and PTEN were also found expressed highly in them. Moreover, CD133+ cells were more resistant to hypoxia, irradiations and some chemotherapeutics than CD133-cells. So we suggested that glioblastoma stem like cells were existed in CD133+ cells in U251 cell line with characteristics of self-renew and generation of an unlimited progeny of non-tumorigenic cells. Molecular and functional characterization of such a tumorigenic population may be exploited in the development of novel cancer therapeutic drugs.

Raso, A., F. Negri, et al. (2008). "Successful isolation and long-term establishment of a cell line with stem cell-like features from an anaplastic medulloblastoma." Neuropathol Appl Neurobiol **34**(3): 306-15.

AIMS: Herein we report on the successful isolation and establishment of a novel, long-term, primary, neurosphere-like cell line called 1603-MED from a 5-year-old boy affected by a highly aggressive anaplastic medulloblastoma. METHODS: Elaboration of the new protocol for neurosphere assay is extensively discussed, together with a complete immuno-histochemical and cytogenetic characterization of 1603-MED. RESULTS: Clinical course and histopathology are briefly discussed. The 1603-MED possesses a high capacity for proliferation, CD133 expression, self-renewal and differentiation, thus indicating that anaplastic medulloblastoma contains a subpopulation of cancer stem cells as observed in classic medulloblastoma. CONCLUSIONS: 1603-MED provides us with the first in vitro model of anaplastic medulloblastoma that may be suitable for studying both tumour progression and the genetic mechanisms related to therapy resistance, and may lead to the development and testing of chemosensitivity and new therapeutic targets.

Rietze, R. L. and B. A. Reynolds (2006). "Neural stem cell isolation and characterization." Methods Enzymol **419**: 3-23.

Throughout the process of development and continuing into adulthood, stem cells function as a reservoir of undifferentiated cell types, whose role is to underpin cell genesis in a variety of tissues and organs. In the adult, they play an essential homeostatic role by replacing differentiated tissue cells "worn off" by physiological turnover or lost to injury or disease. As such, the discovery of such cells in the adult mammalian central nervous system (CNS), an organ traditionally thought to have little or no regenerative capacity, was most unexpected. Nonetheless, by employing a novel serum-free culture system termed the neurosphere assay, Reynolds and Weiss demonstrated the presence of neural stem cells in both the adult (Reynolds and Weiss, 1992) and embryonic mouse brain (Reynolds et al., 1992). Here we describe how to generate, serially passage, and differentiate neurospheres derived from both the developing and adult brain, and provide more technical details that will enable one to achieve reproducible cultures,

which can be passaged over an extended period of time.

Rowley, S. D., M. Loken, et al. (1998). "Isolation of CD34+ cells from blood stem cell components using the Baxter Isolex system." Bone Marrow Transplant **21**(12): 1253-62.

The CD34 antigen is expressed by human hematopoietic progenitor and stem cells. These cells are capable of reconstituting marrow function after marrow-ablative chemo-radiotherapy. Several different technologies have been developed for the separation of CD34+ cells from bone marrow or peripheral blood stem cell (PBSC) components. We used an immunomagnetic separation technique to enrich CD34+ cells from PBSC components in anticipation of autologous transplantation for patients with B lymphoid malignancies. Twenty-nine patients enrolled on this study and received mobilization chemotherapy followed by G-CSF. Of these, 21 achieved a peripheral blood CD34+ cell level of at least 2.0 x 10(4)/l required by protocol for separation of the stem cell components. A median of three components per patient was collected for processing. The average CD34+ cell concentration in the components after apheresis was 1.0 +/- 1.2%. After the CD34+ cell selection, the enriched components contained 0.6 +/- 0.6% of the starting nucleated cells. The recovery of CD34+ cells, however, averaged 58.4 +/- 19.2% of the starting cell number, with a purity of 90.8 +/- 6.5%. Overall depletion of CD34- cells was 99.96 +/- 0.06%. Nineteen patients were treated with marrow-ablative conditioning regimens and received an average of 6.2 +/- 2.0 x 10(6) CD34+ cells/kg body weight. These patients recovered to an ANC >0.5 x 10(9)/l at a median of 11 days (range 8-14), and platelet transfusion independence at a median of 9 days (range 5-13). Four patients died of transplant-related complications or relapse before 100 days after transplantation. No patient required infusion of unseparated cells because of failure of sustained bone marrow function. These data demonstrate that peripheral blood-derived CD34+ cells enriched by use of an immunomagnetic separation technique are capable of rapid engraftment after autologous transplantation.

Rudland, P. S., G. Ollerhead, et al. (1989). "Isolation of simian virus 40-transformed human mammary epithelial stem cell lines that can differentiate to myoepithelial-like cells in culture and in vivo." Dev Biol **136**(1): 167-80.

Simian Virus 40 (SV40) transformation of primary cultures of human mammary epithelial cells has yielded a cloned epithelial-like cell line and a representative, single-cell subclone. Although apparently homogeneous, both cloned cell lines can also yield small numbers of three other cell types. The more-elongated cell type can be obtained directly by replating cells from the medium of the epithelial-like cell cultures or by picking and culturing single cells to form representative lines. Immunofluorescent and immunocytochemical analysis of these cell lines growing on plastic or as tumor-nodules in nude mice for epithelial membrane antigens, various cytokeratins, various actins, laminin, Type IV collagen, the common acute lymphoblastic leukemia antigen (CALLA), and a 135-kDa glycoprotein confirm the epithelial nature of the epithelial-like cells and suggest a myoepithelial origin for the more-elongated cell type. Ultrastructural analysis largely confirms the results, although the myofilamental bundles can be scanty in the growing myoepithelial-like cells. The other two cell types are possibly related to the keratinizing and casein-secreting cells seen in the epithelial tumor-nodules before and after mating the mice, respectively. The myoepithelial-like cells produce 5- to 17-fold more laminin, Type IV collagen, CALLA, and the 135-kDa glycoprotein than the epithelial cells, and all of these antigens are preferentially found on myoepithelial cells in vivo. It is suggested that the SV40-transformed epithelial cell is an immortalized form of human mammary stem cell which can differentiate in culture and in vivo to myoepithelial-like cells.

Russell, J. A., A. Chaudhry, et al. (2000). "Early outcomes after allogeneic stem cell transplantation for leukemia and myelodysplasia without protective isolation: a 10-year experience." Biol Blood Marrow Transplant **6**(2): 109-14.

Although it is common practice to use some form of isolation to protect allogeneic stem cell transplant patients from infection, the necessity for these practices in all environments has not been demonstrated. The current study evaluated patterns of infection and 100-day transplant-related mortality in 288 patients with myelodysplasia and leukemia transplanted without isolation. Patients were allowed out of hospital at any time within constraints of the medication schedule. Fever, foci of infection, and positive cultures within 28 days and death within 100 days because of the transplant procedure were recorded. Fever occurred in 57% of patients, and 10% had a clinical or radiographic focus of infection. Most infections were apparently endogenous; blood cultures from 24% of recipients grew organisms, 87% of which were gram-positive bacteria. Four patients (1%) died with aspergillus infection in circumstances indicating that isolation would not have been helpful. Twenty percent of patients remained without evidence of infection throughout. Transplant-related mortality

at 100 days was 1% for 108 patients with early leukemia receiving transplants from matched siblings. For patients at higher risk, by virtue of donor and/or disease status, mortality was 21%. These figures compare favorably with those reported to the International Bone Marrow Transplant Registry, the majority of patients having been subjected to some form of isolation. We conclude that allogeneic stem cell transplantation can be safely performed in some environments without confining patients continuously to the hospital.

Safdar, A., S. Singhal, et al. (2004). "Clinical significance of non-Candida fungal blood isolation in patients undergoing high-risk allogeneic hematopoietic stem cell transplantation (1993-2001)." Cancer **100**(11): 2456-61.

BACKGROUND: The clinical relevance of mold isolated from blood cultures, even in severely immunosuppressed allogeneic hematopoietic stem cell transplantation (HSCT) recipients, remains uncertain. The authors hypothesized that isolation of non-Candida fungi from blood cultures in patients undergoing high-risk HSCT would have clinical significance. METHODS: The authors reviewed the records of 73 allogeneic HSCT recipients between January 1, 1993 and January 1, 2001 in whom fungal species were isolated from blood cultures. RESULTS: Fifty-two episodes of non-Candida fungemia occurred in 48 patients (66%) after a median of 10 days (range, 2-341) after transplantation. All 48 patients had indwelling intravascular catheters, and 23 patients (48%) had profound neutropenia. Thirty-five of 48 patients had received partially matched, related donor stem cell grafts (19 patients had 3-antigen-mismatched grafts); 35 patients had undergone T-cell depleted transplantation and 9 patients were receiving treatment for acute graft-versus-host disease. In 5 of 48 patients (10%), death was attributed to fungemia that occurred 8-11 days after the initial fungal blood culture was obtained; all 5 patients were age > 30 years. No deaths occurred in the younger age group (n = 22 patients; P = 0.05). In the 24 patients who did not receive systemic antifungal therapy, 4 deaths (17%) were attributed to infections with Penicillium (n = 2 patients), Epicoccum (n = 1 patient), or Penicillium plus Cladosporium species (n = 1 patient). Of the 24 patients who received amphotericin B, only 1 patient (4%) died as a result of a probable hematogenous Aspergillus species infection; this difference in outcome, however, was not significant (P = 0.2). CONCLUSIONS: Most of the non-Candida fungal blood culture isolates in recipients of high-risk, mismatched donor transplantation were clinically nonsignificant. However, because these low-virulence saprophytes occasionally may cause life-threatening disease, a reevaluation of the existing diagnostic paradigm is needed so that clinically significant fungemia may be differentiated from pseudofungemia.

Salaway, T. and D. Ilic (2008). "Logistics of stem cell isolation, preparation and delivery for heart repair: concerns of clinicians, manufacturers, investors and public health." Regen Med **3**(1): 83-91.

Recent developments in stem cell (SC) research challenge the long-held paradigm that the human heart cannot be repaired. While SC therapies for cardiac disease may not be available as soon as the public believes, it is important for investors, providers and clients to begin considering the expertise and facilities SC therapies may eventually require. Here we review several logistical issues that are integral to the development and delivery of SC therapy for cardiac disease. In the near future, quality control measures and sources of progenitor cells will be key determinants of treatment costs and clinical and research infrastructures. SC research and therapeutic development will yield greatest payoffs for patients and investors if insurance coverage can be obtained for therapeutic applications. This will require rigorous FDA review and approval for therapeutic use and Centers for Medicare and Medicaid Services coverage decisions.

Shpall, E. J., A. Gee, et al. (1995). "Stem cell isolation." Curr Opin Hematol **2**(6): 452-9.

High-dose chemotherapy with autologous hematopoietic progenitor cell support is increasingly used to treat a variety of malignant diseases. A drawback of this technique is the potential for infusing clonogenic tumor cells with the autograft, producing relapse of the disease in the patient. The use of positive selection techniques to isolate stem cells and thus reduce or eliminate tumor cell contamination has been extensively studied over the past few years. Preliminary clinical results have demonstrated that these procedures deplete 2 to 7 logs of tumor cells and do not impair engraftment. It is too early to assess the ultimate clinical benefit of this strategy. Additional applications of CD34-selection include ex vivo expansion of and gene transfer into hematopoietic progenitor cells and T-cell depletion of allogeneic grafts to reduce the incidence of graft-versus-host disease.

Singhatanadgit, W., N. Donos, et al. (2009). "Isolation and characterization of stem cell clones from adult human ligament." Tissue Eng Part A **15**(9): 2625-36.

Cells derived from the periodontal ligament (PDL) have previously been reported to have stem cell-like characteristics and to play an important part in re-building damaged tissue, including alveolar

bone. However, these populations have been heterogeneous, and thus far no highly purified periodontal stem cell (PSC) clone has yet been established from adult human PDL tissue. The present study was therefore carried out to isolate single cell-derived PDL clones and to delineate their phenotypic and functional characteristics. In this report we have obtained four homogeneous and distinct clones--namely, C5, C6, C7, and C8--and have found these to be highly proliferative and to express the stromal cell markers CD29 and CD44. In particular, C7 showed stem cell-like characteristics of small cell size with reduced cytoplasm, clonogenicity, and multilineage potential, including osteogenic activity in forming bone-like tissue in organoid micromass cultures. Clones C5 and C6 possessed osteoprogenitor features with mineralized matrix-forming activity, whereas C8 did not undergo osteogenic, adipogenic, or chondrogenic differentiation. The present study thus reports, for the first time, the isolation and cellular and molecular characterization of highly purified putative PSC and osteoprogenitors in adult human PDL, based on clonogenicity and multilineage differentiation potential, with PSC-C7 capable of bone formation in vitro, suggesting that such cells may have potential value for stem cell-based bone tissue engineering in vivo.

Stemple, D. L. and D. J. Anderson (1992). "Isolation of a stem cell for neurons and glia from the mammalian neural crest." Cell **71**(6): 973-85.

We have isolated mammalian neural crest cells using a monoclonal antibody to the low affinity NGF receptor, and established conditions for the serial propagation of these cells in clonal culture to assess their developmental potential. This analysis indicates that, first, single mammalian neural crest cells are multipotent, able to generate at least neurons and Schwann cells like their avian counterparts. Second, multipotent neural crest cells generate multipotent progeny, indicating that they are capable of self-renewal and therefore are stem cells. Third, multipotent neural crest cells also generate some clonal progeny that form only neurons or glia, suggesting the production of committed neuroblasts and glioblasts. Manipulation of the substrate alters the fate of the multipotent cells. These findings have implications for models of neural crest development in vivo, and establish a system for studying the generation of cellular diversity by a multipotent stem cell in vitro.

Stewart, M. H., M. Bosse, et al. (2006). "Clonal isolation of hESCs reveals heterogeneity within the pluripotent stem cell compartment." Nat Methods **3**(10): 807-15.

Human embryonic stem cell (hESC) lines are known to be morphologically and phenotypically heterogeneous. The functional nature and relationship of cells residing within hESC cultures, however, has not been evaluated because isolation of single hESCs is limited to drug or manual selection. Here we provide a quantitative method using flow cytometry to isolate and clonally expand hESCs based on undifferentiated markers, alone or in combination with a fluorescent reporter. This method allowed for isolation of stage-specific embryonic antigen-3-positive (SSEA-3+) and SSEA-3- cells from hESC cultures. Although both SSEA-3+ and SSEA-3- cells could initiate pluripotent hESC cultures, we show that they possess distinct cell-cycle properties, clonogenic capacity and expression of ESC transcription factors. Our study provides formal evidence for heterogeneity among self-renewing pluripotent hESCs, illustrating that this isolation technique will be instrumental in further dissecting the biology of hESC lines.

Strom, S., J. Inzunza, et al. (2007). "Mechanical isolation of the inner cell mass is effective in derivation of new human embryonic stem cell lines." Hum Reprod **22**(12): 3051-8.

BACKGROUND: For clinical grade human embryonic stem cell (hESC) lines, a robust derivation system without any substances having animal origin would be required. We have gradually improved our hESC derivations. Human skin fibroblasts were used as feeder cells in derivation of all our 25 permanent fully characterized hESC lines. In the first four derivations, fetal calf serum was used as a supplement in the medium, thereafter, serum replacement medium was used. Immunosurgery generally used for isolation of the inner cell mass (ICM) still involves animal serum and complement. METHODS: We developed a practical mechanical isolation method for the ICM. Two flexible metal needles with sharpened tips, 0.125 mm in diameter, were used to open the zona pellucida and extract the ICM under a stereomicroscope. Immunohistochemical and karyotype characterization of the new hESC lines was carried out, and pluripotency was tested in vitro (immunocytochemistry and RT-PCR) and in vivo (teratoma growth). RESULTS: Five hESC lines were obtained from 19 supernumerary blastocysts collected in 2005-2006 (26%), whereas in similar conditions, we obtained 16 lines from 100 blastocysts (16%) using immunosurgery in 2003-2005. The new lines had a normal karyotype and tissues originating from the three embryonic germ cell layers were present. CONCLUSIONS: Mechanical isolation of the ICM proved to be an effective way to derive new hESC lines. The technique is fast, does not require any extra

investment and the xeno-components of immunosurgery could be avoided.

Sukoyan, M. A., A. N. Golubitsa, et al. (1992). "Isolation and cultivation of blastocyst-derived stem cell lines from American mink (Mustela vison)." Mol Reprod Dev **33**(4): 418-31.

Ten embryonic stem (ES) cell lines from mink blastocysts were isolated and characterized. All the lines had a normal diploid karyotype; of the ten lines studied, five had the XX and five had the XY constitution. Testing of the pluripotency of the ES-like cells demonstrated that 1) among four lines of genotype XX, and X was late-replicating in three; both Xs were active in about one-third of cells of line MES8, and analysis of glucose-6-phosphate dehydrogenase revealed no dosage compensation for the X-linked gene; 2) when cultured in suspension, the majority of lines were capable of forming "simple" embryoid bodies (EB), and two only showed the capacity for forming "cystic" multilayer EBs. However, formation of ectoderm or foci of yolk sac hematopoiesis, a feature of mouse ES cells, was not observed in the "cystic" EB; 3) when cultured as a monolayer without feeder, the ES cells differentiated into either vimentin-positive fibroblast-like cells or cytokeratin-positive epithelial-like cells (less frequently); neural cells appeared in two lines; 4) when injected into athymic mice, only one of the four tested lines gave rise to tumors. These were fibrosarcomas composed of fibroblast-like cells, with an admixture of smooth muscular elements and stray islets of epithelial tissue; (5) when the ES cells of line MES1 were injected into 102 blastocyst cavities and subsequently transplanted into foster mothers, we obtained 30 offspring. Analysis of the biochemical markers and coat color did not demonstrate the presence of chimaeras among offspring. Thus the cell lines derived from mink blastocysts are true ES cells. However, their pluripotential capacities are restricted.

Szilvassy, S. J., P. M. Lansdorp, et al. (1989). "Isolation in a single step of a highly enriched murine hematopoietic stem cell population with competitive long-term repopulating ability." Blood **74**(3): 930-9.

A simple procedure is described for the quantitation and enrichment of murine hematopoietic cells with the capacity for long-term repopulation of lymphoid and myeloid tissues in lethally irradiated mice. To ensure detection of the most primitive marrow cells with this potential, we used a competitive assay in which female recipients were injected with male "test" cells and 1 to 2 x 10(5) "compromised" female marrow cells with normal short-term repopulating ability, but whose long-term repopulating ability had been reduced by serial transplantation. Primitive hematopoietic cells were purified by flow cytometry and sorting based on their forward and orthogonal light-scattering properties, and Thy-1 and H-2K antigen expression. Enrichment profiles for normal marrow, and marrow of mice injected with 5-fluorouracil (5-FU) four days previously, were established for each of these parameters using an in vitro assay for high proliferative potential, pluripotent colony-forming cells. When all four parameters were gated simultaneously, these clonogenic cells were enriched 100-fold. Both day 9 and day 12 CFU-S were copurified; however, the purity (23%) and enrichment (75-fold) of day 12 CFU-S in the sorted population was greater with 5-FU-treated cells. Five hundred of the sorted 5-FU marrow cells consistently repopulated recipient lymphoid and myeloid tissues (greater than 50% male, 1 to 3 months post-transplant) when co-injected with 1 to 2 x 10(5) compromised female marrow cells, and approximately 100 were sufficient to achieve the same result in 50% of recipients under the same conditions. This relatively simple purification and assay strategy should facilitate further analysis of the heterogeneity and regulation of stem cells that maintain hematopoiesis in vivo.

Takahashi, T., T. Kawai, et al. (2006). "Identification and isolation of embryonic stem cell-derived target cells by adenoviral conditional targeting." Mol Ther **14**(5): 673-83.

The technical limitations of isolating target cells have restricted the utility of pluripotent embryonic stem (ES) cells. For example, early cardiac (i.e., precontractile) cells have not been isolated from ES cells. Here, we find that direct expression of reporter genes under cell-specific promoters-the currently available strategy for isolating cells lacking cell-specific surface markers-is ineffective for isolating progenitor cells. This was due to the weak activity of cell-specific promoters, particularly in ES cells at early stages. We show that adenoviral conditional targeting efficiently isolates viable ES cell-derived target cells without harmful effects. In this strategy, we employ the alpha-myosin heavy chain and Nkx2.5 promoter to visualize and purify efficiently differentiated and primitive cells of the cardiac lineage, respectively. While the former cells predominantly expressed sarcomeric proteins and maintained contractile function, the latter demonstrated neither of these features, but rather exhibited expression patterns characteristic of a mixture of primitive cells and cardiomyocytes. Interestingly, smooth muscle actin was predominantly expressed in the latter cells, and both functionally known and unknown genes were systematically identified, demonstrating the benefits of this system.

Thus, our method facilitates molecular and cellular studies of development and ES cell-derived cell therapy.

Thomson, J. A., J. Kalishman, et al. (1995). "Isolation of a primate embryonic stem cell line." Proc Natl Acad Sci U S A **92**(17): 7844-8.

Embryonic stem cells have the ability to remain undifferentiated and proliferate indefinitely in vitro while maintaining the potential to differentiate into derivatives of all three embryonic germ layers. Here we report the derivation of a cloned cell line (R278.5) from a rhesus monkey blastocyst that remains undifferentiated in continuous passage for > 1 year, maintains a normal XY karyotype, and expresses the cell surface markers (alkaline phosphatase, stage-specific embryonic antigen 3, stage-specific embryonic antigen 4, TRA-1-60, and TRA-1-81) that are characteristic of human embryonal carcinoma cells. R278.5 cells remain undifferentiated when grown on mouse embryonic fibroblast feeder layers but differentiate or die in the absence of fibroblasts, despite the presence of recombinant human leukemia inhibitory factor. R278.5 cells allowed to differentiate in vitro secrete bioactive chorionic gonadotropin into the medium, express chorionic gonadotropin alpha- and beta-subunit mRNAs, and express alpha-fetoprotein mRNA, indicating trophoblast and endoderm differentiation. When injected into severe combined immunodeficient mice, R278.5 cells consistently differentiate into derivatives of all three embryonic germ layers. These results define R278.5 cells as an embryonic stem cell line, to our knowledge, the first to be derived from any primate species.

Tilesi, F., G. Coata, et al. (2000). "A new methodology of fetal stem cell isolation, purification, and expansion: preliminary results for noninvasive prenatal diagnosis." J Hematother Stem Cell Res **9**(4): 583-90.

We developed a combined methodological approach to enrich and to proliferate in vitro fetal CD34+ stem progenitor cells. Using a magnetic cell-sorting technique, CD34+ cells from pregnant women at the early-second trimester were isolated and enriched and compared to those isolated from blood of nonpregnant women. The number and frequency of CD34+ cells were significantly higher ($p < 0.001$) in the pregnant women. Unenriched peripheral blood mononuclear cells (PBMC) and enriched CD34+ cells were cultured in a methylcellulose system to evaluate the cloning potential of progenitor cells. After culture, the numbers of burst-forming units erythroid/colony-forming units erythroid (BFU-E/CFU-E) and colony-forming units granulocyte-macrophage (CFU-GM) colonies were increased by 33 and 16 times, respectively. Finally, to distinguish between fetal and maternal cells, four cases of cultured cells were hybridized with specific probes for X and Y chromosomes and two cases with a specific probe for chromosome 21. In normal pregnancies, we identified a high number of male fetal cells and an elevated fetal/maternal ratio. When we analyzed blood samples from pregnancies with trisomic fetuses, we scored a high ratio of trisomic cells respect to maternal cells that was significantly different from the ratio of pregnancies with normal fetuses. Our results demonstrate fetal progenitor cells may be cultured and detected successfully with an appropriate combined methodological approach, which may significantly increase the feasibility of noninvasive prenatal diagnosis.

Verma, V., S. K. Gautam, et al. (2007). "Isolation and characterization of embryonic stem cell-like cells from in vitro-produced buffalo (Bubalus bubalis) embryos." Mol Reprod Dev **74**(4): 520-9.

This study was carried out to isolate and characterize buffalo embryonic stem (ES) cell-like cells from in vitro-produced embryos. Inner cell mass (ICM) cells were isolated either mechanically or by enzymatic digestion from 120 blastocysts whereas 28 morulae were used for the isolation of blastomeres mechanically. The ICM cells/ blastomeres were cultured on mitomycin-C-treated feeder layer. Primary cell colony formation was higher ($P < 0.05$) for hatched blastocysts (73.1%, 30/41) than that for early/expanded blastocysts (25.3%, 20/79). However, no primary cell colonies were formed when blastomeres obtained from morulae were cultured. Primary colonies were formed in 14.1% (12/85) of intact blastocyst culture, which was significantly lower ($P < 0.05$) than that of 41.6% for ICM culture. These colonies were separated by enzymatic or mechanical disaggregation. Using mechanical disaggregation method, the cells remained undifferentiated and two buffalo ES cell-like cell lines (bES1, bES2) continued to grow in culture up to eight passages. However, disassociation through enzymatic method resulted in differentiation. Undifferentiated cells exhibited stem cell morphological features, normal chromosomal morphology, and expressed specific markers such as alkaline phosphatase (AP) and Oct-4. Cells formed embryoid bodies (EBs) in suspension culture; extended culture of EBs resulted in formation of cystic EBs. Following prolonged in vitro culture, these cells differentiated into several types of cells including neuron-like and epithelium-like cells. Furthermore, the vitrified-thawed ES cell-like cells also exhibited typical stem cell characteristics. In conclusion, buffalo ES cell-like

cells could be isolated from in vitro-produced blastocysts and maintained in vitro for prolonged periods of time.

Vescovi, A. L., E. A. Parati, et al. (1999). "Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation." Exp Neurol **156**(1): 71-83.

Stem cells that can give rise to neurons, astroglia, and oligodendroglia have been found in the developing and adult central nervous system (CNS) of rodents. Yet, their existence within the human brain has not been documented, and the isolation and characterization of multipotent embryonic human neural stem cells have proven difficult to accomplish. We show that the developing human CNS embodies multipotent precursors that differ from their murine counterpart in that they require simultaneous, synergistic stimulation by both epidermal and fibroblast growth factor-2 to exhibit critical stem cell characteristics. Clonal analysis demonstrates that human C NS stem cells are multipotent and differentiate spontaneously into neurons, astrocytes, and oligodendrocytes when growth factors are removed. Subcloning and population analysis show their extensive self-renewal capacity and functional stability, their ability to maintain a steady growth profile, their multipotency, and a constant potential for neuronal differentiation for more than 2 years. The neurons generated by human stem cells over this period of time are electrophysiologically active. These cells are also cryopreservable. Finally, we demonstrate that the neuronal and glial progeny of long-term cultured human CNS stem cells can effectively survive transplantation into the lesioned striatum of adult rats. Tumor formation is not observed, even in immunodeficient hosts. Hence, as a consequence of their inherent biology, human CNS stem cells can establish stable, transplantable cell lines by epigenetic stimulation. These lines represent a renewable source of neurons and glia and may significantly facilitate research on human neurogenesis and the development of clinical neural transplantation.

Vindigni, V., L. Michelotto, et al. (2009). "Isolation method for a stem cell population with neural potential from skin and adipose tissue." Neurol Res.

OBJECTIVE: In recent years, research on stem cells has been focused on the development of personalized cell-based therapies. Owing to their homing properties, adult human stem cells are a promising source of autologous cells to be used as therapeutic vehicles. Multiple potential sources for clinically useful stem and progenitor cells have been identified, including autologous and allogenic embryonic, fetal and adult somatic cells from neural, adipose and mesenchymal tissue. In the present report, we describe a simple protocol to obtain an enriched culture of adult stem cells organized in neurospheres from two post-natal tissues: skin and adipose tissue. METHODS: Adult stem cells isolated from skin and adipose tissue derived from the same adult donor were amplified under varying conditions related to the coating of the chamber slide and the presence of serum and/or growth factors, such as with EGF and FGF2. Neurospheres were then expanded and evaluated in terms of proliferation and gene expression. RESULTS: Adipose and skin derived neurospheres were comparable in size, quantity of cells and genes expressed. Cells from both types of tissue grew optimally without slide coating, in the presence of serum and with the combined addition of FGF2 and EGF. DISCUSSION: We describe a method for isolating and improving a population of multipotent adult precursor cells from the two most accessible adult tissue sources: skin and adipose tissue. This autologous adult stem cell population could be used for cell replacement or cell therapies.

Waddington, R. J., S. J. Youde, et al. (2009). "Isolation of distinct progenitor stem cell populations from dental pulp." Cells Tissues Organs **189**(1-4): 268-74.

The present study compared the cellular characteristics of progenitor stem cell populations present in adult dental pulp, isolated by different methods utilizing 2 different features of stem cell biology. One population expressing high levels of beta1 integrin was isolated by preferential selection of adherent cells to fibronectin over 20 min. In an alternative approach, cells expressing the embryonic neural crest cell marker, low-affinity nerve growth factor receptor (LANGFR), were selected by magnetic-activated cell sorting. For each method, clonal cell lines were established and expanded in culture. One clone derived via the respective methods was examined for embryonic/progenitor cell markers by immunocytochemistry and RT-PCR. Both clonal populations demonstrated the expression of stro-1 and stained positive for vimentin, demonstrating mesenchymal lineage. Of note, cells selected for LANGFR cells demonstrated the additional expression of CD105 and Notch 2. For both clonal populations, expanded cultures demonstrated the ability to differentiate into osteoblasts, adipocytes and chondrocytes. These results would suggest the potential isolation of 2 progenitor cell populations exhibiting different cellular characteristics in terms of their embryonic nature. The potential for both cell populations to derive from a common origin is discussed.

Wagner, J. E., D. Collins, et al. (1995). "Isolation of small, primitive human hematopoietic stem cells: distribution of cell surface cytokine receptors and growth in SCID-Hu mice." Blood **86**(2): 512-23.

Human CD34+ cells were subfractionated into three size classes using counterflow centrifugal elutriation followed by immunoadsorption to polystyrene cell separation devices. The three CD34+ cell fractions (Fr), Fr 25/29, Fr 33/37, and Fr RO, had mean sizes of 8.5, 9.3 and 13.5 microns, respectively. The majority of cells in the large Fr RO CD34+ cell population expressed the committed stage antigens CD33, CD19, CD38, or HLA-DR and contained the majority of granulocyte-macrophage colony-forming units (CFU-GM), burst-forming units-erythroid (BFU-E), and CFU-mixed lineage (GEMM). In contrast, the small Fr 25/29 CD34+ cells were devoid of committed cell surface antigens and lacked colony-forming activity. When seeded to allogeneic stroma, Fr RO CD34+ cells produced few CFU-GM at week 5, whereas cells from the Fr 25/29 CD34+ cell population showed a 30- to 55-fold expansion of myeloid progenitors at this same time point. Furthermore, CD34+ cells from each size fraction supported ontogeny of T cells in human thymus/liver grafts in severe combined immunodeficient (SCID) mice. Upon cell cycle analyses, greater than 97% of the Fr 25/29 CD34+ cells were in G0/G1 phase, whereas greater proportions of the two larger CD34+ cell fractions were in active cell cycle. Binding of the cytokines interleukin (IL)-1 alpha, IL-3, IL-6, stem cell factor (SCF), macrophage inhibitory protein (MIP)-1 alpha, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage (GM)-CSF to these CD34+ cell populations was also analyzed by flow cytometry. As compared with the larger CD34+ cell fractions, cells in the small Fr 25/29 CD34+ cell population possessed the highest numbers of receptors for SCF, MIP1 alpha, and IL-1 alpha. Collectively, these results indicate that the Fr 25/29 CD34+ cell is a very primitive, quiescent progenitor cell population possessing a high number of receptors for SCF and MIP1 alpha and capable of yielding both myeloid and lymphoid lineages when placed in appropriate in vitro or in vivo culture conditions.

Wolosin, J. M. (2006). "Cell markers and the side population phenotype in ocular surface epithelial stem cell characterization and isolation." Ocul Surf **4**(1): 10-23.

The ocular surface is covered by tworapidly renewing and embryologically-related linings, the corneal and conjunctival epithelia. The long-term survival of thesetissues is ultimately dependent on their respective resident stem cells. In the corneal epithelium, the stem cells and their early precursors are exclusively circumscribed to the narrow vasscularize limbal rim that provides epithelial precursor cells to the critically transparent central cornea. Limbal damage causes an interruption of this essential cell supply and allows the invasion of the corneal surface by the conjunctival epithelium, an event that ultimately leads to corneal scarring. The limited supply of immunocompatible tissue is a major hindrance to efforts to develop effective procedures for ocular surface reconstruction. This review describes some of the current work and strategies being developed to achieve the isolation of the limbal stem cell and define its genetic, biochemical, and functional make-up. The study of isolated ocular surface stem cells will foster basic understanding of the environmentalrequisites for their survival and proliferation in a self-replicative mode, leading eventually to advances in therapeutic approaches.

Wu, J., P. F. Mu, et al. (2005). "Parental experience of family stress during hematopoietic stem cell transplantation of pediatric patients in germ-free isolation in Taiwan." Cancer Nurs **28**(5): 363-71.

This qualitative inquiry study used convenience sampling through in-depth interviews to obtain life experiences and feelings of parents while their children underwent hematopoietic stem cell transplantation. Eleven parents of children younger than 18 years undergoing hematopoietic stem cell transplantation in a medical center in north Taiwan consented to participate in this study and provide oral and written responses in Mandarin or Taiwanese. Semistructured interviews lasting 60 minutes were conducted privately and audiotaped. Verbatim transcriptions of the interviews were examined by the content analysis method. The trustworthiness of the data was examined by Lincoln and Guba (Naturalistic Inquiry. Newbury Park, Calif: Sage; 1985) principles. Results indicated that family stress experiences of parents of children undergoing hematopoietic stem cell transplants include 4 themes: parental psychological distress, family lifestyle disturbances, parents' coping patterns, and family resources. The results of this study provide evidence of the nature of parents' experiences while their children undergo hematopoietic stem cell transplantation and provide guidelines for family-centered nursing care.

Yang, Y. C., S. W. Wang, et al. (2007). "Isolation and characterization of human gastric cell lines with stem cell phenotypes." J Gastroenterol Hepatol **22**(9): 1460-8.

AIM: The aim of this study was to develop an in vitro human gastric stem and/or progenitor cell model that may be used to study the mechanism of

gastric carcinogenesis induced by Helicobacter pylori infection. METHODS: Human gastric biopsy was minced and digested with collagenase and dispase and cultured in a low-calcium medium (serum-free keratinocyte medium; keratinocyte-SFM) supplemented with N-acetyl-L-cysteine and L-ascorbic acid 2-phosphate. Actively proliferating epithelial colonies with sustained growth were isolated and characterized for karyotype and phenotypes related to stem cell characteristics including proliferation and differentiation potential, ability of anchorage-independent growth (AIG), gap junctional intercellular communication (GJIC) and the expression of Oct-4, a transcription factor previously shown to be expressed in embryonic stem cells, adult stem cells and undifferentiated tumor cells. To study the carcinogenic effect of H. pylori infection, gastric stem and/or progenitor cells were incubated with H. pylori culture products and/or N-methyl-N-nitro-N-nitrosoguanidine (MNNG), a chemical carcinogen, to see the telomerase activation. RESULTS: Multiple cell lines with stem cell features were isolated by this new cell culture method. The results based on detailed characterization of one cell clone, KMU-GI2, revealed stem cell features of these cells. The initial clone contained mostly undifferentiated epithelial-like cells, which, upon subculture and propagation, gave rise to a heterogeneous cell population. Single cell-derived subclones, similar to the parental population, retained high differentiation potential and were capable of giving rise to many morphologically different cell types (i.e. epithelial-like, glial or neuron-like, round and various peculiar-shaped cells). Although these cells were normal in karyotype and competent in GJIC, they had the ability to grow in soft agar. Cells expressing epithelial membrane antigen (EMA), mucin 5AC, glial fibrillary acidic protein (GFAP), cytokeratin-18 (CK-18), trefoil factor 1 (TFF-1) and Oct-4 were found in the cell culture, but not E-cadherin-, gastrin- or telomerase-expressing cells. Furthermore, spontaneously immortalized non-tumorigenic clones could be derived from the cell population. After treating these cell cultures with the chemical carcinogen, MNNG and H. pylori culture products for 5 days, telomerase activity and telomerase mRNA expression were significantly elevated, while treatment with either of them showed no effect. CONCLUSION: The new cell culture method can be used to develop gastric epithelial cell clones with sustained growth from endoscopic biopsy. The gastric cell clone showed several stem and/or progenitor cell phenotypes (i.e. the ability of AIG, high differentiation capacity, high susceptibility to spontaneous immortalization and the expression of Oct-4). The telomerase expression in these gastric stem and/or progenitor cells can be upregulated by exposure to H. pylori culture products and MNNG, an important step in neoplastic transformation. These results show that putative human gastric stem and/or progenitor cell clones can be developed by our method and these cells could be useful for studying the mechanisms of human gastric carcinogenesis including the mechanism of action of H. pylori, as well as the regulation of the proliferation and differentiation of human gastric mucosa.

Yi, L., Z. H. Zhou, et al. (2007). "Isolation and characterization of stem cell-like precursor cells from primary human anaplastic oligoastrocytoma." Mod Pathol **20**(10): 1061-8.

A small population of stem cell-like precursors in solid tumors are linked to histological composition, progression, angiogenesis, metastasis, recurrence and drug resistance of a variety of malignant tumors. Oligoastrocytoma is the most common brain mixed glioma composed of mixed cells of oligodendroglial and astrocytic phenotypes. Identification and characterization of stem cell-like precursors in oligoastrocytoma may shed light on the oncogenesis of this unique type of tumor and assist in the design of novel therapeutic strategy. Here, tumor stem cell-like precursors were identified from primary human anaplastic oligoastrocytomas by labeling of the tumor sections with nestin and CD133. Tumor cells were cultured in vitro in stem cell medium with growth factors and the capacity of the surviving stem cell-like precursors to form tumor spheres was tested. The tumor spheres were further injected subcutaneously into nude mice to observe the contribution of stem cell-like precursors to histological composition and tumor progression. We found that primary human oligoastrocytoma tissues contained nestin+/CD133+ stem cell-like precursors. These cells differentiated into tumor cells with both oligodendroglial and astrocytic characteristics and formed tumor spheres in vitro, which upon implantation in nude mice, grew into tumor nodules containing nestin+/CD133+ cells at levels higher than in the primary tumor tissues. This study revealed for the first time that anaplastic human oligoastrocytomas contained stem cell-like precursors, which exhibit neural stem cell properties with tumorigenicity. These stem cell-like precursors may be responsible for the oligodendroglial and astrocytic components of human oligoastrocytoma and should be considered as therapeutic targets.

Yu, S. C., Y. F. Ping, et al. (2008). "Isolation and characterization of cancer stem cells from a human glioblastoma cell line U87." Cancer Lett **265**(1): 124-34.

A variety of malignant cancers have been found to contain a subpopulation of stem cell-like tumor cells, or cancer stem cells (CSCs). However, the existence of CSCs in U87, a most commonly used glioma cell line, is still controversial. In this study, we demonstrate that U87 cell line contained a fraction of tumor cells that could form tumor spheres and were enriched by progressively increasing the concentration of serum-free neural stem cell medium with or without low dose vincristine. These cells possessed the ability of self-renewal and multipotency, the defined characteristics of CSCs. Moreover, the tumors formed by the secondary spheres displayed typical histological features of human glioblastoma, including cellular pleomorphism, pseudopalisades surrounding necrosis, hyperchromatic nuclei, high density of microvessels and invasion to the brain parenchyma. These results indicate that gradually increasing the concentration of serum-free neural stem cell culture medium with or without vincristine is a simple and effective method for isolation of CSCs to study the initiation and progression of human glioblastoma.

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