Stem Cell Marker Literatures

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

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

Literature

Agbalika, F., J. Larghero, et al. (2007). "Epstein-Barr virus early-antigen antibodies before allogeneic haematopoietic stem cell transplantation as a marker of risk of post-transplant lymphoproliferative disorders." Br J Haematol **136**(2): 305-8.

The occurrence of post-transplant lymphoproliferative disorders (PTLDs) after allogeneic haematopoietic stem cell transplantation (allo-HSCT) represents a clinical problem. Pretransplant Epstein-Barr virus serological status and viral load was determined in 21 recipients and 28 control transplanted patients, with (+) and without (-) PTLD, respectively. Early-antigen immunoglobulin G (EA-IgG) was detected in 12/21 PTLD+ patients, but only 2/28 PTLD patients (P = 0.00023, Odds ratio = 17.42). High viral load was detected in peripheral blood mononuclear cells at PTLD diagnosis, independently of deleted LMP1. Detection of EA-IgG in allo-HSCT recipients pretransplantation might be considered as risk factor for PTLD development.

Ahmad, S., S. Kolli, et al. (2008). "A putative role for RHAMM/HMMR as a negative marker of stem cell-containing population of human limbal epithelial cells." Stem Cells **26**(6): 1609-19.

The corneal epithelium is maintained by stem cells located at the periphery of the cornea in a region known as the limbus. Depletion of limbal stem cells (LSCs) results in limbal stem cell deficiency. Treatments for this disease are based on limbal replacement or transplantation of ex vivo expanded LSCs. It is, therefore, crucial to identify cell surface markers for LSCs that can be used for their enrichment and characterization. Aldehyde dehydrogenases (ALDHs) are enzymes which protect cells from the toxic effects of peroxidic aldehydes. In this manuscript, we show for the first time that ALDH1 is absent from the basal cells of the limbal and corneal epithelium. We separated limbal epithelial cells on the basis of ALDH activity and showed that ALDH(dim) cells expressed significantly higher levels of DeltaNp63 and ABCG2 as well as having a greater colony forming efficiency (CFE) when compared to ALDH(bright) cells. Large scale transcriptional analysis of these two populations led to identification of a new cell surface marker, RHAMM/HMMR, which is located in all layers of corneal epithelium and in the suprabasal layers of the limbal epithelium but is completely absent from the basal layer of the limbus. Our studies indicate that absence of RHAMM/HMMR expression is correlated with properties associated with LSCs. RHAMM/HMMR- limbal epithelial cells are smaller in size, express negligible CK3, have higher levels of DeltaNp63 and have a higher CFE compared to RHAMM/HMMR+ cells. Taken together these results suggest a putative role for RHAMM/ HMMR as a negative marker of stem cell containing limbal epithelial cells. Cell selection based on Hoechst exclusion and lack of cell surface RHAMM/HMMR expression resulted in increased colony forming efficiency compared to negative selection using RHAMM/HMMR alone or positive selection using Hoechst on its own. Combination of these two cell selection methods presents a novel method for LSC enrichment and characterization. Disclosure of potential conflicts of interest is found at the end of this article.

Aisa, Y., T. Mori, et al. (2007). "Blood eosinophilia as a marker of favorable outcome after allogeneic stem cell transplantation." Transpl Int **20**(9): 761-70.

Eosinophilia is observed in a variety of disorders including acute and chronic graft-versus-

host disease (GVHD). The clinical records of 237 patients who underwent allogeneic stem cell transplantation (allo-SCT) were retrospectively reviewed. Eosinophilia, defined as a relative eosinophil count>4% within the first 100 days, was observed in 135 patients (57%). The incidence of grades II-IV acute GVHD was significantly higher in patients without eosinophilia than in those with eosinophilia (68% vs. 43%; P<0.001). The incidence of chronic GVHD was significantly higher in patients without eosinophilia than in those with eosinophilia (73% vs. 56%; P=0.011). Relapse rate was similar between patients with and without eosinophilia (33% vs. 27%; P=0.438). The probability of nonrelapse mortality was 10% in patients with eosinophilia, which was significantly lower than that in patients without eosinophilia (31%; P<0.001), and the overall survival (OS) at 3 years was 67% in patients with eosinophilia, which was significantly higher than that in patients without eosinophilia (51%; P=0.003). Multivariate analysis identified older age, high-risk disease, acute GVHD, sex disparity between patient and donor, and the absence of eosinophilia as significant factors for reduced OS. These data lead us to conclude that eosinophilia after allo-SCT may serve as a favorable prognostic marker.

Arnes, J. B., K. Collett, et al. (2008). "Independent prognostic value of the basal-like phenotype of breast cancer and associations with EGFR and candidate stem cell marker BMI-1." Histopathology **52**(3): 370-80.

Aims: To study the relationship between basal-like breast cancers, epidermal growth factor receptor (EGFR) and candidate stem cell markers (BMI-1, EZH2, Oct-4) in a population-based setting. Methods and results: Immunohistochemistry was evaluated in a series of 190 breast cancers. Basal-like phenotype (BLP) 1-5 was found in 4.3-14.3% of cases. EGFR was expressed in 9% of cases and associated with cytokeratin (CK) 5 and P-cadherin positivity, but not with survival; 28% of CK5+ cases were EGFR+. On multivariate analysis, basal-like differentiation and lymph node status were independent prognostic factors of comparable strength. BMI-1 positivity (42.6%) was associated with absence of basal-like features, oestrogen receptor positivity and low Ki67, but not related to survival. BMI was not associated with EZH2 expression, and these markers tended to show opposite associations with other variables, suggesting different roles in breast cancer. Oct-4 expression was not detected in this series. Conclusions: Basal-like features and lymph node status were strong and independent prognostic factors in this population-based series of breast cancer. Neither EGFR nor BMI-1 had significant prognostic impact, whereas EZH2 expression was associated with decreased survival. BMI-1 was inversely related to basal-like factors, and a stem cell phenotype of the basal-like subgroup could not be verified by this marker.

Asari, S., S. Okada, et al. (2004). "Beta-galactosidase of ROSA26 mice is a useful marker for detecting the definitive erythropoiesis after stem cell transplantation." Transplantation **78**(4): 516-23.

BACKGROUND: Hematopoietic reconstitution after stem cell transplantation has been analyzed by using stem cells of Ly5 congenic mice. However, the early erythropoiesis has never been analyzed because this marker is not expressed on all of the erythroid lineage cells. The transgenic mouse expressing beta-galactosidase (beta-gal) or green fluorescent protein (GFP) has been reported. Using these markers, we analyzed the early erythropoiesis after stem cell transplantation. METHODS: The beta-gal activity and GFP were examined in the hematopoietic cells of ROSA26 and GFP transgenic mice, respectively, by flow cytometry. The primitive hematopoietic stem cell fraction (Lin(-)c-kit(+)Sca-1(+)) in bone marrow (BM) cells of ROSA26 mice was transferred into lethally irradiated mice. The kinetics of hematopoietic reconstitution was analyzed in the BM and spleen after transplantation. RESULTS: The beta-gal activity, but not the GFP and Ly5, was detected in all of the erythroid (TER119+) cells. The beta-gal activity was also detected in the donor-derived myeloid (Mac-1+), B lymphoid (B220+), and T lymphoid (Thy-1+) cells in the BM and spleen after stem cell transplantation. The kinetics of the hematopoietic reconstitution demonstrated that early erythroid (TER119(low)CD71(med)) cells were developed in the BM and spleen within 2 days after transplantation before development of proerythroblasts (TER119(+)CD71(high)), and that massive erythropoiesis and myelopoiesis were observed in the spleen until 2 and 4 weeks after transplantation, respectively. Conclusions. The beta-gal of ROSA26 mice can be a useful marker to identify the donor-derived hematopoietic cells, including early erythroid cells, and the first major wave of erythropoiesis occurring in the spleen after stem cell transplantation.

Atlasi, Y., S. J. Mowla, et al. (2007). "OCT-4, an embryonic stem cell marker, is highly expressed in bladder cancer." Int J Cancer **120**(7): 1598-602.

OCT-4 (also known as POU5F1) is a key regulator of self-renewal in embryonic stem cells. Regarding the new cancer stem cell concept, the expression of such genes is potentially correlated with tumorigenesis and can affect some aspects of tumor

behavior, such as tumor recurrence or resistance to therapies. Although OCT-4 has been introduced as a molecular marker for germ cell tumors, little is known about its expression in somatic cancers. Here, we have investigated the potential expression of OCT-4 in bladder cancer. We used semiquantitative RT-PCR to examine the expression of OCT-4 in 32 tumors, 13 apparently nontumor tissues taken from the margin of tumors and 9 normal urothelial tissues. The expression of OCT-4 at protein level was further determined by Western blotting and immunohistochemical (IHC) analysis. OCT-4 expression was detected in almost all examined tumors (31/32), but at much lower level ($p<0.001$) in some nonneoplastic samples (6/22). A significantly strong correlation of 0.6 has been observed between OCT-4 expression and the presence of tumors ($p<0.001$). Western blot analysis further confirmed the expression of OCT-4 in tumor biopsies. According to IHC results, OCT-4 is primarily localized in the nuclei of tumor cells, with no or low immunoreactivity in nontumor cells. Our study demonstrated, for the first time, the expression of OCT-4 in bladder cancer and a further clue to the involvement of embryonic genes in carcinogenesis.

Becker, L., Q. Huang, et al. (2008). "Immunostaining of Lgr5, an intestinal stem cell marker, in normal and premalignant human gastrointestinal tissue." ScientificWorldJournal **8**: 1168-76.

Lgr5 has recently been identified as a murine marker of intestinal stem cells. Its expression has not been well characterized in human gastrointestinal tissues, but has been reported in certain cancers. With the increasing appreciation for the role of cancer stem cells or tumor-initiating cells in certain tumors, we sought to explore the expression of Lgr5 in normal and premalignant human gastrointestinal tissues. Using standard immunostaining, we compared expression of Lgr5 in normal colon and small intestine vs. small intestinal and colonic adenomas and Barrett's esophagus. In the normal tissue, Lgr5 was expressed in the expected stem cell niche, at the base of crypts, as seen in mice. However, in premalignant lesions, Lgr5+ cells were not restricted to the crypt base. Additionally, their overall numbers were increased. In colonic adenomas, Lgr5+ cells were commonly found clustered at the luminal surface and rarely at the crypt base. Finally, we compared immunostaining of Lgr5 with that of CD133, a previously characterized marker for tumor-initiating cells in colon cancer, and found that they identified distinct subpopulations of cells that were in close proximity, but did not costain. Our findings suggest that (1) Lgr5 is a potential marker of intestinal stem cells in humans and (2) loss of restriction to the stem cell niche is an early event in the premalignant transformation of stem cells and may play a role in carcinogenesis.

Bickenbach, J. R., V. Vormwald-Dogan, et al. (1998). "Telomerase is not an epidermal stem cell marker and is downregulated by calcium." J Invest Dermatol **111**(6): 1045-52.

The ribonucleoprotein complex telomerase, which was found to be active in germ line, immortal, and tumor cells, and in cells from continuously renewing normal tissues such as epidermis or bone marrow, is thought to be correlated with an indefinite life span. Therefore, it has been postulated that in the normal tissues, telomerase activity may be restricted to stem cells, the possible precursors of tumor cells. Here, we demonstrate that a 56% enriched population of epidermal stem cells exhibited less telomerase activity than the more actively proliferating transit amplifying cells, which are destined to differentiate after a finite number of cell divisions. Thus telomerase is not a stem cell marker. In human epidermis we found a heterogeneous expression of the telomerase RNA component (hTR) within the basal layer, with clusters of hTR-positive cells showing variable activities. Histone-3 expressing S-phase basal cells were distributed evenly, illustrating that hTR upregulation may not strictly be correlated with proliferation. We further show for human epidermal cells that differentiation-dependent downregulation of telomerase correlates with Ca++-induced cell differentiation and that increasing the amount of Ca++ but not Mg++ or Zn++ reduced telomerase activity in a dose-dependent manner in a cell-free system (differentiation-independent). Furthermore, addition of ethyleneglycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid completely reversed this Ca++-induced inhibition. These data indicate that Ca++ is not only an important regulator of epidermal differentiation but also a key regulator of telomerase.

Boiani, M., L. Gentile, et al. (2005). "Variable reprogramming of the pluripotent stem cell marker Oct4 in mouse clones: distinct developmental potentials in different culture environments." Stem Cells **23**(8): 1089-104.

A prevailing view of cloning by somatic-cell nuclear transfer is that reprogramming of gene expression occurs during the first few hours after injection of the nucleus into an oocyte, that the process is stochastic, and that the type of reprogramming needed for cloning success is foreign and unlikely to be readily achieved in the ooplasm. Here, we present evidence that the release of reprogramming capacity is contingent on the culture environment of the clone while the contribution of aneuploidy to altered gene expression is marginal. In

particular, the rate of blastocyst formation in clones and the regional distribution of mRNA for the pluripotent stem cell marker Oct4 in clonal blastocysts was highly dependent on the culture environment after cumulus cell nuclear transfer, unlike that in genetically equivalent zygotes. Epigenetic modifications of genetically identical somatic nuclei continue after the first cell division of the clones and are amenable to a degree of experimental control, and their development to the blastocyst stage and appropriate expression of Oct4 predict further outcome, such as derivation of embryonic stem (ES) cells, but not fetal development. This observation indicates that development to the blastocyst stage is not equivalent to full reprogramming and lends support to the novel concept that ES cells are not the equivalent of the inner cell mass, hence the discrepancy between ES cell derivability and fetal development of clones.

Boivin, D., D. Labbe, et al. (2009). "The stem cell marker CD133 (prominin-1) is phosphorylated on cytoplasmic tyrosine-828 and tyrosine-852 by Src and Fyn tyrosine kinases." Biochemistry **48**(18): 3998-4007.

CD133 (prominin-1) is a transmembrane glycoprotein expressed at the surface of normal and cancer stem cells, progenitor cells, rod photoreceptor cells, and a variety of epithelial cells. Although CD133 is widely used as a marker of various somatic and putative cancer stem cells, its contribution to fundamental properties of stem cells such as self-renewal and differentiation remains unknown. CD133 contains a short C-terminal cytoplasmic domain with five tyrosine residues, including a consensus tyrosine phosphorylation site that has not yet been investigated. In this study, we show that CD133 is phosphorylated in human medulloblastoma D283 and Daoy cells, in a Src family kinase-dependent manner. The cytoplasmic domain of CD133 is tyrosine phosphorylated in Daoy cells overexpressing Src and Fyn tyrosine kinases, as well as in vitro using recombinant proteins. Deletion of the C-terminal cytoplasmic domain of CD133 considerably reduced its phosphorylation by Src. To identify the tyrosine phosphorylation sites in CD133, we used matrix-assisted laser desorption/ionization quadrupole time-of-flight (MALDI Q-TOF) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Analysis of tyrosine-phosphorylated CD133 by mass spectrometry and site-directed mutagenesis identified tyrosine-828 and the nonconsensus tyrosine-852 as the major tyrosine phosphorylation sites both in vitro and in intact cells. Identification of CD133 as a substrate for Src-family tyrosine kinases suggests that the cytoplasmic domain of CD133 might play an important role in the regulation of its functions.

Bourguignon, L. Y., K. Peyrollier, et al. (2008). "Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells." J Biol Chem **283**(25): 17635-51.

Hyaluronan (HA) is a major glycosaminoglycan in the extracellular matrix whose expression is tightly linked to multidrug resistance and tumor progression. In this study we investigated HA-induced interaction between CD44 (a HA receptor) and Nanog (an embryonic stem cell transcription factor) in both human breast tumor cells (MCF-7 cells) and human ovarian tumor cells (SK-OV-3.ipl cells). Using a specific primer pair to amplify Nanog by reverse transcriptase-PCR, we detected the expression of Nanog transcript in both tumor cell lines. In addition, our results reveal that HA binding to these tumor cells promotes Nanog protein association with CD44 followed by Nanog activation and the expression of pluripotent stem cell regulators (e.g. Rex1 and Sox2). Nanog also forms a complex with the "signal transducer and activator of transcription protein 3" (Stat-3) in the nucleus leading to Stat-3-specific transcriptional activation and multidrug transporter, MDR1 (P-glycoprotein) gene expression. Furthermore, we observed that HA-CD44 interaction induces ankyrin (a cytoskeletal protein) binding to MDR1 resulting in the efflux of chemotherapeutic drugs (e.g. doxorubicin and paclitaxel (Taxol)) and chemoresistance in these tumor cells. Overexpression of Nanog by transfecting tumor cells with Nanog cDNA stimulates Stat-3 transcriptional activation, MDR1 overexpression, and multidrug resistance. Down regulation of Nanog signaling or ankyrin function (by transfecting tumor cells with Nanog small interfering RNA or ankyrin repeat domain cDNA) not only blocks HA/CD44-mediated tumor cell behaviors but also enhances chemosensitivity. Taken together, these findings suggest that targeting HA/CD44-mediated Nanog-Stat-3 signaling pathways and ankyrin/cytoskeleton function may represent a novel approach to overcome chemotherapy resistance in some breast and ovarian tumor cells displaying stem cell marker properties during tumor progression.

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

Multidrug resistance and disease relapse is a challenging clinical problem in the treatment of breast cancer. In this study, we investigated the hyaluronan (HA)-induced interaction between CD44 (a primary HA receptor) and protein kinase Cepsilon (PKCepsilon), which regulates a number of human breast tumor cell functions. Our results indicate that HA binding to CD44 promotes PKCepsilon activation, which, in turn, increases the phosphorylation of the stem cell marker, Nanog, in the breast tumor cell line MCF-7. Phosphorylated Nanog is then translocated from the cytosol to the nucleus and becomes associated with RNase III DROSHA and the RNA helicase p68. This process leads to microRNA-21 (miR-21) production and a tumor suppressor protein (e.g. PDCD4 (program cell death 4)) reduction. All of these events contribute to up-regulation of inhibitors of apoptosis proteins (IAPs) and MDR1 (multidrug-resistant protein), resulting in anti-apoptosis and chemotherapy resistance. Transfection of MCF-7 cells with PKCepsilon or Nanog-specific small interfering RNAs effectively blocks HA-mediated PKCepsilon-Nanog signaling events, abrogates miR-21 production, and increases PDCD4 expression/eIF4A binding. Subsequently, this PKCepsilon-Nanog signaling inhibition causes IAP/MDR1 down-regulation, apoptosis, and chemosensitivity. To further evaluate the role of miR-21 in oncogenesis and chemoresistance, MCF-7 cells were also transfected with a specific anti-miR-21 inhibitor in order to silence miR-21 expression and inhibit its target functions. Our results indicate that anti-miR-21 inhibitor not only enhances PDCD4 expression/eIF4A binding but also blocks HA-CD44-mediated tumor cell behaviors. Thus, this newly discovered HA-CD44 signaling pathway should provide important drug targets for sensitizing tumor cell apoptosis and overcoming chemotherapy resistance in breast cancer cells.

Brenner, M. K. (1995). "The contribution of marker gene studies to hemopoietic stem cell therapies." Stem Cells **13**(5): 453-61.

Although the transfer of "therapeutic" genes into hemopoietic stem cells (HSC) offers many opportunities to treat a wide range of human disease, the low efficiency of transfer and limited expression of the transferred gene have so far largely prevented any direct beneficial effect from being obtained. However, gene marker studies in which the transferred genes are used simply to track the individual components of the infused HSC have already shown their utility. Genetic marking may be used to identify cells capable of causing relapse after autologous bone marrow transplantation and to distinguish cells in the graft capable of preventing malignant disease. Marking may also be used to analyze the consequences of ex vivo or in vivo manipulations of the HSC which are intended to accelerate engraftment or augment gene transfer efficiencies. Information obtained from these studies should therefore not only improve the outcome of HSC based therapies, but also aid in the introduction of successful gene therapy protocols.

Buescher, E. S., D. W. Alling, et al. (1985). "Use of an X-linked human neutrophil marker to estimate timing of lyonization and size of the dividing stem cell pool." J Clin Invest **76**(4): 1581-4.

In families with X-linked chronic granulomatous disease (CGD), heterozygous females have two stable populations of polymorphonuclear leukocytes (PMN) in their blood; one normal, the other, deficient in oxygen metabolism. The two types of PMN can be distinguished by the ability or lack of ability to reduce nitroblue tetrazolium dye. The variation in the percent normal PMN among 11 CGD heterozygotes was shown to follow a binomial distribution based on eight independent trials and a chance of success of 50%. This is consistent with the occurrence of X-chromosome inactivation (lyonization) when eight embryonic founder cells for the hematopoietic system are present. Serial determinations of the percent normal PMN in individual heterozygotes showed very limited variability (standard deviations ranged from 2.0% to 5.2%) most of which could be ascribed to experimental error. An estimate of the remaining variation (residual variance) was introduced into a well-known formula to calculate the appropriate number of pluripotent stem cells necessary to support hematopoiesis and a figure exceeding 400 was obtained. Thus, the data indicate that in humans there is a highly polyclonal system of hematopoiesis.

Chen, S., M. Takahara, et al. (2008). "Increased expression of an epidermal stem cell marker, cytokeratin 19, in cutaneous squamous cell carcinoma." Br J Dermatol **159**(4): 952-5.

BACKGROUND: Cytokeratin 19 (CK19) has been considered to be a putative marker for epidermal stem cells in the hair follicle bulge. Cumulative reports have shown that epidermal stem cells play an important role in skin carcinogenesis. However, to date there has been no report on the clinical alteration of the stem cells in squamous cell carcinoma (SCC). OBJECTIVES: To investigate alteration of the stem cells and proliferating cells and to assess their relationship and potential contribution to SCC. METHODS: Thirty paraffin-embedded neoplastic skin lesions, consisting of 10 cases each of actinic keratosis (AK), Bowen disease (BD) and SCC,

were examined immunohistologically for CK19 and Ki-67. RESULTS: Positive reactivity for CK19 was seen in 30% of AK, 50% of BD and 80% of SCC lesions. There was significantly higher expression levels of CK19 in SCC than in AK and BD ($P < 0.05$). In addition, BD lesions harboured a significantly higher number of CK19-positive cells than did AK lesions ($P < 0.05$). There were significant differences in Ki-67 labelling indices between AK and BD and between AK and SCC ($P < 0.001$), but not between BD and SCC ($P > 0.05$). Furthermore, a serial section comparison study showed that there was a minor population of cells co-expressing CK19 and Ki-67 in a subset of the tumour cells of SCC samples. The percentage of CK19+ cells significantly correlated with that of Ki67+ cells in all examined neoplastic skin lesions. CONCLUSIONS: These results suggest that CK19 expression may be associated with the retention of stem cell characteristics or a state that is uncommitted to terminal squamous differentiation.

Chen, Z., W. H. Evans, et al. (2006). "Gap junction protein connexin 43 serves as a negative marker for a stem cell-containing population of human limbal epithelial cells." Stem Cells **24**(5): 1265-73.

This study evaluated whether the gap junction protein connexin (Cx) 43 could serve as a negative cell surface marker for human corneal epithelial stem cells. Cx43 expression was evaluated in corneo-limbal tissue and primary limbal epithelial cultures. Immunofluorescent staining and laser scanning confocal microscopy showed that Cx43 was strongly expressed in the corneal and limbal suprabasal epithelial cells, but the basal cells of the limbal epithelium were negative. Cx43 antibody stained mainly large cells but not small cells in primary limbal epithelial cultures. As determined by semiquantitative reverse transcription polymerase chain reaction (PCR) and real-time PCR, Cx43 mRNA was more abundant in the corneal than limbal epithelia, and it was expressed in higher levels in mature limbal epithelial cultures. Using GAP11, a rabbit polyclonal antibody against the Cx32 extracellular loop 2 (151-187), a sequence that is highly homologous in Cx43, the Cx43(dim) and Cx43(bright) cells were selected from primary limbal epithelial cultures by fluorescence-activated cell sorting and were evaluated for stem cell properties. These Cx43(dim) and Cx43(bright) cells were confirmed by their expression levels of Cx43 protein and mRNA. The Cx43(dim) cells were found to contain higher percentages of slow-cycling bromodeoxyuridine (BrdU)-label retaining cells and the cells that were positive for stem cell-associated markers p63, ABCG2, and integrin beta1 and negative for differentiation markers K3 and involucrin. The Cx43(dim) cells possessed a greater proliferative potential than Cx43(bright) cells and nonfractionated cells as evaluated by BrdU incorporation, colony-forming efficiency, and growth capacity. Our findings suggest that human limbal basal cells do not express connexin 43, which could serve as a negative cell surface marker for the stem cell-containing population of human limbal epithelial cells.

Cheng, J. X., B. L. Liu, et al. (2009). "How powerful is CD133 as a cancer stem cell marker in brain tumors?" Cancer Treat Rev **35**(5): 403-8.

Cancer stem cells (CSCs) have been identified in a growing number of hematopoietic and solid tissue malignancies and are typically recognized by virtue of the expression of cell surface markers. CD133, a stem cell marker, is now extensively used as a surface marker to identify and isolate brain tumor stem cells (BTSCs) in malignant brain tumors. However, CD133 as the marker to sort BTSCs suffered some controversies. In this review, we reviewed the rise of CD133, analyzed the efficiency of CD133 on identification and isolation of BTSCs, explained some controversial study results and summed up the role of CD133 and other effective CSCs markers in sorting CSCs in other tumors. We analyzed current limited reports and found that the expression of CD133 was correlated with poor clinical prognosis in brain tumors. Finally, we summarized the mechanisms of chemo- and radio- resistance of CD133+ brain tumor cell, especially emphasized that the aberrant activation of development pathways in BTSCs can be potential targets to BTSCs, and outlined current preclinical studies on killing BTSCs or sensitizing BTSCs to chemo- and radio-therapies.

Cheng, L., R. E. Reiter, et al. (2003). "Immunocytochemical analysis of prostate stem cell antigen as adjunct marker for detection of urothelial transitional cell carcinoma in voided urine specimens." J Urol **169**(6): 2094-100.

PURPOSE: Prostate stem cell antigen (PSCA), a homologue of the Ly-6/Thy-1 family of cell surface antigens, has been shown to be increased in a majority of human transitional cell carcinomas. We tested the possibility of using PSCA as an adjunct marker for urine cytology. MATERIALS AND METHODS: Immunocytochemical analysis was performed on 44 archived voided urine samples obtained from 3 groups of patients based on initial voided urine cytological results and subsequent followup biopsy findings. Group 1 (14 of 44 patients) had positive findings on cytology and histology, group 2 (16 of 44) had negative cytology but positive histology, and group 3 (14 of 44) had negative findings on cytology and histology. Cytological slides

prepared from 10 fresh voided urine samples were also analyzed. Papanicoloau stained archived urine slides were de-stained and re-stained immunocytochemically with a monoclonal antibody against PSCA. Immunofluorescence followed by laser scanning cytometer analysis was also performed on archived slides from 2 representative cases. RESULTS: Sensitivity and specificity were 46.7% and 100% for cytology alone, respectively, and 80% and 85.7% for PSCA alone, respectively. PSCA immunostaining was positive in 92.8% group 1, 68.8% group 2 and only 14.3% group 3 samples. The difference in positive PSCA findings in groups 2 and 3 were statistically significant at $p < 0.01$ by chi-square test. Whereas some superficial umbrella cells showed slight staining by immunocytochemistry, it was feasible to distinguish the expression levels between tumor and normal superficial umbrella cells quantitatively using immunofluorescence coupled with laser scan cytometry analysis. CONCLUSIONS: Immunocytochemical analysis of PSCA on archived voided urine samples may provide a simple and quantitative adjunct marker for cytological diagnosis of urothelial carcinoma.

Christensen, J. L. and I. L. Weissman (2001). "Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells." Proc Natl Acad Sci U S A **98**(25): 14541-6.

Clonogenic multipotent mouse hematopoietic stem cells (HSCs) and progenitor cells are contained within the c-kit(+) (K) lineage(-/lo) (L) Sca-1(+) (S) population of hematopoietic cells; long-term (LT) and short-term (ST) HSCs are Thy-1.1(lo). c-kit is a member of the receptor tyrosine kinase family, a class of receptors that are important in the proliferation and differentiation of hematopoietic cells. To establish whether the Flk-2/Flt3 receptor tyrosine kinase was expressed on the most primitive LT-HSCs, we sorted highly purified multipotent stem and progenitor cells on the basis of Flk-2 surface expression and used them in competitive reconstitution assays. Low numbers of Flk-2(-) HSCs gave rise to long-term multilineage reconstitution in the majority of recipients, whereas the transfer of Flk-2(+) multipotent cells resulted in mostly short-term multilineage reconstitution. The KLS subset of adult mouse bone marrow was analyzed for Flk-2 and Thy-1.1 expression. Three phenotypically and functionally distinct populations were isolated: Thy(lo) Flk-2(-) (LT-HSCs), Thy(lo) Flk-2(+) (ST-HSCs), and Thy(-) Flk-2(+) multipotent progenitors. The loss of Thy-1.1 and gain of Flk-2 expression marks the loss of self-renewal in HSC maturation. The addition of Flk-2 antibody to the lineage mix allows direct isolation of LT-HSC from adult bone marrow as c-kit(+) lin(-) Sca-1(+) Flk-2(-) from many strains of mice. Fetal liver HSCs are contained within Flk-2(-) and Flk-2(+) KTLS cells.

Colitti, M. and M. Farinacci (2009). "Expression of a putative stem cell marker, Musashi 1, in mammary glands of ewes." J Mol Histol **40**(2): 139-49.

Several recent studies demonstrated that development, function and remodelling of mammary glands involved multipotent cells, but no specific molecular markers for mammary epithelial stem cells were revealed. These studies principally concerned human and mouse mammary tissue, but mammary stem cells could be a valuable tool in agricultural production and bioengineering in farm animals. The Musashi-1 (Msi 1) gene encodes an RNA binding protein, which is likely to be associated with self-renewal of neural, intestinal and mammary progenitor cells and is believed to influence the Notch signalling pathway. In this study Musashi-1 expression was detected using immunohistochemistry and in situ hybridisation analysis on mammary glands of ewes at different developmental stages. The protein expression was observed in the epithelial cells at all stages examined. In situ hybridization analysis showed that Msi 1 mRNA has an expression pattern similar to the encoded protein, with positive staining in both nuclei and cytoplasm of ductal, secretory and stromal cells. Ultrastructural in situ analysis confirmed the nuclear and cytoplasmatic expression of Msi. Quantitative analysis of Msi 1 gene expression showed a strong correlation with that of Ki-67, that is a marker of cell proliferation. This is the first report outlining expression of Msi 1 in ovine mammary glands during a complete cycle of lactation.

Constantinescu, D., H. L. Gray, et al. (2006). "Lamin A/C expression is a marker of mouse and human embryonic stem cell differentiation." Stem Cells **24**(1): 177-85.

Nuclear lamins comprise the nuclear lamina, a scaffold-like structure that lines the inner nuclear membrane. B-type lamins are present in almost all cell types, but A-type lamins are expressed predominantly in differentiated cells, suggesting a role in maintenance of the differentiated state. Previous studies have shown that lamin A/C is not expressed during mouse development before day 9, nor in undifferentiated mouse embryonic carcinoma cells. To further investigate the role of lamins in cell phenotype maintenance and differentiation, we examined lamin expression in undifferentiated mouse and human embryonic stem (ES) cells. Wide-field and confocal immunofluorescence microscopy and semiquantitative reverse transcription-polymerase chain reaction analysis revealed that undifferentiated mouse and human ES cells express lamins B1 and B2 but not

lamin A/C. Mouse ES cells display high levels of lamins B1 and B2 localized both at the nuclear periphery and throughout the nucleoplasm, but in human ES cells, B1 and B2 expression is dimmer and localized primarily at the nuclear periphery. Lamin A/C expression is activated during human ES cell differentiation before downregulation of the pluripotency marker Oct-3/4 but not before the downregulation of the pluripotency markers Tra-1-60, Tra-1-81, and SSEA-4. Our results identify the absence of A-type lamin expression as a novel marker for undifferentiated ES cells and further support a role for nuclear lamins in cell maintenance and differentiation.

Corbeil, D., A. Joester, et al. (2009). "Expression of distinct splice variants of the stem cell marker prominin-1 (CD133) in glial cells." Glia **57**(8): 860-74.

Prominin-1 (CD133) is a cholesterol-interacting pentaspan membrane glycoprotein specifically associated with plasma membrane protrusions. Prominin-1 is expressed by various stem and progenitor cells, notably neuroepithelial progenitors found in the developing embryonic brain. Here, we further investigated its expression in the murine brain. Biochemical analyses of brain membranes at early stages of development revealed the expression of two distinct splice variants of prominin-1, s1 and s3, which have different cytoplasmic C-terminal domains. The relative abundance of the s3 variant increased toward adulthood, whereas the opposite was observed for the s1 variant. Our combined in situ hybridization and immunohistochemistry revealed the expression of prominin-1 in a subpopulation of Olig-2-positive oligodendroglial cells present within white matter tracts of postnatal and adult brain. Furthermore, immunohistological and biochemical characterization suggested strongly that the s3 variant is a novel component of myelin. Consistent with this, the expression of prominin-1.s3 was significantly reduced in the brain of myelin-deficient mice. Finally, oligodendrocytes expressed selectively the s3 variant whereas GFAP-positive astrocytes expressed the s1 variant in primary glial cell cultures derived from embryonic brains. Collectively, our data demonstrate a complex expression pattern of prominin-1 molecules in developing adult brain. Given that prominin-1 is thought to act as an organizer of plasma membrane protrusions, they further suggest that a specific prominin-1 splice variant might play a role in morphogenesis and/or maintenance of the myelin sheath.

David, R., M. Groebner, et al. (2005). "Magnetic cell sorting purification of differentiated embryonic stem cells stably expressing truncated human CD4 as surface marker." Stem Cells **23**(4): 477-82.

Embryonic stem (ES) cells offer great potential in regenerative medicine and tissue engineering. Clinical applications are still hampered by the lack of protocols for gentle, high-yield isolation of specific cell types for transplantation expressing no immunogenic markers. We describe labeling of stably transfected ES cells expressing a human CD4 molecule lacking its intracellular domain (DeltaCD4) under control of the phosphoglycerate kinase promoter for magnetic cell sorting (MACS). To track the labeled ES cells, we fused DeltaCD4 to an intracellular enhanced green fluorescent protein domain (DeltaCD4EGFP). We showed functionality of the membrane-bound fluorescent fusion protein and its suitability for MACS leading to purities greater than 97%. Likewise, expression of DeltaCD4 yielded up to 98.5% positive cells independently of their differentiation state. Purities were not limited by the initial percentage of DeltaCD4(+) cells, ranging from 0.6%-16%. The viability of MACS-selected cells was demonstrated by reaggregation and de novo formation of embryoid bodies developing all three germ layers. Thus, expression of DeltaCD4 in differentiated ES cells may enable rapid, high-yield purification of a desired cell type for tissue engineering and transplantation studies.

de Jong, J. and L. H. Looijenga (2006). "Stem cell marker OCT3/4 in tumor biology and germ cell tumor diagnostics: history and future." Crit Rev Oncog **12**(3-4): 171-203.

The transcription factor OCT3/4 (also known as POU5F1 and Oct4) is regarded as one of the key regulators of pluripotency. Expression in nonmalignant cells is restricted to the pluripotent cells in the embryo and the primordial germ cells that will pass pluripotency to future generations via the gametes. Although major progress has been made in successfully identifying other players in the pluripotency network by using high-throughput screening methods, the exact mechanisms involved in regulation of OCT3/4 in vivo remain largely to be elucidated. In human tumors, OCT3/4 is the most informative marker in germ cell tumors diagnostics and is expressed in the precursor lesions gonadoblastoma and carcinoma in situ, as well as in invasive embryonal carcinoma and seminomatous tumors. Currently, the application of OCT3/4 for screening in high-risk patient populations is the novel focus of study. This article reviews OCT3/4 expression in normal development and germ cell tumors. In addition, expression in adult tissues and

nongerm cell tumor malignancies in relation to splice variants and pseudogenes is discussed. An overview of the upstream and downstream factors in the OCT3/4 pathway as well as the epigenetic regulation of the gene is summarized and the possible role in oncogenesis considered.

Dubreuil, V., A. M. Marzesco, et al. (2007). "Midbody and primary cilium of neural progenitors release extracellular membrane particles enriched in the stem cell marker prominin-1." J Cell Biol **176**(4): 483-95.

Expansion of the neocortex requires symmetric divisions of neuroepithelial cells, the primary progenitor cells of the developing mammalian central nervous system. Symmetrically dividing neuroepithelial cells are known to form a midbody at their apical (rather than lateral) surface. We show that apical midbodies of neuroepithelial cells concentrate prominin-1 (CD133), a somatic stem cell marker and defining constituent of a specific plasma membrane microdomain. Moreover, these apical midbodies are released, as a whole or in part, into the extracellular space, yielding the prominin-1-enriched membrane particles found in the neural tube fluid. The primary cilium of neuroepithelial cells also concentrates prominin-1 and appears to be a second source of the prominin-1-bearing extracellular membrane particles. Our data reveal novel origins of extracellular membrane traffic that enable neural stem and progenitor cells to avoid the asymmetric inheritance of the midbody observed for other cells and, by releasing a stem cell membrane microdomain, to potentially influence the balance of their proliferation versus differentiation.

Eiges, R., M. Schuldiner, et al. (2001). "Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells." Curr Biol **11**(7): 514-8.

Human embryonic stem (ES) cells are pluripotent cell lines that have been derived from the inner cell mass (ICM) of blastocyst stage embryos [1--3]. They are characterized by their ability to be propagated indefinitely in culture as undifferentiated cells with a normal karyotype and can be induced to differentiate in vitro into various cell types [1, 2, 4--6]. Thus, human ES cells promise to serve as an unlimited cell source for transplantation. However, these unique cell lines tend to spontaneously differentiate in culture and therefore are difficult to maintain. Furthermore, colonies may contain several cell types and may be composed of cells other than pluripotent cells [1, 2, 6]. In order to overcome these difficulties and establish lines of cells with an undifferentiated phenotype, we have introduced a reporter gene that is regulated by a promoter of an ES cell-enriched gene into the cells. For the introduction of DNA into human ES cells, we have established a specific transfection protocol that is different from the one used for murine ES cells. Human ES cells were transfected with enhanced green fluorescence protein (EGFP), under the control of murine Rex1 promoter. The transfected cells show high levels of GFP expression when in an undifferentiated state. As the cells differentiate, this expression is dramatically reduced in monolayer cultures as well as in the primitive endoderm of early stage (simple) embryoid bodies (EBs) and in mature EBs. The undifferentiated cells expressing GFP can be analyzed and sorted by using a Fluorescence Activated Cell Sorter (FACS). Thus, we have established lines of human ES cells in which only undifferentiated cells are fluorescent, and these cells can be followed and selected for in culture. We also propose that the pluripotent nature of the culture is made evident by the ability of the homogeneous cell population to form EBs. The ability to efficiently transfect human ES cells will provide the means to study and manipulate these cells for the purpose of basic and applied research.

Erlecke, J., I. Hartmann, et al. (2009). "Automated detection of residual cells after sex-mismatched stem-cell transplantation - evidence for presence of disease-marker negative residual cells." Mol Cytogenet **2**: 12.

BACKGROUND: A new chimerism analysis based on automated interphase fluorescence in situ hybridization (FISH) evaluation was established to detect residual cells after allogene sex-mismatched bone marrow or blood stem-cell transplantation.Cells of 58 patients were characterized as disease-associated due to presence of a bcr/abl-gene-fusion or a trisomy 8 and/or a simultaneous hybridization of gonosome-specific centromeric probes. The automatic slide scanning platform Metafer with its module MetaCyte was used to analyse 3,000 cells per sample. RESULTS: Overall 454 assays of 58 patients were analyzed. 13 of 58 patients showed residual recipient cells at one stage of more than 4% and 12 of 58 showed residual recipient cells less than 4%, respectively. As to be expected, patients of the latter group were associated with a higher survival rate (48 vs. 34 month). In only two of seven patients with disease-marker positive residual cells between 0.1-1.3% a relapse was observed. Besides, disease-marker negative residual cells were found in two patients without relapse at a rate of 2.8% and 3.3%, respectively. CONCLUSION: The definite origin and meaning of disease-marker negative residual cells is still unclear. Overall, with the presented automatic chimerism analysis of interphase FISH slides, a sensitive method for detection of disease-marker positive residual cells is on hand.

Ernst, C. and B. R. Christie (2006). "The putative neural stem cell marker, nestin, is expressed in heterogeneous cell types in the adult rat neocortex." Neuroscience **138**(1): 183-8.

Nestin is a putative neural stem cell marker that is expressed in different areas of the adult mammalian brain that are known to support mitotic activity. Recently the neocortex has been proposed to support neurogenesis, however little is known of the expression pattern of nestin in neocortex. In the present study, we demonstrate that cells that express nestin can be found throughout the neocortex, and that these cells are morphologically heterogeneous. Some nestin-expressing cells had one extension arising from the cell body, reminiscent of the nestin-expressing cells that are thought to be young neurons in the hippocampus. The frequency of single extension cells in the neocortex was approximately one cell per 50,000 microm(2). Other cells had numerous extensions arising from the cell body. In all cases, cells that expressed nestin were also found to co-label with the glial marker glial fibrillary acidic protein. In addition, nestin-expressing cells in the neocortex did not express the cell cycle marker, Ki-67, indicating they were not actively engaged in mitotic activity. When small lesions were made in cortex, nestin could also be observed in reactive astrocytes as part of the inflammatory response. Approximately 94% of reactive astrocytes expressed Ki-67. These results demonstrate that there are different populations of cells in the neocortex that can express nestin, but that only reactive astrocytes in this region are mitotically active.

Fehr, A., A. Meyer, et al. (2009). "A link between the expression of the stem cell marker HMGA2, grading, and the fusion CRTC1-MAML2 in mucoepidermoid carcinoma." Genes Chromosomes Cancer **48**(9): 777-85.

Recently, the concept of cancer stem cells and their expression of embryonic stem cell markers has gained considerable experimental support. In this study, we examined the expression of one such marker, the high-mobility group AT-hook 2 gene (HMGA2) mRNA, in 53 formalin-fixed, paraffin-embedded mucoepidermoid carcinomas (MEC) and four normal parotid tissues using quantitative real-time RT-PCR (qPCR). MECs are often characterized by the fusion gene CRTC1-MAML2, the detection of which is an important tool for the diagnosis and prognosis of MEC. For detection of the CRTC1-MAML2 fusion transcript, we performed RT-PCR. The mean expression level of HMGA2 was higher in fusion negative (302.8 +/- 124.4; n = 14) than in positive tumors (67.3 +/- 13.1; n = 39). Furthermore, the fusion-negative tumors were often high-grade tumors and the HMGA2 expression level rose with the tumor grade (low: 43.7 +/- 11.0, intermediate: 126.2 +/- 28.3, and high: 271.2 +/- 126.5). A significant difference was found in the HMGA2 expression levels between the different grading groups (one-way ANOVA, P = 0.04) and among the fusion-negative and -positive tumors (t-test, P = 0.05), indicating that the expression level of HMGA2 was closely linked to grading, the presence/absence of the CRTC1-MAML2 fusion, and the tumor behavior of MECs. These findings offer further evidence for the theory that the MEC group comprises two subgroups: one group with the CRTC1-MAML2 fusion, which is a group with a moderate aggressiveness and prognosis, and the other group lacking that fusion corresponding to an increased stemness, and thus, higher aggressiveness and worse prognosis.

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

Human prominin-1/CD133 has been reported to be expressed in neural and hematopoietic stem/progenitor cells and in embryonic, but not adult, epithelia. This lack of detection of human prominin-1, as defined by its glycosylation-dependent AC133 epitope, is surprising given the expression of the murine ortholog in adult epithelia. Here, we demonstrate, by using a novel prominin-1 antiserum (alphahE2), that the decrease of AC133 immunoreactivity observed during differentiation of the colonic adenocarcinoma-derived Caco-2 cells is not paralleled by a down-regulation of prominin-1. We have also shown that alphahE2 immunoreactivity, but not AC133 immunoreactivity, is present in several adult human tissues, such as kidney proximal tubules and the parietal layer of Bowman's capsule of juxtamedullary nephrons, and in lactiferous ducts of the mammary gland. These observations suggest that only the AC133 epitope is down-regulated upon cell differentiation. Furthermore, alphahE2 immunoreactivity has been detected in several kidney carcinomas derived from proximal tubules, independent of their grading. Interestingly, in one particular case, the AC133 epitope, which is restricted to stem cells in normal adult tissue, was up-regulated in the vicinity of the tumor. Our data thus show that (1) in adults, the expression of human prominin-1 is not limited to stem and progenitor cells, and (2) the epitopes of prominin-1 might be useful for investigating solid cancers.

Fujisawa, M., M. Kanzaki, et al. (1998). "Stem cell factor in human seminal plasma as a marker for spermatogenesis." Urology **51**(3): 460-3.

OBJECTIVES: To measure the level of stem cell factor (SCF) in human seminal plasma to determine whether SCF may be useful in evaluating the ability to produce sperm and search the role of SCF in the testes. METHODS: We measured the level of SCF in seminal plasma obtained from 108 males, including idiopathic azoospermia due to germ cell aplasia (n = 10), oligospermia (n = 50), asthenospermia (n = 31), and normospermia (n = 1 7). The expression of SCF messenger ribonucleic acid in the human testis was evaluated by reverse transcriptase polymerase chain reaction (RT-PCR). The correlation between its level and clinical findings was also evaluated. RESULTS: RT-PCR showed a larger form that encoded the soluble protein and a smaller form that encoded the membrane-associated form of SCF in the human testis. The similar ratio of the larger form to the smaller one was observed both in the testis of normal and oligospermic men. The level of SCF is significantly correlated with the sperm count (r = 0.214; P < 0.05). CONCLUSIONS: The level of SCF in seminal plasma appeared to predict the ability to produce sperm. Thus, this factor may play an important role in spermatogenesis.

Ghaffari, S. H., B. Chahardouli, et al. (2008). "Evaluation of hematopoietic chimerism following allogeneic peripheral blood stem cell transplantation with amelogenin marker." Arch Iran Med **11**(1): 35-41.

BACKGROUND: Monitoring the engraftment of donor cells after allogeneic stem cell transplantation is important for the early diagnosis of graft failure or relapse of disease. The objective of the present study was to evaluate the application of the amelogenin gene for the assessment of chimerism in samples of patients who had received a sex-mismatched stem cell transplantation. METHODS: A polymerase chain reaction technique was developed using a set of amelogenin gene primers alone and/or in combination with short tandem repeats primers and was preformed on blood and/or bone marrow aspiration samples of 30 recipient patients after transplantation. The technique was then set up as a routine procedure, from September 2000 through April 2006, more than 1400 samples taken from 300 stem cell transplantation patients suffering from different types of leukemia and nonmalignant hematologic disorders were evaluated for detection of chimerism after transplantation. RESULTS: The sensitivity of the test was as low as 1 - 2%. The ratio of X/Y fragments was as the mixed chimerism. In 90% of the patients, amelogenin marker was as informative as short tandem repeats markers, as confirmed by the clinical outcome. In 5% of the patients, when there was no pre- bone marrow transplantation sample from either donor or recipient, the applicability of this assay became crucial to our treating physicians. CONCLUSION: The application of the amelogenin marker alone or in combination with the short tandem repeats system can be used for relative quantitative analysis of mixed chimerism and for observing kinetics of engraftment in patients who have sex-mismatched bone marrow transplantation. Amelogenin polymerase chain reaction analysis showed an excellent correlation with the short tandem repeats-polymerase chain reaction results.

Gilner, J. B., W. G. Walton, et al. (2007). "Antibodies to stem cell marker antigens reduce engraftment of hematopoietic stem cells." Stem Cells **25**(2): 279-88.

Hematopoietic stem cells (HSCs) have enormous potential for use in transplantation and gene therapy. However, the frequency of repopulating HSCs is often very low; thus, highly effective techniques for cell enrichment and maintenance are required to obtain sufficient cell numbers for therapeutic use and for studies of HSC physiology. Common methods of HSC enrichment use antibodies recognizing HSC surface marker antigens. Because antibodies are known to alter the physiology of other cell types, we investigated the effect of such enrichment strategies on the physiology and lineage commitment of HSCs. We sorted HSCs using a method that does not require antibodies: exclusion of Hoechst 33342 to isolate side population (SP) cells. To elucidate the effect of antibody binding on this HSC population, we compared untreated SP cells with SP cells treated with the Sca-1(+)c-Kit(+)Lin(-) (SKL) antibody cocktail prior to SP sorting. Our findings revealed that HSCs incubated with the antibody cocktail had decreased expression of the stem cell-associated genes c-Kit, Cd34, Tal-1, and Slamf1 relative to untreated SP cells or to cells treated with polyclonal isotype control antibodies. Moreover, SKL antibodies induced cycling in SP cells and diminished their ability to confer long-term hematopoietic engraftment in lethally irradiated mice. Taken together, these data suggest that antibody-based stem cell isolation procedures can have negative effects on HSC physiology.

Glazer, R. I., X. Y. Wang, et al. (2008). "Musashi1: a stem cell marker no longer in search of a function." Cell Cycle **7**(17): 2635-9.

One of the earliest genes identified with stem and early progenitor cells is the RNA-binding protein, Musashi1 (Msi1). Through gene profiling of mammary epithelial cells transduced with Msi1, a

unique autocrine signaling pathway was identified that activates both the Wnt and Notch pathways. This process was associated with increased secretion of the growth factor, PLF1 and inhibition of the secreted Wnt pathway inhibitor, DKK3. Identification of PLF1 as an effector of these pathways in the absence of the DKK3 tumor suppressor provides a new avenue for investigating differences between normal and malignant tissues, and potentially targeting tumor stem cells.

Gotte, M., M. Wolf, et al. (2008). "Increased expression of the adult stem cell marker Musashi-1 in endometriosis and endometrial carcinoma." J Pathol **215**(3): 317-29.

Adult stem cells are thought to be responsible for the high regenerative capacity of the human endometrium, and have been implicated in the pathology of endometriosis and endometrial carcinoma. The RNA-binding protein Musashi-1 is associated with maintenance and asymmetric cell division of neural and epithelial progenitor cells. We investigated expression and localization of Musashi-1 in endometrial, endometriotic and endometrial carcinoma tissue specimens of 46 patients. qPCR revealed significantly increased Musashi-1 mRNA expression in the endometrium compared to the myometrium. Musashi-1 protein expression presented as nuclear or cytoplasmic immunohistochemical staining of single cells in endometrial glands, and of single cells and cell groups in the endometrial stroma. Immunofluorescence microscopy revealed colocalization of Musashi-1 with its molecular target Notch-1 and telomerase. In proliferative endometrium, the proportion of Musashi-1-positive cells in the basalis layer was significantly increased 1.5-fold in the stroma, and three-fold in endometrial glands compared to the functionalis. The number of Musashi-1 expressing cell groups was significantly increased (four-fold) in proliferative compared to secretory endometrium. Musashi-1 expressing stromal cell and cell group numbers were significantly increased (five-fold) in both endometriotic and endometrial carcinoma tissue compared to secretory endometrium. A weak to moderate, diffuse cytoplasmic glandular staining was observed in 50% of the endometriosis cases and in 75% of the endometrioid carcinomas compared to complete absence in normal endometrial samples. Our results emphasize the role of Musashi-1-expressing endometrial progenitor cells in proliferating endometrium, endometriosis and endometrioid uterine carcinoma, and support the concept of a stem cell origin of endometriosis and endometrial carcinoma.

Grubbs, E. G., Z. Abdel-Wahab, et al. (2006). "Utilizing quantitative polymerase chain reaction to evaluate prostate stem cell antigen as a tumor marker in pancreatic cancer." Ann Surg Oncol **13**(12): 1645-54.

BACKGROUND: Real-time quantitative polymerase chain reaction (qPCR) may prove to be a sensitive technique by which to evaluate potential tumor markers in pancreatic cancer. METHODS: The prostate stem cell antigen (PSCA) gene was identified as a marker highly expressed in pancreatic adenocarcinoma and not normal pancreas. RNA from pancreatic and nonpancreatic cancer cell lines as well as tissue and blood from pancreatic cancer and control patients was reverse-transcribed and PSCA quantified by qPCR. RESULTS: Individual operator experience affects the results of qPCR, with significantly different copy numbers at experiment numbers 5, 15, and 40. Five of six pancreatic cell lines had PSCA/actin ratios 10-fold greater than nonpancreatic cancer lines. Mean PSCA expression in pancreatic tumor tissue was significantly higher ($P < 0.05$, Student's t-test) than in the tissue of benign pancreatic processes. The close correlation of PSCA/actin copy number with number of tumor cells in the blood was demonstrated by regression analysis ($r = 0.768$, $P = 0.0001$). PSCA copy number was significantly higher in the blood of patients with metastatic pancreatic cancer than in that of normal patients ($P < 0.05$, Student's t-test). CONCLUSIONS: Such trends suggest that PSCA may prove to be a valuable pancreatic cancer tumor marker. More generally, the technique of qPCR is shown to provide a sensitive method of evaluating markers in cancer patients.

Hardingham, J. E., D. Kotasek, et al. (1995). "Significance of molecular marker-positive cells after autologous peripheral-blood stem-cell transplantation for non-Hodgkin's lymphoma." J Clin Oncol **13**(5): 1073-9.

PURPOSE: To evaluate the significance of molecular marker-positive cells in a cohort of non-Hodgkin's lymphoma (NHL) patients undergoing high-dose chemotherapy and autologous peripheral-blood stem-cell transplantation (PBSCT). PATIENTS AND METHODS: Twenty-eight PBSC transplants have been performed in 24 patients with poor-prognosis NHL. Molecular analysis of the t(14;18) (q32;q21) translocation (bcl-2/immunoglobulin [Ig] heavy-chain joining locus [JH] fusion) or antigen receptor gene rearrangements was performed to determine the presence of lymphoma cells at presentation, in PBSC harvests, and before and after autologous PBSCT. Kaplan-Meier estimates of survival and Cox regression analyses were used to test the effect of bone marrow involvement, tumor-cell contamination of PBSCs, disease stage, and chemotherapy sensitivity at transplantation, and

presence of marker-positive cells post-PBSCT on disease-free and overall survival. RESULTS: Thirteen of 24 patients (54%) are alive following PBSCT at a median follow-up time of 654 days (range, 193 to 1,908). Nine patients are in complete remission (CR) at day 216 to 1,799 (median, 805) and four are alive following relapse (day 440, 573, 1,188, and 1,908). Eleven patients (46%) have died: three of transplant-related complications at day 0, 1, and 13, and eight of recurrent disease (day 132 to 1,330; median, 451). Longitudinal marker studies post-PBSCT showed that of 16 relapse events, 13 (81%) were positive for the lymphoma marker at or before clinically documented relapse. Marker studies became negative post-PBSCT in nine of nine patients who entered and remained in CR. Disease-free survival (DFS) was significantly shortened in patients in whom marker-positive cells were detected in serial samples posttransplantation (P = .006). Cox regression analysis showed that patients in this group had a 24 times higher risk of relapse (P = .03). CONCLUSION: The results show that the reappearance or persistence of marker-positive cells after autologous PBSCT is strongly associated with relapse.

Hayry, V., O. Tynninen, et al. (2008). "Stem cell protein BMI-1 is an independent marker for poor prognosis in oligodendroglial tumours." Neuropathol Appl Neurobiol **34**(5): 555-63.

AIMS: The polycomb factor BMI-1 has recently been implicated in tumorigenesis of the central nervous system in several experimental animal models. However, the significance of BMI-1 in human glioma has not been investigated. Here we describe expression of the polycomb protein BMI-1 and its downstream targets p16(Ink4a) and MDM2 in both high- and low-grade human glioma. METHODS: Tumour samples were collected from 305 adult patients treated for primary grades 2-4 gliomas between 1980 and 2006 in Finland and Germany. BMI-1, p16 and MDM2 expression was evaluated using immunohistochemistry in representative paraffin-embedded tumour tissue. The significance of observed immunoreactivity, age at onset, gender, histopathological findings and proliferative index was analysed in univariate and multivariate survival models. RESULTS: BMI-1 was expressed in all histologic types of diffuse gliomas. We found a significant correlation (P = 0.007) between the frequency of BMI-1 immunoreactive tumour cells and poor survival in World Health Organization grades II-III oligodendrogliomas and oligoastrocytomas (n = 62). The median survival of patients grouped by low, intermediate or high frequency of BMI-1 immunoreactive tumour cells was 191 months, 151 months and 68 months, respectively. This association was also significant in the Cox multivariate regression model. Nuclear p16 immunopositivity predicted better survival in astrocytomas and an inverse correlation between p16 expression and the Ki-67 mitotic index was also observed. CONCLUSIONS: BMI-1 is found in all histological types of gliomas and the relative protein expression of BMI-1 is a novel independent prognostic marker in oligodendroglial tumours.

Heng, B. C. and T. Cao (2005). "Immunoliposome-mediated delivery of neomycin phosphotransferase for the lineage-specific selection of differentiated/committed stem cell progenies: potential advantages over transfection with marker genes, fluorescence-activated and magnetic affinity cell-sorting." Med Hypotheses **65**(2): 334-6.

A major challenge in the therapeutic application of stem cells in regenerative medicine is the lineage-specific selection of their committed/differentiated progenies for transplantation. This is necessary to avoid engraftment of undesired lineages at the transplantation site, i.e. fibroblastic scar tissue, as well as to enhance the efficacy of transplantation therapy. Commonly used techniques for lineage-specific selection of committed/differentiated stem cell progenies include marker gene transfection, fluorescence-activated (FACS) and magnetic-affinity (MACS) cell-sorting. Nevertheless, these have their disadvantages for therapeutic applications. Marker gene transfection invariably leads to permanent genetic modification of stem cells, which in turn limits their use in human clinical therapy due to overwhelming ethical and safety concerns. FACS requires expensive instrumentation and highly-skilled personnel, and is unsuited for handling bulk quantities of cells that would almost certainly be required for transplantation therapy. MACS is a cheaper alternative, but the level of purity attained is also reduced. A possible novel approach that has yet to be investigated is immunoliposome-mediated delivery of neomycin phosphotranferase (NPT) for lineage-specific selection of stem cell progenies. This would avoid permanent genetic modification to the cell, unlike recombinant NPT expression linked to activation of specific promoter sequences. Moreover, it could potentially provide a much more practical and cost-effective alternative for handling bulk quantities of cells that would be required for transplantation therapy, as compared to FACS or MACS. As such, this alternative approach needs to be rigorously investigated, in view of its potentially useful applications in stem cell therapeutics.

Hirano, K., Y. Shishido-Hara, et al. (2008). "Expression of stem cell factor (SCF), a KIT ligand, in

gastrointestinal stromal tumors (GISTs): a potential marker for tumor proliferation." Pathol Res Pract **204**(11): 799-807.

Gastrointestinal stromal tumors (GISTs) show a high incidence of gain-of-function mutations of the c-kit gene, which encodes a receptor tyrosine kinase KIT. This mutation is seen independently of metastasis and/or recurrence of tumors; thus, the factors involved in tumor proliferation rate and malignancy are still not known. Some mesenchymal and epithelial tumors have been reported to co-express KIT and its ligand, stem cell factor (SCF), for autonomous proliferation by the autocrine mechanism. The purpose of this study is to clarify whether GIST cells produce SCF, despite mutated KIT with constitutive activation. Immunohistochemically, we examined the co-expression of KIT and SCF in 36 GIST cases. All cases were found to be KIT-positive, and of these, 21 cases, including four recurrent or metastatic GISTs, showed co-expression of SCF. MIB-1 labeling index was significantly higher, and the average tumor size was larger in SCF-positive cases. By confocal microscopy, KIT was expressed on the cellular membrane, around which SCF was distributed less densely. Western blot analysis revealed that the membrane-bound SCF of 31 kDa was found to be approximately 10 times more abundant than the soluble SCF of 18.5 kDa, suggesting continuous KIT activation. These results indicate that proliferation of GIST cells can be caused not only by the gain-of-function mutation of c-kit, but also by the autocrine mechanism of the SCF/KIT system. Thus, SCF expression would be a useful marker for tumor proliferation.

Horst, D., S. K. Scheel, et al. (2009). "The cancer stem cell marker CD133 has high prognostic impact but unknown functional relevance for the metastasis of human colon cancer." J Pathol **219**(4): 427-34.

In colon cancer, CD133 has recently been used to enrich for a subset of tumour cells with tumour-initiating capabilities and was therefore suggested to mark colon cancer stem cells. However, this molecule has surprisingly been shown to lack functional importance for tumour initiation itself. Herein, we investigated whether CD133 may be relevant for colon cancer metastasis in patients, and as metastasis requires several additional biological characteristics besides tumour initiation, we examined the effects of knocking down CD133 expression in colon cancer cell lines on proliferation, migration, invasion, and colony formation. We demonstrate that high CD133 expression correlates strongly with synchronous liver metastasis in a matched case-control collection, while siRNA-mediated knock down of this factor has no significant effect on the mentioned biological characteristics. Thus, we conclude that CD133 expression is a marker with high prognostic impact for colon cancer, while it seems to have no obvious functional role as a driving force of this malignancy.

Hosen, N., C. Y. Park, et al. (2007). "CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia." Proc Natl Acad Sci U S A **104**(26): 11008-13.

Permanent cure of acute myeloid leukemia (AML) by chemotherapy alone remains elusive for most patients because of the inability to effectively eradicate leukemic stem cells (LSCs), the self-renewing component of the leukemia. To develop therapies that effectively target LSC, one potential strategy is to identify cell surface markers that can distinguish LSC from normal hematopoietic stem cells (HSCs). In this study, we employ a signal sequence trap strategy to isolate cell surface molecules expressed on human AML-LSC and find that CD96, which is a member of the Ig gene superfamily, is a promising candidate as an LSC-specific antigen. FACS analysis demonstrates that CD96 is expressed on the majority of CD34(+)CD38(-) AML cells in many cases (74.0 +/- 25.3% in 19 of 29 cases), whereas only a few (4.9 +/- 1.6%) cells in the normal HSC-enriched population (Lin(-)CD34(+)CD38(-)CD90(+)) expressed CD96 weakly. To examine whether CD96(+) AML cells are enriched for LSC activity, we separated AML cells into CD96(+) and CD96(-) fractions and transplanted them into irradiated newborn Rag2(-/-) gamma(c)(-/-) mice. In four of five samples, only CD96(+) cells showed significant levels of engraftment in bone marrow of the recipient mice. These results demonstrate that CD96 is a cell surface marker present on many AML-LSC and may serve as an LSC-specific therapeutic target.

Huttner, H. B., P. Janich, et al. (2008). "The stem cell marker prominin-1/CD133 on membrane particles in human cerebrospinal fluid offers novel approaches for studying central nervous system disease." Stem Cells **26**(3): 698-705.

Cerebrospinal fluid (CSF) is routinely used for diagnosing and monitoring neurological diseases. The CSF proteins used so far for diagnostic purposes (except for those associated with whole cells) are soluble. Here, we show that human CSF contains specific membrane particles that carry prominin-1/CD133, a neural stem cell marker implicated in brain tumors, notably glioblastoma. Differential and equilibrium centrifugation and detergent solubility analyses showed that these membrane particles were similar in physical properties and microdomain

organization to small membrane vesicles previously shown to be released from neural stem cells in the mouse embryo. The levels of membrane particle-associated prominin-1/CD133 declined during childhood and remained constant thereafter, with a remarkably narrow range in healthy adults. Glioblastoma patients showed elevated levels of membrane particle-associated prominin-1/CD133, which decreased dramatically in the final stage of the disease. Hence, analysis of CSF for membrane particles carrying the somatic stem cell marker prominin-1/CD133 offers a novel approach for studying human central nervous system disease.

Ikeda, J. I., E. Morii, et al. (2006). "Epigenetic regulation of the expression of the novel stem cell marker CDCP1 in cancer cells." J Pathol **210**(1): 75-84.

CDCP1 is a novel stem cell marker that is expressed in several types of cancer. The mechanisms by which CDCP1 expression is regulated, and the clinical implications of this marker, have not been clarified. In this report, we examine the epigenetic regulation of CDCP1 expression in cell lines and clinical samples from patients with breast cancer. Many CpG sequences were localized around the transcription initiation site of CDCP1. These CpG motifs were found to be poorly methylated in cell lines with high levels of CDCP1 expression and heavily methylated in cell lines with low levels of CDCP1 expression. The in vitro methylation of CpG sites decreased CDCP1 promoter activity, and the addition of a demethylating reagent restored activity. In 25 breast cancer samples, an inverse correlation was noted between the CDCP1 expression level and the proportion of methylated to non-methylated CpG sites. Tumours with high-level CDCP1 expression showed higher levels of proliferation, as revealed by immunohistochemical detection of the MIB-1 antigen, than tumours with low-level CDCP1 expression. These findings indicate that the expression of CDCP1 is regulated by methylation of its promoter region in tumours. CDCP1 expression may prove to be useful in the further characterization of cancers.

Iki, K. and P. M. Pour (2006). "Expression of Oct4, a stem cell marker, in the hamster pancreatic cancer model." Pancreatology **6**(4): 406-13.

BACKGROUND: Oct4 has been shown to present a stem cell marker that is expressed in embryonic cells and in germ cell tumors. Recently, its expression in a few human tissues and cancer cells has been reported. Because in the hamster pancreatic cancer model most tumors develop from within islets presumably from stem cells, we investigated the expression of Oct4 in this model. METHODS: Two normal pancreases and 15 pancreatic cancers induced by N-nitrosobis(2-oxypropyl)amine (BOP) were processed for immunohistochemistry using a monoclonal Oct4 antibody at a concentration of 1:500. RESULTS: In the normal pancreas, Oct4 was expressed only in islet cells in a diffuse cytoplasmic pattern. No nuclear staining was found in any cells. In 14 of the pancreatic cancers, nuclear staining was detected in many cells or in small foci. Diffuse cytoplasmic but no nuclear staining was found in one tumor and a mixed Golgi type and nuclear staining in two cases. Nuclear staining was also identified in early intrainsular ductular and in Ca in situ lesions. CONCLUSIONS: BOP reactivates the Oct4 gene and can be considered an early tumor marker in this model.

Imamura, M., K. Miura, et al. (2006). "Transcriptional repression and DNA hypermethylation of a small set of ES cell marker genes in male germline stem cells." BMC Dev Biol **6**: 34.

BACKGROUND: We previously identified a set of genes called ECATs (ES cell-associated transcripts) that are expressed at high levels in mouse ES cells. Here, we examine the expression and DNA methylation of ECATs in somatic cells and germ cells. RESULTS: In all ECATs examined, the promoter region had low methylation levels in ES cells, but higher levels in somatic cells. In contrast, in spite of their lack of pluripotency, male germline stem (GS) cells expressed most ECATs and exhibited hypomethylation of ECAT promoter regions. We observed a similar hypomethylation of ECAT loci in adult testis and isolated sperm. Some ECATs were even less methylated in male germ cells than in ES cells. However, a few ECATs were not expressed in GS cells, and most of them targets of Oct3/4 and Sox2. The Octamer/Sox regulatory elements were hypermethylated in these genes. In addition, we found that GS cells express little Sox2 protein and low Oct3/4 protein despite abundant expression of their transcripts. CONCLUSION: Our results suggest that DNA hypermethylation and transcriptional repression of a small set of ECATs, together with post-transcriptional repression of Oct3/4 and Sox2, contribute to the loss of pluripotency in male germ cells.

Immervoll, H., D. Hoem, et al. (2008). "Expression of the "stem cell marker" CD133 in pancreas and pancreatic ductal adenocarcinomas." BMC Cancer **8**: 48.

BACKGROUND: It has been suggested that a small population of cells with unique self-renewal properties and malignant potential exists in solid tumors. Such "cancer stem cells" have been isolated

by flow cytometry, followed by xenograft studies of their tumor-initiating properties. A frequently used sorting marker in these experiments is the cell surface protein CD133 (prominin-1). The aim of this work was to examine the distribution of CD133 in pancreatic exocrine cancer. METHODS: Fifty-one cases of pancreatic ductal adenocarcinomas were clinically and histopathologically evaluated, and immunohistochemically investigated for expression of CD133, cytokeratin 19 and chromogranin A. The results were interpreted on the background of CD133 expression in normal pancreas and other normal and malignant human tissues. RESULTS: CD133 positivity could not be related to a specific embryonic layer of organ origin and was seen mainly at the apical/endoluminal surface of non-squamous, glandular epithelia and of malignant cells in ductal arrangement. Cytoplasmic CD133 staining was observed in some non-epithelial malignancies. In the pancreas, we found CD133 expressed on the apical membrane of ductal cells. In a small subset of ductal cells and in cells in centroacinar position, we also observed expression in the cytoplasm. Pancreatic ductal adenocarcinomas showed a varying degree of apical cell surface CD133 expression, and cytoplasmic staining in a few tumor cells was noted. There was no correlation between the level of CD133 expression and patient survival. CONCLUSION: Neither in the pancreas nor in the other investigated organs can CD133 membrane expression alone be a criterion for "stemness". However, there was an interesting difference in subcellular localization with a minor cell population in normal and malignant pancreatic tissue showing cytoplasmic expression. Moreover, since CD133 was expressed in shed ductal cells of pancreatic tumors and was found on the surface of tumor cells in vessels, this molecule may have a potential as clinical marker in patients suffering from pancreatic cancer.

Inoue, M., F. Koga, et al. (2008). "False tumor marker surge evoked by peripheral blood stem cell transplantation." Oncologist **13**(5): 526-9.

Peripheral blood stem cell transplantation (PBSCT) allows multiple intensive chemotherapy treatments and hematopoietic progenitor cell rescues for poor-risk patients with a variety of malignancies. We report false surges of a tumor marker, serum human chorionic gonadotropin (hCG), evoked by PBSCT in the course of chemotherapy for a poor-risk testicular cancer patient. We confirmed that this phenomenon resulted from reinfusion of peripheral blood stem cells (PBSCs) containing hCG at a high concentration, collected when the patient's serum hCG levels were remarkably elevated. This is the first report to demonstrate false tumor marker surges caused by PBSCT. Because a rapid rise in tumor markers may demand an immediate change in the therapeutic strategy, physicians should be aware of the possibility of this phenomenon when treating poor-risk cancer patients whose tumor markers are remarkably elevated at the time of PBSC harvest.

Ioffe, E., Y. Liu, et al. (1995). "WW6: an embryonic stem cell line with an inert genetic marker that can be traced in chimeras." Proc Natl Acad Sci U S A **92**(16): 7357-61.

Mutant mice produced by gene targeting in embryonic stem (ES) cells often have a complex or embryonic lethal phenotype. In these cases, it would be helpful to identify tissues and cell types first affected in mutant embryos by following the contribution to chimeras of ES cells homozygous for the mutant allele. Although a number of strategies for following ES cell development in vivo have been reported, each has limitations that preclude its general application. In this paper, we describe ES cell lines that can be tracked to every nucleated cell type in chimeras at all developmental stages. These lines were derived from blastocysts of mice that carry an 11-Mb beta-globin transgene on chromosome 3. The transgene is readily detected by DNA in situ hybridization, providing an inert, nuclear-localized marker whose presence is not affected by transcriptional or translational controls. The "WW" series of ES lines possess the essential features of previously described ES lines, including giving rise to a preponderance of male chimeras, all of which have to date exhibited germ-line transmission. In addition, clones selected for single or double targeting events form strong chimeras, demonstrating the feasibility of using WW6 cells to identify phenotypes associated with the creation of a null mutant.

Ishimura, D., N. Yamamoto, et al. (2008). "Differentiation of adipose-derived stromal vascular fraction culture cells into chondrocytes using the method of cell sorting with a mesenchymal stem cell marker." Tohoku J Exp Med **216**(2): 149-56.

The incidence of arthritic diseases is rapidly increasing in most advanced countries. Articular cartilage, which is the most important tissue in the joint, consists of chondrocytes and abundant extracellular matrix, including aggrecan, and shows poor self-repair. We studied the potential of stem cells in mouse subcutaneous adipose tissue as a source of cells to regenerate cartilage tissue. Analysis of adipose-derived stromal vascular fraction culture cells (ADSVFs) using mesenchymal stem cell markers showed that CD90-positive cells accounted for 93.8%, CD105-positive cells for 68.5%, and p75 neurotrophin receptor (p75NTR, CD271)-positive cells for 36.1%.

These results indicate that cells positive for mesenchymal stem cell markers are present in ADSVFs. The CD105-positive or -negative cells were isolated from ADSVFs by magnetic cell separation (MACS), and the efficiency of differentiation into chondrocytes was compared with using three methods of pellet method, gel-coating method, and gel-embedding sheet method. Using the CD105-positive cells and the gel-embedding sheet method, aggrecan mRNA was detected about three times higher than pellet and gel-coating methods. The above data suggest that ADSVFs could be differentiated into chondrocyte-like cells in the gel-embedding sheet method and could be useful in regenerative medicine to treat cartilage defects or cartilage degenerative disease. The use of cells sorted by mesenchymal stem cell markers from adipose tissue would gain position in the repair of cartilage tissue.

Izumi, M., B. J. Pazin, et al. (2009). "Quantitative comparison of stem cell marker-positive cells in fetal and term human amnion." J Reprod Immunol **81**(1): 39-43.

Scattered in the amniotic epithelium of the human term placenta are pluripotent stem cell marker-positive cells. Unlike other parts of the placenta, amniotic epithelial (AE) cells are derived from pluripotent epiblasts. It is hypothesized that most epiblast-derived fetal AE cells are positive for stem cell markers at the beginning of pregnancy and that the stem cell marker-positive cells scattered through the term amnion are remaining, epiblast-like stem cells. To test this hypothesis, human fetal amnia from early-stage pregnancies were evaluated for expression of the stem cell-specific cell surface markers TRA 1-60 and TRA 1-81 and of the pluripotent stem cell marker genes Oct4, Nanog, and Sox2. Whole-mount immunohistochemical analysis demonstrated that a greater proportion of AE cells in the 17-19 week human fetal amnion are positive for both TRA 1-60 and TRA 1-81 than in the term amnion. Quantitative real-time RT-PCR analysis confirmed that the fetal AE cells exhibit greater stem cell marker gene expression than those in term placentae. Expression of both Nanog and Sox2 mRNAs were significantly higher in the fetal amnion, while Oct4 mRNA expression was not significantly changed. These differences in abundance of stem cell marker-positive cells and stem cell marker gene expression together indicate that some stem cell marker-positive cells are conserved over the course of pregnancy. The results suggest that stem cell marker-positive AE cells in the term amnion are retained from epiblast-derived fetal AE cells.

Jiang, F., Q. Qiu, et al. (2009). "Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer." Mol Cancer Res **7**(3): 330-8.

Tumor contains small population of cancer stem cells (CSC) that are responsible for its maintenance and relapse. Analysis of these CSCs may lead to effective prognostic and therapeutic strategies for the treatment of cancer patients. We report here the identification of CSCs from human lung cancer cells using Aldefluor assay followed by fluorescence-activated cell sorting analysis. Isolated cancer cells with relatively high aldehyde dehydrogenase 1 (ALDH1) activity display in vitro features of CSCs, including capacities for proliferation, self-renewal, and differentiation, resistance to chemotherapy, and expressing CSC surface marker CD133. In vivo experiments show that the ALDH1-positive cells could generate tumors that recapitulate the heterogeneity of the parental cancer cells. Immunohistochemical analysis of 303 clinical specimens from three independent cohorts of lung cancer patients and controls show that expression of ALDH1 is positively correlated with the stage and grade of lung tumors and related to a poor prognosis for the patients with early-stage lung cancer. ALDH1 is therefore a lung tumor stem cell-associated marker. These findings offer an important new tool for the study of lung CSCs and provide a potential prognostic factor and therapeutic target for treatment of the patients with lung cancer.

Kania, G., D. Corbeil, et al. (2005). "Somatic stem cell marker prominin-1/CD133 is expressed in embryonic stem cell-derived progenitors." Stem Cells **23**(6): 791-804.

Prominin-1/CD133 is a plasma membrane marker found in several types of somatic stem cells, including hematopoietic and neural stem cells. To study its role during development and with differentiation, we analyzed its temporal and spatial expression (mRNA and protein) in preimplantation embryos, undifferentiated mouse embryonic stem (ES) cells, and differentiated ES cell progeny. In early embryos, prominin-1 was expressed in trophoblast but not in cells of the inner cell mass; however, prominin-1 transcripts were detected in undifferentiated ES cells. Both ES-derived cells committed to differentiation and early progenitor cells coexpressed prominin-1 with early lineage markers, including the cytoskeletal markers (nestin, cytokeratin 18, desmin), fibulin-1, and valosin-containing protein. After spontaneous differentiation at terminal stages, prominin-1 expression was downregulated and no coexpression with markers characteristic for neuroectodermal, mesodermal, and endodermal cells was found. Upon induction of neuronal differentiation,

some prominin-1-positive cells, which coexpressed nestin and showed the typical morphology of neural progenitor cells, persisted until terminal stages of differentiation. However, no coexpression of prominin-1 with markers of differentiated neural cells was detected. In conclusion, we present the somatic stem cell marker prominin-1 as a new parameter to define ES-derived committed and early progenitor cells.

Kanoh, M., Y. Amoh, et al. (2008). "Expression of the hair stem cell-specific marker nestin in epidermal and follicular tumors." Eur J Dermatol **18**(5): 518-23.

Nestin, a marker of neural stem cells, is expressed in the stem cells of the mouse hair follicle. The nestin-expressing hair follicle stem cells give rise to the outer-root sheath. Nestin-expressing hair follicle stem cells that are negative for the keratinocyte marker keratin 15 (K15) can differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. Recent studies suggest that the epithelial stem cells are important in tumorigenesis. In this study, we immunohistochemically examined the expression of three hair follicle stem cell and progenitor cell markers, nestin, K15, and CD34, in normal human epidermis and hair follicles and in epidermal and follicular tumors, trichilemmoma, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC). In normal human skin, the cells in the epidermal basal layer were positive for K15 and negative for nestin and CD34. The hair follicle cells below the sebaceous glands were also positive for nestin and K15 and negative for CD34. The outer-root sheath cells under this area could be divided into three parts: an upper part of the outer-root sheath cells that was partially positive for nestin and positive for K15 and negative for CD34; a middle part that was CD34-positive and K15-negative; and a lower part that was positive for K15 and negative for CD34. In the tumor tissues, nestin immunoreactivity was observed in trichilemmoma but not in BCC. Also, immunoreactivity for K15 was strong in BCC and weak in trichilemmoma, and SCC was negative for nestin and partially positive for K15. No CD34 immunoreactivity was observed in any of the cases. These results suggested that trichilemmoma originates in the nestin-positive/K15-positive/CD34-negative outer-root sheath cells below sebaceous glands, BCC tumor cells from the more mature nestin-negative/K15-positive/CD34-negative outer-root sheath cells, and SCC from the nestin-negative/K15-positive/CD34-negative keratinocytes of the basal cell layer in the epidermis.

Karbanova, J., E. Missol-Kolka, et al. (2008). "The stem cell marker CD133 (Prominin-1) is expressed in various human glandular epithelia." J Histochem Cytochem **56**(11): 977-93.

Human prominin-1 (CD133) is expressed by various stem and progenitor cells originating from diverse sources. In addition to stem cells, its mouse ortholog is expressed in a broad range of adult epithelial cells, where it is selectively concentrated in their apical domain. The lack of detection of prominin-1 in adult human epithelia might be explained, at least in part, by the specificity of the widely used AC133 antibody, which recognizes an epitope that seems dependent on glycosylation. Here we decided to re-examine its expression in adult human tissues, particularly in glandular epithelia, using a novel monoclonal antibody (80B258) generated against the human prominin-1 polypeptide. In examined tissues, we observed 80B258 immunoreactivity at the apical or apicolateral membranes of polarized cells. For instance, we found expression in secretory serous and mucous cells as well as intercalated ducts of the large salivary and lacrimal glands. In sweat glands including the gland of Moll, 80B258 immunoreactivity was found in the secretory (eccrine and apocrine glands) and duct (eccrine glands) portion. In the liver, 80B258 immunoreactivity was identified in the canals of Hering, bile ductules, and small interlobular bile ducts. In the uterus, we detected 80B258 immunoreactivity in endometrial and cervical glands. Together these data show that the overall expression of human prominin-1 is beyond the rare primitive cells, and it seems to be a general marker of apical or apicolateral membrane of glandular epithelia. This manuscript contains online supplemental material at http://www.jhc.org. Please visit this article online to view these materials.

Katoh, Y. and M. Katoh (2007). "Comparative genomics on PROM1 gene encoding stem cell marker CD133." Int J Mol Med **19**(6): 967-70.

Stem cells are characterized by self-renewal and multipotency to produce multiple lineages of progenitor and differentiated cells. PROM1 gene encodes CD133 protein, which is a cell surface marker of hematopoietic stem cells, prostatic epithelial stem cells, pancreatic stem cells, leukemic stem cells, liver cancer stem cells, and colorectal cancer stem cells. Here, comparative integromics analyses on PROM1 orthologs were performed. Human PROM1 RefSeq NM_006017.1 was a truncated transcript, while AK027422.1 was the representative human PROM1 cDNA. Chimpanzee PROM1 gene, consisting of 27 exons, was identified within NW_001234057.1 genome sequence. Chimpanzee 5-transmembrane protein CD133 showed 99.2% and 60.9% total-amino-acid identity with human and mouse CD133 orthologs,

respectively. Only 2 of 8 Asn-linked glycosylation sites in primate CD133 orthologs were conserved in rodent CD133 orthologs. Comparative proteomics revealed that CD133 orthologs were relatively divergent between primates and rodents. PROM1 mRNA was expressed in human embryonic stem (ES) cells, trachea, small intestine, NT2 cells, diffuse-type gastric cancer, and colorectal cancer. Human PROM1 mRNA transcribed from exon 1A was the major transcript. Comparative genomics revealed that the region around exon 1A corresponding to 5'-UTR of human PROM1 mRNA was not conserved in mouse and rat. Intron 2 of PROM1 orthologs was relatively well conserved among mammals. Tandem TCF/LEF-binding sites with 7-bp spacing within intron 2 were conserved among human, chimpanzee, mouse, and rat PROM1 orthologs. Together these facts indicate that canonical WNT signaling activation is implicated in CD133 expression in ES cells, adult stem cells, and cancer stem cells.

Kim, M. K., S. Kim, et al. (2007). "A randomized comparison of peripheral blood hematopoietic progenitor cell level of 5/mm3 versus 50/mm3 as a surrogate marker to initiate efficient autologous blood stem cell collection." J Clin Apher **22**(5): 277-82.

We previously showed that at least 5/mm(3) hematopoietic progenitor cells (HPCs) could be used as a marker for initiating autologous blood stem cell collection (ABSCC). However, the timing of efficient ABSCC following mobilization is still to be determined. We conducted a prospective, randomized comparison of 5/mm(3) versus 50/mm(3) peripheral blood (PB) HPCs as a surrogate marker to initiate efficient ABSCC. Forty-five consecutive patients, 26 with multiple myeloma (MM) and 19 with non-Hodgkin's lymphoma (NHL), were enrolled between October 2004 and October 2006. Chemotherapy was cyclophosphamide 4 g/m(2) for MM and ESHAP (etoposide, methylprednisolone, high-dose cytarabine, and cisplatin), with or without Rituximab, for NHL. Circulating HPCs were monitored daily with the Sysmex SE9000 automated hematology analyzer, and harvested CD34+ cells were counted by flow cytometry. ABSCC was initiated when HPC levels reached at least 5/mm(3) (HPC5 group) or 50/mm(3) (HPC50 group). The median number of harvested CD34+ cells was 15.0 x 10(6)/kg and 21.0 x 10(6)/kg in the HPC5 and HPC50 groups, respectively (P = 0.23). Optimal collection (>5 x 10(6) CD34+ cells/kg) in a single session (day 1) was attained in 15 HPC5 patients (63%) and in 14 HPC50 patients (67%), and targeted collection of 5 x 10(6) CD34+ cells/kg was achieved in 100 and 95% of HPC5 and HPC50 patients, respectively (P = 0.47), with a median number of 1 apheresis in both groups (P = 0.58). There were no between group differences in optimal collection rate on day 1, median number of aphereses to achieve optimal collection, and overall optimal collection rate. HPC > or = 5/mm(3) and > or =50/mm(3) are both reliable indices for the timing of ABSCC.

Kim, T. H., H. M. Lee, et al. (2009). "Expression and distribution patterns of the stem cell marker, nestin, and the stem cell renewal factor, BMI-1, in normal human nasal mucosa and nasal polyps." Acta Otolaryngol **129**(9): 996-1001.

CONCLUSIONS: These results suggest that nestin and BMI-1 are candidates for stem cell markers and renewal factors in human nasal mucosa, may contribute to tissue homeostasis and differentiation in the epithelium and submucosal glands of normal nasal mucosa, and may play a role in proliferation of nasal polyps. OBJECTIVES: The stem cell marker, nestin, and the stem cell renewal factor, BMI-1, have been identified in a variety of inflammatory and normal tissues, implicating these markers in tissue regeneration. MATERIALS AND METHODS: We investigated the expression and distribution of nestin and BMI-1 in normal nasal mucosa and nasal polyps, using RT-PCR, immunohistochemistry, and Western blotting. RESULTS: Nestin and BMI-1 were localized to the epithelium and submucosal glands of normal nasal mucosa and nasal polyps. The expression of nestin was confined to the plasma membrane and cytoplasm, whereas BMI-1 showed a nuclear staining pattern. In normal nasal mucosa and nasal polyps, nestin and BMI-1 expression was strongest in the basal portion of the epithelial layer, and decreased toward the upper portion. In the submucosal glands, weak to strong expression was commonly detected in the glandular acini. There was no significant difference in the level of expression of nestin and BMI-1 between normal nasal mucosa and nasal polyps.

Kimura, H., E. Morii, et al. (2006). "Role of DNA methylation for expression of novel stem cell marker CDCP1 in hematopoietic cells." Leukemia **20**(9): 1551-6.

CDCP1, a novel stem cell marker, is expressed in hematopoietic cell line K562 but not in Jurkat. When CDCP1 promoter was transfected exogenously, Jurkat showed comparable promoter activity with K562, suggesting that the factor to enhance transcription was present but interfered to function in Jurkat. The reporter assay and si-RNA-mediated knockdown experiment revealed that zfp67, a zinc-finger protein, enhanced CDCP1 transcription. Amount of zfp67 in Jurkat was comparable with K562, but chromatin immunoprecipitation showed

that zfp67 bound to CDCP1 promoter in K562 but not in Jurkat. There are CpG sequences around the promoter of CDCP1, which were heavily methylated in Jurkat but not in K562. Addition of demethylating reagent to Jurkat induced CDCP1 expression, and increased the zfp67 binding to CDCP1 promoter. Among normal hematopoietic cells such as CD34+CD38- cells, lymphocytes and granulocytes, inverse correlation between proportion of methylated CpG sequences and CDCP1 expression level was found. Demethylation of CpG sequences in lymphocytes, in which CpG sequences were heavily methylated, induced CDCP1 expression and its expression level further increased through zfp67 overexpression. The methylation of DNA appeared to regulate the cell-type-specific expression of CDCP1 through the control of interaction between chromatin DNA and transcription factors.

Koch, L. K., H. Zhou, et al. (2008). "Stem cell marker expression in small cell lung carcinoma and developing lung tissue." Hum Pathol **39**(11): 1597-605.

Histopathologic and clinical findings suggest that small cell lung cancer is derived from a multipotent proximal airway epithelial cell. In order to investigate the histogenetic origin of small cell lung cancer, we compared stem cell marker expression in human fetal lung tissue, human adult bronchial tissue, and a cohort of 64 small cell lung cancers. Supporting derivation of a multipotent precursor cell, 87.5% (56/64) of small cell lung cancers showed a dot-like expression of podocalyxin-like protein 1 (PODXL-1), a marker of embryonic and hematopoetic stem cells. Of small cell lung cancers, 98.4% (63/64) ubiquitously expressed Bmi-1, a key player in self-renewal of stem cells. Oct4 and AP2gamma were not expressed. Although podocalyxin-like protein 1 did not correlate with p53 or Wilms tumor suppressor 1, known regulators of podocalyxin-like protein 1, we could demonstrate demethylated CpG islands in the podocalyxin-like protein 1 promoter in small cell lung cancer, indicating epigenetic regulation. During fetal lung development and within adult bronchial mucosa, Bmi-1 was expressed ubiquitously. In contrast, podocalyxin-like protein 1 was detected in few stromal cells during the pseudoglandular phase (n = 7) and, importantly, in clustered epithelial cells within proximal bronchi and the trachea during the canalicular phase (n = 10). Interestingly, podocalyxin-like protein 1 was not expressed in normal or metaplastic adult bronchial epithelium (n = 36) but was expressed in sparse epithelial cells in half of the cases of normal tumor adjacent bronchial mucosa (20/40). Taken together, we show that small cell lung cancers and clustered epithelial cells in developing proximal bronchi share the expression of stem cell markers, suggesting a possible histogenetic link.

Krahl, D. and K. Sellheyer (2009). "The neuroepithelial stem cell protein nestin is a marker of the companion cell layer of the adult and developing human hair follicle." Br J Dermatol **161**(3): 678-82.

BACKGROUND: The interface between the inner root sheath (IRS) and the outer root sheath (ORS) represents a slippage plane for the hair shaft to evolve from the pilar canal to the skin surface. Interposed between the IRS and ORS is a single cell layer which is believed to represent the angle point of that slippage plane, termed the companion cell layer (CCL). The CCL is cited in most of the literature as part of the ORS. OBJECTIVES: To describe the expression pattern of nestin, a neuroepithelial stem cell protein, in the adult and developing human hair follicle. METHODS: Immunohistochemical evaluation with a monoclonal antibody against nestin was performed using standard techniques. RESULTS: Nestin is selectively expressed in the CCL of the adult anagen and late stage fetal hair follicles. Early stages of hair follicle development are negative for nestin expression. CONCLUSIONS: The selective demarcation of the CCL by nestin highlights the unique feature of this follicular cell layer and raises the question of whether the CCL should not be better conceptualized as a part of the IRS rather than the ORS. The results of the present study, together with published ultrastructural data, also suggest that the slippage plane for the evolving hair shaft may be located at the interface between the CCL and the ORS.

Krishnamurthy, P., D. D. Ross, et al. (2004). "The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with heme." J Biol Chem **279**(23): 24218-25.

Our studies demonstrate that the ABC transporter and marker of stem and progenitor cells known as the breast cancer resistance protein (BCRP or ABCG2) confers a strong survival advantage under hypoxic conditions. We show that, under hypoxia, progenitor cells from Bcrp(-)/(-)mice have a reduced ability to form colonies as compared with progenitor cells from Bcrp(+/+) mice. Blocking BCRP function in Bcrp(+/+) progenitor cells markedly reduces survival under hypoxic conditions. However, blocking heme biosynthesis reverses the hypoxic susceptibility of Bcrp(-/-) progenitor cells, a finding that indicates that heme molecules (i.e. porphyrins) are detrimental to Bcrp(-/-) cells under hypoxia. BCRP specifically binds heme, and cells lacking BCRP accumulate porphyrins. Finally, Bcrp expression is up-regulated by hypoxia, and we demonstrate that this up-regulation involves the hypoxia-inducible

transcription factor complex HIF-1. Collectively, our findings suggest that cells can, upon hypoxic demand, use BCRP to reduce heme or porphyrin accumulation, which can be detrimental to cells. Our findings have implications for the survival of stem cells and tumor cells in hypoxic environments.

Lahad, J. P., G. B. Mills, et al. (2005). "Stem cell-ness: a "magic marker" for cancer." J Clin Invest **115**(6): 1463-7.

Transcriptional profiling of patient tumors is a much-heralded advancement in cancer therapy, as it provides the opportunity to identify patients who would benefit from more or less aggressive therapy and thus allows the development of individualized treatment. However, translation of this promise into patient benefit has proven challenging. In this issue of the JCI, Glinsky and colleagues used human and murine models to identify a potential stem cell mRNA signature, based on the hypothesis that tumors with stem cell-like characteristics are likely to have a poor prognosis. Remarkably, an 11-gene "expression signature" associated with "stem cell-ness" separated patients with different cancers into good- and poor-prognosis groups. Such a "magic marker" would, if validated, have a major impact on patient care. However, there remain challenges incumbent with creating and validating such signatures.

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

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

Larouche, D., C. Hayward, et al. (2005). "Keratin 19 as a stem cell marker in vivo and in vitro." Methods Mol Biol **289**: 103-10.

The skin is a dynamic tissue in which terminally differentiated keratinocytes are replaced by the proliferation of new epithelial cells that will undergo differentiation. The rapid and continual turnover of skin throughout life depends on a cell population with unique characteristics: the stem cells. These cells are relatively undifferentiated, retain a high capacity for self-renewal throughout their lifetime, have a large proliferative potential, and are normally slow cycling. The long-term regeneration of grafted cultured epidermis indicates that epidermal stem cells are maintained in cultures. In animals they can be identified with 3H-thymidine or bromodeoxyuridine based on their property of slow cycling. The development of markers such as keratin 19 also permits their study in human tissues. In this chapter, protocols to study skin stem cells using their property of slow cycling and their expression of keratin 19 will be described in detail. The methods include the double labeling of tissues for keratin 19 and label-retaining cells (auto radiography of 3H-thymidine) in situ. The labeling of keratin 19 by immunofluorescence of by flow cytometry is described for cells in vitro.

Leung, N., A. Dispenzieri, et al. (2005). "Renal response after high-dose melphalan and stem cell transplantation is a favorable marker in patients with primary systemic amyloidosis." Am J Kidney Dis **46**(2): 270-7.

BACKGROUND: Primary systemic (AL) amyloidosis is a rare plasma cell disorder characterized by soft-tissue deposition of monoclonal light chain fragments. High-dose melphalan followed by autologous stem cell transplantation currently has become the treatment of choice. Favorable outcome is ensured with achievement of hematologic response, but little is known about organ response. This study was undertaken to determine the prognostic importance of renal response after high-dose melphalan and stem cell transplantation. METHODS: All patients with AL amyloidosis who underwent autologous stem cell transplantation between 1996 and December 2002 were selected for study. Renal response is defined as a 50% or greater reduction in proteinuria with less than 25% decline in renal function. Exclusion criteria included pretransplantation dialysis therapy or dialysis dependence posttransplantation, treatment mortality,

lack of proteinuria assessment posttransplantation, and baseline proteinuria with protein less than 1 g/d. RESULTS: Of 105 patients, 47 were excluded for stated reasons. Renal response was achieved in 60.3% of evaluated patients. Proteinuria was reduced by greater than 90% in 37.9% and returned to normal in 15.5%. Median response time was 12 months. Renal response was associated with a greater increase in serum albumin level ($P = 0.001$), maintenance of renal function ($P < 0.001$), and better survival ($P = 0.0003$). Renal responders had better survival regardless of hematologic response ($P = 0.01$ to 0.05). CONCLUSION: Currently, high-dose melphalan followed by stem cell transplantation is the most effective treatment for AL amyloidosis for those who are eligible. Our data show that renal response after high-dose melphalan followed by stem cell transplantation is associated with improved survival. Renal response is an independent marker of treatment success and can be used in cases in which determination of hematologic response is difficult.

Lioznov, M. V., P. Freiberger, et al. (2005). "Aldehyde dehydrogenase activity as a marker for the quality of hematopoietic stem cell transplants." Bone Marrow Transplant **35**(9): 909-14.

Taking advantage of fluorescent substrates for their metabolic marker aldehyde dehydrogenase (ALDH), hematopoietic stem cells (HSC) were defined as SSC(lo)ALDH(br) - reflecting their low orthogonal light scattering and bright fluorescence intensity in flow cytometry. Based thereon, we investigated the usefulness of ALDH activity for characterizing HSC graft quality, particularly under stress conditions. We first compared the expression of ALDH vs CD34 in bone marrow and peripheral blood stem cell (PBSC) samples over 7 days. We noted that (i) only ALDH activity but not CD34 expression strongly reflected colony-forming ability over time, and that (ii) PBSC grafts stored at room temperature lost most of their progenitor cells within just 48 h. We then retrospectively related ALDH and CD34 expression as well as granulocyte-macrophage colony-forming units (CFU-GM) potential for 19 cryopreserved allogeneic PBSC grafts to engraftment data. Strikingly, in all six patients who received markedly decreased numbers of SSC(lo)ALDH(br) cells, this was associated not only with almost complete loss of CFU-GM potential but also with delayed establishment/permanent absence of full hematopoietic donor cell chimerism, whereas all other patients showed early complete donor chimerism. In conclusion, we suggest to measure ALDH activity as a surrogate marker for HSC activity, and to transport and store PBSC under controlled cooling conditions.

Lobo, M. V., M. I. Arenas, et al. (2004). "Nestin, a neuroectodermal stem cell marker molecule, is expressed in Leydig cells of the human testis and in some specific cell types from human testicular tumours." Cell Tissue Res **316**(3): 369-76.

The intermediate filament protein nestin is predominantly expressed in some stem/progenitor cells and appears to be a useful molecular tool to characterise tumours originating from precursor cells of neuroectodermal and mesenchymal lineages. Leydig cells originate in the adult testis by differentiation from stem cells and express a variety of neural and neuroendocrine markers. The possible expression of the neural stem cell marker nestin in Leydig cells and testicular tumour cells was determined by analysing the patterns of nestin expression in normal and pathological human testes by Western blot and immunohistochemical methods. In normal testis, nestin was found in some vascular endothelial cells, a subset of peritubular spindle-shaped cells and some Leydig cells; spermatogenic and Sertoli cells were unstained. In normal Leydig cells, nestin was distributed in the perinuclear cytoplasm and accumulated in the crystalloids of Reinke with ageing. In non-tumour pathologies (cryptorchidism, impaired spermatogenesis), the seminiferous tubules were immunonegative, whereas hyperplastic Leydig cells showed cytoplasmic immunolabelling. In testicular malignancies, nestin was localised in the Sertoli cells of the seminiferous tubules affected with intratubular germ cell neoplasia, in the hyperplastic Leydig cells associated with these tumours and in some components (mesenchymal and neuroepithelial cells) of teratomas; spermatocytic and non-spermatocytic seminomas were unstained. Some vascular endothelial cells were immunolabelled in all tumour samples. Thus, nestin is expressed in a population of normal and hyperplastic Leydig cells and in Sertoli cells in the presence of intratubular germ-cell neoplasia. Nestin may be a good marker for identifying components of testicular teratomas.

Machida, K., H. Tsukamoto, et al. (2009). "Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog." Proc Natl Acad Sci U S A **106**(5): 1548-53.

Alcohol synergistically enhances the progression of liver disease and the risk for liver cancer caused by hepatitis C virus (HCV). However, the molecular mechanism of this synergy remains unclear. Here, we provide the first evidence that Toll-like receptor 4 (TLR4) is induced by hepatocyte-specific transgenic (Tg) expression of the HCV nonstructural protein NS5A, and this induction mediates synergistic liver damage and tumor

formation by alcohol-induced endotoxemia. We also identify Nanog, the stem/progenitor cell marker, as a novel downstream gene up-regulated by TLR4 activation and the presence of CD133/Nanog-positive cells in liver tumors of alcohol-fed NS5A Tg mice. Transplantation of p53-deficient hepatic progenitor cells transduced with TLR4 results in liver tumor development in mice following repetitive LPS injection, but concomitant transduction of Nanog short-hairpin RNA abrogates this outcome. Taken together, our study demonstrates a TLR4-dependent mechanism of synergistic liver disease by HCV and alcohol and an obligatory role for Nanog, a TLR4 downstream gene, in HCV-induced liver oncogenesis enhanced by alcohol.

Mangiola, A., G. Lama, et al. (2007). "Stem cell marker nestin and c-Jun NH2-terminal kinases in tumor and peritumor areas of glioblastoma multiforme: possible prognostic implications." Clin Cancer Res **13**(23): 6970-7.

PURPOSE: It has been hypothesized that brain tumors are derived from stem cell or transiently dividing precursor transformation. Furthermore, c-Jun NH(2)-terminal kinases (JNKs) have been involved in gliomagenesis. This study analyzes stem cell marker nestin and JNK expression in glioblastoma multiforme (GBM) and peritumor tissue and assesses their possible prognostic implications. EXPERIMENTAL DESIGN: Nestin and both total JNK (tJNK) and phosphorylated JNK (pJNK) expression was investigated by immunohistochemistry in 20 GBMs. Samples were derived from tumors (first area), from tissues at a distance <1 cm (second area), and between 1 and 3.5 cm (third area) from the macroscopic tumor border. The relationships between patients' age, Karnofsky performance status, gender, protein expression, and survival were analyzed. RESULTS: Nestin cytoplasmic immunoreactivity was observed in the majority of cells in tumor but infrequently in peritumor areas. tJNK, observed in the nucleus and cytoplasm, was widely expressed in the three areas; pJNK, mostly located in the nuclei, was found in a variable percentage of cells in the tumor and peritumor tissue. Nestin and JNK expression in peritumor areas was independent of the presence of neoplastic cells. Univariate analysis indicated that survival was longer (19 versus 12 months; P = 0.01) for patients whose pJNK/nestin and (pJNK/tJNK)/nestin ratios in the second area were > or =2.619 and > or =0.026, respectively. The same variables showed an independent prognostic value in multivariate analysis. CONCLUSIONS: Nestin and JNK expression indicates that peritumor tissue, independently of the presence of neoplastic cells, may present signs of transformation. Moreover, pJNK/nestin and (pJNK/tJNK)/nestin ratios in that tissue seem to have some prognostic implications in GBM patients.

Mao, X. G., X. Zhang, et al. (2009). "Brain Tumor Stem-Like Cells Identified by Neural Stem Cell Marker CD15." Transl Oncol **2**(4): 247-57.

In recent years, a small number of cells that have stem cell properties were identified in human gliomas called brain tumor stem cells (BTSCs), which were thought to mainly contribute to the initiation and development of gliomas and could be identified by the surface marker CD133. However, recent studies indicated that the expression of CD133 might be regulated by environmental conditions such as hypoxia and that there might be CD133(-) BTSCs. Genetic mouse models demonstrated that some gliomas originated from transformed neural stem cells (NSCs). Therefore, we investigated the expression of CD15, a surface marker for NSCs, in tumor spheres derived from astrocytoma and ependymoma. CD15(+) cells isolated from these tumor spheres had properties of BTSCs including self-renewal, multidifferentiation, and the ability to recapitulate the phenocopy of primary tumors. CD15 exhibited stable expression in long-term cultured tumor spheres, which sustained BTSCs properties, whereas CD133 expression decreased significantly in late passages. Furthermore, CD15(+)CD133(-) cells isolated from early or late passages of tumor spheres showed similar characteristics of BTSCs. Examination of glioma samples by immunohistochemistry showed that CD15 was expressed in a subset of human brain tumors. Therefore, CD15 can be used as a marker of stem-like cells derived from brain tumors that might contain CD133(-) BTSCs.

Marzesco, A. M., P. Janich, et al. (2005). "Release of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133) from neural progenitors and other epithelial cells." J Cell Sci **118**(Pt 13): 2849-58.

Apical plasma membrane constituents of mammalian neural stem/progenitor cells have recently been implicated in maintaining their stem/progenitor cell state. Here, we report that in the developing embryonic mouse brain, the fluid in the lumen of the neural tube contains membrane particles carrying the stem cell marker prominin-1 (CD133), a pentaspan membrane protein found on membrane protrusions of the apical surface of neuroepithelial cells. Two size classes of prominin-1-containing membrane particles were observed in the ventricular fluid: approximately 600-nm particles, referred to as P2 particles, and 50-80-nm vesicles, referred to as P4 particles. The P2 and P4 particles appeared in the ventricular fluid at the

very onset and during the early phase of neurogenesis, respectively. Concomitant with their appearance, the nature of the prominin-1-containing apical plasma membrane protrusions of neuroepithelial cells changed, in that microvilli were lost and large pleiomorphic protuberances appeared. P4 particles were found in various body fluids of adult humans, including saliva, seminal fluid and urine, and were released by the epithelial model cell line Caco-2 upon differentiation. Importantly, P4 particles were distinct from exosomes. Our results demonstrate the widespread occurrence of a novel class of extracellular membrane particles containing proteins characteristic of stem cells, and raise the possibility that the release of the corresponding membrane subdomains from the apical surface of neural progenitors and other epithelial cells may have a role in tissue development and maintenance. Moreover, the presence of prominin-1-containing membrane particles in human body fluids may provide the basis for a protein-based diagnosis of certain diseases.

May, R., T. E. Riehl, et al. (2008). "Identification of a novel putative gastrointestinal stem cell and adenoma stem cell marker, doublecortin and CaM kinase-like-1, following radiation injury and in adenomatous polyposis coli/multiple intestinal neoplasia mice." Stem Cells **26**(3): 630-7.

In the gut, tumorigenesis arises from intestinal or colonic crypt stem cells. Currently, no definitive markers exist that reliably identify gut stem cells. Here, we used the putative stem cell marker doublecortin and CaM kinase-like-1 (DCAMKL-1) to examine radiation-induced stem cell apoptosis and adenomatous polyposis coli (APC)/multiple intestinal neoplasia (min) mice to determine the effects of APC mutation on DCAMKL-1 expression. Immunoreactive DCAMKL-1 staining was demonstrated in the intestinal stem cell zone. Furthermore, we observed apoptosis of the cells negative for DCAMKL-1 at 6 hours. We found DNA damage in all the cells in the crypt region, including the DCAMKL-1-positive cells. We also observed stem cell apoptosis and mitotic DCAMKL-1-expressing cells 24 hours after irradiation. Moreover, in APC/min mice, DCAMKL-1-expressing cells were negative for proliferating cell nuclear antigen and nuclear beta-catenin in normal-appearing intestine. However, beta-catenin was nuclear in DCAMKL-1-positive cells in adenomas. Thus, nuclear translocation of beta-catenin distinguishes normal and adenoma stem cells. Targeting DCAMKL-1 may represent a strategy for developing novel chemotherapeutic agents.

Mercati, F., L. Pascucci, et al. (2009). "Expression of mesenchymal stem cell marker CD90 on dermal sheath cells of the anagen hair follicle in canine species." Eur J Histochem **53**(3): 159-66.

The dermal sheath (DS) of the hair follicle is comprised by fibroblast-like cells and extends along the follicular epithelium, from the bulb up to the infundibulum. From this structure, cells with stem characteristics were isolated: they have a mesenchymal origin and express CD90 protein, a typical marker of mesenchymal stem cells. It is not yet really clear in which region of hair follicle these cells are located but some experimental evidence suggests that dermal stem cells are localized prevalently in the lower part of the anagen hair follicle. As there are no data available regarding DS stem cells in dog species, we carried out a morphological analysis of the hair follicle DS and performed both an immunohistochemical and an immunocytochemical investigation to identify CD90+ cells. We immunohistochemically evidenced a clear and abundant positivity to CD90 protein in the DS cells located in the lower part of anagen hair follicle. The positive cells showed a typical fibroblast-like morphology. They were flat and elongated and inserted among bundles of collagen fibres. The whole structure formed a close and continuous sleeve around the anagen hair follicle. Our immunocytochemical study allowed us to localize CD90 protein at the cytoplasmic membrane level.

Michur, H., K. Maslanka, et al. (2008). "Reticulated platelets as a marker of platelet recovery after allogeneic stem cell transplantation." Int J Lab Hematol **30**(6): 519-25.

Reticulated platelets (RP) are the youngest forms of platelets in blood and reflect the rate of bone marrow platelet production. In the present study, we used flow cytometric analysis to determine the percentage of RPs in patients undergoing allogeneic stem cell transplantation. We investigated 10 patients after transplantation from HLA identical siblings: five with acute myeloid leukemia (AML), four with chronic myeloid leukemia (CML), and one patient with myelodysplastic syndrome (MDS). Of the patients examined, four patients underwent allogeneic bone marrow transplantation and six patients underwent peripheral blood stem cell transplantation. It was observed that the initially reduced percentage of RPs (2.9 +/- 1.7%; mean +/- SD) was significantly higher (P = 0.0109) in all patients (13.6 +/- 6.4%) in the following 10-26 days. The RP percentage peak preceded the recovery of peripheral platelet count up to 45.6 x 10(9)/l on average by 3 days. We found no difference in RP% between the AML and CML patients but we did observe that in CML patients the RP percentage increased on average 7 days earlier than in AML patients. The elevated RP percentage

reflects increased bone marrow regeneration and can be considered an additional marker of thrombopoietic recovery in the patients undergoing allogeneic stem cell transplantation.

Miki, T., K. Mitamura, et al. (2007). "Identification of stem cell marker-positive cells by immunofluorescence in term human amnion." J Reprod Immunol **75**(2): 91-6.

The placenta contains different populations of stem/progenitor cells such as mesenchymal, hematopoietic, trophoblastic and pluripotent stem cells. Although some tissue-specific stem cells are restricted to particular parts of the placenta, the localization of embryonic stem cell-like cells in term human placenta has not been determined. We have used immunofluorescence staining techniques with antibodies to pluripotent stem cell antigens, SSEA-3, SSEA-4, TRA 1-60 and TRA 1-81, and confocal microscopic analysis to identify and localize stem cells within the placenta. Stem cell marker-positive cells were found in amnion but not in choriodecidua, tissues known to contain hematopoietic and trophoblastic stem cells. Amniotic mesenchymal cells did not react with these pluripotent stem cell markers, while all amniotic epithelial cells reacted with at least one antibody. The TRA 1-60 and TRA 1-81 positive cells were solitary and present throughout the surface of amniotic membrane without a specific pattern of distribution, whereas SSEA-3 was negative and SSEA-4 was weakly positive on all amniotic epithelial cells. These data suggest that the human amnion contains stem cell-like cells at different states of differentiation. Human term amnion may be useful source of pluripotent stem cells for regenerative medicine.

Mongan, N. P., K. M. Martin, et al. (2006). "The putative human stem cell marker, Rex-1 (Zfp42): structural classification and expression in normal human epithelial and carcinoma cell cultures." Mol Carcinog **45**(12): 887-900.

Human Rex-1 (hRex-1) (also referred to as zinc-finger protein-42, Zfp42) encodes a zinc finger protein expression of which is believed to be characteristic of pluripotent stem cells. We have applied bioinformatics to classify the relationship of human, rat, and mouse REX1 proteins in the C2H2 family of zinc finger proteins and demonstrate that REX1 is a member of the YY1 sub-family of transcription factors, which includes the Drosophila pleiohomeotic (Pho) protein. We have generated a molecular model of the human REX1 zinc finger domains based on the crystal structure of the YY1 transcription factor. To date, expression of hRex-1 and its extensively studied mouse homolog mRex-1, has been reported only in embryonic and adult stem cells and in differentiated spermatocytes. In this study, reverse transcription-PCR and Western analysis were employed to assay for hRex-1 expression in cultured normal human epithelial cells and human carcinoma cell lines. Expression of hRex-1 mRNA was detected in normal human epidermal keratinocytes, normal prostate epithelial cells (PrEC), bronchial, and small airway lung epithelial cells. Other stem cell markers, such as Oct 4, DAB2, and cMyc were also detected in normal human epidermal keratinocyte cultures. Expression of hRex-1 was also detected in some human tumor cell lines including MDA-MB-468 mammary carcinoma, SCC-15 head and neck squamous cell carcinoma, and N-TERA2 human teratocarcinoma cells. Western analyses confirmed expression of the human REX1 (ZFP42) protein in MDA-MB-468 cells and normal human keratinocytes. This research has identified model human cell culture systems, in addition to embryonic stem (ES) cells, in which Rex-1 is expressed, and this should enable the characterization of REX1 functions in normal adult epithelial cells and tumorigenic stem cells.

Morimoto, K., S. J. Kim, et al. (2009). "Stem cell marker aldehyde dehydrogenase 1-positive breast cancers are characterized by negative estrogen receptor, positive human epidermal growth factor receptor type 2, and high Ki67 expression." Cancer Sci **100**(6): 1062-8.

Recently, aldehyde dehydrogenase (ALDH) 1 has been identified as a reliable marker for breast cancer stem cells. The aim of our study was to investigate the clinicopathological characteristics of breast cancers with ALDH1+ cancer stem cells. In addition, the distribution of ALDH1+ tumor cells was compared on a cell-by-cell basis with that of estrogen receptor (ER)+, Ki67+, or human epidermal growth factor receptor type 2 (HER2)+ tumor cells by means of double immunohistochemical staining. Immunohistochemical staining of ALDH1 was applied to 203 primary breast cancers, and the results were compared with various clinicopathological characteristics of breast cancers including tumor size, histological grade, lymph node metastases, lymphovascular invasion, ER, progesterone receptor, HER2, Ki67, and topoisomerase 2A as well as prognosis. Immunohistochemical double staining of ALDH1 and ER, Ki67, or HER2 was also carried out to investigate their distribution. Of the 203 breast cancers, 21 (10%) were found to be ALDH1+, and these cancers were significantly more likely to be ER- ($P = 0.004$), progesterone receptor- ($P = 0.025$), HER2+ ($P = 0.001$), Ki67+ ($P < 0.001$), and topoisomerase 2A+ tumors ($P = 0.012$). Immunohistochemical double staining studies showed

that ALDH1+ tumor cells were more likely to be ER-, Ki67-, and HER2+ tumor cells. Patients with ALDH1 (score 3+) tumors showed a tendency ($P = 0.056$) toward a worse prognosis than did those with ALDH1- tumors. Breast cancers with ALDH1+ cancer stem cells posses biologically aggressive phenotypes that tend to have a poor prognosis, and ALDH1+ cancer stem cells are characterized by ER-, Ki67-, and HER2+.

Murata, H., S. Tsuji, et al. (2008). "Helicobacter pylori infection induces candidate stem cell marker Musashi-1 in the human gastric epithelium." Dig Dis Sci **53**(2): 363-9.

Musashi-1 (Msi-1), a mammalian neural RNA-binding protein, has been found to play important roles in the maintenance of stem cell states and differentiation in neural stem cells and mouse intestinal cells. We explored Msi-1 expression and its potential implications in the human stomach. Reverse transcription-PCR revealed that Msi-1 levels were significantly higher in the corpus than in antrum in Helicobacter pylori (Hp)-infected patients ($n = 49$) ($P < 0.00001$) in paired biopsy samples, whereas they were low and comparable at these two sites in Hp-negative patients ($n = 31$). Msi-1 levels were significantly higher in the Hp-infected corpus ($n = 107$) than in the Hp-negative corpus ($n = 69$) ($P < 0.00000001$). Immunohistochemistry and in situ hybridization demonstrated that Msi-1 was expressed at the base and neck/isthmus region of the fundic glands and partly co-expressed in Ki-67-positive cells in the corpus and antrum. Msi-1 levels correlated with Hp density ($P < 0.05$). Based on these results, we conclude that Hp infection strongly induces Msi-1 in the corpus. Given its expression in dividing cells, Msi-1 may modulate the state of gastric progenitor cells.

Nagasawa, M., T. Isoda, et al. (2006). "Analysis of serum granulysin in patients with hematopoietic stem-cell transplantation: its usefulness as a marker of graft-versus-host reaction." Am J Hematol **81**(5): 340-8.

Granulysin is a newly identified CTL/NK cell-related cytotoxic protein, which is secreted in both constitutive and Ca-dependent manner. To evaluate its significance in stem-cell transplantation (SCT), serum granulysin was measured by newly established ELISA method in 26 patients undergoing SCT (21 allogeneic and 5 autologous). In the allogeneic SCT, granulysin was transiently increased in 3 weeks, which was not observed in autologous SCT. In acute GVHD, serum granulysin was markedly increased and correlated with the severity of GVHD. Elevation of granulysin was not necessarily associated with increase of sIL2R or IFN-gamma. In vitro, allospecific T cells released granulysin in an allo-specific manner, and it was correlated with allo-specific cytotoxic activity. These results indicate that increased release of granulysin presents alloreactivity and serum granulysin is useful as a marker of GVHD in SCT.

Nagy, M., J. Rascon, et al. (2006). "Evaluation of whole-genome amplification of low-copy-number DNA in chimerism analysis after allogeneic stem cell transplantation using STR marker typing." Electrophoresis **27**(15): 3028-37.

Whole-genome DNA amplification (WGA) is a promising method that generates large amounts of DNA from samples of limited quantity. We investigated the accuracy of a multiplex PCR approach to WGA over STR loci. The amplification bias within a locus and over all analyzed loci was investigated in relation to the amount of template in the WGA reaction, the specific STR locus, and allele length. We observed reproducible error-free STR profiles with 10 ng down to 1 ng of DNA template. The amplification deviation at a locus and between loci was within the intra-method reproducibility. WGA is the method of choice for amplifying nanogram amounts of genomic DNA for different applications. We detected unbalanced STR amplifications at one locus and between loci, allelic drop-outs, and additional alleles after WGA of low-copy-number DNA. We found that the high number of drop-outs and drop-ins could be eradicated using pooled DNA from separate WGA reactions even with as little as 100 pg of starting template. Nevertheless, the quality of the results was still not sufficient for use in routine chimerism analysis of limited specific cell populations after allogeneic stem cell transplantation.

Newman, R. A., P. J. Klein, et al. (1979). "Binding of peanut lectin to breast epithelium, human carcinomas, and a cultured rat mammary stem cell: use of the lectin as a marker of mammary differentiation." J Natl Cancer Inst **63**(6): 1339-46.

We investigated the binding of fluorescence-labeled peanut agglutinin (PNA) to breast epithelium. Specific binding of PNA to the mammary glands of female Sprague-Dawley rats increased as the gland matured. Sexually immature rats showed relatively little fluorescence, but this increased in mature and pregnant animals. A maximum was reached in lactating rats in which significant labeling of material within the lumen was observed. PNA was bound exclusively to the epithelial and not the myoepithelial or mesenchymal cells. In tissue culture, a rat mammary epithelial stem cell line, which can be stimulated to differentiate to alveolus-like secretory or myoepithelial cells, showed evidence of PNA binding only on the secretory cells and not on unstimulated or

myoepithelial cells. Fibroblast cultures also failed to show significant binding of PNA. Receptor sites on the secretory cells were masked mainly by sialic acid. Human breast sections, like those of the rat, showed fluorescent labeling at the apical region of the epithelial cells; this labeling increased if the tissue had prior treatment with neuraminidase. Breast carcinomas that were morphologically differentiated showed more labeling with PNA than did undifferentiated tumors, which often had weak or sometimes negative labeling. When significant fluorescence was observed, it was localized mainly in the cytoplasm. By contrast, labeling was restricted to the cell periphery in differentiated carcinomas. The use of PNA as a marker for breast epithelial cell differentiation is therefore proposed.

Nishimura, S., N. Wakabayashi, et al. (2003). "Expression of Musashi-1 in human normal colon crypt cells: a possible stem cell marker of human colon epithelium." Dig Dis Sci **48**(8): 1523-9.

Musashi has been identified as an RNA-binding protein thought to be involved in asymmetric divisions during Drosophila neural development. To analyze expression patterns of mammalian Musashi homolog Musashi-1 in human normal colon crypt, 155 colon crypts separated from biopsy specimens of normal colonic mucosa were evaluated. Specimens were fixed, microdissected to isolate a few crypts, immunostained with anti-Musashi-1 antibody (14H1), and examined under confocal laser scan microscopy. The number of Musashi-1-positive cells in each crypt was 19.0 +/- 7.53 (mean +/- SD). Most Musashi-1 positive cells were located at the crypt base, between cell positions 1 and 10. Distribution of Musashi-1-positive cells corresponded with that of stem cells, as outlined in previous reports, implying that Musashi-1 is a key control element of asymmetrical division within the colon crypt. This is the first report outlining expression of Musashi-1 in human colon crypt cells.

Noda, S., K. Horiguchi, et al. (2008). "Repopulating activity of ex vivo-expanded murine hematopoietic stem cells resides in the CD48-c-Kit+Sca-1+lineage marker- cell population." Stem Cells **26**(3): 646-55.

A better understanding of the biology of cultured hematopoietic stem cells (HSCs) is required to achieve ex vivo expansion of HSCs. In this study, clonal analysis of the surface phenotype and repopulating activity of ex vivo-expanded murine HSCs was performed. After 7 days of culture with stem cell factor, thrombopoietin, fibroblast growth factor-1, and insulin-like growth factor-2, single CD34-/lowc-Kit+Sca-1+lineage marker- (CD34-KSL) cells gave rise to various numbers of cells. The proportion of KSL cells decreased with increasing number of expanded cells. Transplantation studies revealed that the progeny containing a higher percentage of KSL cells tended to have enhanced repopulating potential. We also found that CD48 was heterogeneously expressed in the KSL cell population after culture. Repopulating activity resided only in the CD48-KSL cell population, which had a relatively long intermitotic interval. Microarray analysis showed surprisingly few differences in gene expression between cultured CD48-KSL cells (cycling HSCs) and CD48+KSL cells (cycling non-HSCs) compared with freshly isolated CD34-KSL cells (quiescent HSCs), suggesting that the maintenance of stem cell activity is controlled by a relatively small number of genes. These findings should lead to a better understanding of ex vivo-expanded HSCs.

Ooi, A. G., H. Karsunky, et al. (2009). "The adhesion molecule esam1 is a novel hematopoietic stem cell marker." Stem Cells **27**(3): 653-61.

Hematopoietic stem cells (HSCs) have been highly enriched using combinations of 12-14 surface markers. Genes specifically expressed by HSCs as compared with other multipotent progenitors may yield new stem cell enrichment markers, as well as elucidate self-renewal and differentiation mechanisms. We previously reported that multiple cell surface molecules are enriched on mouse HSCs compared with more differentiated progeny. Here, we present a definitive expression profile of the cell adhesion molecule endothelial cell-selective adhesion molecule (Esam1) in hematopoietic cells using reverse transcription-quantitative polymerase chain reaction and flow cytometry studies. We found Esam1 to be highly and selectively expressed by HSCs from mouse bone marrow (BM). Esam1 was also a viable positive HSC marker in fetal, young, and aged mice, as well as in mice of several different strains. In addition, we found robust levels of Esam1 transcripts in purified human HSCs. Esam1(-/-) mice do not exhibit severe hematopoietic defects; however, Esam1(-/-) BM has a greater frequency of HSCs and fewer T cells. HSCs from Esam1(-/-) mice give rise to more granulocyte/monocytes in culture and a higher T cell:B cell ratio upon transplantation into congenic mice. These studies identify Esam1 as a novel, widely applicable HSC-selective marker and suggest that Esam1 may play roles in both HSC proliferation and lineage decisions.

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

Vascular endothelial Flt-1 and other stem cell markers are variably expressed in vascular smooth

muscle cells (SMCs) during normal and pathological conditions, but their biological role remains uncertain. In normal rat aorta, rare flt-1+ and c-kit+ SMCs were detected. Fifteen days after injury, 61.8 +/- 3.8, 45.7 +/- 3% of the intimal cells resulted flt-1+ and c-kit+ and expressed low level of alpha-smooth muscle actin; CD133+ cells were 5.6 +/- 0.7%. BrDU+/flt-1+ largely predominated in the neointima, whereas BrDU+/CD133+ cells were rare. Forty-five and sixty days after injury, intimal proliferation such as BrDU+ cells was greatly reduced. After sixty days, intimal stem marker expression had almost disappeared whereas alpha-smooth muscle actin was restored. Flk-1 and Oct-4 SMC immunodection was consistently negative. In vitro, intimal cells obtained fifteen days after injury exhibited an epithelioid phenotype and increased flt-1 and c-kit protein and mRNA and low smooth muscle markers compared to spindle-shaped medial and intimal SMCs obtained after sixty days. Epithelioid clones, independently from layer of origin, were similar in stem cell marker expression. The anti-flt-1 blocking antibody added to epithelioid SMC cultures reduced serum-deprived apoptosis and migration but not PDGF-BB-induced proliferation, and increased cell-populated collagen lattice contraction. In conclusion, vascular SMC stem marker expression was variable, chronologically modulated and prevalent in epithelioid populations and clones; among stem markers, flt-1 expression critically regulates intimal SMC response to microenviromental changes.

Palapattu, G. S., C. Wu, et al. (2009). "Selective expression of CD44, a putative prostate cancer stem cell marker, in neuroendocrine tumor cells of human prostate cancer." Prostate **69**(7): 787-98.

BACKGROUND: Hormonal therapy is effective for advanced prostate cancer (PC) but the disease often recurs and becomes hormone-refractory. It is hypothesized that a subpopulation of cancer cells, that is, cancer stem cells (CSCs), survives hormonal therapy and leads to tumor recurrence. CD44 expression was shown to identify tumor cells with CSC features. PC contains secretory type epithelial cells and a minor population of neuroendocrine cells. Neuroendocrine cells do not express androgen receptor and are quiescent, features associated with CSCs. The purpose of the study was to determine the expression of CD44 in human PC and its relationship to neuroendocrine tumor cells. METHODS: Immunohistochemistry and immunofluorescence were performed to study CD44 expression in PC cell lines, single cells from fresh PC tissue and archival tissue sections of PC. We then determined if CD44+ cells represent neuroendocrine tumor cells. RESULTS: In human PC cell lines, expression of CD44 is associated with cells of NE phenotype. In human PC tissues, NE tumor cells are virtually all positive for CD44 and CD44+ cells, excluding lymphocytes, are all NE tumor cells. CONCLUSIONS: Selective expression of the stem cell-associated marker CD44 in NE tumor cells of PC, in combination with their other known features, further supports the significance of such cells in therapy resistance and tumor recurrence.

Petersen, B. E., J. P. Goff, et al. (1998). "Hepatic oval cells express the hematopoietic stem cell marker Thy-1 in the rat." Hepatology **27**(2): 433-45.

Hepatic oval cells (HOC) are a small subpopulation of cells found in the liver when hepatocyte proliferation is inhibited and followed by some type of hepatic injury. HOC can be induced to proliferate using a 2-acetylaminofluorene (2-AAF)/hepatic injury (i.e., CCl4, partial hepatectomy [PHx]) protocol. These cells are believed to be bipotential, i.e., able to differentiate into hepatocytes or bile ductular cells. In the past, isolation of highly enriched populations of these cells has been difficult. Thy-1 is a cell surface marker used in conjunction with CD34 and lineage-specific markers to identify hematopoietic stem cells. Thy-1 antigen is not normally expressed in adult liver, but is expressed in fetal liver, presumably on the hematopoietic cells. We report herein that HOC express high levels of Thy-1. Immunohistochemistry revealed that the cells expressing Thy-1 were indeed oval cells, because they also expressed alpha-fetoprotein (AFP), gamma-glutamyl transpeptidase (GGT), cytokeratin 19 (CK-19), OC.2, and OV-6, all known markers for oval cell identification. In addition, the Thy-1+ cells were negative for desmin, a marker specific for Ito cells. Using Thy-1 antibody as a new marker for the identification of oval cells, a highly enriched population was obtained. Using flow cytometric methods, we isolated a 95% to 97% pure Thy-1+ oval cell population. Our results indicate that cell sorting using Thy-1 could be an attractive tool for future studies, which would facilitate both in vivo and in vitro studies of HOC.

Petrenko, O., A. Beavis, et al. (1999). "The molecular characterization of the fetal stem cell marker AA4." Immunity **10**(6): 691-700.

We have identified and characterized the stem cell antigen AA4. This molecule is a type I transmembrane protein whose overall structure suggests a role in cell adhesion. During fetal ontogeny (days 9-14 of development), AA4 is expressed in three major cell types: vascular endothelial cells, aorta-associated hematopoietic clusters, and primitive fetal liver hematopoietic progenitors. In the adult, AA4 is abundant in lung, heart, and whole bone marrow. In

the adult hematopoietic compartment, aa4 transcripts are present in bone marrow CD34(-/lo) Lin- Sca-1+ c-Kit+ and CD34hi Lin- Sca-1+ c-Kit+ stem and progenitor cell subsets. Our observations suggest that AA4 plays a role in cell-cell interactions during hematopoietic and vascular development.

Plotton, I., P. Sanchez, et al. (2005). "Quantification of stem cell factor mRNA levels in the rat testis: usefulness of clusterin mRNA as a marker of the amount of mRNA of Sertoli cell origin in post pubertal rats." J Endocrinol **186**(1): 131-43.

Spermatogenesis is a complex cellular process regulated by gonadotrophins and local cell-cell interactions. Stem cell factor (SCF) is one of the paracrine factors, produced by the Sertoli cells, involved in the local regulation of spermatogenesis. Measurement of its testicular level is important for addressing its role in testis physiopathology. However, the relative cell composition of experimental and pathological testis samples may lead to misinterpretation in relating SCF mRNA levels to the amount of RNA extracted from the whole tissue sample. Taking into account the relative RNA content of Sertoli cell origin should provide more significant data. In the present study, three sets of experiments were intended for modifying the proportion of RNA of Sertoli cell origin in RNA extracted from whole testis tissue samples: during postnatal development; following methoxy-acetic acid (MAA) administration; and after injecting a long-acting gonadotrophin-releasing hormone agonist (GnRHa). In a first step, we demonstrated clusterin mRNA level stability in purified Sertoli cell preparations between 20 days and adulthood, and following MAA or GnRHa treatment. In a second step, we used a competitive RT-PCR assay to measure SCF and clusterin mRNA levels and expressed the amount of SCF mRNA relative to the amount of clusterin mRNA under the above experimental conditions. The SCF/clusterin mRNA level ratio was found to remain roughly stable from 20 days post-partum to adulthood; i.e. during the development of spermatogenesis. MAA administration led to an overall increase in the SCF/clusterin mRNA level ratio between 7 and 14 days after administration, consistent with the replenishment of the testis with pachytene spermatocytes and round spermatids. Conversely, after long-acting GnRHa injection, the SCF/clusterin mRNA level ratio decreased only slightly from day 21 onward. Hence, the present studies indicate that, under physiopathological conditions, the amount of clusterin mRNA is a good marker of the amount of RNA of Sertoli cell origin in testis samples at day 20 or later; different experimental alterations of spermatogenesis are associated with different patterns of SCF mRNA levels; the relationship between FSH and SCF in vivo is not as simple as that described in vitro.

Pode-Shakked, N., S. Metsuyanim, et al. (2009). "Developmental tumourigenesis: NCAM as a putative marker for the malignant renal stem/progenitor cell population." J Cell Mol Med **13**(8B): 1792-808.

During development, renal stem cells reside in the nephrogenic blastema. Wilms' tumour (WT), a common childhood malignancy, is suggested to arise from the nephrogenic blastema that undergoes partial differentiation and as such is an attractive model to study renal stem cells leading to cancer initiation and maintenance. Previously we have made use of blastema-enriched WT stem-like xenografts propagated in vivo to define a 'WT-stem' signature set, which includes cell surface markers convenient for cell isolation (frizzled homolog 2 [Drosophila] - FZD2, FZD7, G-protein coupled receptor 39, activin receptor type 2B, neural cell adhesion molecule - NCAM). We show by fluorescenceactivated cell sorting analysis of sphere-forming heterogeneous primary WT cultures that most of these markers and other stem cell surface antigens (haematopoietic, CD133, CD34, c-Kit; mesenchymal, CD105, CD90, CD44; cancer, CD133, MDR1; hESC, CD24 and putative renal, cadherin 11), are expressed in WT cell sub-populations in varying levels. Of all markers, NCAM, CD133 and FZD7 were constantly detected in low-to-moderate portions likely to contain the stem cell fraction. Sorting according to FZD7 resulted in extensive cell death, while sorted NCAM and CD133 cell fractions were subjected to clonogenicity assays and quantitative RT-PCR analysis, exclusively demonstrating the NCAM fraction as highly clonogenic, overexpressing the WT 'stemness' genes and topoisomerase2A (TOP2A), a bad prognostic marker for WT. Moreover, treatment of WT cells with the topoisomerase inhibitors, Etoposide and Irinotecan resulted in down-regulation of TOP2A along with NCAM and WT1. Thus, we suggest NCAM as a marker for the WT progenitor cell population. These findings provide novel insights into the cellular hierarchy of WT, having possible implications for future therapeutic options.

Potten, C. S., C. Booth, et al. (2003). "Identification of a putative intestinal stem cell and early lineage marker; musashi-1." Differentiation **71**(1): 28-41.

There are few reliable markers for adult stem cells and none for those of the intestinal epithelium. Previously, indirect experimental approaches have predicted stem cell position and numbers. The Musashi-1 (Msi-1) gene encodes an RNA binding protein associated with asymmetric divisions in neural progenitor cells. Two-day-old, adult, and 4.5 h, 1-, 2-,

4- and 12-day post-irradiation samples of BDF1 mouse small intestine, together with some samples of mouse colon were stained with a rat monoclonal antibody to Musashi-1 (14 H-1). Min (+ / -) mice with small intestinal adenomas of varying sizes were also analysed. Samples of human small and large bowel were also studied but the antibody staining was weak. Musashi-1 expression was observed using immunohistochemistry in neonatal, adult, and regenerating crypts with a staining pattern consistent with the predicted number and distribution of early lineage cells including the functional stem cells in these situations. Early dysplastic crypts and adenomas were also strongly Musashi-1 positive. In situ hybridization studies showed similar expression patterns for the Musashi mRNA and real-time quantitative RT-PCR showed dramatically more Msi-1 mRNA expression in Min tumours compared with adjacent normal tissue. These observations suggest that Musashi-1 is a marker of stem and early lineage progenitor cells in murine intestinal tissue.

Pries, R., N. Witrkopf, et al. (2008). "Potential stem cell marker CD44 is constitutively expressed in permanent cell lines of head and neck cancer." In Vivo **22**(1): 89-92.

BACKGROUND: Despite significant advances in the use of diagnosis and therapy to treat head and neck squamous cell carcinoma (HNSCC), the prognosis has improved only marginally in the last decades. Thus, there is an enormous need for better understanding of tumor biology and reversely novel immunotherapeutic approaches. It is becoming increasingly obvious that stem cells play an important role in tumor development and progression. The identity of these cells and the underlying cellular and molecular mechanisms are mostly unknown in HNSCC to date. MATERIALS AND METHODS: Solid HNSCC tumors, as well as permanent HNSCC cell lines, were analyzed by flow cytometry concerning the expression of different putative stem cell marker proteins. RESULTS: Distinct populations of CD44 expressing potential stem cells could be identified in solid tumors of HNSCC patients with strong individual deviations. Surprisingly, the potential stem cell marker CD44 was found to be constitutively expressed on the surface of all the permanent HNSCC cell lines analyzed. CONCLUSION: CD44+ 'tumor stem cells' may play a key role in the establishment of permanent HNSCC cell lines, selecting especially robust cell entities that might drive the progression and metastasis of HNSCC. Individual analysis of 'tumor stem cell' markers will be an important tool for innovative therapies and for determining the prognosis of patients with HNSCC.

Prusa, A. R., E. Marton, et al. (2003). "Stem cell marker expression in human trisomy 21 amniotic fluid cells and trophoblasts." J Neural Transm Suppl(67): 235-42.

Down Syndrome is the most frequent genetic cause of mental retardation. Deregulation of specific differentiation processes is a major cause for the neuropathological cell features typical for this syndrome. The molecular mechanisms leading to Down Syndrome are likely to be operative from the very earliest time of embryonic/fetal development. We therefore analysed human amniotic fluid cell samples and cytotrophoblastic cells from placental biopsies, both with normal karyotypes and with trisomy 21, for the mRNA expression of stem cell marker genes. Here we describe for the first time that these human primary cell sources contain cells that express telomerase reverse transcriptase, leukemia inhibitory factor receptor, and bone morphogenetic protein receptor II. A specific difference between aneuploid and normal cells could not be detected. These data provide evidence that human amniotic fluid and cytotrophoblastic cell cultures might provide a new source for research on primary cell systems expressing these stem cell markers. In addition, it is suggested that early deregulation of the expression of these genes in the here analysed cell sources does not contribute to the molecular development of Down Syndrome.

Qureshi, M. A., R. E. Girgis, et al. (2004). "Increased exhaled nitric oxide following autologous peripheral hematopoietic stem-cell transplantation: a potential marker of idiopathic pneumonia syndrome." Chest **125**(1): 281-7.

BACKGROUND: Increased production of nitric oxide (NO) and oxidative stress following bone marrow transplantation may play a role in the pathogenesis of idiopathic pneumonia syndrome (IPS). We hypothesize that patients who received high-dose chemotherapy followed by autologous peripheral hematopoietic stem-cell transplantation (APHSCT) have increased exhaled NO. METHOD: We measured exhaled lower respiratory tract NO concentration with a chemiluminescent NO analyzer during a slow vital capacity maneuver against a positive pressure of 16 cm H(2)O at an expiratory flow rate of 50 mL/s in 20 female patients who received high-dose chemotherapy (cyclophosphamide, carmustine, and cisplatin) followed by APHSCT for the treatment of stage III or IV breast carcinoma. Pulmonary function tests were performed, and exhaled NO measurements and clinical and laboratory data were obtained before transplantation and at every 6-week visit after transplantation for 24 weeks.

RESULTS: All study patients had evidence of IPS with dyspnea and reduction in diffusion capacity of the lung for carbon monoxide (DLCO). Lower respiratory tract exhaled NO was significantly higher after APHSCT and during the 6 months of follow-up. Mean (+/- SD) exhaled NO increased from (mean +/- SD) 12.54 +/- 1.32 parts per billion (ppb) before APHSCT to 21.26 +/- 1.94 ppb at 6 weeks (p = 0.099), 21.26 +/- 1.94 ppb (p = 0.006) at 12 weeks, 24.62 +/- 2.55 ppb (p = 0.012) at 18 weeks, and 25.28 +/- 3.31 ppb (p = 0.013) at 24 weeks (all p values were compared to baseline). There was a strong negative correlation between DLCO and exhaled NO (regression coefficient - 0.60, p = 0.01). CONCLUSION: Lower respiratory tract concentration of exhaled NO is significantly increased following APHSCT and correlates with reduction in DLCO. Increase in lower respiratory tract concentration of NO is a potential marker of IPS.

Ragone, G., A. Bresin, et al. (2009). "The Tcl1 oncogene defines secondary hair germ cells differentiation at catagen-telogen transition and affects stem-cell marker CD34 expression." Oncogene **28**(10): 1329-38.

Overexpression of the TCL1 gene family plays a role in the onset of T-cell leukemias in mice and in humans. The Tcl1 gene is tightly regulated during early embryogenesis in which it participates in embryonic stem (ES)-cells proliferation and during lymphoid differentiation. Here, we provide evidences that Tcl1 is also important in mouse hair follicle (HF) and skin homeostasis. We found that Tcl1(-/-) adult mice exhibit hair loss, leading to alopecia with extensive skin lesions. By analysing Tcl1 expression in the wild-type (wt) skin through different stages of hair differentiation, we observe high levels in the secondary hair germ (HG) cells and hair bulges, during early anagen and catagen-telogen transition phases. The loss of Tcl1 does not result in apparent skin morphological defects during embryonic development and at birth, but its absence causes a reduction of proliferation in anagen HFs. Importantly, we show the that absence of Tcl1 induces a significant loss of the stem-cell marker CD34 (but not alpha6-integrin) expression in the bulge cells, which is necessary to maintain stem-cell characteristics. Therefore, our findings indicate that Tcl1 gene(s) might have important roles in hair formation, by its involvement in cycling and self-renewal of transient amplifying (TA) and stem-cell (SC) populations.

Raman, J. D., N. P. Mongan, et al. (2006). "Decreased expression of the human stem cell marker, Rex-1 (zfp-42), in renal cell carcinoma." Carcinogenesis **27**(3): 499-507.

The Rex-1 (Zfp-42) gene encodes a zinc finger family transcription factor which is highly expressed in mouse and human embryonic stem cells. It is one of several gene markers used to identify human stem cells. While several organs are known to harbor adult human stem cells, the presence and distribution of stem cells in both the normal and neoplastic adult kidney remains largely unknown. In this study we evaluated Rex-1 mRNA and protein expression in normal and malignant kidney tissue specimens from human patients. Rex-1 mRNA expression was determined using both reverse transcription and real-time PCR. REX1 protein expression was assessed by western analysis and immunohistochemistry, using an affinity-purified, polyclonal antibody to the REX1 protein. We found that 14 of 15 (93%) non-tumor renal parenchymal specimens demonstrated Rex-1 mRNA, compared with 5 of 14 (36%) renal tumors (P < 0.005). REX1 protein expression was detected in 21 of 23 (91%) non-tumor and in 7 of 19 (37%) tumor specimens (P < 0.001). Furthermore, in six of these seven renal tumor specimens where REX1 protein expression was detected, the levels were at least 3-fold lower than those in adjacent, normal kidney tissue. There were no differences in Rex-1 mRNA or protein expression among the various histologic subtypes of renal tumors (clear cell carcinoma, papillary carcinoma, chromophobe carcinoma and oncocytoma). Immunohistochemical staining confirmed the absence of REX1 in three renal tumor specimens (two clear cell and one papillary carcinoma), while the REX1 protein was detected in a small percentage of proximal tubular cells in normal renal tissue. Immunohistochemical staining of another stem cell marker, OCT4, demonstrated a similar pattern of protein expression in a small percentage of normal renal proximal tubular cells. In summary, we were able to detect Rex-1 mRNA and protein expression in over 90% of normal renal parenchymal specimens and we observed a significant reduction in REX1 expression in renal tumor specimens of all histologic subtypes.

Reiter, R. E., Z. Gu, et al. (1998). "Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer." Proc Natl Acad Sci U S A **95**(4): 1735-40.

The identification of cell surface antigens is critical to the development of new diagnostic and therapeutic modalities for the management of prostate cancer. Prostate stem cell antigen (PSCA) is a prostate-specific gene with 30% homology to stem cell antigen 2, a member of the Thy-1/Ly-6 family of glycosylphosphatidylinositol (GPI)-anchored cell surface antigens. PSCA encodes a 123-aa protein with

an amino-terminal signal sequence, a carboxyl-terminal GPI-anchoring sequence, and multiple N-glycosylation sites. PSCA mRNA expression is prostate-specific in normal male tissues and is highly up-regulated in both androgen-dependent and -independent prostate cancer xenografts. In situ mRNA analysis localizes PSCA expression in normal prostate to the basal cell epithelium, the putative stem cell compartment of the prostate. There is moderate to strong PSCA expression in 111 of 126 (88%) prostate cancer specimens examined by in situ analysis, including high-grade prostatic intraepithelial neoplasia and androgen-dependent and androgen-independent tumors. Flow cytometric analysis demonstrates that PSCA is expressed predominantly on the cell surface and is anchored by a GPI linkage. Fluorescent in situ hybridization analysis localizes the PSCA gene to chromosome 8q24.2, a region of allelic gain in more than 80% of prostate cancers. A mouse homologue with 70% amino acid identity and similar genomic organization to human PSCA has also been identified. These results support PSCA as a target for prostate cancer diagnosis and therapy.

Riekstina, U., I. Cakstina, et al. (2009). "Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis." Stem Cell Rev **5**(4): 378-86.

Mesenchymal stem cells (MSCs) have been isolated from a variety of human tissues, e.g., bone marrow, adipose tissue, dermis, hair follicles, heart, liver, spleen, dental pulp. Due to their immunomodulatory and regenerative potential MSCs have shown promising results in preclinical and clinical studies for a variety of conditions, such as graft versus host disease (GvHD), Crohn's disease, osteogenesis imperfecta, cartilage damage and myocardial infarction. MSC cultures are composed of heterogeneous cell populations. Complications in defining MSC arise from the fact that different laboratories have employed different tissue sources, extraction, and cultivation methods. Although cell-surface antigens of MSCs have been extensively explored, there is no conclusive evidence that unique stem cells markers are associated with these adult cells. Therefore the aim of this study was to examine expression of embryonic stem cell markers Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4 in adult mesenchymal stem cell populations derived from bone marrow, adipose tissue, dermis and heart. Furthermore, we tested whether human mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions. We found that bone marrow MSCs express embryonic stem cell markers Oct4, Nanog, alkaline phosphatase and SSEA-4, adipose tissue and dermis MSCs express Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4, whereas heart MSCs express Oct4, Nanog, SOX2 and SSEA-4. Our results also indicate that human adult mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions during early passages, as shown by distinct germ layer and embryonic stem cell marker expression patterns. Studies are now needed to determine the functional role of embryonic stem cell markers Oct4, Nanog and SOX2 in adult human MSCs.

Sakata, N., M. Yasui, et al. (2001). "Kinetics of plasma cytokines after hematopoietic stem cell transplantation from unrelated donors: the ratio of plasma IL-10/sTNFR level as a potential prognostic marker in severe acute graft-versus-host disease." Bone Marrow Transplant **27**(11): 1153-61.

The plasma levels of a panel of cytokines and cytokine-associated molecules (IL-1alpha, IL-2, IL-4, IL-6, IL-10, IL-12, IL-15, macrophage colony-stimulating factor (M-CSF), interferon-gamma (IFN-gamma), tumor necrosis factor-alpha (TNF-alpha), soluble IL-2 receptor (sIL-2R), soluble tumor necrosis factor receptor I or II (sTNFRI or II)) were assessed in 56 plasma samples of 13 pediatric patients undergoing hematopoietic stem cell transplantation (HSCT, bone marrow in 12 and cord blood in one) from unrelated donors. Eight patients developed severe (grade III-IV) acute GVHD (aGVHD). The plasma IL-6, IL-10, M-CSF, sTNFRI and II levels were significantly high in the severe aGVHD group compared to the mild aGVHD group (grade 0-II). The plasma IL-15 level increased transiently in the early period following HSCT and remained high in the severe aGVHD group even after 4 weeks following HSCT. Based on analysis of the correlations between the kinetics of the plasma cytokine levels after HSCT and the clinical manifestations of aGVHD, IL-15 and/or M-CSF were involved in the development of aGVHD, following elevation of the plasma IL-10 and sTNFRI or II levels. These kinetics suggest that IL-10 and sTNFRs worked as suppressor cytokines and seemed to suppress clinical manifestations of aGVHD. Furthermore, it seemed that the plasma ratio of IL-10/sTNFRII from 5 to 12 weeks following HSCT was linked to the poor outcome in the patients with severe aGVHD, suggesting that IL-10 plays an important role in protecting hosts from transplantation-related complications, including GVHD.

Salnikov, A. V., A. Groth, et al. (2009). "Targeting of cancer stem cell marker EpCAM by bispecific antibody EpCAMxCD3 inhibits pancreatic carcinoma." J Cell Mol Med **13**(9B): 4023-33.

Patients with pancreatic cancer have a poor survival rate, and new therapeutic strategies are needed. Epithelial cell adhesion molecule (EpCAM), suggested as a marker for cancer stem cells, is over-expressed on most pancreatic tumour cells but not on normal cells and may be an ideal therapeutic target. We evaluated the anti-tumour efficiency of bispecific EpCAMxCD3 antibody linking tumour cells and T lymphocytes. In NOD SCID mice, EpCAMxCD3 had a long serum half-life (t(1/2) approximately 7 days). EpCAMxCD3 significantly retarded growth of BxPC-3 pancreatic carcinoma xenografts. For mimicking a pancreatic cancer microenvironment in vitro, we used a three-dimensional tumour reconstruct system, in which lymphocytes were co-cultured with tumour cells and fibroblasts in a collagen matrix. In this in vivo-like system, EpCAMxCD3 potently stimulated production of the effector cytokines IFN-gamma and TNF-alpha by extracorporally pre-activated lymphocytes. Moreover, compared with a bivalent anti-CD3 antibody, EpCAMxCD3 more efficiently activated the production of TNF-alpha and IFN-gamma by non-stimulated peripheral blood mononuclear cells. Most excitingly, we demonstrate for the first time that EpCAMxCD3 induces prolonged contacts between lymphocytes and tumour cells, which may be the main reason for the observed anti-tumour effects. As an important prerequisite for future use in patients, EpCAMxCD3 did not alter lymphocyte migration as measured by time-lapse video microscopy. Our data may open a way to improve the immune response and treatment outcome in patients with pancreatic cancer.

Samuel, J., A. A. Noujaim, et al. (1989). "A novel marker for basal (stem) cells of mammalian stratified squamous epithelia and squamous cell carcinomas." Cancer Res **49**(9): 2465-70.

We have developed a monoclonal antibody (174H.64) which selectively recognizes antigens shared by the basal cells of mammalian stratified squamous epithelium and squamous cell carcinoma (SCC). Histopathological studies of the frozen tissue sections demonstrated selective binding of this antibody to SCCs of human, bovine, canine, feline, and murine origin. Tumors of other histological types did not show reactivity with the antibody. In well-differentiated SCCs the peripheral layer of the tumor showed preferential binding of the antibody, suggesting that the antigens are associated with the proliferative compartment of the tumor. Studies on normal human tissues showed selective binding of the antibody to the basal layer of stratified squamous epithelia, thymic epithelial cells, and myoepithelial cells around breast ducts, while no antibody binding was observed for the suprabasal layers of stratified epithelia, simple epithelia, or tissues of nonepithelial origin. A similar pattern of antibody binding was also observed for bovine and murine skin with staining of the basal layer. The antigens detected by monoclonal antibody 174H.64 were characterized from cytoskeletal protein extracts of normal human keratinocytes as well as human and bovine SCC tissues by using an immunoblotting technique. The antigens detected in normal human keratinocytes consisted of two major protein bands of approximate molecular weights of 48,000-50,000 and 57,000. In bovine SCC tumor the antigen detected was the Mr 48,000-50,000 band and in the human SCC tumor it was the Mr 57,000 band. A murine lung SCC model was developed with a murine SCC cell line KLN-205. The lung tumor obtained was reactive against the antibody and showed selective staining of the peripheral layer of the tumor containing the stem cell population. The antigens described by monoclonal antibody 174H.64 appear to be molecules associated with the stem cell populations of normal stratified epithelium and squamous cell carcinoma.

Sato, T., R. Kobayashi, et al. (2005). "Significance of eosinophilia after stem cell transplantation as a possible prognostic marker for favorable outcome." Bone Marrow Transplant **36**(11): 985-91.

Although eosinophilia after stem cell transplantation (SCT) has been addressed in recent reports, the significance of eosinophilia in disease outcome after SCT has not been well studied. In this study, we investigate the frequency of eosinophilia after SCT to determine its prognostic value. The subjects were 113 patients with malignant or nonmalignant diseases who underwent SCT treatment. In these patients, eosinophilia was detected in 44 cases (38.9%), on average 67.5 days after transplantation, and the mean maximum absolute eosinophil count was 840.5 x 10(6)/l. To study the basis of eosinophilia after SCT, various serum cytokine levels during SCT in patients both with and without eosinophilia were analyzed. Statistical analysis indicated that the overall patient survival rates improved in those with eosinophilia compared to those without eosinophilia (88.7 vs 43.0%, P=0.0034). In particular, in patients with malignant diseases, those with eosinophilia showed a higher event-free survival (81.1 vs 44.6%, P=0.0025) and a lower relapse rate (16.0 vs 43.0%, P=0.0287) than those without eosinophilia. In conclusion, we propose that eosinophilia after SCT could be a useful prognostic marker for determining favorable outcomes in patients with malignant diseases. The reasons for this good prognosis in SCT patients with eosinophilia are discussed.

Seidel, M. G., U. Ernst, et al. (2006). "Expression of the putatively regulatory T-cell marker FOXP3 by CD4(+)CD25+ T cells after pediatric hematopoietic stem cell transplantation." Haematologica **91**(4): 566-9.

FOXP3 has been proposed to be critical for the regulatory function of CD4(+)CD25+ T cells and it has been reported that its expression correlates with protection from graft-versus-host-disease (GvHD) after allogeneic hematopoietic stem cell transplantation (HSCT). Here, by monitoring 28 pediatric HSCT recipients, we found that the levels of FOXP3-mRNA expression in highly enriched CD4(+)CD25+ cells were identical to those in healthy controls irrespective of GvHD status. Moreover, FOXP3-mRNA was abundant in recently in vitro stimulated CD4(+)CD25+ cells that lacked regulatory function. Together these findings suggest that FOXP3-mRNA expression primarily reflects CD4(+)CD25+ cell frequency rather than defining the regulatory potential of CD4(+)CD25+ T cells and GvHD risk after HSCT.

Smalley, M. J. and R. B. Clarke (2005). "The mammary gland "side population": a putative stem/progenitor cell marker?" J Mammary Gland Biol Neoplasia **10**(1): 37-47.

Hematopoietic Stem Cells have been isolated by their ability to pump out Hoechst 33342 dye and form a distinct population definable by flow cytometry--the Side Population (SP). The membrane pump Bcrp has been identified as the molecular determinant of the SP phenotype. An SP population with Bcrp activity has been defined in a number of tissues, including mouse mammary and human breast epithelium, and it has been proposed that the SP phenotype is a universal stem cell marker. Studies of mouse and human breast SP suggest that the population is undifferentiated but capable of differentiating into epithelial structures of both luminal and myoepithelial lineages both in vitro and in vivo. However, evidence that the SP is enriched for stem cells is, at the moment, only correlative, and there are potentially confounding technical issues. We still await formal proof that the SP contains a stem cell population.

Song, W., H. Li, et al. (2008). "Expression and clinical significance of the stem cell marker CD133 in hepatocellular carcinoma." Int J Clin Pract **62**(8): 1212-8.

BACKGROUND: Although the primitive haematopoietic and neuronal stem cell marker CD133 is known to be present in cancer stem cells (CSCs) in hepatocellular carcinoma (HCC), the postresection prognostic impact of CD133 in HCC patients remains limited. METHODS: Sixty-three resected specimens were collected from HCC patients. The expression of CD133 protein was analysed by immunohistochemistry and the association of CD133 expression with clinicopathological characteristics, tumour recurrence and survival of the patients was evaluated. RESULTS: Immunohistochemical analysis of 63 HCC tissue specimens revealed that CD133 positive tumour cells were frequently present in HCC. Increased CD133 immunostaining was found in 26 specimens (41.3%). Increased CD133 expression levels were correlated with increased tumour grade, advanced disease stage, and elevated serum alpha-fetoprotein levels. Kaplan-Meier analysis indicated that patients with increased CD133 levels had shorter overall survival and higher recurrence rates compared with patients with low CD133 expression. Multivariate analyses revealed that increased CD133 expression was an independent prognostic factor for survival and tumour recurrence in patients with HCC. CONCLUSIONS: These findings suggest that reactivated CD133 positive cells are frequently present in HCC. Additionally, increased CD133 expression corresponds with higher stage tumours in HCC, thus indicating a poor prognosis for patients. These data support the CSC hypothesis.

Sottile, V., M. Li, et al. (2006). "Stem cell marker expression in the Bergmann glia population of the adult mouse brain." Brain Res **1099**(1): 8-17.

Recent evidence suggests that the postnatal cerebellum contains cells with characteristics of neural stem cells, which had so far only been identified in the subventricular zone of the lateral ventricles and the subdentate gyrus of the hippocampus. In order to investigate the identity of these cells in the adult cerebellum, we have analyzed the expression of Sox1, a transcription factor from the SoxB1 subgroup and widely used marker of neural stem cells. In situ hybridization and the use of a transgenic mouse model show that, in the adult cerebellum, Sox 1 is only expressed in the Bergmann glia, a population of radial glia present in the Purkinje cell layer. Furthermore, another neural stem cell marker, Sox2 (also member of the SoxB1 subgroup), is also expressed in the Bergmann glia. We have previously shown that these same cells express Sox9, a member of the SoxE subgroup known for its role in glial development. Here we show that Sox9 is in fact also expressed in other regions harboring adult neural stem cells, suggesting that Sox9 represents a novel stem cell marker. Finally, using a Sox1-null mouse, we show that the formation of this Sox2/Sox9 positive Bergmann glia population does not require the presence of a functional Sox1. Our results identify these radial glia as a previously unreported

Sox1/Sox2/Sox9 positive adult cell population, suggesting that these cells may represent the recently reported stem cells in the adult cerebellum.

Spooncer, E., N. Brouard, et al. (2008). "Developmental fate determination and marker discovery in hematopoietic stem cell biology using proteomic fingerprinting." Mol Cell Proteomics **7**(3): 573-81.

In hematopoiesis, co-expression of Sca-1 and c-Kit defines cells (LS(+)K) with long term reconstituting potential. In contrast, poorly characterized LS(-)K cells fail to reconstitute lethally irradiated recipients. Relative quantification mass spectrometry and transcriptional profiling were used to characterize LS(+)K and LS(-)K cells. This approach yielded data on >1200 proteins. Only 32% of protein changes correlated to mRNA modulation demonstrating post-translational protein regulation in early hematopoietic development. LS(+)K cells had lower expression of protein synthesis proteins but did express proteins associated with mature cell function. Major increases in erythroid development proteins were observed in LS(-)K cells; based on this assessment of erythroid potential we showed them to be principally erythroid progenitors, demonstrating effective use of discovery proteomics for definition of primitive cells.

Steckel, N. K., M. Koldehoff, et al. (2007). "Use of the activating gene mutation of the tyrosine kinase (VAL617Phe) JAK2 as a minimal residual disease marker in patients with myelofibrosis and myeloid metaplasia after allogeneic stem cell transplantation." Transplantation **83**(11): 1518-20.

Here we report on the use of a new real-time polymerase chain reaction (PCR) method to detect and quantify the activating gene mutation of the tyrosine kinase JAK2. We evaluated patients with myelofibrosis with myeloid metaplasia (MMM; n=25) for the gene mutation prior to allogeneic stem cell transplantation and monitored them in the long-term follow up of 125 months (median 15, range 4-125) after transplant. The results obtained were correlated to the chimerism status of these patients. The JAK2 gene mutation was detected in 15 of 25 analyzed patients prior to transplant. Three patients who were again positive for JAK2 after transplant also had mixed chimerism status. These three patients relapsed from MMM shortly after JAK2 gene mutation was detected for the first time after transplant. Our presented data shows the feasibility of the detection of JAK2 gene mutation by real-time PCR as a minimal residual disease marker after transplant.

Stoop, H., F. Honecker, et al. (2008). "Stem cell factor as a novel diagnostic marker for early malignant germ cells." J Pathol **216**(1): 43-54.

Carcinoma in situ (CIS) of the testis is the pre-invasive stage of type II testicular germ cell tumours (TGCTs) of adolescents and adults. These tumours are the most frequently diagnosed cancer in Caucasian adolescents and young adults. In dysgenetic gonads, the precursor of type II GCTs can be either CIS or a lesion known as gonadoblastoma (GB). CIS/GB originates from a primordial germ cell (PGC)/gonocyte, ie an embryonic cell. CIS can be cured by local low-dose irradiation, with limited side effects on hormonal function. Therefore, strategies for early diagnosis of CIS are essential. Various markers are informative to diagnose CIS in adult testis by immunohistochemistry, including c-KIT, PLAP, AP-2gamma, NANOG, and POU5F1 (OCT3/4). OCT3/4 is the most informative and consistent in presence and expression level, resulting in intense nuclear staining. In the case of maturational delay of germ cells, frequently present in gonads of individuals at risk for type II (T)GCTs, use of these markers can result in overdiagnosis of malignant germ cells. This demonstrates the need for a more specific diagnostic marker to distinguish malignant germ cells from germ cells showing maturation delay. Here we report the novel finding that immunohistochemical detection of stem cell factor (SCF), the c-KIT ligand, is informative in this context. This was demonstrated in over 400 cases of normal (fetal, neonatal, infantile, and adult) and pathological gonads, as well as TGCT-derived cell lines, specifically in cases of CIS and GB. Both membrane-bound and soluble SCF were expressed, suggestive of an autocrine loop. SCF immunohistochemistry can be a valuable diagnostic tool, in addition to OCT3/4, to screen for precursor lesions of TGCTs, especially in patients with germ cell maturation delay.

Sugano, Y., M. Takeuchi, et al. (2008). "Junctional adhesion molecule-A, JAM-A, is a novel cell-surface marker for long-term repopulating hematopoietic stem cells." Blood **111**(3): 1167-72.

Junctional adhesion molecule-A (JAM-A/JAM-1/F11R) is a cell adhesion molecule expressed in epithelial and endothelial cells, and also hematopoietic cells, such as leukocytes, platelets, and erythrocytes. Here, we show that JAM-A is expressed at a high level in the enriched hematopoietic stem cell (HSC) fraction; that is, CD34(+)c-Kit(+) cells in embryonic day 11.5 (E11.5) aorta-gonod-mesonephros (AGM) and E11.5 fetal liver (FL), as well as c-Kit(+)Sca-1(+)Lineage(-) (KSL) cells in E14.5 FL, E18.5FL, and adult bone marrow (BM). Although the percentage of JAM-A(+) cells in those tissues

decreases during development, the expression in the HSC fraction is maintained throughout life. Colony-forming assays reveal that multilineage colony-forming activity in JAM-A(+) cells is higher than that in JAM-A(-) cells in the enriched HSC fraction in all of those tissues. Transplantation assays show that long-term reconstituting HSC (LTR-HSC) activity is exclusively in the JAM-A(+) population and is highly enriched in the JAM-A(+) cells sorted directly from whole BM cells by anti-JAM-A antibody alone. Together, these results indicate that JAM-A is expressed on hematopoietic precursors in various hematopoietic tissues and is an excellent marker to isolate LTR-HSCs.

Taieb, N., M. Maresca, et al. (2009). "The first extracellular domain of the tumour stem cell marker CD133 contains an antigenic ganglioside-binding motif." Cancer Lett **278**(2): 164-73.

Prominin 1/CD133 is a marker of transplantable cancer stem cells. We have generated anti-peptide antibodies against a N-terminal epitope of CD133 belonging to a ganglioside-binding domain. The labelling of colon cancer cells with these antibodies was inhibited by various gangliosides including GM1 and GD3, but not GT1b. CD133 immunolabelling progressively decreased to undetectable levels in post-confluent cultures, possibly through ganglioside-mediated epitope masking since the staining was partially recovered after chemical disruption of lipid rafts. We suggest that selected gangliosides could modulate the accessibility of CD133 and regulate cell-cell contacts involving CD133(+) stem cells at the earliest steps of tumour development.

Takaishi, S., T. Okumura, et al. (2009). "Identification of gastric cancer stem cells using the cell surface marker CD44." Stem Cells **27**(5): 1006-20.

Cancer stem cells (CSCs) have been defined as a unique subpopulation in tumors that possess the ability to initiate tumor growth and sustain tumor self-renewal. Although the evidence has been provided to support the existence of CSCs in various solid tumors, the identity of gastric CSCs has not been reported. In this study, we have identified gastric cancer-initiating cells from a panel of human gastric cancer cell lines using cell surface marker CD44. Among six gastric cancer cell lines, three lines MKN-45, MKN-74, and NCI-N87 had a sizeable subpopulation of CD44(+) cells, and these cells showed spheroid colony formation in serum-free media in vitro as well as tumorigenic ability when injected into stomach and skin of severe combined immunodeficient (SCID) mice in vivo. The CD44(+) gastric cancer cells showed the stem cell properties of self-renewal and the ability to form differentiated progeny and gave rise to CD44(-) cells. CD44 knockdown by short hairpin RNA resulted in much reduced spheroid colony formation and smaller tumor production in SCID mice, and the CD44(-) populations had significantly reduced tumorigenic ability in vitro and in vivo. Other potential CSC markers, such as CD24, CD133, CD166, stage-specific embryonic antigen-1 (SSEA-1), and SSEA-4, or sorting for side population did not show any correlation with tumorigenicity in vitro or in vivo. The CD44(+) gastric cancer cells showed increased resistance for chemotherapy- or radiation-induced cell death. These results support the existence of gastric CSCs and may provide novel approaches to the diagnosis and treatment of gastric cancer.

Teng, L., J. Y. Cheng, et al. (2004). "[Establishing mouse embryonic stem cell line carrying a fluorescent undifferentiated marker]." Yi Chuan Xue Bao **31**(10): 1061-5.

To label mouse ES cells,a cell line derived from the inner cell mass of 3.5-day blastocysts,with enhanced green fluorescent protein (EGFP), the vector of pRex-1-EGFP was transferred into mouse ES cells by electroporation. The expressions of Rex-1 in undifferentiated and differentiated ES cells were detected by the microscopic observation of EGFP and by RT-PCR. The results showed that the EGFP gene was transferred into the mouse ES cell line, and the transfected cells in undifferentiated state showed high levels of EGFP expression. When the cells began to differentiate, the EGFP expressions were gradually reduced. A mouse ES cell line expressing EGFP under the control of Rex-1 gene promoter was generated. The cell line provides a powerful approach for the research of the process of mammalian development and for the screening of small molecules that can regulate this process.

Tiede, B. J., L. A. Owens, et al. (2009). "A novel mouse model for non-invasive single marker tracking of mammary stem cells in vivo reveals stem cell dynamics throughout pregnancy." PLoS One **4**(11): e8035.

Mammary stem cells (MaSCs) play essential roles for the development of the mammary gland and its remodeling during pregnancy. However, the precise localization of MaSCs in the mammary gland and their regulation during pregnancy is unknown. Here we report a transgenic mouse model for luciferase-based single marker detection of MaSCs in vivo that we used to address these issues. Single transgene expressing mammary epithelial cells were shown to reconstitute mammary glands in vivo while immunohistochemical staining identified MaSCs in basal and luminal locations, with preponderance

towards the basal position. By quantifying luciferase expression using bioluminescent imaging, we were able to track MaSCs non-invasively in individual mice over time. Using this model to monitor MaSC dynamics throughout pregnancy, we found that MaSCs expand in both total number and percentage during pregnancy and then drop down to or below baseline levels after weaning. However, in a second round of pregnancy, this expansion was not as extensive. These findings validate a powerful system for the analysis of MaSC dynamics in vivo, which will facilitate future characterization of MaSCs during mammary gland development and breast cancer.

Tong, Q. S., L. D. Zheng, et al. (2008). "Expression and clinical significance of stem cell marker CD133 in human neuroblastoma." World J Pediatr **4**(1): 58-62.

BACKGROUND: Recent evidences indicate that CD133, a kind of transmembrane protein, can be used as a marker to isolate stem cells from tumors originating from neural crest. This study was undertaken to explore the expression and clinical significance of stem cell marker CD133 in neuroblastoma (NB). METHODS: Immunohistochemical staining was used to detect the expression of CD133 in 32 patients with NB and 8 patients with ganglioneuroblastoma (GNB). The relationships were analyzed among CD133 expression, international neuroblastoma staging system (INSS) stages, pathological classification, and postoperative survival time of NB patients. RESULTS: The expression rates of CD133 in NB and GNB were 46.9% (15/32) and 37.5% (3/8) respectively, mainly in cytoplasm of neuroblastoma cells. The expression rates of stage 1-2, stage 3-4 and stage 4S were 30.7%, 57.9% and 37.5%, respectively. The differences in various stages were significant (P<0.05). The positive rate of CD133 in patients with unfavorable histology (52.4%) was significantly higher than that in patients with favorable histology (36.8%) (P=0.007). The survival time of CD133 negative patients was significantly longer than that of CD133 positive patients (P=0.026). CONCLUSIONS: CD133 which might be correlated with the development and progression of NB can serve as one of the important indicators for prognosis of NB.

Tran, C. P., C. Lin, et al. (2002). "Prostate stem cell antigen is a marker of late intermediate prostate epithelial cells." Mol Cancer Res **1**(2): 113-21.

Prostate stem cell antigen (PSCA, named for its strong sequence homology to the thymocyte marker stem cell antigen 2) is a cell surface antigen expressed in normal prostate and associated with human and murine prostate cancer. To begin to investigate a possible link between PSCA expression in normal prostate and prostate carcinogenesis, we characterized the phenotype and proliferative behavior of normal PSCA-expressing prostate epithelial cells (PrEC) in tissue culture. PSCA was expressed in a subset of prostate epithelial cells that coexpress basal and secretory cytokeratins. PSCA-positive cells were the direct progeny of PSCA-negative cells and were characterized by a more differentiated morphology and a slower proliferative rate than PSCA-negative cells. Although PSCA-positive cells continued to express basal cell markers such as CD44, they lost expression of the basal cell marker p63. In contrast, expression of prostate specific antigen and androgen receptor transcripts was detectable in PSCA-positive PrEC. These findings suggest that PSCA is a unique marker of an intermediate subpopulation of PrEC in transition from a basal to a terminally differentiated secretory phenotype and may be a useful marker for the study of normal and malignant prostate development.

Tsujimura, A., K. Fujita, et al. (2007). "Prostatic stem cell marker identified by cDNA microarray in mouse." J Urol **178**(2): 686-91.

PURPOSE: Identifying prostatic stem cells is important to elucidate the mechanisms by which the prostate develops and control prostate cancer. We recently reported that the proximal region of the mouse prostate contains a population of stem cells. However, to our knowledge the specific marker of stem cells in the proximal region remains unknown. MATERIALS AND METHODS: We performed cDNA microarray analysis of cells obtained from the proximal region and from the remaining regions in dorsal prostates to identify several candidate stem cell markers. After we focused on 1 candidate among them we confirmed the expression of this candidate gene by reverse transcriptase-polymerase chain reaction analysis and immunohistochemistry. We also investigated the relation between positive cells for this marker and those for telomerase reverse transcriptase. Finally, we investigated the functional potential of prominin positive cells in 3-dimensional culture. RESULTS: Seven of 4,800 genes analyzed showed proximal/remaining ratios greater than 20. Of these genes we focused on prominin because it is a cell surface marker widely used to identify and isolate stem cells from various organs. We found a prominin positive cell population enriched in the basal cell layer in the proximal region, and the coincidence of prominin and telomerase reverse transcriptase immunostaining. We also found that prominin positive cells gave rise to numerous and large-branched ducts, whereas prominin negative cells formed far fewer such structures in 3-dimensional culture. CONCLUSIONS: A small population of prominin

positive cells in the mouse prostate basal layer of the proximal region represents a stem cell population.

Ueberham, E., T. Aigner, et al. (2007). "E-cadherin as a reliable cell surface marker for the identification of liver specific stem cells." J Mol Histol **38**(4): 359-68.

Oval cells are liver-specific bipotent stem cells which accumulate in injured liver when proliferation of mature hepatocytes and/or cholangiocytes is impaired. They represent an intermediary cell type with phenotypical characteristics of both, hepatocytes and cholangiocytes. Oval cells express specific cell surface proteins allowing their identification in situ. Most of these cell surface proteins, however, are recognized by antibodies in mouse liver tissue that are not commercially available or work only on frozen sections. We show herein the unequivocal identification of oval cells in paraffin-embedded mouse liver samples based on strong E-cadherin expression different from that of hepatocytes and bile duct cells. By comparing the pattern of E-cadherin expression with that of both, A6-antigen and CD44, we suggest a tight control of E-cadherin expression depending on the differentiation stage of the progenitor cells. In human cirrhotic liver samples E-cadherin expression was found as a common feature of both, typical and atypical reactions, and, thus, can also serve as an indication of the progenitor cell compartment activation.

van der Lugt, N., E. R. Maandag, et al. (1991). "A pgk::hprt fusion as a selectable marker for targeting of genes in mouse embryonic stem cells: disruption of the T-cell receptor delta-chain-encoding gene." Gene **105**(2): 263-7.

We have constructed a hypoxanthine phosphoribosyl transferase-selectable marker (hprt) under the control of the phosphoglycerate kinase (pgk) promoter. This construct permits cell growth in hypoxanthine/aminopterin/thymidine media and confers 6-thioguanine sensitivity upon mouse Hprt- embryonic stem cells, allowing either positive or negative selection in gene-targeting experiments. We have successfully targeted the gene encoding the T-cell receptor delta-chain using the pgk::hprt fusion for counterselection.

van Rhenen, A., B. Moshaver, et al. (2007). "Aberrant marker expression patterns on the CD34+CD38- stem cell compartment in acute myeloid leukemia allows to distinguish the malignant from the normal stem cell compartment both at diagnosis and in remission." Leukemia **21**(8): 1700-7.

Acute myeloid leukemia (AML) is generally regarded as a stem cell disease. In CD34-positive AML, the leukemic stem cell has been recognized as CD38 negative. This CD34+CD38- population survives chemotherapy and is most probable the cause of minimal residual disease (MRD). The outgrowth of MRD causes relapse and MRD can therefore serve as a prognostic marker. The key role of leukemogenic CD34+CD38- cells led us to investigate whether they can be detected under MRD conditions. Various markers were identified to be aberrantly expressed on the CD34+CD38- population in AML and high-risk MDS samples at diagnosis, including C-type lectin-like molecule-1 and several lineage markers/marker-combinations. Fluorescent in situ hybridization analysis revealed that marker-positive cells were indeed of malignant origin. The markers were neither expressed on normal CD34+CD38- cells in steady-state bone marrow (BM) nor in BM after chemotherapy. We found that these markers were indeed expressed in part of the patients on malignant CD34+CD38- cells in complete remission, indicating the presence of malignant CD34+CD38- cells. Thus, by identifying residual malignant CD34+CD38- cells after chemotherapy, MRD detection at the stem cell level turned out to be possible. This might facilitate characterization of these chemotherapy-resistant leukemogenic cells, thereby being of help to identify new targets for therapy.

Walsh, M. J., T. G. Fellous, et al. (2008). "Fourier transform infrared microspectroscopy identifies symmetric PO(2)(-) modifications as a marker of the putative stem cell region of human intestinal crypts." Stem Cells **26**(1): 108-18.

Complex biomolecules absorb in the mid-infrared (lambda = 2-20 microm), giving vibrational spectra associated with structure and function. We used Fourier transform infrared (FTIR) microspectroscopy to "fingerprint" locations along the length of human small and large intestinal crypts. Paraffin-embedded slices of normal human gut were sectioned (10 microm thick) and mounted to facilitate infrared (IR) spectral analyses. IR spectra were collected using globar (15 microm x 15 microm aperture) FTIR microspectroscopy in reflection mode, synchrotron (<or=10 microm x 10 microm aperture) FTIR microspectroscopy in transmission mode or near-field photothermal microspectroscopy. Dependent on the location of crypt interrogation, clear differences in spectral characteristics were noted. Epithelial-cell IR spectra were subjected to principal component analysis to determine whether wavenumber-absorbance relationships expressed as single points in "hyperspace" might on the basis of multivariate distance reveal biophysical differences along the length of gut crypts. Following spectroscopic analysis, plotted clusters and their

loadings plots pointed toward symmetric (nu(s))PO(2)(-) (1,080 cm(-1)) vibrations as a discriminating factor for the putative stem cell region; this proved to be a more robust marker than other phenotypic markers, such as beta-catenin or CD133. This pattern was subsequently confirmed by image mapping and points to a novel approach of nondestructively identifying a tissue's stem cell location. nu(s)PO(2)(-), probably associated with DNA conformational alterations, might facilitate a means of identifying stem cells, which may have utility in other tissues where the location of stem cells is unclear.

Wang, Q., Z. G. Chen, et al. (2009). "Cancer stem cell marker CD133+ tumour cells and clinical outcome in rectal cancer." Histopathology **55**(3): 284-93.

AIMS: The CD133 antigen has been identified as a putative stem cell marker in colorectal cancer tissues. According to the cancer stem cell hypothesis, CD133+ cells determine long-term tumour growth and are therefore suspected of influencing clinical outcome. The aim was to investigate the prognostic value of CD133 expression in rectal cancer patients after preoperative radiation and curative resection. METHODS AND RESULTS: The expression of the CD133 stem cell antigen in a series of 73 patients with rectal cancer of various ypTNM stages was analysed by immunohistochemistry on formalin-fixed paraffin-embedded sections. The prognostic value of CD133 expression and other clinicopathological factors was evaluated. On multivariate survival analysis, the proportion of CD133+ cells was a significant ($P < 0.05$) prognostic factor for adverse disease-free survival and overall survival independent of ypTNM stage, tumour differentiation or lymphovascular invasion. CONCLUSIONS: CD133 stem cell antigen expression correlates with patient survival in rectal cancer, lending support to the current cancer stem cell hypothesis.

Wang, X. and J. T. Hsieh (1994). "Androgen repression of cytokeratin gene expression during rat prostate differentiation: evidence for an epithelial stem cell-associated marker." Chin Med Sci J **9**(4): 237-41.

Cytokeratin (CK) 8 mRNA expression in developing and degenerating rat prostate was studied using in situ hybridization with an antisense RNA-probe. It was found that: 1) the CK 8 antisense probe was accumulated only within prostatic epithelial cells; 2) after castration, CK 8 mRNA signals in ventral prostate (VP) sections were significantly increased, and elevated CK 8 mRNA expression persisted even long after prostate involution was complete; and 3) during prostate development, the strongest CK 8 mRNA staining was found in the early neonatal prostatic epithelia which were composed mainly of prostatic stem cells. Thereafter, a shift of CK 8 mRNA staining to peripheral regions and decreased overall CK 8 mRNA levels were noted. These data indicate that excessive expression of CK 8 mRNA is a characteristic of prostatic stem cells, and CK molecules are excellent markers for determining the hierarchical pathway of cell differentiation in prostate epithelium.

Webster, J. D., V. Yuzbasiyan-Gurkan, et al. (2007). "Expression of the embryonic transcription factor Oct4 in canine neoplasms: a potential marker for stem cell subpopulations in neoplasia." Vet Pathol **44**(6): 893-900.

Neoplastic cells and stem cells share several phenotypic characteristics. Recently, numerous studies have identified adult stem cells that have been hypothesized to be the cellular origin for cancer in several tissues. Oct4 has been consistently associated with pluripotent or stemlike cells, and it is hypothesized that Oct4 is necessary for the maintenance of pluripotency. We hypothesize that Oct4-positive cells are present in all canine neoplasms and that these subpopulations of neoplastic cells might represent "cancer stem" cells. To test this hypothesis, 83 canine neoplasms representing 21 neoplastic diseases were evaluated for Oct4 expression using immunohistochemistry. The results of this study showed that all tumors included in this study contained a subpopulation of Oct4-positive cells, although the proportion of Oct4-positive cells and the intensity of immunoreactivity varied both within and between tumor types. Subpopulations of Oct4-positive cells identified in these tumors are likely to represent "cancer stem" cells and therefore might be responsible for the maintenance and propagation of the tumors. If these cells represent cancer stem cells, and are therefore responsible for the maintenance and growth of the neoplastic cellular population, then these cells should serve as relevant therapeutic targets and offer the greatest potential for curative treatment.

Wei, X. D., L. Zhou, et al. (2009). "In vivo investigation of CD133 as a putative marker of cancer stem cells in Hep-2 cell line." Head Neck **31**(1): 94-101.

BACKGROUND: Mounting evidence suggests that most tumors consist of a heterogeneous population of cells with a subset population that has the exclusive tumorigenic ability. They are called cancer stem cells (CSCs). CSCs can self-renew to generate additional CSCs and also differentiate to generate phenotypically diverse cancer cells with limited proliferative potential. They have been

identified in a variety of tumors. In this study, we identify the marker of CSCs in the established human laryngeal tumor Hep-2 cell line in vivo. Our in vitro experiment shown as CD133, a 5-transmembrane glycoprotein expressed in Hep-2 cell line. CD133 was supposed as a candidate of CSC in laryngeal carcinoma. In this study, the expression of CD133 was detected in a Hep-2 cell line. Applying the magnetic cell sorting (MACS) technology, we reported the results of purifying CD133 positive cells from a Hep-2 cell line. Three-type cells' tumor-forming ability was examined in vivo to identify the marker of CSCs in Hep-2 cell line. METHODS: CD133 was selected as a putative marker of CSC in laryngeal carcinoma, Hep-2 cell lines. Flow cytometry was used to detect the expression of CD133 in the Hep-2 cell line. Immunomagnetic beads were applied to purify CD133-positive cells. CD133(+), CD133(-) tumor cells, and unsorted Hep-2 cells were injected into severe combined immune deficiency (SCID) mice individually to observe tumor-forming ability. RESULTS: Only a small proportion (3.15% +/- 0.83%) of cells in the Hep-2 cell line express the CD133 marker. In comparison with CD133(-) tumor cells and unsorted cells, CD133(+) cells possess a marked capacity for tumor formation in vivo ($p < .05$). CONCLUSION: CD133 is 1 of the markers for CSCs in human laryngeal tumors of the Hep-2 cell line. Work on the characterization of these cells provides a powerful tool to investigate the tumorigenic process in the larynx and to develop therapies targeting the CSC.

Woywodt, A., J. Scheer, et al. (2004). "Circulating endothelial cells as a marker of endothelial damage in allogeneic hematopoietic stem cell transplantation." Blood **103**(9): 3603-5.

Damage to endothelial cells is the common feature of vascular disorders associated with hematopoietic stem cell transplantation (HSCT). Elevated numbers of circulating endothelial cells reflect the extent of endothelial damage in a variety of disorders but their use in HSCT has not been investigated so far. We studied 39 patients undergoing allogeneic HSCT with different conditioning regimens and 22 healthy controls. Circulating endothelial cells were enumerated with immunomagnetic isolation during the course of HSCT. After conditioning, cell numbers were significantly elevated (median 44 cells/mL) compared with baseline (median 16 cells/mL) and controls (median 8 cells/mL). Patients who received radiation had an earlier peak when compared with patients who received chemotherapy. Patients who received reduced-intensity conditioning had significantly lower cell numbers (median 24 cells/mL) than those who received standard conditioning. These observations provide a novel marker to investigate microvascular endothelial damage and the effects of different conditioning regimens in patients undergoing HSCT.

Yamashita, T., A. Budhu, et al. (2007). "Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma." Cancer Res **67**(22): 10831-9.

The heterogeneous nature of hepatocellular carcinoma (HCC) and the lack of appropriate biomarkers have hampered patient prognosis and treatment stratification. Using a gene expression profiling approach, we recently identified a novel prognostic HCC subtype that resembles hepatic progenitor cells with the activation of stem cell markers and Wnt-beta-catenin signaling, based on EpCAM (epithelial cell adhesion molecule, a hepatic stem cell marker) expression. In this study, we investigated whether the activation of the Wnt-beta-catenin pathway regulates EpCAM expression. We found that nuclear accumulation of beta-catenin induced, whereas the degradation of beta-catenin or inhibition of Tcf/beta-catenin complex formation reduced EpCAM gene expression in cultured normal human hepatocytes and HCC cell lines. We identified two Tcf binding elements in the EpCAM promoter that specifically bound to Tcf-4 in an electrophoretic mobility shift assay. EpCAM promoter luciferase activity was down-regulated by the degradation of beta-catenin or inhibition of Tcf/beta-catenin complex formation. Furthermore, we found that EpCAM-positive HCC is much more sensitive to Tcf/beta-catenin binding inhibitors than EpCAM-negative HCC in vitro. Taken together, our data indicate that EpCAM is a Wnt-beta-catenin signaling target gene and may be used to facilitate HCC prognosis by enabling effective stratification of patients with predicted pharmacologic responses to Wnt-beta-catenin signaling antagonists.

Ye, K., S. Jin, et al. (2004). "Genetically engineered fluorescent cell marker for labeling CD34+ hematopoietic stem cells." Biotechnol Prog **20**(2): 561-5.

To address the challenge of labeling and tracking stem cells in vivo, we have engineered a fluorescent cell marker CD34EGFP by utilizing the mechanism of the cell-specific activity of CD34 promoter in CD34(+) stem cells. A retroviral vector derived from a murine stem cell virus was constructed to integrate the CD34EGFP gene into the genome of the cells for labeling. Our experiment demonstrates that the 454 bp segment upstream of the murine CD34 cDNA sequence has full function of promoter activity and can serve as a cell-specific promoter for driving the expression of EGFP in CD34(+) hematopoietic

stem cells (HSC), providing a living color for labeling stem cells. The CD34EGFP marker was tested in various types of cells, including terminally differentiated cells, CD34(+) mouse myeloid leukemia progenitor cells, CD34(-) hematopoietic cells, and CD34(+) HSCs. We show that the engineered CD34EGFP cell marker is expressed in the CD34(+) stem or progenitor cells but not in CD34(-) or terminally differentiated cells. RT-PCR assay indicates that the transcription level of the CD34EGFP gene from CD34 promoter is almost the same as that from CMV promoter in CD34(+) progenitor cells. The approach we present here offers a framework for genetic engineering of fluorescent cell markers for labeling and tracking stem cells in vivo. We anticipate that a variety of cell markers could be generated by coupling variants of fluorescent proteins with various cell-specific promoters.

Yeo, S., S. Jeong, et al. (2007). "Characterization of DNA methylation change in stem cell marker genes during differentiation of human embryonic stem cells." Biochem Biophys Res Commun **359**(3): 536-42.

Pluripotent human embryonic stem cells (hESCs) have the distinguishing feature of innate capacity to allow indefinite self-renewal. This attribute continues until specific constraints or restrictions, such as DNA methylation, are imposed on the genome, usually accompanied by differentiation. With the aim of utilizing DNA methylation as a sign of early differentiation, we probed the genomic regions of hESCs, particularly focusing on stem cell marker (SCM) genes to identify regulatory sequences that display differentiation-sensitive alterations in DNA methylation. We show that the promoter regions of OCT4 and NANOG, but not SOX2, REX1 and FOXD3, undergo significant methylation during hESCs differentiation in which SCM genes are substantially repressed. Thus, following exposure to differentiation stimuli, OCT4 and NANOG gene loci are modified relatively rapidly by DNA methylation. Accordingly, we propose that the DNA methylation states of OCT4 and NANOG sequences may be utilized as barometers to determine the extent of hESC differentiation.

Ying, Z., J. Gonzalez-Martinez, et al. (2005). "Expression of neural stem cell surface marker CD133 in balloon cells of human focal cortical dysplasia." Epilepsia **46**(11): 1716-23.

PURPOSE: Focal cortical dysplasia (CD) is characterized by the presence of dysmorphic neurons, laminar and columnar disorganization. A few patients with CD have balloon cells intermixed with dysmorphic neurons. The cellular characteristics of balloon cells remain unknown. This study was intended to determine further the cellular characteristics of balloon cells. Balloon cells (BCs) were found to be immunoreactive to Bcl-2 (46%), vimentin (41%), Nestin (28%), CD133 (28%), MAP2 (27%), GFAP (14%), and TUJ1 (10%). An extremely small number of BCs were immunopositive for NeuN. Confocal double labeling showed that balloon cells were dually immunopositive for CD133/nestin; CD133/GFAP; CD133/Bcl-2, and nestin/GFAP. CONCLUSIONS: These results show that balloon cells are heterogeneous cell populations expressing cell-surface markers for pluripotential stem cells and proteins for multipotent progenitors, or immature neurons/glia. The presence of stem cell/progenitor markers in the balloon cells could be due to a persistent postnatal neurogenesis or early embryonic insult that resulted in arrest of proliferation/differentiation at their early stages. Additionally, the coexpression of Bcl-2 in CD133-positive balloon cells suggests that a resistance to programmed cell death may be involved in the pathogenesis of cortical dysplasia.

Zangrossi, S., M. Marabese, et al. (2007). "Oct-4 expression in adult human differentiated cells challenges its role as a pure stem cell marker." Stem Cells **25**(7): 1675-80.

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

new insights into the role of Oct-4 in fully differentiated cells. Disclosure of potential conflicts of interest is found at the end of this article.

Zeilstra, J., S. P. Joosten, et al. (2008). "Deletion of the WNT target and cancer stem cell marker CD44 in Apc(Min/+) mice attenuates intestinal tumorigenesis." Cancer Res **68**(10): 3655-61.

Mutation of the genes encoding the WNT signaling components adenomatous polyposis coli or beta-catenin plays a critical role in the initiation of colorectal cancer. These mutations cause constitutively active beta-catenin/TCF-mediated transcription, driving the transformation of intestinal crypts to colorectal cancer precursor lesions, called dysplastic aberrant crypt foci. CD44 is a prominent WNT signaling target in the intestine and is selectively expressed on the renewing epithelial cells lining the crypts. The expression of CD44 is dramatically increased in aberrant crypt foci in both humans and tumor-susceptible Apc(Min/+) mice, suggesting a role for CD44 in intestinal tumorigenesis. To study this role, we crossed C57BL/6J-Cd44(-/-) mice with C57BL/6J-Apc(Min/+) mice. Compared with C57BL/6J-Cd44(+/+)/Apc(Min/+) mice, C57BL/6J-Cd44(-/-)/Apc(Min/+) mice showed an almost 50% reduction in the number of intestinal adenomas. This reduction was primarily caused by a decrease in the formation of aberrant crypts, implying the involvement of CD44 in tumor initiation. The absence of CD44 in the normal (nonneoplastic) crypts of Cd44(-/-)/Apc(Min/+) mice did not alter the proliferative capacity and size of the intestinal stem cell and transit-amplifying compartments. However, compared with Cd44(+/+)/Apc(Min/+) mice, Cd44(-/-)/Apc(Min/+) showed an increase in the number of apoptotic epithelial cells at the base of the crypt which correlated with an increased expression of the proapoptotic genes Bok and Dr6. Our results show an important role for CD44 in intestinal tumorigenesis and suggest that CD44 does not affect proliferation but is involved in the control of the balance between survival and apoptosis in the intestinal crypt.

Zeppernick, F., R. Ahmadi, et al. (2008). "Stem cell marker CD133 affects clinical outcome in glioma patients." Clin Cancer Res **14**(1): 123-9.

PURPOSE: The CD133 antigen has been identified as a putative stem cell marker in normal and malignant brain tissues. In gliomas, it is used to enrich a subpopulation of highly tumorigenic cancer cells. According to the cancer stem cell hypothesis, CD133-positive cells determine long-term tumor growth and, therefore, are suspected to influence clinical outcome. To date, a correlation between CD133 expression in primary tumor tissues and patients' prognosis has not been reported. RESULTS: By multivariate survival analysis, we found that both the proportion of CD133-positive cells and their topological organization in clusters were significant (P < 0.001) prognostic factors for adverse progression-free survival and overall survival independent of tumor grade, extent of resection, or patient age. Furthermore, proportion of CD133-positive cells was an independent risk factor for tumor regrowth and time to malignant progression in WHO grade 2 and 3 tumors. CONCLUSIONS: These findings constitute the first conclusive evidence that CD133 stem cell antigen expression correlates with patient survival in gliomas, lending support to the current cancer stem cell hypothesis.

Zhang, X. Y., H. K. Pfeiffer, et al. (2008). "USP22, an hSAGA subunit and potential cancer stem cell marker, reverses the polycomb-catalyzed ubiquitylation of histone H2A." Cell Cycle **7**(11): 1522-4.

Initial studies of the mammalian hSAGA transcriptional coactivator complex identified the acetyltransferase hGCN5/PCAF as the only known enzymatic subunit. Recently we demonstrated that the ubiquitin hydrolase USP22 comprises a second enzymatic subunit of hSAGA, and that is required for activator-driven transcription. USP22 is expressed with polycomb ubiquitin ligases in an 11 gene signature that defines therapy-resistant tumors. At the biochemical level, these Polycomb proteins function as global transcriptional repressors by catalyzing the ubiquitylation of histone H2A. In yeast, the USP22 homolog functions as a transcriptional coactivator by removing ubiquitin from a distinct core histones, H2B. Given that USP22 is expressed in cancer as part of an 11 gene signature that includes transcriptional repressors which ubiquitylate H2A, it seemed possible that USP22 might activate transcription in part via the deubiquitylation of this same substrate. As reported here, biochemical analysis of the substrate specificity of USP22 reveals that it deubiquitylates histone H2A in addition to H2B. This finding supports a model in which the H2A ubiquitin hydrolase USP22 is coordinately expressed with Polycomb H2A ubiquitin ligases in order that the transcription of certain critical transforming genes be maintained in the face of the global repression mediated by Polycomb.

Zhang, X. Y., M. Varthi, et al. (2008). "The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression." Mol Cell **29**(1): 102-11.

Polycomb genes encode critical regulators of both normal stem cells and cancer stem cells. A gene signature that includes Polycomb genes and additional genes coregulated with Polycomb genes was recently

identified. The expression of this signature has been reported to identify tumors with the cancer stem cell phenotypes of aggressive growth, metastasis, and therapy resistance. Most members of this 11 gene signature encode proteins with well-defined roles in human cancer. However, the function of the signature member USP22 remains unknown. We report that USP22 is a previously uncharacterized subunit of the human SAGA transcriptional cofactor complex. Within SAGA, USP22 deubiquitylates histone H2B. Furthermore, USP22 is recruited to specific genes by activators such as the Myc oncoprotein, where it is required for transcription. In support of a functional role within the Polycomb/cancer stem cell signature, USP22 is required for appropriate progression through the cell cycle.

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