Cancer Stem Cells

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Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the cancer stem cells.
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1. Introduction

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into many different types of cells in life bodies, to be many different tissues and organs. Stem cells can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003). Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situition 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.

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

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

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

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

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

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

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

Normally to say that somatic stem cells differentiate only into specific tissue cells wherein they reside. However, somatic stem cells can differentiate into cells other than those of their tissue

of origin. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart and peripheral blood possess stem or progenitor cells with the capacity to transdifferentiate. Due to this developmental plasticity, somatic stem cells may have potential in autologous regenerative medicine, circumventing problems like rejection and the ethically challenged use of embryocyte stem cells.

Cancer stem cell (CSC) is cancer cell that has two facts: they exist in cancers (or tumors) and have the characteristics of stem cells. CSCs may generate tumors. Such cells could stay in in tumor or cause rise to new tumors. To treat cancer stem cell is important in the clinical cancer biology, even CSCs form only a small proportion of the tumor. Chemotherapies kill differentiated or differentiating cells that form the bulk of the tumor, but are unable to generate new cells. A population of CSCs, which gave rise to it, could remain untouched and cause a relapse of the disease.

Aldehyde dehydrogenase (ALDH) expressed in liposarcoma patient body (Stratford, Castro et al.).

In different cancer subtypes, cells in the tumor perform the functional heterogeneity, and tumors are formed from various proliferative and differentiate cells. This functional heterogeneity in cancer cells has 2 characterizations normally: the CSC and clonal evolution models. The CSCs can self-renew and are able to generate the diverse tumor cells. The tumor population is hierarchically arranged with CSCs lying at the apex of the hierarchy. Cancer cells must be capable of continuous proliferation and self-renewal in order to retain the many mutations required for carcinogenesis.

There are questions on the cell of origin of CSCs - whether they originate from normal stem cells that have lost the ability to regulate proliferation, or from more differentiated population of progenitor cells that have acquired abilities to self-renew.

In 1997, Bonnet and Dick isolated a subpopulation of leukaemic cells that expressed a specific surface marker CD34, but lacked the CD38 marker. Bonnet and Dick concluded that the $CD34^{+}/CD38^{-}$ subpopulation is capable of initiating tumors in NOD/SCID mice that are histologically similar to the donor (Bonnet D and Dick JE, 1997).

In cancer biology studies, tumor cells are sometimes injected into experimental animals to induce tumors. Cancer progression is followed in time and drug candidates are tested for their ability to inhibit the cancer growth. However, efficient cancer formation requires thousands of cells to be injected. It is possible that only a small fraction of the injected cells CSCs have the potential to generate cancer. Many cancers are very heterogeneous and contain multiple cell types native to the host organ. Heterogeneity is commonly retained by cancer metastases. The cell that produced cancer have the capacity to generate multiple cell types.

The cancer stem cell (CSC) hypothesis proposes that tumour growth is maintained by a distinct subpopulation of 'CSC'. Whilst cell lines are valuable in assay development, primary cells may provide a more rewarding model for studying tumour heterogeneity in the context of CSC (Blacking, Waterfall et al.).

CSC can be generated as: mutants of developing progenitor cells, developing stem cells, adult progenitor cells, or adult stem cells. One possibility is that the cancer stem cell is generated by a mutation of stem cell niche during cell development. The logical progression claims that these developing stems are mutated and then expand and the mutation is shared by many of the descendants of the mutated stem cells. These daughter stem cells are more easy to become into tumors. There are more chance of a mutation that can cause cancer.

The cancer is a heterogeneous population of mutant cells. The tumor is made up of several types of stem cells. Some cells can become more adaption to certain environments including adaptation to cancer treatment, so that more severe for the patients.

CSCs are found in most human cancers. For the CSC isolation for the researches and diagnosis, fluorescence-activated cell sorting (FACS) with antibodies directed at cell-surface markers and functional approaches including SP analysis (side population assay) or Aldefluor assay can be considered. The CSC-enriched population purified by these approaches is then implanted, at various cell doses. This in vivo assay is called limiting dilution assay. The cancer cell subsets that can initiate cancer development at low cell numbers are further tested for self-renewal capacity in serial cancer capacity. CSC can be identified by efflux of incorporated Hoechst dyes via multidrug resistance (MDR) and ATP-binding cassette (ABC) Transporters. Another method used for identification of cell subset enriched with in CSCs in vitro is sphere-forming assays. Many normal stem cells such as hematopoietics or stem cells from tissues are capable, under special culture conditioned, to form three-dimensional spheres, which can differentiate into multiple cell types. Similarly as normal stem cells, the CSCs isolated from brain or prostate tumors has also ability to form anchorage-independent spheres (Nicolis, 2007).

Many cell surface markers have been used for isolation of subsets enriched CSC, such as CD133 (i.e. PROM1), CD44, CD24, EpCAM (epithelial cell adhesion molecule, i.e. epithelial specific antigen, ESA), THY1 and ATP-binding cassette B5 (ABCB5), etc.

CD133 (prominin 1) is a five-transmembrane domain glycoprotein expressed on $CD34^+$ stem and progenitor cells, in endothelial precursors and fetal neural stem cells. It has been detected using its glycosylated epitope know as AC133.

EpCAM (epithelial cell adhesion molecule, ESA, TROP1) is hemophilic CA^{2+}-independent cell adhesion molecule expressed on the basolateral surface of most epithelial cells.

CD90 (THY1) is a glycosylphosphatidylinositol glycoprotein anchored in the plasma membrane and involved in signal transduction. It may also mediate adhesion between thymocytes and thymic stroma.

CD44 (PGP1) is an adhesion molecule that has pleiotropic roles in cell signaling, migration and homing. It has multiple isoforms, including CD44H, which exhibits high affinity for hyaluronate, and CD44V which has metastatic properties.

CD24 (HSA) is a glycosylated glycosylphosphatidylinositol-anchored adhesion molecule, which has co-stimulatory role in B and T cells.

ALDH is a ubiquitous aldehyde dehydrogenase family of enzymes, which catalyzes the oxidation of aromatic aldehydes to carboxyl acids. For instance, it has role in conversion of retinol to retinoic acid, which is essential for survival.

The first isolated and identified CSC was from breast cancer. Breast CSCs have been enriched in $CD44^+CD24^{-/low}$, SP, $ALDH^+$. Breast CSCs are very phenotypically diverse. Both $CD44^+CD24^-$ and $CD44^+CD24^+$ cell populations are tumor initiating cells. CSC are most highly enriched using the $CD44^+CD49f^{hi}CD133/2^{hi}$ as marker. Stem-like cancer cells have been identified using cell surface markers including CD44, CD133, EGFR and SSEA-1 (stage-specific embryonic antigen-1).

CSCs have also been found in human colon cancer. For their identification, cell surface markers such as ABCB5, CD44, CD133 were used. Multiple CSCs have been fould in prostate, lung and many other organs, including liver, pancreas, kidney or ovary. In prostate cancer, the tumor-initiating cells have been identified in $CD44^+$ cell subset as $CD44^+\alpha2\beta1^+$, TRA-1-$60^+CD151^+CD166^+$ or $ALDH^+$ cell populations.

Drug resistance of cancer stem/initiating cells has been considered to be one of the main reasons for tumor relapse. The breast cancer cell line MDA-MB-468 was cultured with 5-fluorouracil and serially passaged. The drug resistance of cancer cells is mainly due to tumor stem/initiating cells, and that under conditions of persistent chemotherapy, the quantity or function of breast cancer stem/initiating cells increases and decreases alternately.(Lu, Deng et al.)

The existence of CSCs has several implications in terms of future cancer treatment and therapies. These include disease identification, selective drug targets, prevention of metastasis, and development of new intervention strategies.

The epithelial-mesenchymal transition plays a crucial role in the progression of pancreatic cancer. Pancreatic cancer stem-like cells exhibit greater invasion and migration activity in vitro compared to the CD44(-)CD24(-) cells. A direct link between epithelial-mesenchymal transition and cancer stem-like cells in pancreatic cancer (Wang, Wu et al.).

Normal somatic stem cells are naturally resistant to chemotherapeutic agents. CSCs that developed from normal stem cells may also produced these proteins that could increase their resistance towards chemotherapeutic agents. The cell surface receptor interleukin-3 receptor-alpha (CD123) was found to be overexpressed on CD34+CD38- leukemic stem cells (LSCs) in acute myelogenous leukemia (AML) but not on normal CD34+CD38- bone marrow cells.

The design of new drugs for the treatment of CSCs will likely require an understanding of the cellular mechanisms that regulate cell proliferation. A normal stem cell may be transformed into a cancer stem cell through disregulation of the proliferation and differentiation pathways controlling it or by inducing oncoprotein activity.

The Polycomb group transcriptional repressor Bmi-1 was discovered as a common oncogene activated in lymphoma and later shown to specifically regulate HSCs. The role of Bmi-1 has also been illustrated in neural stem cells. The pathway appears to be active in CSCs of pediatric brain tumors.

The role of the Notch pathway in control of stem cell proliferation has been demonstrated for several cell types including hematopoietic, neural and mammary stem cells. Components of the Notch pathway have been proposed to act as oncogenes in mammary. A particular branch of the Notch signaling pathway that involves the transcription factor Hes3 has been shown to regulate a number of cultured cells with cancer stem cell characteristics obtained from glioblastoma patients.

Sonic hedgehog (SHH) and Wnt pathways are commonly hyperactivated in cancer and are required to sustain cancer growth. The Gli transcription factors that are regulated by SHH take their name from gliomas, where they are commonly expressed at high levels. A degree of crosstalk exists between the two pathways. Metastasis is the most serious problem and lethality for the cancer patients.

Application of Stem Cells in Clinical Medicine

There are over four thousand registered diseases specifically linked to genetic abnormalities. Although

stem cells are unlikely to provide powerful treatment for these diseases, they are unique in their potential application to these diseases.

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

Debates on Stem Cell Research

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

Literatures:

Akunuru, S., J. Palumbo, et al. "Rac1 targeting suppresses human non-small cell lung adenocarcinoma cancer stem cell activity." PLoS One **6**(2): e16951.

The cancer stem cell (CSC) theory predicts that a small fraction of cancer cells possess unique self-renewal activity and mediate tumor initiation and propagation. However, the molecular mechanisms involved in CSC regulation remains unclear, impinging on effective targeting of CSCs in cancer therapy. Here we have investigated the hypothesis that Rac1, a Rho GTPase implicated in cancer cell proliferation and invasion, is critical for tumor initiation and metastasis of human non-small cell lung adenocarcinoma (NSCLA). Rac1 knockdown by shRNA suppressed the tumorigenic activities of human NSCLA cell lines and primary patient NSCLA specimens, including effects on invasion, proliferation, anchorage-independent growth, sphere formation and lung colonization. Isolated side population (SP) cells representing putative CSCs from human NSCLA cells contained elevated levels of Rac1-GTP, enhanced in vitro migration, invasion, increased in vivo tumor initiating and lung colonizing activities in xenografted mice. However, CSC activity was also detected within the non-SP population, suggesting the importance of therapeutic targeting of all cells within a tumor. Further, pharmacological or shRNA targeting of Rac1 inhibited the tumorigenic activities of both SP and non-SP NSCLA cells. These studies indicate that Rac1 represents a useful target in NSCLA, and its blockade may have therapeutic value in suppressing CSC proliferation and metastasis.

Ali, H. R., S. J. Dawson, et al. "Cancer stem cell markers in breast cancer: pathological, clinical and prognostic significance." Breast Cancer Res **13**(6): R118.

INTRODUCTION: The cancer stem cell (CSC) hypothesis states that tumours consist of a cellular hierarchy with CSCs at the apex driving tumour recurrence and metastasis. Hence, CSCs are potentially of profound clinical importance. We set out to establish the clinical relevance of breast CSC markers by profiling a large cohort of breast tumours in tissue microarrays (TMAs) using immunohistochemistry (IHC). METHODS: We included 4, 125 patients enrolled in the SEARCH population-based study with tumours represented in TMAs and classified into molecular subtype according to a validated IHC-based five-marker scheme. IHC was used to detect CD44/CD24, ALDH1A1, aldehyde dehydrogenase family 1 member A3 (ALDH1A3) and integrin alpha-6 (ITGA6). A 'Total CSC' score representing expression of all four CSC markers was also investigated. Association with breast cancer specific survival (BCSS) at 10 years was assessed using a Cox proportional-hazards model. This study was complied with REMARK criteria. RESULTS: In ER negative cases, multivariate analysis showed that ITGA6 was an independent prognostic factor with a time-dependent effect restricted to the first two years of follow-up (hazard ratio (HR) for 0 to 2 years follow-up, 2.4; 95% confidence interval (95% CI), 1.2 to 4.8; P = 0.009). The composite 'Total CSC' score carried independent prognostic significance in ER negative

cases for the first four years of follow-up (HR for 0 to 4 years follow-up, 1.3; 95% CI, 1.1 to 1.6; P = 0.006). CONCLUSIONS: Breast CSC markers do not identify identical subpopulations in primary tumours. Both ITGA6 and a composite Total CSC score show independent prognostic significance in ER negative disease. The use of multiple markers to identify tumours enriched for CSCs has the greatest prognostic value. In the absence of more specific markers, we propose that the effective translation of the CSC hypothesis into patient benefit will necessitate the use of a panel of markers to robustly identify tumours enriched for CSCs.

Ali, N., H. Allam, et al. "Hepatitis C virus-induced cancer stem cell-like signatures in cell culture and murine tumor xenografts." J Virol **85**(23): 12292-303.
Hepatitis C virus (HCV) infection is a prominent risk factor for the development of hepatocellular carcinoma (HCC). Similar to most solid tumors, HCCs are believed to contain poorly differentiated cancer stem cell-like cells (CSCs) that initiate tumorigenesis and confer resistance to chemotherapy. In these studies, we demonstrate that the expression of an HCV subgenomic replicon in cultured cells results in the acquisition of CSC traits. These traits include enhanced expression of doublecortin and CaM kinase-like-1 (DCAMKL-1), Lgr5, CD133, alpha-fetoprotein, cytokeratin-19 (CK19), Lin28, and c-Myc. Conversely, curing of the replicon from these cells results in diminished expression of these factors. The putative stem cell marker DCAMKL-1 is also elevated in response to the overexpression of a cassette of pluripotency factors. The DCAMKL-1-positive cells isolated from hepatoma cell lines by fluorescence-activated cell sorting (FACS) form spheroids in Matrigel. The HCV RNA abundance and NS5B levels are significantly reduced by the small interfering RNA (siRNA)-led depletion of DCAMKL-1. We further demonstrate that HCV replicon-expressing cells initiate distinct tumor phenotypes compared to the tumors initiated by parent cells lacking the replicon. This HCV-induced phenotype is characterized by high-level expression/coexpression of DCAMKL-1, CK19, alpha-fetoprotein, and active c-Src. The results obtained by the analysis of liver tissues from HCV-positive patients and liver tissue microarrays reiterate these observations. In conclusion, chronic HCV infection appears to predispose cells toward the path of acquiring cancer stem cell-like traits by inducing DCAMKL-1 and hepatic progenitor and stem cell-related factors. DCAMKL-1 also represents a novel cellular target for combating HCV-induced hepatocarcinogenesis.

Alvero, A. B., M. K. Montagna, et al. "Targeting the mitochondria activates two independent cell death pathways in ovarian cancer stem cells." Mol Cancer Ther **10**(8): 1385-93.
Cancer stem cells are responsible for tumor initiation and chemoresistance. In ovarian cancer, the CD44+/MyD88+ ovarian cancer stem cells are also able to repair the tumor and serve as tumor vascular progenitors. Targeting these cells is therefore necessary to improve treatment outcome and patient survival. The previous demonstration that the ovarian cancer stem cells are resistant to apoptotic cell death induced by conventional chemotherapy agents suggests that other forms of targeted therapy should be explored. We show in this study that targeting mitochondrial bioenergetics is a potent stimulus to induce caspase-independent cell death in a panel of ovarian cancer stem cells. Treatment of these cells with the novel isoflavone derivative, NV-128, significantly depressed mitochondrial function exhibited by decrease in ATP, Cox-I, and Cox-IV levels, and by increase in mitochondrial superoxide and hydrogen peroxide. This promotes a state of cellular starvation that activates two independent pathways: (i) AMPKalpha1 pathway leading to mTOR inhibition; and (ii) mitochondrial MAP/ERK kinase/extracellular signal-regulated kinase pathway leading to loss of mitochondrial membrane potential. The demonstration that a compound can specifically target the mitochondria to induce cell death in this otherwise chemoresistant cell population opens a new venue for treating ovarian cancer patients.

Antonarakis, E. S., M. A. Carducci, et al. "Phase I rapid dose-escalation study of AGS-1C4D4, a human anti-PSCA (prostate stem cell antigen) monoclonal antibody, in patients with castration-resistant prostate cancer: a PCCTC trial." Cancer Chemother Pharmacol **69**(3): 763-71.
PURPOSE: AGS-1C4D4 is a human monoclonal antibody against prostate stem cell antigen (PSCA), a cell-surface protein expressed by most prostate cancers. AGS-1C4D4 is produced in Chinese hamster ovary (CHO) cells and has an identical sequence to AGS-PSCA, an anti-PSCA antibody produced in mouse hybridoma cells that has completed Phase I testing. Preclinical studies demonstrated comparability of AGS-1C4D4 to AGS-PSCA with respect to pharmacokinetics (PK) and tumor inhibition. However, because of differences in antibody-dependent cellular cytotoxicity between AGS-PSCA and AGS-1C4D4, a limited Phase I trial using AGS-1C4D4 was performed evaluating safety and PK. PATIENTS AND METHODS: Thirteen patients with metastatic castration-resistant prostate cancer were enrolled. AGS-1C4D4 was administered

intravenously every 3 weeks for four planned doses at 6, 12, 24, or 48 mg/kg. Primary endpoints were safety and PK. Secondary endpoints were immunogenicity and clinical activity. Disease assessments were conducted every 12 weeks and included radiographic and PSA evaluations. Patients with stable disease could receive extended treatment beyond four infusions. RESULTS: Adverse events were primarily grade 1-2, without any grade 3-4 drug-related toxicities or infusion reactions. Anti-AGS-1C4D4 antibodies were not detected. Similar to AGS-PSCA, serum AGS-1C4D4 concentrations declined biphasically and elimination was characterized by slow clearance (CL) and a long terminal half-life (t (1/2)). Median CL for the four dose levels ranged from 0.10 to 0.14 ml/h kg, and t (1/2) ranged from 2.2 to 2.9 weeks. No PSA reductions >/=50% were observed. Six patients (46%) had radiographically stable disease, lasting a median of 24 weeks. CONCLUSION: AGS-1C4D4 was well-tolerated and demonstrated linear PK. Despite preclinical differences in antibody-dependent cellular cytotoxicity, AGS-1C4D4 and AGS-PSCA have similar safety and PK profiles. The recommended Phase II dose is 48 mg/kg.

Arima, Y., N. Hayashi, et al. "Loss of p16 expression is associated with the stem cell characteristics of surface markers and therapeutic resistance in estrogen receptor-negative breast cancer." Int J Cancer **130**(11): 2568-79.
Triple-negative breast cancer [TNBC, which is negative for the estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2] is a high-risk form of the disease without a specific therapy. DNA microarray and immunohistochemical analyses have shown that most TNBCs fall within the basal-like histological subset of breast cancers, which frequently exhibit inactivation of the retinoblastoma tumor suppressor (Rb) and upregulation of the cyclin-dependent kinase inhibitor p16(INK4a) (p16). However, downregulation of p16 expression has been observed in some basal-like breast cancer cell lines, suggesting that such cells can be divided into two groups according to Rb and p16 status. We now show that cells that are CD44(+) and CD24(-) , a phenotype associated with stem-like breast cancer cells, are more abundant in ER(-) /p16(-) breast cancer cell lines than in ER(-) /p16(+) lines. It was also found that p16 expression was downregulated in mammospheres from an ER-negative breast cancer cell line. Depletion of p16 by RNA interference in ER-negative breast cancer cells increased the percentage of CD44(+) /CD24(-) cells and increased the expression of mRNA of the ES-like genes Nanog, Oct4, and Sox2 through an Rb-independent pathway. Furthermore, such depletion of p16 reduced chemosensitivity. The loss of p16 expression may thus reduce the response of ER-negative breast cancer cells to chemotherapy by conferring cancer stem cell-like properties. Consistent with this conclusion, immunohistochemical analysis of the clinical samples suggests that low p16 expression in TNBC is associated with resistance to preoperative chemotherapy.

Bae, K. M., N. N. Parker, et al. "E-cadherin plasticity in prostate cancer stem cell invasion." Am J Cancer Res **1**(1): 71-84.
Prostate cancer that has progressed to metastatic disease remains largely untreatable. Nearly 90% of patients with advanced prostate cancer develop skeletal metastases, resulting in a substantial reduction in the quality of life and a drastic worsening of patient prognosis. The mechanisms involved in prostate cancer cell dissemination, however, remain poorly understood. We previously reported the identification of a highly tumorigenic E-cadherin positive prostate tumor stem cell subpopulation that expressed the embryonic stem cell markers SOX2 and OCT3/4. We herein demonstrate that this subpopulation is also highly invasive and, importantly, is capable of altering its E-cadherin expression during the process of invasion. The non-tumorigenic E-cadherin negative subpopulation which minimally expresses SOX2 or OCT3/4 was found to be poorly invasive. In addition, targeted knockdown of SOX2 or OCT3/4 markedly suppressed the invasion of prostate cancer cells. Taken together, these findings indicate that the expression of SOX2 or OCT3/4 is required for invasive cell capacity, but the ability to modulate E-cadherin is the key permissive factor enabling cancer stem cell invasion in vitro. We therefore propose a model in which the post-epithelial to mesenchymal transition phenotype progresses to a frank, aggressive, and invasive phenotype by a process requiring the acquisition of E-cadherin plasticity. Considering the clinical significance of the metastatic complications of prostate adenocarcinoma, the identification of factors that promote the dissemination of the malignant prostate phenotype is essential to establish effective therapies to combat this disease in future.

Bao, B., Z. Wang, et al. "Over-expression of FoxM1 leads to epithelial-mesenchymal transition and cancer stem cell phenotype in pancreatic cancer cells." J Cell Biochem **112**(9): 2296-306.
FoxM1 is known to play important role in the development and progression of many malignancies including pancreatic cancer. Studies have shown that the acquisition of epithelial-to-mesenchymal transition (EMT) phenotype and induction of cancer stem cell (CSC) or cancer stem-like cell phenotypes are highly

inter-related, and contributes to drug resistance, tumor recurrence, and metastasis. The molecular mechanism(s) by which FoxM1 contributes to the acquisition of EMT phenotype and induction of CSC self-renewal capacity is poorly understood. Therefore, we established FoxM1 over-expressing pancreatic cancer (AsPC-1) cells, which showed increased cell growth, clonogenicity, and cell migration. Moreover, over-expression of FoxM1 led to the acquisition of EMT phenotype by activation of mesenchymal cell markers, ZEB1, ZEB2, Snail2, E-cadherin, and vimentin, which is consistent with increased sphere-forming (pancreatospheres) capacity and expression of CSC surface markers (CD44 and EpCAM). We also found that over-expression of FoxM1 led to decreased expression of miRNAs (let-7a, let-7b, let-7c, miR-200b, and miR-200c); however, re-expression of miR-200b inhibited the expression of ZEB1, ZEB2, vimentin as well as FoxM1, and induced the expression of E-cadherin, leading to the reversal of EMT phenotype. Finally, we found that genistein, a natural chemo-preventive agent, inhibited cell growth, clonogenicity, cell migration and invasion, EMT phenotype, and formation of pancreatospheres consistent with reduced expression of CD44 and EpCAM. These results suggest, for the first time, that FoxM1 over-expression is responsible for the acquisition of EMT and CSC phenotype, which is in part mediated through the regulation of miR-200b and these processes, could be easily attenuated by genistein.

Bao, B., Z. Wang, et al. "Notch-1 induces epithelial-mesenchymal transition consistent with cancer stem cell phenotype in pancreatic cancer cells." Cancer Lett **307**(1): 26-36.
Activation of Notch-1 is known to be associated with the development and progression of human malignancies including pancreatic cancer. Emerging evidence suggest that the acquisition of epithelial-mesenchymal transition (EMT) phenotype and induction of cancer stem cell (CSC) or cancer stem-like cell phenotype are interrelated and contributes to tumor recurrence and drug resistance. The molecular mechanism(s) by which Notch-1 contributes to the acquisition of EMT phenotype and CSC self-renewal capacity has not been fully elucidated. Here we show that forced over-expression of Notch-1 leads to increased cell growth, clonogenicity, migration and invasion of AsPC-1 cells. Moreover, over-expression of Notch-1 led to the induction of EMT phenotype by activation of mesenchymal cell markers such as ZEB1, CD44, EpCAM, and Hes-1. Here we also report, for the first time, that over-expression of Notch-1 leads to increased expression of miR-21, and decreased expression of miR-200b, miR-200c, let-7a, let-7b, and let-7c. Re-expression of miR-200b led to decreased expression of ZEB1, and vimentin, and increased expression of E-cadherin. Over-expression of Notch-1 also increased the formation of pancreatospheres consistent with expression of CSC surface markers CD44 and EpCAM. Finally, we found that genistein, a known natural anti-tumor agent inhibited cell growth, clonogenicity, migration, invasion, EMT phenotype, formation of pancreatospheres and expression of CD44 and EpCAM. These results suggest that the activation of Notch-1 signaling contributes to the acquisition of EMT phenotype, which is in part mediated through the regulation of miR-200b and CSC self-renewal capacity, and these processes could be attenuated by genistein treatment.

Beattie, S. and S. Lebel "The experience of caregivers of hematological cancer patients undergoing a hematopoietic stem cell transplant: a comprehensive literature review." Psychooncology **20**(11): 1137-50.
OBJECTIVE: Hematopoietic stem cell transplant (HSCT) is a demanding procedure with associated physical and psychological sequelae that affects patients and their families. Caregivers to HSCT patients not only have to cope with the life-threatening nature of the disease and treatment, but they also have care-giving responsibilities. This study reviews the literature on the psychosocial impact of being a caregiver to a HSCT patient. METHODS: A critical review of the literature published before June 2010 was conducted. Databases searched included CINAHL, Medline, PsycInfo, and Academic Search Complete, as well as a comprehensive reference review. Studies that pertained to caregivers of children (under the age of 18) undergoing a HSCT or caregivers to patients with non-hematological malignancies were excluded. Sixteen quantitative research articles and three qualitative research articles were reviewed and analyzed. RESULTS: Caregiver distress is highest pre-transplant and decreases over time, and caregivers display distress levels comparable to or higher than patients' reported distress levels. Predictors of caregiver distress include female gender, elevated subjective burden, and higher patient symptom distress. Caregivers reported uncertainty, fear of the future, juggling patients' needs with their own, and difficulties adapting to role changes; however, they also reported positive aspects to care giving, such as personal growth and developing a more positive relationship with the patient. CONCLUSIONS: There are many limitations to the current research and future directions should include both members of the dyad to evaluate the reciprocal relation between caregiver and patient

variables, as well as theory-driven research and research with direct clinical applications.

Beck, S., X. Jin, et al. "Telomerase activity-independent function of TERT allows glioma cells to attain cancer stem cell characteristics by inducing EGFR expression." Mol Cells **31**(1): 9-15.
Telomerase reverse transcriptase (TERT), the catalytic subunit of the enzyme telomerase, is robustly expressed in cancer cells. TERT enables cells to avoid chromosome shortening during repeated replication by maintaining telomere length. However, several lines of evidence indicate that many cancer cells exhibit shorter telomere length than normal tissues, implying an additional function of TERT in tumor formation and progression. Here, we report a telomerase activity-independent function of TERT that induces cancer stemness in glioma cells. Overexpression of TERT712, a telomerase activity-deficient form of TERT, in U87MG cells promoted cell self-renewal in vitro, and induced EGFR expression and formation of gliomas exhibiting cellular heterogeneity in vivo. In patients with glioblastoma multiforme, TERT expression showed a high correlation with EGFR expression, which is closely linked to the stemness gene signature. Induction of differentiation and TERT-knockdown in glioma stem cells led to a marked reduction in EGFR expression, cancer stemness, and anticancer drug resistance. Together, our findings indicate that TERT plays a crucial role in tumor progression by promoting cancer stemness through expression of EGFR.

Bednar, F. and D. M. Simeone "Pancreatic cancer stem cell biology and its therapeutic implications." J Gastroenterol **46**(12): 1345-52.
Pancreatic cancer remains one of the most difficult malignancies to treat. Significant developments in our understanding of pancreatic cancer biology have occurred over the past decade. One of the key advances has been the formulation of the cancer stem cell model of tumor growth and subsequent experimental proof of pancreatic cancer stem cell existence. Cancer stem cells contribute to pancreatic tumor growth and progression and are at least partially responsible for the relative resistance of the tumor to systemic chemotherapy and radiation. Significant questions remain about how the mutational profile of the tumor, the tumor microenvironment, and normal pancreatic developmental pathways contribute to pancreatic cancer stem cell biology. Answers to these questions will likely yield new therapeutic approaches for this deadly disease.

Beier, F., C. P. Beier, et al. "Identification of CD133(-)/telomerase(low) progenitor cells in glioblastoma-derived cancer stem cell lines." Cell Mol Neurobiol **31**(3): 337-43.
Glioblastoma multiforme (GBM) is paradigmatic for the investigation of cancer stem cells (CSC) in solid tumors. The CSC hypothesis implies that tumors are maintained by a rare subpopulation of CSC that gives rise to rapidly proliferating progenitor cells. Although the presence of progenitor cells is crucial for the CSC hypothesis, progenitor cells derived from GBM CSC are yet uncharacterized. We analyzed human CD133(+) CSC lines that were directly derived from CD133(+) primary astrocytic GBM. In these CSC lines, CD133(+)/telomerase(high) CSC give rise to non-tumorigenic, CD133(-)/telomerase(low) progenitor cells. The proliferation of the progenitor cell population results in significant telomere shortening as compared to the CD133(+) compartment comprising CSC. The average difference in telomere length as determined by a modified multi-color flow fluorescent in situ hybridization was 320 bp corresponding to 4-8 cell divisions. Taken together, we demonstrate that CD133(+) primary astrocytic GBM comprise proliferating, CD133(-)/telomerase(low) progenitor cell population characterized by low telomerase activity and shortened telomeres as compared to CSC.

Berry, D. A., N. T. Ueno, et al. "High-dose chemotherapy with autologous stem-cell support as adjuvant therapy in breast cancer: overview of 15 randomized trials." J Clin Oncol **29**(24): 3214-23.
PURPOSE: Adjuvant high-dose chemotherapy (HDC) with autologous hematopoietic stem-cell transplantation (AHST) for high-risk primary breast cancer has not been shown to prolong survival. Individual trials have had limited power to show overall benefit or benefits within subsets. METHODS: We assembled individual patient data from 15 randomized trials that compared HDC versus control therapy without stem-cell support. Prospectively defined primary end points were relapse-free survival (RFS) and overall survival (OS). We compared the effect of HDC versus control by using log-rank tests and proportional hazards regression, and we adjusted for clinically relevant covariates. Subset analyses were by age, number of positive lymph nodes, tumor size, histology, hormone receptor (HmR) status, and human epidermal growth factor receptor 2 (HER2) status. RESULTS: Of 6,210 total patients (n = 3,118, HDC; n = 3,092 control), the median age was 46 years; 69% were premenopausal, 29% were postmenopausal, and 2% were unknown menopausal status; 49.5% were HmR positive; 33.5% were HmR negative, and 17% were unknown HmR status. The median follow-up was 6 years. After analysis was adjusted for covariates, HDC was found to prolong

relapse-free survival (RFS; hazard ratio [HR], 0.87; 95% CI, 0.81 to 0.93; P < .001) but not overall survival (OS; HR, 0.94; 95% CI, 0.87 to 1.02; P = .13). For OS, no covariates had statistically significant interactions with treatment effect, and no subsets evinced a significant effect of HDC. Younger patients had a significantly better RFS on HDC than did older patients. CONCLUSION: Adjuvant HDC with AHST prolonged RFS in high-risk primary breast cancer compared with control, but this did not translate into a significant OS benefit. Whether HDC benefits patients in the context of targeted therapies is unknown.

Berry, D. A., N. T. Ueno, et al. "High-dose chemotherapy with autologous hematopoietic stem-cell transplantation in metastatic breast cancer: overview of six randomized trials." J Clin Oncol **29**(24): 3224-31.
PURPOSE: High doses of effective chemotherapy are compelling if they can be delivered safely. Substantial interest in supporting high-dose chemotherapy with bone marrow or autologous hematopoietic stem-cell transplantation in the 1980s and 1990s led to the initiation of randomized trials to evaluate its effect in the treatment of metastatic breast cancer. METHODS: We identified six randomized trials in metastatic breast cancer that evaluated high doses of chemotherapy with transplant support versus a control regimen without stem-cell support. We assembled a single database containing individual patient information from these trials. The primary analysis of overall survival was a log-rank test comparing high dose versus control. We also used Cox proportional hazards regression, adjusting for known covariates. We addressed potential treatment differences within subsets of patients. RESULTS: The effect of high-dose chemotherapy on overall survival was not statistically different (median, 2.16 v 2.02 years; P = .08). A statistically significant advantage in progression-free survival (median, 0.91 v 0.69 years) did not translate into survival benefit. Subset analyses found little evidence that there are groups of patients who might benefit from high-dose chemotherapy with hematopoietic support. CONCLUSION: Overall survival of patients with metastatic breast cancer in the six randomized trials was not significantly improved by high-dose chemotherapy; any benefit from high doses was small. No identifiable subset of patients seems to benefit from high-dose chemotherapy.

Bhaijee, F., D. J. Pepper, et al. "Cancer stem cells in head and neck squamous cell carcinoma: a review of current knowledge and future applications." Head Neck **34**(6): 894-9.
Head and neck squamous cell carcinoma (HNSCC) is a major cause of morbidity and mortality, and the alleviation thereof requires greater understanding of the pathobiologic behavior of HNSCC. Although the existence of cancer stem cells (CSCs) in most solid tumors has not been formally proven, application of the CSC concept has certainly enhanced understanding of HNSCC heterogeneity and progression. Recent data support the role of ALDH1(+) CD44(+) CSC in HNSCC, since the implantation of a few ALDH1(+) CD44(+) cells consistently gives rise to tumors that can be serially passaged in vivo. In addition to CSC biomarkers, recent explorations of CSC signaling pathways, gene expression, and localization in HNSCC carry significant clinical and therapeutic implications. Identification and characterization of CSC populations that regulate HNSCC growth, metastasis, and treatment resistance will facilitate development of novel diagnostic, therapeutic, and prognostic strategies. Furthermore, advances in multimodal imaging and nanotechnology, in conjunction with CSC models, may better elucidate the regulatory mechanisms that govern CSC biology in vivo, as well as develop platforms for targeted theragnostics. It is hoped that the promising applications of the CSC model in HNSCC will eventually alleviate the morbidity and mortality of this pervasive disease.

Biava, P. M., M. Basevi, et al. "Cancer cell reprogramming: stem cell differentiation stage factors and an agent based model to optimize cancer treatment." Curr Pharm Biotechnol **12**(2): 231-42.
The recent tumor research has lead scientists to recognize the central role played by cancer stem cells in sustaining malignancy and chemo-resistance. A model of cancer presented by one of us describes the mechanisms that give rise to the different kinds of cancer stem-like cells and the role of these cells in cancer diseases. The model implies a shift in the conceptualization of the disease from reductionism to complexity theory. By exploiting the link between the agent-based simulation technique and the theory of complexity, the medical view is here translated into a corresponding computational model. Two main categories of agents characterize the model, 1) cancer stem-like cells and 2) stem cell differentiation stage factors. Cancer cells agents are then distinguished based on the differentiation stage associated with the malignancy. Differentiation factors interact with cancer cells and then, with varying degrees of fitness, induce differentiation or cause apoptosis. The model inputs are then fitted to experimental data and numerical simulations carried out. By performing virtual experiments on the model's choice variables a decision-maker (physician) can obtains insights on the

progression of the disease and on the effects of a choice of administration frequency and or dose. The model also paves the way to future research, whose perspectives are discussed.

Biddle, A., X. Liang, et al. "Cancer stem cells in squamous cell carcinoma switch between two distinct phenotypes that are preferentially migratory or proliferative." Cancer Res **71**(15): 5317-26.
Epithelial-to-mesenchymal transition (EMT) is an important driver of tumor invasion and metastasis, which causes many cancer deaths. Cancer stem cells (CSC) that maintain and initiate tumors have also been implicated in invasion and metastasis, but whether EMT is an important contributor to CSC function is unclear. In this study, we investigated whether a population of CSCs that have undergone EMT (EMT CSCs) exists in squamous cell carcinoma (SCC). We also determined whether a separate population of CSCs that retain epithelial characteristics (non-EMT CSCs) is also present. Our studies revealed that self-renewing CSCs in SCC include two biologically-distinct phenotypes. One phenotype, termed CD44(high)ESA(high), was proliferative and retained epithelial characteristics (non-EMT CSCs), whereas the other phenotype, termed CD44(high)ESA(low), was migratory and had mesenchymal traits characteristic of EMT CSCs. We found that non-EMT and EMT CSCs could switch their epithelial or mesenchymal traits to reconstitute the cellular heterogeneity which was characteristic of CSCs. However, the ability of EMT CSCs to switch to non-EMT character was restricted to cells that were also ALDH1(+), implying that only ALDH1(+) EMT cells had the ability to seed a new epithelial tumor. Taken together, our findings highlight the identification of two distinct CSC phenotypes and suggest a need to define therapeutic targets that can eradicate both of these variants to achieve effective SCC treatment.

Bishop, M. R., E. P. Alyea, 3rd, et al. "National Cancer Institute's First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation: summary and recommendations from the organizing committee." Biol Blood Marrow Transplant **17**(4): 443-54.
The National Cancer Institute's First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation was organized and convened to identify, prioritize, and coordinate future research activities related to relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Each of the Workshop's 6 Working Committees has published individual reports of ongoing basic, translational, and clinical research and recommended areas for future research related to the areas of relapse biology, epidemiology, prevention, and treatment. This document summarizes each committee's recommendations and suggests 3 major initiatives for a coordinated research effort to address the problem of relapse after allo-HSCT: (1) to establish multicenter correlative and clinical trial networks for basic/translational, epidemiologic, and clinical research; (2) to establish a network of biorepositories for the collection of samples before and after allo-HSCT to aid in laboratory and clinical studies; and (3) to further refine, implement, and study the Workshop-proposed definitions for disease-specific response and relapse and recommendations for monitoring of minimal residual disease. These recommendations, in coordination with ongoing research initiatives and transplantation organizations, provide a research framework to rapidly and efficiently address the significant problem of relapse after allo-HSCT.

Blacking, T. M., M. Waterfall, et al. "CD44 is associated with proliferation, rather than a specific cancer stem cell population, in cultured canine cancer cells." Vet Immunol Immunopathol **141**(1-2): 46-57.
BACKGROUND: The cancer stem cell hypothesis proposes that tumours are maintained by a population of cancer stem cells (CSC), which must be eradicated to prevent disease relapse after treatment. Cells expressing high levels of CD44 have been identified as candidate CSC in a variety of human tumours. This study sought to investigate CD44 expression and its potential as a CSC marker in canine cancer. METHODS: CD44 expression in several canine cancer cell lines was determined by flow cytometry. Cells with low and high levels of CD44 expression were examined for differences in growth characteristics, colony forming ability, drug sensitivity and cell cycle profile. RESULTS: CD44(High) cells demonstrated enhanced growth and colony forming capacity, under both adherent and low-density serum free ("tumoursphere") conditions. However, no difference in sensitivity to doxorubicin was seen between the two populations. Moreover, whilst most CD44(Low) cells were in resting or G(1) growth phase, an increased proportion of CD44(High) cells were in G(2)M phase of the cell cycle. Upon proliferation in culture, both populations gave rise to progeny with a full spectrum of CD44 expression. CONCLUSION: CD44 expression is associated with proliferation in cultured canine cancer cells, but transient and fluctuating expression may limit its utility as a CSC marker.

Blacking, T. M., M. Waterfall, et al. "Flow cytometric techniques for detection of candidate cancer stem cell subpopulations in canine tumour models." Vet Comp Oncol **10**(4): 252-73.
The cancer stem cell (CSC) hypothesis proposes that tumour growth is maintained by a distinct subpopulation of 'CSC'. This study applied flow cytometric methods, reported to detect CSC in both primary and cultured cancer cells of other species, to identify candidate canine subpopulations. Cell lines representing diverse canine malignancies, and cells derived from spontaneous canine tumours, were evaluated for expression of stem cell-associated surface markers (CD34, CD44, CD117 and CD133) and functional properties [Hoecsht 33342 efflux, aldehyde dehydrogenase (ALDH) activity]. No discrete marker-defined subsets were identified within established cell lines; cells derived directly from spontaneous tumours demonstrated more heterogeneity, although this diminished upon in vitro culture. Functional assays produced variable results, suggesting context-dependency. Flow cytometric methods may be adopted to identify putative canine CSC. Whilst cell lines are valuable in assay development, primary cells may provide a more rewarding model for studying tumour heterogeneity in the context of CSC. However, it will be essential to fully characterize any candidate subpopulations to ensure that they meet CSC criteria.

Blagoev, K. B. "Organ aging and susceptibility to cancer may be related to the geometry of the stem cell niche." Proc Natl Acad Sci U S A **108**(48): 19216-21.
Telomere loss at each cell replication limits the proliferative capacity of normal cells, including adult stem cells. Entering replicative senescence protects dividing cells from neoplastic transformation, but also contributes to aging of the tissue. Recent experiments have shown that intestinal mouse stem cells divide symmetrically, at random make decisions to remain stem cells or to differentiate, and gradually lose telomeric DNA. A cell's decision whether to differentiate or to remain a stem cell depends on the local cellular and chemical environment and thus tissue architecture is expected to play role in cell proliferation dynamics. To take into account the structure of the stem cell niche in determining its proliferative potential and susceptibility to cancer, a theoretical model is introduced and the niche proliferative potential is quantified for different architectures. The niche proliferative potential is quantitatively related to the proliferative potential of the individual stem cells for different structural classes of the stem cell niche. Stem cells at the periphery of a niche are under pressure to divide and to differentiate, as well as to maintain the stem cell niche boundary, and thus the geometry of the stem cell niche is expected to play a role in determining the stem cell division sequence and differentiation. Smaller surface-to-volume ratio is associated with higher susceptibility to cancer, higher tissue renewal capacity, and decreased aging rate. Several testable experimental predictions are discussed, as well the presence of stochastic effects.

Bonafe, M., G. Storci, et al. "Inflamm-aging of the stem cell niche: breast cancer as a paradigmatic example: breakdown of the multi-shell cytokine network fuels cancer in aged people." Bioessays **34**(1): 40-9.
Inflamm-aging is a relatively new terminology used to describe the age-related increase in the systemic pro-inflammatory status of humans. Here, we represent inflamm-aging as a breakdown in the multi-shell cytokine network, in which stem cells and stromal fibroblasts (referred to as the stem cell niche) become pro-inflammatory cytokine over-expressing cells due to the accumulation of DNA damage. Inflamm-aging self-propagates owing to the capability of pro-inflammatory cytokines to ignite the DNA-damage response in other cells surrounding DNA-damaged cells. Macrophages, the major cellular player in inflamm-aging, amplify the phenomenon, by broadcasting pro-inflammatory signals at both local and systemic levels. On the basis of this, we propose that inflamm-aging is a major contributor to the increase in cancer incidence and progression in aged people. Breast cancer will be presented as a paradigmatic example for this relationship.

Borovski, T., E. M. F. De Sousa, et al. "Cancer stem cell niche: the place to be." Cancer Res **71**(3): 634-9.
Tumors are being increasingly perceived as abnormal organs that, in many respects, recapitulate the outgrowth and differentiation patterns of normal tissues. In line with this idea is the observation that only a small fraction of tumor cells is capable of initiating a new tumor. Because of the features that these cells share with somatic stem cells, they have been termed cancer stem cells (CSC). Normal stem cells reside in a "stem cell niche" that maintains them in a stem-like state. Recent data suggest that CSCs also rely on a similar niche, dubbed the "CSC niche," which controls their self-renewal and differentiation. Moreover, CSCs can be generated by the microenvironment through induction of CSC features in more differentiated tumor cells. In addition to a role in CSC maintenance, the microenvironment is hypothesized to be involved in metastasis by induction of the epithelial-mesenchymal transition, leading to dissemination and invasion of tumor cells. The localization of secondary tumors also seems to be

orchestrated by the microenvironment, which is suggested to form a premetastatic niche. Thus, the microenvironment seems to be of crucial importance for primary tumor growth as well as metastasis formation. Combined with its role in the protection of CSCs against genotoxic insults, these data strongly put forward the niche as an important target for novel therapies.

Bourseau-Guilmain, E., J. Bejaud, et al. "Development and characterization of immuno-nanocarriers targeting the cancer stem cell marker AC133." Int J Pharm **423**(1): 93-101.
In the context of targeted therapy, we addressed the possibility of developing a drug delivery nanocarrier capable to specifically reach cancer cells that express the most prominent marker associated with cancer stem cell (CSC) phenotype, AC133. For this purpose, 100nm lipid nanocapsules (LNCs) were functionalized with a monoclonal antibody (mAb) directed against AC133 according to two distinct methods: firstly, post-insertion within 100nm LNCs of a lipid poly(ethylene glycol) functionalized with reactive-sulfhydryl maleimide groups (DSPE-PEG(2000)-maleimide) followed by thiolated mAb coupling, and, secondly, creation of a thiolated lipo-immunoglobulin between DSPE-PEG(2000)-maleimide and AC133, then post-inserted within LNCs. Due to the reduced number of purification steps, lower amounts of DSPE-PEG(2000)-maleimide that were necessary as well as lower number of free maleimide functions present onto the surface of immuno-LNC, the second method was found to be more appropriate. Thus, 126nm AC133-LNC with a zeta potential of -22mV while keeping a narrow distribution were developed. Use of the IgG1kappa isotype control-immunoglobulins produced similar control IgG1-LNCs. Micro-Bradford colorimetric assay indicated a fixation of about 40 immunoglobulins per LNC. Use of human Caco-2 cells that constitutively express AC133 (Caco-2-AC133(high)) allowed addressing the behavior of the newly functionalized immuno-LNCs. siRNA knockown strategy permitted to obtain Caco-2-AC133(low) for comparison. Immunofluorescence-combined flow cytometry analysis demonstrated that the epitope-recognition function of AC133 antibody was preserved when present on immuno-LNCs. Although grafting of immunoglobulins onto the surface of LNCs repressed their internalization within Caco-2 cells as evaluated by flow cytometry, AC133-specific cellular binding was obtained with AC133-LNC as assessed by computer-assisted fluorescence microscopy. In conclusion, interest of AC133-LNCs as niche carriers is discussed toward the development of CSC targeted chemo- or radio-nanomedicines.

Bourseau-Guilmain, E., A. Griveau, et al. "The importance of the stem cell marker prominin-1/CD133 in the uptake of transferrin and in iron metabolism in human colon cancer Caco-2 cells." PLoS One **6**(9): e25515.
As the pentaspan stem cell marker CD133 was shown to bind cholesterol and to localize in plasma membrane protrusions, we investigated a possible function for CD133 in endocytosis. Using the CD133 siRNA knockdown strategy and non-differentiated human colon cancer Caco-2 cells that constitutively over-expressed CD133, we provide for the first time direct evidence for a role of CD133 in the intracellular accumulation of fluorescently labeled extracellular compounds. Assessed using AC133 monoclonal antibody, CD133 knockdown was shown to improve Alexa488-transferrin (Tf) uptake in Caco-2 cells but had no impact on FITC-dextran or FITC-cholera-toxin. Absence of effect of the CD133 knockdown on Tf recycling established a role for CD133 in inhibiting Tf endocytosis rather than in stimulating Tf exocytosis. Use of previously identified inhibitors of known endocytic pathways and the positive impact of CD133 knockdown on cellular uptake of clathrin-endocytosed synthetic lipid nanocapsules supported that CD133 impact on endocytosis was primarily ascribed to the clathrin pathway. Also, cholesterol extraction with methyl-beta-cyclodextrine up regulated Tf uptake at greater intensity in the CD133(high) situation than in the CD133(low) situation, thus suggesting a role for cholesterol in the inhibitory effect of CD133 on endocytosis. Interestingly, cell treatment with the AC133 antibody down regulated Tf uptake, thus demonstrating that direct extracellular binding to CD133 could affect endocytosis. Moreover, flow cytometry and confocal microscopy established that down regulation of CD133 improved the accessibility to the TfR from the extracellular space, providing a mechanism by which CD133 inhibited Tf uptake. As Tf is involved in supplying iron to the cell, effects of iron supplementation and deprivation on CD133/AC133 expression were investigated. Both demonstrated a dose-dependent down regulation here discussed to the light of transcriptional and post-transciptional effects. Taken together, these data extend our knowledge of the function of CD133 and underline the interest of further exploring the CD133-Tf-iron network.

Bozcuk, H. and M. Ozdogan "The predictors of the efficacy of high-dose chemotherapy and stem cell support in the management of metastatic germ cell cancer." Bratisl Lek Listy **112**(5): 296-304.
OBJECTIVES: We aimed to analyze the predictors of outcome in metastatic germ cell cancer (MGCC)

patients treated with High-dose Chemotherapy (HDC) and stem cell rescue. BACKGROUND: Various prognostic factors have been suggested in the treatment of metastatic germ cell cancer. However, there is no comprehensive evaluation of independent prognostic factors for the efficacy of HDC in published patient cohorts. METHODS: Thirty-two published patient cohorts with MGCC (encompassing 2176 patients; 510 patients treated upfront and 1666 at relapse) were identified from PUBMED and Cochrane Registry of Clinical Trials. Weighted Regression Analyses of these trials were conducted to define prognosticators. RESULTS: Independent correlates of overall survival (OAS) when all trials were considered were line of chemotherapy index, an indicator of line of HDC utilization (1st line: 71% vs 2nd or higher line: 40%, $p < 0.001$), and number of HDC cycles administered (1 cycle: 43%, 1 to 2 cycles: 43%, 2 or more cycles: 64%, $p = 0.021$). In cohorts having HDC for relapsed disease, lower line of chemotherapy index again ($p = 0.004$), and higher median age ($p = 0.023$) were independently associated with better OAS. In trials utilizing upfront HDC, higher number of chemotherapeutics in the HDC regimen was marginally linked with improved OAS ($p = 0.047$). CONCLUSION: The efficacy of various forms of HDC in MGCC patients with diverse prognostic factors may vary both as an initial or salvage therapy. Clinicians need to be aware of these factors for optimal patient selection for HDC in MGCC (Tab. 3, Fig. 2, Ref. 54).

Broadley, K. W., M. K. Hunn, et al. "Side population is not necessary or sufficient for a cancer stem cell phenotype in glioblastoma multiforme." Stem Cells **29**(3): 452-61.
There is strong evidence for the existence of cancer stem cells (CSCs) in the aggressive brain tumor glioblastoma multiforme (GBM). These cells have stem-like self-renewal activity and increased tumor initiation capacity and are believed to be responsible for recurrence due to their resistance to therapy. Several techniques have been used to enrich for CSC, including growth in serum-free defined media to induce sphere formation, and isolation of a stem-like cell using exclusion of the fluorescent dye Hoechst 33342, the side population (SP). We show that sphere formation in GBM cell lines and primary GBM cells enriches for a CSC-like phenotype of increased self-renewal gene expression in vitro and increased tumor initiation in vivo. However, the SP was absent from all sphere cultures. Direct isolation of the SP from the GBM lines did not enrich for stem-like activity in vitro, and tumor-initiating activity was lower in sorted SP compared with non-SP and parental cells. Transient exposure to doxorubicin enhanced both CSC and SP frequency. However, doxorubicin treatment altered the cytometric profile and obscured the SP demonstrating the difficulty of identifying SP in cells under stress. Doxorubicin-exposed cells showed a transient increase in SP, but the doxorubicin-SP cells were still not enriched for a stem-like self-renewal phenotype. These data demonstrate that the GBM SP does not necessarily contribute to self-renewal or tumor initiation, key properties of a CSC, and we advise against using SP to enumerate or isolate CSC.

Bubalo, J. S., G. Cherala, et al. "Aprepitant pharmacokinetics and assessing the impact of aprepitant on cyclophosphamide metabolism in cancer patients undergoing hematopoietic stem cell transplantation." J Clin Pharmacol **52**(4): 586-94.
Aprepitant, a neurokinin antagonist, is an effective antiemetic agent in chemotherapy for delayed nausea and vomiting. The study objective was to evaluate the pharmacokinetics of aprepitant and concurrent cyclophosphamide (CY), often a component of hematopoietic stem cell transplant (HSCT) conditioning regimen, in cancer patients undergoing HSCT. Forty subjects were randomized to either aprepitant or placebo in addition to standard antiemetics. Aprepitant or placebo was started 1 hour before the first chemotherapy or radiation dose for HSCT conditioning and administered daily until 4 days after infusion of the hematopoietic cell graft (for a total of 10-12 days). Serial blood samples were collected for aprepitant and CY plus 2 important CY metabolites. The results indicate that aprepitant is well absorbed and does not auto-induce its metabolism. No significant drug interaction was observed with CY or its metabolites. A significant portion of the patients had subtherapeutic aprepitant concentrations; however, chemotherapy-induced nausea and vomiting were effectively managed. No dosage adjustment was necessary, and administration of aprepitant in HSCT at the prescribed dosage of 125 mg orally on day 1 and 80 mg orally on each consecutive day through day +4 after HSCT was well tolerated with no significant changes in CY pharmacokinetic parameters.

Buijs, J. T., G. van der Horst, et al. "The BMP2/7 heterodimer inhibits the human breast cancer stem cell subpopulation and bone metastases formation." Oncogene **31**(17): 2164-74.
Accumulating evidence suggests that a subpopulation of breast cancer cells, referred to as cancer stem cells (CSCs), have the ability to propagate a tumor and potentially seed new metastases. Furthermore, stimulation of an epithelial-to-mesenchymal transition by factors like transforming growth factor-beta (TGFbeta) is accompanied with the generation of

breast CSCs. Previous observations indicated that bone morphogenetic protein-7 (BMP7) antagonizes the protumorigenic and prometastatic actions of TGFbeta, but whether BMP7 action is mechanistically linked to breast CSCs has remained elusive. Here, we have studied the effects of BMP7, BMP2 and a BMP2/7 heterodimer on the formation of human breast CSCs (ALDH(hi)/CD44(hi)/CD24(-/low)) and bone metastases formation in a preclinical model of intra-cardiac injection of MDA-MB-231 cells in athymic nude (Balb/c nu/nu) mice. The BMP2/7 heterodimer was the most efficient stimulator of BMP signaling and very effectively reduced TGFbeta-driven Smad signaling and cancer cell invasiveness. The tested BMPs-particularly the heterodimeric BMP2/7-strongly reduced the size of the ALDH(hi)/CD44(hi)/CD24(-/low) CSC subpopulation. In keeping with these in vitro observations, pretreatment of cancer cells with BMPs for 72 h prior to systemic inoculation of the cancer cells inhibited the formation of bone metastases. Collectively, our data support the notion that breast CSCs are involved in bone metastasis formation and describe heterodimeric BMP2/7 as a powerful TGFbeta antagonist with anti-metastatic potency.

Cabarcas, S. M., L. A. Mathews, et al. "The cancer stem cell niche--there goes the neighborhood?" Int J Cancer **129**(10): 2315-27.
The niche is the environment in which stem cells reside and is responsible for the maintenance of unique stem cell properties such as self-renewal and an undifferentiated state. The heterogeneous populations which constitute a niche include both stem cells and surrounding differentiated cells. This network of heterogeneity is responsible for the control of the necessary pathways that function in determining stem cell fate. The concept that cancer stem cells, a subpopulation of cells responsible for tumor initiation and formation, reside in their own unique niche is quickly evolving and it is of importance to understand and identify the processes occurring within this environment. The necessary intrinsic pathways that are utilized by this cancer stem cell population to maintain both self-renewal and the ability to differentiate are believed to be a result of the environment where cancer stem cells reside. The ability of a specific cancer stem cell niche to provide the environment in which this population can flourish is a critical aspect of cancer biology that mandates intense investigation. This review focuses on current evidence demonstrating that homeostatic processes such as inflammation, epithelial to mesenchymal transition, hypoxia and angiogenesis contribute to the maintenance and control of cancer stem cell fate by providing the appropriate signals within the microenvironment. It is necessary to understand the key processes occurring within this highly specialized cancer stem cell niche to identify potential therapeutic targets that can serve as the basis for development of more effective anticancer treatments.

Cao, L., M. Shao, et al. "Tissue transglutaminase links TGF-beta, epithelial to mesenchymal transition and a stem cell phenotype in ovarian cancer." Oncogene **31**(20): 2521-34.
Tissue transglutaminase (TG2), an enzyme involved in cell proliferation, differentiation and apoptosis is overexpressed in ovarian carcinomas, where it modulates epithelial-to-mesenchymal transition (EMT) and promotes metastasis. Its regulation in ovarian cancer (OC) remains unexplored. Here, we show that transforming growth factor (TGF)-beta, a cytokine involved in tumor dissemination is abundantly secreted in the OC microenvironment and induces TG2 expression and enzymatic activity. This is mediated at transcriptional level by SMADs and by TGF-beta-activated kinase 1-mediated activation of the nuclear factor-kappaB complex. TGF-beta-stimulated OC cells aggregate as spheroids, which enable peritoneal dissemination. We show that TGF-beta-induced TG2 regulates EMT, formation of spheroids and OC metastasis. TG2 knock-down in OC cells decreases the number of cells harboring a cancer stem cell phenotype (CD44+/CD117+). Furthermore, CD44+/CD117+ cells isolated from human ovarian tumors express high levels of TG2. In summary, TGF-beta-induced TG2 enhances ovarian tumor metastasis by inducing EMT and a cancer stem cell phenotype.

Cao, L., Y. Zhou, et al. "Sphere-forming cell subpopulations with cancer stem cell properties in human hepatoma cell lines." BMC Gastroenterol **11**: 71.
BACKGROUND: Cancer stem cells (CSCs) are regarded as the cause of tumor formation and recurrence. The isolation and identification of CSCs could help to develop novel therapeutic strategies specifically targeting CSCs. METHODS: Human hepatoma cell lines were plated in stem cell conditioned culture system allowed for sphere forming. To evaluate the stemness characteristics of spheres, the self-renewal, proliferation, chemoresistance, tumorigenicity of the PLC/PRF/5 sphere-forming cells, and the expression levels of stem cell related proteins in the PLC/PRF/5 sphere-forming cells were assessed, comparing with the parental cells. The stem cell RT-PCR array was performed to further explore the biological properties of liver CSCs. RESULTS: The PLC/PRF/5, MHCC97H and HepG2 cells could form clonal nonadherent 3-D spheres and be serially passaged.

The PLC/PRF/5 sphere-forming cells possessed a key criteria that define CSCs: persistent self-renewal, extensive proliferation, drug resistance, overexpression of liver CSCs related proteins (Oct3/4, OV6, EpCAM, CD133 and CD44). Even 500 sphere-forming cells were able to form tumors in NOD/SCID mice, and the tumor initiating capability was not decreased when spheres were passaged. Besides, downstream proteins DTX1 and Ep300 of the CSL (CBF1 in humans, Suppressor of hairless in Drosophila and LAG1 in C. elegans) -independent Notch signaling pathway were highly expressed in the spheres, and a gamma-secretase inhibitor MRK003 could significantly inhibit the sphere formation ability. CONCLUSIONS: Nonadherent tumor spheres from hepatoma cell lines cultured in stem cell conditioned medium possess liver CSC properties, and the CSL-independent Notch signaling pathway may play a role in liver CSCs.

Capittini, C., P. Bergamaschi, et al. "Birth-weight as a risk factor for cancer in adulthood: the stem cell perspective." Maturitas **69**(1): 91-3.
The 'stem cell burden' hypothesis represents a plausible explanation for the association between birth-weight and the risk of breast cancer in adulthood. The size of the overall stem cell pool would be expected to affect organ size and consequently birth-weight, making birth-weight a proxy for the overall number of fetal stem cells. As stem cells are self-renewing, the greater their number is at birth, the higher will be the chance that one of them will undergo carcinogenesis over the years. To investigate the correlation between birth-weight and stem cell burden, we examined the cord blood hematopoietic CD34+ stem cell population as an indicator of the overall fetal stem cell number. We measured both the CD34+ level (by flow cytometry) and the CD34+ proliferative potential (by the GM-CFU culture), in a sample of 1037 healthy newborn cord blood donors. We found that heavier babies had a significantly greater CD34+ stem cell concentration ($p<0.001$) and a higher GM-CFU number than lighter babies ($p<0.001$). Thus, a high birth-weight was positively associated with a high concentration of CD34+ stem cells and also with a qualitatively higher "stemness" of this pool. Therefore, our data support the theory that birth-weight reflects the number of fetal stem cells.

Chandrasekaran, S. and L. A. DeLouise "Enriching and characterizing cancer stem cell sub-populations in the WM115 melanoma cell line." Biomaterials **32**(35): 9316-27.
Cutaneous melanoma is an increasingly common and potentially lethal malignancy of melanocytes, the melanin producing cells normally located in the basal layer of the skin epidermis. Despite major advances in cancer chemotherapeutics and immunotherapy, the success in treating metastatic melanoma remains poor. The notion that cancer stem cells (CSCs) play a key role in melanoma progression is well received. Therefore, isolating and characterizing CSCs is of critical importance for designing new therapeutic strategies that target this unique tumor initiating cell sub-population. In this work, we present a simple in vitro method, employing cell culture on polydimethylsiloxane (PDMS) and transfer back onto standard tissue culture plate, to enrich a non-adherent spheroid (NA/S) forming and an adherent monolayer (AM) cell sub-populations from the tumorigenic WM115 melanoma cell line. The phenotypes of the morphologically distinct NA/S and AM sub-populations were further characterized by quantifying the expression of stem cell markers, CD20 and CD271. Flow cytometric analysis found 2.32% of the cells in the NA/S sub-population were CD20+ CD271+ whereas only 0.27% of the cells in the AM sub-population were CD20+ CD271+. When the NA/S sub-population was cultured back onto PDMS it resulted in the further enrichment of CD20+ CD271+ cells to 14.7%. We used microbubble arrays to quantify the in vitro clonogenic potential of the NA/S and AM cell sub-populations. Microbubbles are spherical cavities, ~160 mum in diameter with 60 mum circular openings, formed in PDMS using the gas expansion molding (GEM) process. Cells from each sub-population were seeded, under limiting dilution conditions, onto separate arrays containing 1215 microbubble wells. After five days in culture, wells seeded with 1, 2, 3 and >3 cells per microbubble well were inspected for cell proliferation. The Extreme Limiting Dilutions Analysis (ELDA) determined a ~58% clonal survival (1 in every 1.72 cells) for the NA/S sub-population and ~25% clonal survival (1 in every 3.93 cells) for the AM sub-population (= 176, p = 4.41e(-40)). These findings taken together add to the existing evidence that melanoma cells propagating as non-adherent/spheroids represent a more aggressive phenotype due to the greater presence of tumor initiating cells.

Che, S. M., X. Z. Zhang, et al. "The radiosensitization effect of NS398 on esophageal cancer stem cell-like radioresistant cells." Dis Esophagus **24**(4): 265-73.
This study aimed to investigate the cancer stem cell (CSC) properties of radioresistant esophageal cancer cells and the radiosensitization effect of NS398, a cyclooxygenase (COX)-2 inhibitor, on them. Fractionated irradiation was applied to acquire radioresistant esophageal cancer cells. Clone

formation assay was employed to detect cell radiosensitivity and cloning formation ability. Cell viability was determined by methyl tetrazolium colorimetry assay. Cell cycle distribution and apoptosis were detected by flow cytometry. Tumorigenicity was investigated by xenograft tumorigenicity assay. Expression levels of beta-catenin were detected by reverse transcription polymerase chain reaction or Western blot. As results, radioresistant Eca109R50Gy cells were obtained through fractional irradiation from Eca109 cells; Eca109R50Gy cells displayed higher ability of proliferation, colony-formation, and 40 times tumorigenic ability as high as that of the Eca109 cells in vivo. Meantime stem cell marker beta-catenin was elevated in Eca109R50Gy cells. All of the above implied that Eca109R50Gy cells have some properties of CSCs. NS398 enhanced the radiosensitivity of Eca109R50Gy cells accompanied by down-regulating the expression of beta-catenin. In conclusion, radioresistant Eca109R50Gy cells carried some CSC-like properties; NS398 enhanced the radiosensitivity of CSC-like Eca109R50Gy cells and this function may partly through down-regulating the expression of beta-catenin. These findings both stress the important role of CSCs in esophageal cancer radioresistance and provide new insight on possible application of COX-2 inhibitors on CSCs.

Chefetz, I., J. C. Holmberg, et al. "Inhibition of Aurora-A kinase induces cell cycle arrest in epithelial ovarian cancer stem cells by affecting NFkB pathway." Cell Cycle **10**(13): 2206-14.
Recurrent ovarian cancer is resistant to conventional chemotherapy. A sub-population of ovarian cancer cells, the epithelial ovarian cancer stem cells (EOC stem cells) have stemness properties, constitutive NFkappaB activity, and represent the chemoresistant population. Currently, there is no effective treatment that targets these cells. Aurora-A kinase (Aurora-A) is associated with tumor initiation and progression and is overexpressed in numerous malignancies. The aim of this study is to determine the effect of Aurora-A inhibition in EOC stem cells. EOC stem cells were treated with the Aurora-A inhibitor, MK-5108. Cell growth was monitored by Incucyte real-time imaging system, cell viability was measured using the Celltiter 96 assay and cytokine levels were quantified using xMAP technology. The intracellular changes associated with MK-5108 treatment are: (1) polyploidy and cell cycle arrest; (2) inhibition of NFkappaB activity; (3) decreased cytokine production; and (4) nuclear accumulation of IkappaBalpha. Thus, inhibition of Aurora-A decreases cell proliferation in the EOC stem cells by inducing cell cycle arrest and affecting the NFkappaB pathway. As EOC stem cells represent a source of recurrence and chemoresistance, these results suggest that Aurora-A inhibition may effectively target the cancer stem cell population in ovarian cancer.

Chen, C., Y. Wei, et al. "Evidence for epithelial-mesenchymal transition in cancer stem cells of head and neck squamous cell carcinoma." PLoS One **6**(1): e16466.
Initiation, growth, recurrence, and metastasis of head and neck squamous cell carcinomas (HNSCC) have been related to the behavior of cancer stem cells (CSC) that can be identified by their aldehyde-dehydrogenase-isoform-1 (ALDH1) activity. We quantified and enriched ALDH1(+) cells within HNSCC cell lines and subsequently characterized their phenotypical and functional properties like invasion capacity and epithelial-mesenchymal transition (EMT). Spheroid culture enriched CSC from five HNSCC cell lines by up to 5-fold. In spheroid-derived cells (SDC) and the parental monolayer-derived cell line ALDH1, CD44, CD24, E-Cadherin, alpha-SMA, and Vimentin expression was compared by flow-cytometry and immunofluorescence together with proliferation and cell cycle analysis. Invasion activity was evaluated by Matrigel assay and expression of stemness-related transcription factors (TF) Nanog, Oct3/4, Sox2 and EMT-related genes Snail1 and 2, and Twist by real-time PCR. All cell lines formed spheroids that could self-renew and be serially re-passaged. ALDH1 expression was significantly higher in SDC. ALDH1(+) cells showed increased colony-formation. The proportion of cells with a putative CSC marker constellation of CD44(+)/CD24(-) was highly variable (0.5% to 96%) in monolayer and spheroid cultures and overlapped in 0%-33% with the CD44(+)/CD24(-)/ALDH1(+) cell subset. SDC had significantly higher invading activity. mRNA of the stemness-related genes Sox2, Nanog, and Oct3/4 was significantly increased in SDC of all cell lines. Twist was significantly increased in two while Snail2 showed a significant increase in one and a significant decrease in SDC of two cell lines. SDC had a higher G0 phase proportion, showed high-level expression of alpha-SMA and Vimentin, but significantly decreased E-Cadherin expression. HNSCC-lines harbor potential CSC, characterized by ALDH1 and stemness marker TF expression as well as properties like invasiveness, quiescence, and EMT. CSC can be enriched by anchorage-independent culture techniques, which may be important for the investigation of their contribution to therapy resistance, tumor recurrence and metastasis.

Chen, H. C., A. S. Chou, et al. "Induction of metastatic cancer stem cells from the NK/LAK-

resistant floating, but not adherent, subset of the UP-LN1 carcinoma cell line by IFN-gamma." Lab Invest **91**(10): 1502-13.

As an advanced status of cancer stem cells (CSCs), metastatic CSCs (mCSCs) have been proposed to be the essential seeds that initiate tumor metastasis. However, the biology of mCSCs is poorly understood. In this study, we used a lymph node (LN) metastatic CEA-producing carcinoma cell line, UP-LN1, characterized by the persistent appearance of adherent (A) and floating (F) cells in culture, to determine the distribution of CSCs and mechanisms for the induction of mCSCs. F and A cells displayed distinct phenotypes, CD44(high)/CD24(low) and CD44(low)/CD24(high), respectively. The CSC-rich nature of F cells was typified by stronger expression of multiple drug resistance genes and a 7.8-fold higher frequency of tumor-initiating cells in NOD/SCID mice when compared with A cells. F cells showed a greater depression in HLA class I expression and an extreme resistance to NK/LAK-mediated cytolysis. Moreover, the NK/LAK-resistant F cells were highly susceptible to IFN-gamma-mediated induction of surface CXCR4, with concomitant downregulation of cytoplasmic CXCL12 expression, whereas these two parameters remained essentially unchanged in NK/LAK-sensitive A cells. Following the induction of surface CXCR4, enhanced migratory/invasive potential of F cells was demonstrated by in vitro assays. Confocal immunofluorescence microscopy showed the two distinct phenotypes of F and A cells could be correspondingly identified in monodispersed and compact tumor cell areas within the patient's LN tumor lesion. In response to IFN-gamma or activated NK/LAK cells, the CXCR4(+) mCSCs could be only induced from the CSCs, which were harbored in the highly tumorigenic CD44(high)/CD24(low) F subset. Our results revealed the complexity and heterogeneity of the CSC of this cell line/tumor and the differential immunomodulatory roles of F and A cells. A better understanding of the interactions among different classes of CSCs and their niches may assist us in eradicating the CSCs/mCSCs through targeted immunotherapy, chemotherapy, or both.

Chen, J., T. Guo, et al. "CD133 and CD44 are universally overexpressed in GIST and do not represent cancer stem cell markers." Genes Chromosomes Cancer **51**(2): 186-95.

Although imatinib mesylate has been a major breakthrough in the treatment of advanced gastrointestinal stromal tumors (GIST), complete responses are rare and most patients eventually develop resistance to the drug. Thus, the possibility of an imatinib-insensitive cell subpopulation within GIST tumors, harboring stem cell characteristics, may be responsible for the clinical failures. However, the existence of a cancer stem cell component in GIST has not been yet established. This study was aimed to determine whether expression of commonly used stem cell markers in other malignancies, that is, CD133 and CD44, might identify cells with characteristics of cancer stem/progenitor cells in human GIST. CD133 and CD44 expression in GIST explants was analyzed by flow cytometry, immunofluorescence, and gene expression. Their transcription levels were correlated with clinical and molecular factors in a large, well-annotated cohort of GIST patients. FACS sorted GIST cells based on CD133 and CD44 expression were isolated and used to assess phenotypic characteristics, ability to maintain their surface expression, sensitivity to imatinib, and expression signature. The enrichment in CD133/CD44 cells in the side population (SP) assay was also investigated. CD133 expression was consistently found in GIST. CD133(-) cells formed more colonies, were more invasive in a matrigel assay, and showed enrichment in the SP cells, compared to CD133(+) cells. CD133 expression was also detected in the two imatinib-sensitive GIST cell lines, while was absent in the imatinib-resistant lines. Our results show that CD133 and CD44 are universally expressed in GIST, and may represent a lineage rather than a cancer stem cell marker.

Chen, L., Z. Xiao, et al. "The enhancement of cancer stem cell properties of MCF-7 cells in 3D collagen scaffolds for modeling of cancer and anti-cancer drugs." Biomaterials **33**(5): 1437-44.

Three-dimensional (3D) culture could partially simulate in vivo conditions. In this work, we developed a 3D collagen scaffold to investigate cellular properties of MCF-7 cells. The porous scaffolds not only induced the diversification of cell morphologies but also extended cell proliferation. The expression of pro-angiogenic growth factors and the transcriptions of matrix metalloproteinases (MMPs) were significantly increased in cells cultured in 3D collagen scaffolds. In addition, 3D collagen scaffolds could generate a cell population with the properties of cancer stem cells (CSCs). The upregulation of EMT markers and the downregulation of the epithelial cell marker were observed in cells cultured in collagen scaffolds. The expression of stem cell markers, including OCT4A and SOX2, and breast cancer stem cell signatures, including SOX4, JAG1 and CD49F, was significantly unregulated in 3D collagen scaffolds. The proportion of cells with CSC-like CD44(+)/CD24(-/low) phenotype was notably increased. High-level expression of CSC-associated properties of MCF-7 cells cultured in 3D was further confirmed by high tumorigenicity in vivo. Moreover, xenografts with 3D cells formed larger tumors. The

properties of MCF-7 cells in 3D may have partially simulated their in vivo behaviors. Thus, 3D collagen scaffolds might provide a useful platform for anti-cancer therapeutics and CSC research.

Cheng, L., R. Alexander, et al. "The clinical and therapeutic implications of cancer stem cell biology." Expert Rev Anticancer Ther **11**(7): 1131-43.
Cancer stem cells (CSCs) have provided new insights into the tumorigenesis and metastatic potential of cancer. The discovery of CSCs has provided many new insights into the complexities of cancer therapy: tumor initiation, treatment resistance, metastasis, recurrence, assessment of prognosis and prediction of clinical course. Recent rapid advances in molecular analysis have contributed to the better understanding of the molecular attributes and pathways that give CSCs their unique attributes. Use of these molecular techniques has facilitated elucidation of specific surface markers and pathways that favor propagation of CSCs - allowing for targeted therapy. Furthermore, it has been discovered that a specific microenvironment, or niche, is essential for the genesis of tumors from CSCs. Therapeutic strategies that alter these microenvironments compromise CSC proliferation and constitute another method of targeted cancer therapy. We review the clinical and therapeutic implications of CSCs, with a focus on treatment resistance and metastasis, and the emerging approaches to target CSCs and their microenvironments in order to attain improved outcomes in cancer. It is noteworthy that CSCs are the only cells capable of sustaining tumorigenesis; however, the cell of origin of cancer, in which tumorigenesis is initiated, may be distinct from CSCs that propagate the tumor.

Chikamatsu, K., G. Takahashi, et al. "Immunoregulatory properties of CD44+ cancer stem-like cells in squamous cell carcinoma of the head and neck." Head Neck **33**(2): 208-15.
BACKGROUND: CD44 was found as a surface marker in cancer stem cell (CSC) of squamous cell carcinoma of the head and neck (SCCHN); however, the immunologic properties of such CSCs have not yet been elucidated. METHODS: The immunologic properties of CD44+ cancer stem-like cells were compared with those of CD44- cells using flow cytometry and enzyme-linked immunosorbent assay. RESULTS: CD44+ cells exhibited weak HLA-A2 and class II expression. Interestingly, downregulation of transporter antigen processing (TAP)2 was found in CD44+ cells. The CD44+ cell population produced significantly higher levels of interleukin (IL)-8, granulocyte colony-stimulating factor (G-CSF), and transforming growth factor (TGF)-beta than the CD44- cell population. Moreover, CD44+ cells have been shown to not only more strongly inhibit T-cell proliferation, but also to more efficiently inhibit regulatory T cells (Treg cells) and myeloid-derived suppressor cells (MDSC) as compared with CD44- cells. Additionally, CD44+ cells suppressed Th1 responses and enhanced regulatory T cell responses. CONCLUSION: CSCs might have higher malignant potential with numerous escape strategies from immune attack.

Choijamts, B., S. Jimi, et al. "CD133+ cancer stem cell-like cells derived from uterine carcinosarcoma (malignant mixed Mullerian tumor)." Stem Cells **29**(10): 1485-95.
Cancer stem cells (CSCs) that display tumor-initiating properties have recently been identified. CD133, a surface glycoprotein linked to organ-specific stem cells, has been described as a marker of CSCs in different tumor types. We herein identify and characterize CSCs in human uterine carcinosarcoma (malignant mixed Mullerian tumor), which is one of the most aggressive and therapy-resistant gynecological malignancies and is considered to be of mesodermal origin. The CD133(+) population was increased in uterine carcinosarcoma, and this population showed biphasic properties in the primary tumor. CD133(+) cells predominantly formed spheres in culture and were able to differentiate into mesenchymal lineages. CD133(+) cells were more resistant to cisplatin/paclitaxel-induced cytotoxicity in comparison with CD133(-) cells. A real-time polymerase chain reaction analysis of the genes implicated in stem cell maintenance revealed that CD133(+) cells express significantly higher levels of Oct4, Nanog, Sox2, and Bmi1 than CD133(-) cells. Moreover, CD133(+) cells showed a high expression level of Pax2 and Wnt4, which are genes essential for Mullerian duct formation. These CD133(+) cells form serially transplantable tumors in vivo and the resulting CD133(+) tumors replicated the EpCAM, vimentin, and estrogen and progesterone receptor expression of the parent tumor, indicating that CSCs likely differentiated into cells comprising the uterine carcinosarcoma tissue. Moreover, strong CD133 expression in both epithelial and mesenchymal elements in primary tumor demonstrated significant prognostic value. These findings suggest that CD133(+) cells have the characteristics of CSCs and Mullerian mesenchymal progenitors.

Christ, B., P. Stock, et al. "CD13: Waving the flag for a novel cancer stem cell target." Hepatology **53**(4): 1388-90.
Cancer stem cells (CSCs) are generally dormant or slowly cycling tumor cells that have the ability to

reconstitute tumors. They are thought to be involved in tumor resistance to chemo/radiation therapy and tumor relapse and progression. However, neither their existence nor their identity within many cancers has been well defined. Here, we have demonstrated that CD13 is a marker for semiquiescent CSCs in human liver cancer cell lines and clinical samples and that targeting these cells might provide a way to treat this disease. CD13+ cells predominated in the G0 phase of the cell cycle and typically formed cellular clusters in cancer foci. Following treatment, these cells survived and were enriched along the fibrous capsule where liver cancers usually relapse. Mechanistically, CD13 reduced ROS-induced DNA damage after genotoxic chemo/radiation stress and protected cells from apoptosis. In mouse xenograft models, combination of a CD13 inhibitor and the genotoxic chemotherapeutic fluorouracil (5-FU) drastically reduced tumor volume compared with either agent alone. 5-FU inhibited CD90+ proliferating CSCs, some of which produce CD13+ semiquiescent CSCs, while CD13 inhibition suppressed the self-renewing and tumor-initiating ability of dormant CSCs. Therefore, combining a CD13 inhibitor with a ROS-inducing chemo/radiation therapy may improve the treatment of liver cancer.

Cihova, M., V. Altanerova, et al. "Stem cell based cancer gene therapy." Mol Pharm **8**(5): 1480-7.
The attractiveness of prodrug cancer gene therapy by stem cells targeted to tumors lies in activating the prodrug directly within the tumor mass, thus avoiding systemic toxicity. Suicide gene therapy using genetically engineered mesenchymal stem cells has the advantage of being safe, because prodrug administration not only eliminates tumor cells but consequently kills the more resistant therapeutic stem cells as well. This review provides an explanation of the stem cell-targeted prodrug cancer gene therapy principle, with focus on the choice of prodrug, properties of bone marrow and adipose tissue-derived mesenchymal stem and neural stem cells as well as the mechanisms of their tumor homing ability. Therapeutic achievements of the cytosine deaminase/5-fluorocytosine prodrug system and Herpes simplex virus thymidine kinase/ganciclovir are discussed. In addition, delivery of immunostimulatory cytokines, apoptosis inducing genes, nanoparticles and antiangiogenic proteins by stem cells to tumors and metastases is discussed as a promising approach for antitumor therapy. Combinations of traditional, targeted and stem cell-directed gene therapy could significantly advance the treatment of cancer.

Cioffi, M., J. Dorado, et al. "EpCAM/CD3-Bispecific T-cell engaging antibody MT110 eliminates primary human pancreatic cancer stem cells." Clin Cancer Res **18**(2): 465-74.
PURPOSE: Tumor-initiating cells with stem-like properties, also termed cancer stem cells (CSC), have been shown to sustain tumor growth as well as metastasis and are highly resistant to chemotherapy. Because pancreatic CSCs have been isolated on the basis of EpCAM expression, we investigated whether a targeted immunotherapy to EpCAM using the bispecific T-cell-engaging antibody MT110 is capable of eradicating CSCs. EXPERIMENTAL DESIGN: We studied in vitro and in vivo the effects of MT110 on CSCs using both established cell lines as well as primary cells of human pancreatic cancer. RESULTS: Although established cell lines were more responsive to MT110-engaged T cells, also primary cells showed a time- and dose-dependent response to treatment with the bispecific antibody. In addition, the population of highly tumorigenic CSCs was efficiently targeted by the EpCAM/CD3-bispecific antibody MT110 in vitro and in vivo using a mouse model of established primary pancreatic cancer. Pancreatic cancer cells derived from metastases were slightly more resistant to MT110 treatment on the basis of in vivo tumorigenicity studies. This appeared to be related to a higher frequency of an EpCAM-negative subpopulation of CSCs. CONCLUSIONS: Cytotoxic T cells can be effectively redirected against primary human pancreatic cancer cells by T-cell-engaging BiTE antibody MT110 including a subpopulation of highly tumorigenic CSCs.

Colombel, M., C. L. Eaton, et al. "Increased expression of putative cancer stem cell markers in primary prostate cancer is associated with progression of bone metastases." Prostate **72**(7): 713-20.
BACKGROUND: A number of putative stem cell markers have been associated with aggressiveness of prostate cancer, including alpha 2 and alpha 6 integrin and c-met. The study aimed to test the hypothesis that the development of bone metastasis correlates with the proportion of prostate cancer stem cell-like cells present in the primary tumor. METHODS: Prostate tissue samples were obtained from patients with high-risk prostatic adenocarcinoma. Prostate cancer tumor tissue samples underwent immunohistochemical staining for alpha 2 and alpha 6 integrin and c-met; positive and negative controls were included. Samples were scored as positive if >5% of cells within the sample stained positively. Survival and bone metastasis-free survival curves on the patient cohort were estimated by the actuarial method of Kaplan-Meier. RESULTS: A total of 62 patients were included in the study. Bone metastases progression rate was 46% at 105 months with a median time of 46 months (95% CI: 1-62.5 months); prostate cancer-

specific survival was 33% at 122 months with a median survival time of 69.4 months (95% CI: 63.5-109.4 months). Survival curves show that c-met-, alpha 2, and alpha 6 integrin-positive tumors were positively associated with the occurrence of bone metastasis-free survival. There was a higher level of significance when at least c-met and either alpha 2 or alpha 6 integrin was positive. CONCLUSION: It can be concluded that percentage of stem cell-like prostate cancer cells has a prognostic impact especially on the risk of metastatic bone progression.

Cordenonsi, M., F. Zanconato, et al. "The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells." Cell **147**(4): 759-72.
Cancer stem cells (CSCs) are proposed to drive tumor initiation and progression. Yet, our understanding of the cellular and molecular mechanisms that underlie CSC properties is limited. Here we show that the activity of TAZ, a transducer of the Hippo pathway, is required to sustain self-renewal and tumor-initiation capacities in breast CSCs. TAZ protein levels and activity are elevated in prospective CSCs and in poorly differentiated human tumors and have prognostic value. Gain of TAZ endows self-renewal capacity to non-CSCs. In epithelial cells, TAZ forms a complex with the cell-polarity determinant Scribble, and loss of Scribble--or induction of the epithelial-mesenchymal transition (EMT)--disrupts the inhibitory association of TAZ with the core Hippo kinases MST and LATS. This study links the CSC concept to the Hippo pathway in breast cancer and reveals a mechanistic basis of the control of Hippo kinases by cell polarity.

Cui, F., J. Wang, et al. "CD133 is a temporary marker of cancer stem cells in small cell lung cancer, but not in non-small cell lung cancer." Oncol Rep **25**(3): 701-8.
Lung cancer is the most common cause of cancer-related death worldwide. Current investigations in the field of cancer research have intensively focused on the 'cancer stem cell' or 'tumor-initiating cell'. While CD133 was initially considered as a stem cell marker only in the hematopoietic system and the nervous system, the membrane antigen also identifies tumorigenic cells in certain solid tumors. In this study, we investigated the human lung cancer cell lines A549, H157, H226, Calu-1, H292 and H446. The results of real-time PCR analysis after chemotherapy drug selection and the fluorescence-activated cell sorting analysis showed that CD133 only functioned as a marker in the small cell lung cancer line H446. The sorted CD133+ subset presented stem cell-like features, including self-renewal, differentiation, proliferation and tumorigenic capacity in subsequent assays. Furthermore, a proportion of the CD133+ cells had a tendency to remain stable, which may explain the controversies arising from previous studies. Therefore, the CD133+ subset should provide an enriched source of tumor-initiating cells among H446 cells. Moreover, the antigen could be used as an investigative marker of the tumorigenic process and an effective treatment for small cell lung cancer.

D'Andrea, F. P., A. Safwat, et al. "Cancer stem cell overexpression of nicotinamide N-methyltransferase enhances cellular radiation resistance." Radiother Oncol **99**(3): 373-8.
BACKGROUND: Cancer stem cells are thought to be a radioresistant population and may be the seeds for recurrence after radiotherapy. Using tumorigenic clones of retroviral immortalized human mesenchymal stem cell with small differences in their phenotype, we investigated possible genetic expression that could explain cancer stem cell radiation resistance. METHODS: Tumorigenic mesenchymal cancer stem cell clones BB3 and CE8 were irradiated at varying doses and assayed for clonogenic surviving fraction. Altered gene expression before and after 2 Gy was assessed by Affymetric exon chip analysis and further validated with q-RT-PCR using TaqMan probes. RESULTS: The CE8 clone was more radiation resistant than the BB3 clone. From a pool of 15 validated genes with altered expression in the CE8 clone, we found the enzyme nicotinamide N-methyltransferase (NNMT) more than 5-fold upregulated. In-depth pathway analysis found the genes involved in cancer, proliferation, DNA repair and cell death. CONCLUSIONS: The higher radiation resistance in clone CE8 is likely due to NNMT overexpression. The higher levels of NNMT could affect the cellular damage resistance through depletion of the accessible amounts of nicotinamide, which is a known inhibitor of cellular DNA repair mechanisms.

D'Anselmi, F., A. Cucina, et al. "Zebrafish stem cell differentiation stage factors suppress Bcl-xL release and enhance 5-Fu-mediated apoptosis in colon cancer cells." Curr Pharm Biotechnol **12**(2): 261-7.
Stem cell differentiation stage factors (SCDSF), taken from Zebrafish embryos during the stage in which totipotent stem cells are differentiating into pluripotent stem cells, have been shown to inhibit proliferation and induce apoptosis in colon tumors. In order to ascertain if these embryonic factors could synergistically/additively interact with 5-Fluorouracil (5-Fu), whole cell-count, flow-cytometry analysis and apoptotic parameters were recorded in human colon cancer cells (Caco2) treated with Zebrafish stem cell differentiation stage factors (SCDSF 3 microg/ml) in

association or not with 5-Fu in the sub-pharmacological therapeutic range (0.01 mg/ml). Cell proliferation was significantly reduced by SCDSF, meanwhile SCDSF+5-Fu leads to an almost complete growth-inhibition. SCDSF produces a significant apoptotic effect, meanwhile the association with 5-FU leads to an enhanced additive apoptotic rate at both 24 and 72 hrs. SCDSF alone and in association with 5-Fu trigger both the extrinsic and the intrinsic apoptotic pathways, activating caspase-8, -3 and -7. SCDSF and 5-Fu alone exerted opposite effects on Bax and Bcl-xL proteins, meanwhile SCDSF+5-Fu induced an almost complete suppression of Bcl-xL release and a dramatic increase in the Bax/Bcl-xL ratio. These data suggest that zebrafish embryo factors could improve chemotherapy efficacy by reducing anti-apoptotic proteins involved in drug-resistance processes.

Das, U. N. "Essential fatty acids and their metabolites as modulators of stem cell biology with reference to inflammation, cancer, and metastasis." Cancer Metastasis Rev **30**(3-4): 311-24.
Stem cells are pluripotent and expected to be of benefit in the management of coronary heart disease, stroke, diabetes mellitus, cancer, and Alzheimer's disease in which pro-inflammatory cytokines are increased. Identifying endogenous bioactive molecules that have a regulatory role in stem cell survival, proliferation, and differentiation may aid in the use of stem cells in various diseases including cancer. Essential fatty acids form precursors to both pro- and anti-inflammatory molecules have been shown to regulate gene expression, enzyme activity, modulate inflammation and immune response, gluconeogenesis via direct and indirect pathways, function directly as agonists of a number of G protein-coupled receptors, activate phosphatidylinositol 3-kinase/Akt and p44/42 mitogen-activated protein kinases, and stimulate cell proliferation via Ca(2+), phospholipase C/protein kinase, events that are also necessary for stem cell survival, proliferation, and differentiation. Hence, it is likely that bioactive lipids play a significant role in various diseases by modulating the proliferation and differentiation of embryonic stem cells in addition to their capacity to suppress inflammation. Ephrin Bs and reelin, adhesion molecules, and microRNAs regulate neuronal migration and cancer cell metastasis. Polyunsaturated fatty acids and their products seem to modulate the expression of ephrin Bs and reelin and several adhesion molecules and microRNAs suggesting that bioactive lipids participate in neuronal regeneration and stem cell proliferation, migration, and cancer cell metastasis. Thus, there appears to be a close interaction among essential fatty acids, their bioactive products, and inflammation and cancer growth and its metastasis.

Daugaard, G., I. Skoneczna, et al. "A randomized phase III study comparing standard dose BEP with sequential high-dose cisplatin, etoposide, and ifosfamide (VIP) plus stem-cell support in males with poor-prognosis germ-cell cancer. An intergroup study of EORTC, GTCSG, and Grupo Germinal (EORTC 30974)." Ann Oncol **22**(5): 1054-61.
BACKGROUND: To compare the efficacy of one cycle of standard dose cisplatin, etoposide, and ifosfamide (VIP) plus three cycles of high-dose VIP followed by stem-cell infusion [high-dose chemotherapy (HD-CT arm)] to four cycles of standard cisplatin, etoposide, and bleomycin (BEP) in patients with poor-prognosis germ-cell cancer (GCC). Patient and methods: Patients with poor-prognosis GCC were assigned to receive either BEP or VIP followed by HD-CT. To show a 15% improvement in a 1-year failure-free survival (FFS), the study aimed to recruit 222 patients but closed with 137, due to slow accrual. RESULTS: One hundred thirty-one patients were included in this analysis. The complete response rates in the HD-CT and in the BEP arm did not differ: (intention to treat) 44.6% versus 33.3% (P = 0.18). There was no difference in FFS between the two treatment arms (P = 0.057, 66 events). At 2 years, the FFS rate was 44.8% [95% confidence interval (CI) 32.5-56.4] and 58.2%, respectively (95% CI 48.0-71.9); but this 16.3% (standard deviation 7.5%) difference was not statistically significant (P = 0.060). Overall survival did not differ between the two groups (log-rank P > 0.1, 47 deaths). CONCLUSION: This study could not demonstrate that high-dose chemotherapy given as part of first-line therapy improves outcome in patients with poor-prognosis GCC.

Davies, E. J., V. Marsh, et al. "Origin and maintenance of the intestinal cancer stem cell." Mol Carcinog **50**(4): 254-63.
Colorectal cancer is one of the most common cancers in the western world and its incidence is steadily increasing. Understanding the basic biology of both the normal intestine and of intestinal tumorigenesis is vital for developing appropriate and effective cancer therapies. However, relatively little is known about the normal intestinal stem cell or the hypothetical intestinal cancer stem cell, and there is much debate surrounding these areas. This review briefly describes our current understanding of the properties of both the intestinal stem cell and the intestinal cancer stem cell. We also discuss recent theories regarding the origin of the intestinal cancer stem cell, and the signals required for its maintenance and proliferation. Finally, we

place the relevance of cancer stem cell research into context by discussing potential clinical applications of targeting the intestinal cancer stem cell.

de Sousa, E. M. F., S. Colak, et al. "Methylation of cancer-stem-cell-associated Wnt target genes predicts poor prognosis in colorectal cancer patients." Cell Stem Cell **9**(5): 476-85.
Gene signatures derived from cancer stem cells (CSCs) predict tumor recurrence for many forms of cancer. Here, we derived a gene signature for colorectal CSCs defined by high Wnt signaling activity, which in agreement with previous observations predicts poor prognosis. Surprisingly, however, we found that elevated expression of Wnt targets was actually associated with good prognosis, while patient tumors with low expression of Wnt target genes segregated with immature stem cell signatures. We discovered that several Wnt target genes, including ASCL2 and LGR5, become silenced by CpG island methylation during progression of tumorigenesis, and that their re-expression was associated with reduced tumor growth. Taken together, our data show that promoter methylation of Wnt target genes is a strong predictor for recurrence of colorectal cancer, and suggest that CSC gene signatures, rather than reflecting CSC numbers, may reflect differentiation status of the malignant tissue.

Deleyrolle, L. P., G. Ericksson, et al. "Determination of somatic and cancer stem cell self-renewing symmetric division rate using sphere assays." PLoS One **6**(1): e15844.
Representing a renewable source for cell replacement, neural stem cells have received substantial attention in recent years. The neurosphere assay represents a method to detect the presence of neural stem cells, however owing to a deficiency of specific and definitive markers to identify them, their quantification and the rate they expand is still indefinite. Here we propose a mathematical interpretation of the neurosphere assay allowing actual measurement of neural stem cell symmetric division frequency. The algorithm of the modeling demonstrates a direct correlation between the overall cell fold expansion over time measured in the sphere assay and the rate stem cells expand via symmetric division. The model offers a methodology to evaluate specifically the effect of diseases and treatments on neural stem cell activity and function. Not only providing new insights in the evaluation of the kinetic features of neural stem cells, our modeling further contemplates cancer biology as cancer stem-like cells have been suggested to maintain tumor growth as somatic stem cells maintain tissue homeostasis. Indeed, tumor stem cell's resistance to therapy makes these cells a necessary target for effective treatment. The neurosphere assay mathematical model presented here allows the assessment of the rate malignant stem-like cells expand via symmetric division and the evaluation of the effects of therapeutics on the self-renewal and proliferative activity of this clinically relevant population that drive tumor growth and recurrence.

Dewi, D. L., H. Ishii, et al. "Cancer stem cell theory in gastrointestinal malignancies: recent progress and upcoming challenges." J Gastroenterol **46**(10): 1145-57.
A growing body of evidence supports the notion that malignant tumors are heterogeneous and contain diverse subpopulations of cells with unique characteristics including the ability to initiate a tumor and metastasize. This phenomenon might be explained by the so-called cancer stem cell (CSC) theory. Recent technological developments have allowed a deeper understanding and characterization of CSCs. Even though the application of this theory to hematopoietic malignancies and solid tumors holds promise for new ways to treat cancer, it also brings some skepticism. Efficacious therapeutic approaches targeting the CSC population should be explored to overcome therapeutic failure and improve patient outcomes. This review will focus on the intrinsic and extrinsic regulation of CSCs, as well as the development of therapeutic approaches against CSCs, predominantly focusing on gastrointestinal malignancies.

Dodge, M. E. and L. Lum "Drugging the cancer stem cell compartment: lessons learned from the hedgehog and Wnt signal transduction pathways." Annu Rev Pharmacol Toxicol **51**: 289-310.
Cell-cell communication mediated by the secreted Hedgehog (Hh) and Wnt signaling molecules is essential to the coordination of cell fate decision making throughout the metazoan lifespan. From decades of genetically based interrogation, core components constituting the Hh and Wnt signal transduction pathways have been assembled, and a deep appreciation of how these signals elaborate distinct bodily tissues during development has been established. On the other hand, our incapacity to leverage similar genetic approaches to study adult organ systems has limited our understanding of how these molecules promote tissue renewal and regeneration through stem cell regulation. We discuss recent progress in the use of chemically based approaches to achieve control of these pathway activities in a broad range of biological studies and therapeutic contexts. In particular, we discuss the unique experimental opportunities that chemical

modulators of these pathways afford in exploring the cancer stem cell hypothesis.

Dotto, G. P. "Calcineurin signaling as a negative determinant of keratinocyte cancer stem cell potential and carcinogenesis." Cancer Res **71**(6): 2029-33.
Calcineurin is the only known serine-threonine phosphatase under calcium-calmodulin control and key regulator of the immune system. Treatment of patients with calcineurin-inhibitory drugs like cyclosporin A and FK506 to prevent graft rejection dramatically increases the risk of cutaneous squamous cell carcinoma, which is a major cause of death after organ transplants. Recent evidence indicates that suppression of calcineurin signaling, together with its impact on the immune system, exerts direct tumor-promoting effects in keratinocytes, enhancing cancer stem cell potential. The underlying mechanism involves interruption of a double negative regulatory axis, whereby calcineurin and nuclear factors of activated T-cell signaling inhibits expression of ATF3, a negative regulator of p53. The resulting suppression of keratinocyte cancer cell senescence is of likely clinical significance for the many patients under treatment with calcineurin inhibitors and may be of relevance for other cancer types in which altered calcium-calcineurin signaling plays a role.

Dou, J., C. Jiang, et al. "Using ABCG2-molecule-expressing side population cells to identify cancer stem-like cells in a human ovarian cell line." Cell Biol Int **35**(3): 227-34.
CSCs (cancer stem cells) are a small subset of cells within a tumour that possesses the characteristics of stem cells and are considered to be responsible for resistance to chemoradiation. Identification of CSCs through stem cell characteristics might have relevant clinical implications. In this study, SP (side population) cells were sorted from a human ovarian cancer cell line by FACS to determine whether cancer stem cell-like SP cells were present. A very small fraction of SP cells (2.6%) was detected in A2780 cells. SP cells possessed the following characteristics: highly proliferative activity, marked ability for self-renewal in soft agar and culture medium, high expression of ABCG2, drug resistance to vinblastine in vitro, and strong tumourigenic potential in Balb/c nude mice. It is concluded that there exists in the A2780 cell line a small number of SP cells with high expression of ABCG2. The cells have the characteristics of cancer stem-like cells, and identification and cloning of such human SP cells can help in improving therapeutic approaches to ovarian cancer in patients.

Droste, S., A. Herrmann-Frank, et al. "Ethical issues in autologous stem cell transplantation (ASCT) in advanced breast cancer: a systematic literature review." BMC Med Ethics **12**: 6.
BACKGROUND: An effectiveness assessment on ASCT in locally advanced and metastatic breast cancer identified serious ethical issues associated with this intervention. Our objective was to systematically review these aspects by means of a literature analysis. METHODS: We chose the reflexive Socratic approach as the review method using Hofmann's question list, conducted a comprehensive literature search in biomedical, psychological and ethics bibliographic databases and screened the resulting hits in a 2-step selection process. Relevant arguments were assembled from the included articles, and were assessed and assigned to the question list. Hofmann's questions were addressed by synthesizing these arguments. RESULTS: Of the identified 879 documents 102 included arguments related to one or more questions from Hofmann's question list. The most important ethical issues were the implementation of ASCT in clinical practice on the basis of phase-II trials in the 1990s and the publication of falsified data in the first randomized controlled trials (Bezwoda fraud), which caused significant negative effects on recruiting patients for further clinical trials and the doctor-patient relationship. Recent meta-analyses report a marginal effect in prolonging disease-free survival, accompanied by severe harms, including death. ASCT in breast cancer remains a stigmatized technology. Reported health-related-quality-of-life data are often at high risk of bias in favor of the survivors. Furthermore little attention has been paid to those patients who were dying. CONCLUSIONS: The questions were addressed in different degrees of completeness. All arguments were assignable to the questions. The central ethical dimensions of ASCT could be discussed by reviewing the published literature.

Du, Z., R. Qin, et al. "Pancreatic cancer cells resistant to chemoradiotherapy rich in "stem-cell-like" tumor cells." Dig Dis Sci **56**(3): 741-50.
BACKGROUND: Tumor resistance to chemoradiation therapy is partly attributed to the presence of apoptosis-resistant cancer stem cells (CSCs). Chemoradiation therapy can enrich CSCs by killing apoptosis-susceptible cancer cells. AIM: Our preliminary study showed chemoradiation-resistant pancreatic cancer cells to have some CSC characteristics, and to undergo epithelial-mesenchymal transition (EMT); we aimed to verify that study's implication that chemoradiation-resistant subpopulations are enriched with "stem-cell-like" tumor cells, which may be linked to EMT.

METHODS: Four pancreatic cancer cell lines were cultured in gemcitabine with synchronous radiotherapy to obtain resistant subpopulations. Morphological changes were observed under microscope; migration and invasiveness were assessed by Transwell tests. Protein expression was determined by immunoblotting. Pancreatic CSC markers were studied using fluorescence-activated cell sorting analyses. Colony-formation tests, tumor sphere formation assays, and tumor xenografts in BALB/C nude mice were used to evaluate "stemness" in resistant cells. RESULTS: Resistant cells expressed more antiapoptotic protein Bcl-2, apoptosis-inhibitory protein survivin, and stem cell markers Oct4, ABCG2, CD24, and CD133, were more tumorigenic in vitro and in vivo, and showed phenotypic and molecular changes consistent with EMT, including upregulation of vimentin and downregulation of E-cadherin. They were also more invasive and migratory. CONCLUSIONS: We found chemoradiation-resistant pancreatic cancer cells to be similar to CSCs and to undergo EMT, suggesting that chemoradiation resistance-induced EMT is linked to CSC generation.

Dwyer, R. M., J. Ryan, et al. "Mesenchymal Stem Cell-mediated delivery of the sodium iodide symporter supports radionuclide imaging and treatment of breast cancer." Stem Cells **29**(7): 1149-57.
Mesenchymal Stem Cells (MSCs) migrate specifically to tumors in vivo, and coupled with their capacity to bypass immune surveillance, are attractive vehicles for tumor-targeted delivery of therapeutic agents. This study aimed to introduce MSC-mediated expression of the sodium iodide symporter (NIS) for imaging and therapy of breast cancer. Tumor bearing animals received an intravenous or intratumoral injection of NIS expressing MSCs (MSC-NIS), followed by (99m) Technetium pertechnetate imaging 3-14 days later using a BazookaSPECT gamma-camera. Tissue was harvested for analysis of human NIS (hNIS) expression by relative quantitative-polymerase chain reaction. Therapy animals received an i.p. injection of (131) I or saline 14 days after injection of MSC-NIS, and tumor volume was monitored for 8 weeks. After injection of MSC-NIS, BazookaSPECT imaging revealed an image of animal intestines and chest area at day 3, along with a visible weak tumor image. By day 14, the tumor was visible with a significant reduction in radionuclide accumulation in nontarget tissue observed. hNIS gene expression was detected in the intestines, heart, lungs, and tumors at early time points but later depleted in nontarget tissues and persisted at the tumor site. Based on imaging/biodistribution data, animals received a therapeutic dose of (131) I 14 days after MSC-NIS injection. This resulted in a significant reduction in tumor growth (mean +/- SEM, 236 +/- 62 mm(3) vs. 665 +/- 204 mm(3) in controls). The ability to track MSC migration and transgene expression noninvasively in real time before therapy is a major advantage to this strategy. This promising data supports the feasibility of this approach as a novel therapy for breast cancer.

Eguiara, A., O. Holgado, et al. "Xenografts in zebrafish embryos as a rapid functional assay for breast cancer stem-like cell identification." Cell Cycle **10**(21): 3751-7.
The cancer stem cell is defined by its capacity to self-renew, the potential to differentiate into all cells of the tumor and the ability to proliferate and drive the expansion of the tumor. Thus, targeting these cells may provide novel anti-cancer treatment strategies. Breast cancer stem cells have been isolated according to surface marker expression, ability to efflux fluorescent dyes, increased activity of aldehyde dehydrogenase or the capacity to form spheres in non-adherent culture conditions. In order to test novel drugs directed towards modulating self-renewal of cancer stem cells, rapid, easy and inexpensive assays must be developed. Using 2 days-post-fertilization (dpf) zebrafish embryos as transplant recipients, we show that cells grown in mammospheres from breast carcinoma cell lines migrate to the tail of the embryo and form masses with a significantly higher frequency than parental monolayer populations. When stem-like self-renewal was targeted in the parental population by the use of the dietary supplement curcumin, cell migration and mass formation were reduced, indicating that these effects were associated with stem-like cell content. This is a proof of principle report that proposes a rapid and inexpensive assay to target in vivo cancer stem-like cells, which may be used to unravel basic cancer stem cell biology and for drug screening.

Fan, H. and J. L. Guan "Compensatory function of Pyk2 protein in the promotion of focal adhesion kinase (FAK)-null mammary cancer stem cell tumorigenicity and metastatic activity." J Biol Chem **286**(21): 18573-82.
Mammary cancer stem cells (MaCSCs) have been identified as a rare population of cells capable of self-renewal to drive mammary tumorigenesis and metastasis. Nevertheless, relatively little is known about the intracellular signaling pathways regulating self-renewal and metastatic activities of MaCSCs in vivo. Using a recently developed breast cancer mouse model with focal adhesion kinase (FAK) deletion in mammary tumor cells (MFCKO-MT mice), here we present evidence suggesting a compensatory function

of Pyk2, a FAK-related kinase, in the regulation of MaCSCs and metastasis in these mice. Increased expression of Pyk2 was found selectively in pulmonary metastatic nodules of MFCKO-MT mice, and its inhibition significantly reduced mammary tumor development and metastasis in these mice. Consistent with the idea of metastasis driven by MaCSCs, we detected selective up-regulation of Pyk2 in MaCSCs, but not bulk mammary tumor cells, of primary tumors developed in MFCKO-MT mice. We further showed that inhibition of Pyk2 in FAK-null MaCSCs significantly decreased their tumorsphere formation and migration in vitro as well as self-renewal, tumorigenicity, and metastatic activity in vivo. Last, we identified PI3K/Akt signaling as a major mediator of FAK regulation of MaCSCs as well as a target for the compensatory function of Pyk2 in FAK-null MaCSCs. Together, these results further advance our understanding of FAK and its related tyrosine kinase Pyk2 in regulation of MaCSCs in breast cancer and suggest that pharmaceutically targeting these kinases may hold promise as a novel treatment for the disease by targeting and eradicating MaCSCs.

Fang, X., Y. Cai, et al. "Twist2 contributes to breast cancer progression by promoting an epithelial-mesenchymal transition and cancer stem-like cell self-renewal." Oncogene **30**(47): 4707-20.
The epithelial to mesenchymal transition (EMT) is a highly conserved cellular programme that has an important role in normal embryogenesis and in cancer invasion and metastasis. We report here that Twist2, a tissue-specific basic helix-loop-helix transcription factor, is overexpressed in human breast cancers and lymph node metastases. In mammary epithelial cells and breast cancer cells, ectopic overexpression of Twist2 results in morphological transformation, downregulation of epithelial markers and upregulation of mesenchymal markers. Moreover, Twist2 enhances the cell migration and colony-forming abilities of mammary epithelial cells and breast cancer cells in vitro and promotes tumour growth in vivo. Ectopic expression of Twist2 in mammary epithelial cells and breast cancer cells increases the size and number of their CD44(high)/CD24(low) stem-like cell sub-populations, promotes the expression of stem cell markers and enhances the self-renewal capabilities of stem-like cells. In addition, exogenous expression of Twist2 leads to constitutive activation of STAT3 (signal transducer and activator of transcription 3) and downregulation of E-cadherin. Thus, the overexpression of Twist2 may contribute to breast cancer progression by activating the EMT programme and enhancing the self-renewal of cancer stem-like cells.

Farabaugh, S. M., D. S. Micalizzi, et al. "Eya2 is required to mediate the pro-metastatic functions of Six1 via the induction of TGF-beta signaling, epithelial-mesenchymal transition, and cancer stem cell properties." Oncogene **31**(5): 552-62.
Six1 is a critical regulator of embryonic development that requires interaction with the Eya family of proteins (Eya1-4) to activate the transcription of genes involved in neurogenesis, myogenesis and nephrogenesis. Although expression of Six1 and Eya family members is predominantly observed in development, their overexpression is observed in numerous cancers. Importantly, both Six1 and Eya have independently been shown to mediate breast cancer metastasis, but whether they functionally interact during tumor progression has not been explored. Herein, we demonstrate that knockdown of Eya2 in MCF7 mammary carcinoma cells reverses the ability of Six1 to induce transforming growth factor-beta signaling, as well as to induce characteristics associated with epithelial-mesenchymal transition and cancer stem cells, suggesting that Six1 is dependent on Eya2 to mediate numerous pro-metastatic characteristics. The importance of the Six1-Eya interaction in human breast cancer is underscored by the finding that high levels of Six1 correlate with shortened time to relapse and metastasis as well as decreased survival only when co-expressed with high levels of Eya2. Overall, these data implicate Eya2 as a necessary co-factor for many of the metastasis promoting functions of Six1, suggesting that targeting the Six1-Eya interaction may inhibit breast cancer progression. As Six1 and Eya2 are not highly expressed in most adult tissues, the Six1-Eya interaction may be a valuable future therapeutic target whose inhibition would be expected to impair breast cancer progression while conferring limited side effects.

Felthaus, O., T. Ettl, et al. "Cancer stem cell-like cells from a single cell of oral squamous carcinoma cell lines." Biochem Biophys Res Commun **407**(1): 28-33.
Resistance of oral squamous cell carcinomas (OSCC) to conventional chemotherapy or radiation therapy might be due to cancer stem cells (CSCs). The development of novel anticancer drugs requires a simple method for the enrichment of CSCs. CSCs can be enriched from OSCC cell lines, for example, after cultivation in serum-free cell culture medium (SFM). In our study, we analyzed four OSCC cell lines for the presence of CSCs. CSC-like cells could not be enriched with SFM. However, cell lines obtained from holoclone colonies showed CSC-like properties such as a reduced rate of cell proliferation and a reduced sensitivity to Paclitaxel in comparison to cells from

the parental lineage. Moreover, these cell lines differentially expressed the CSC-marker CD133, which is also upregulated in OSCC tissues. Interestingly, CD133(+) cells in OSCC tissues expressed little to no Ki67, the cell proliferation marker that also indicates reduced drug sensitivity. Our study shows a method for the isolation of CSC-like cell lines from OSCC cell lines. These CSC-like cell lines could be new targets for the development of anticancer drugs under in vitro conditions.

Ferletta, M., J. Grawe, et al. "Canine mammary tumors contain cancer stem-like cells and form spheroids with an embryonic stem cell signature." Int J Dev Biol **55**(7-9): 791-9.
We have investigated the presence of tentative stem-like cells in the canine mammary tumor cell line CMT-U229. This cell line is established from an atypical benign mixed mammary tumor, which has the property of forming duct-like structures in collagen gels. Stem cells in mammary glands are located in the epithelium; therefore we thought that the CMT-U229 cell line would be suitable for detection of tentative cancer stem-like cells. Side population (SP) analyses by flow cytometry were performed with cells that formed spheroids and with cells that did not. Flow cytometric, single sorted cells were expanded and re-cultured as spheroids. The spheroids were paraffin embedded and characterized by immunohistochemistry. SP analyses showed that spheroid forming cells (retenate) as well as single cells (filtrate) contained SP cells. Sca1 positive cells were single cell sorted and thereafter the SP population increased with repeated SP analyses. The SP cells were positively labeled with the cell surface-markers CD44 and CD49f (integrin alpha6); however the expression of CD24 was low or negative. The spheroids expressed the transcription factor and stem cell marker Sox2, as well as Oct4. Interestingly, only peripheral cells of the spheroids and single cells were positive for Oct4 expression. SP cells are suggested to correspond to stem cells and in this study, we have enriched for tentative tumor stem-like cells derived from a canine mammary tumor. All the used markers indicate that the studied CMT-U229 cell line contains SP cells, which in particular have cancer stem-like cell characteristics.

Gadalla, S. E., A. Alexandraki, et al. "Uncoupling of the ERalpha regulated morphological phenotype from the cancer stem cell phenotype in human breast cancer cell lines." Biochem Biophys Res Commun **405**(4): 581-7.
The CD24(low/-)CD44(+)EpCAM(+) phenotype is associated with breast cancer initiating cells. To investigate if these putative breast cancer stem cell markers are regulated by estrogen receptor alpha (ERalpha) we have determined the expression levels of EpCAM, CD44 and CD24 in several well characterized breast cancer cell lines. The expression levels of the three adhesion proteins were quantitatively different in the cell lines but the composite CD24(low/-)CD44(+)EpCAM(+) breast cancer stem cell phenotype was shown to exist as a small fraction, between 0.1% and 1.2%, in all breast cancer cell lines tested. Experimental silencing of ERalpha resulted in a reduced epithelial appearance and partial reduction of CD24 mRNA, while levels of CD44 and EpCAM were unaltered. Moreover, knockdown of ERalpha led to a change in the morphology of the cells similar to the epithelial to mesenchymal transition phenotype and was associated with decreased E-cadherin expression. Our findings offer new insights into the regulation of the breast cancer stem cell phenotype by ERalpha and suggest that treatments targeting the breast cancer stem cell adhesion molecules and the ERalpha pathway may be complementary.

Garcia Campelo, M. R., G. Alonso Curbera, et al. "Stem cell and lung cancer development: blaming the Wnt, Hh and Notch signalling pathway." Clin Transl Oncol **13**(2): 77-83.
Primary lung cancer may arise from the central (bronchial) or peripheral (bronchiolo-alveolar) compartments. However the origins of the different histological types of primary lung cancer are not well understood. Stem cells are believed to be crucial players in tumour development and there is much interest in identifying those compartments that harbour stem cells involved in lung cancer. Although the role of stem cells in carcinogenesis is not well characterised, emerging evidence is providing new insights into this process. Numerous studies have indicated that lung cancer is not a result of a sudden transforming event but a multistep process in which a sequence of molecular changes result in genetic and morphological aberrations. The exact sequence of molecular events involved in lung carcinogenesis is not yet well understood, therefore deeper knowledge of the aberrant stem cell fate signalling pathway could be crucial in the development of new drugs against the advanced setting.

Gavert, N., A. Vivanti, et al. "L1-mediated colon cancer cell metastasis does not require changes in EMT and cancer stem cell markers." Mol Cancer Res **9**(1): 14-24.
Aberrant activation of Wnt/beta-catenin signaling is common in most sporadic and inherited colorectal cancer (CRC) cells leading to elevated beta-catenin/TCF transactivation. We previously identified

the neural cell adhesion molecule L1 as a target gene of beta-catenin/TCF in CRC cells. Forced expression of L1 confers increased cell motility, invasion, and tumorigenesis, and the induction of human CRC cell metastasis to the liver. In human CRC tissue, L1 is exclusively localized at the invasive front of such tumors in a subpopulation of cells displaying nuclear beta-catenin. We determined whether L1 expression confers metastatic capacities by inducing an epithelial to mesenchymal transition (EMT) and whether L1 cosegregates with cancer stem cell (CSC) markers. We found that changes in L1 levels do not affect the organization or expression of E-cadherin in cell lines, or in invading CRC tissue cells, and no changes in other epithelial or mesenchymal markers were detected after L1 transfection. The introduction of major EMT regulators (Slug and Twist) into CRC cell lines reduced the levels of E-cadherin and induced fibronectin and vimentin, but unlike L1, Slug and Twist expression was insufficient for conferring metastasis. In CRC cells L1 did not specifically cosegregate with CSC markers including CD133, CD44, and EpCAM. L1-mediated metastasis required NF-kappaB signaling in cells harboring either high or low levels of endogenous E-cadherin. The results suggest that L1-mediated metastasis of CRC cells does not require changes in EMT and CSC markers and operates by activating NF-kappabeta signaling.

Gerger, A., W. Zhang, et al. "Common cancer stem cell gene variants predict colon cancer recurrence." Clin Cancer Res **17**(21): 6934-43.
PURPOSE: Recent evidence suggests that cancer stem cells (CSC) are responsible for key elements of colon cancer progression and recurrence. Germline variants in CSC genes may result in altered gene function and/or activity, thereby causing interindividual differences in a patient's tumor recurrence capacity and chemoresistance. We investigated germline polymorphisms in a comprehensive panel of CSC genes to predict time to tumor recurrence (TTR) in patients with stage III and high-risk stage II colon cancer. EXPERIMENTAL DESIGN: A total of 234 patients treated with 5-fluorouracil-based chemotherapy at the University of Southern California were included in this study. Whole blood samples were analyzed for germline polymorphisms in genes that have been previously associated with colon CSC (CD44, Prominin-1, DPP4, EpCAM, ALCAM, Msi-1, ITGB1, CD24, LGR5, and ALDH1A1) by PCR-RFLP or direct DNA-sequencing. RESULTS: The minor alleles of CD44 rs8193 C>T, ALCAM rs1157 G>A, and LGR5 rs17109924 T>C were significantly associated with increased TTR (9.4 vs. 5.4 years; HR, 0.51; 95% CI: 0.35-0.93; P = 0.022; 11.3 vs. 5.7 years; HR, 0.56; 95% CI: 0.33-0.94; P = 0.024, and 10.7 vs. 5.7 years; HR, 0.33; 95% CI: 0.12-0.90; P = 0.023, respectively) and remained significant in the multivariate analysis stratified by ethnicity. In recursive partitioning, a specific gene variant profile including LGR5 rs17109924, CD44 rs8193, and ALDH1A1 rs1342024 represented a high-risk subgroup with a median TTR of 1.7 years (HR, 6.71, 95% CI: 2.71-16.63, P < 0.001). CONCLUSION: This is the first study identifying common germline variants in colon CSC genes as independent prognostic markers for stage III and high-risk stage II colon cancer patients.

Ghani, F. I., H. Yamazaki, et al. "Identification of cancer stem cell markers in human malignant mesothelioma cells." Biochem Biophys Res Commun **404**(2): 735-42.
Malignant mesothelioma (MM) is an aggressive and therapy-resistant neoplasm arising from the pleural mesothelial cells and usually associated with long-term asbestos exposure. Recent studies suggest that tumors contain cancer stem cells (CSCs) and their stem cell characteristics are thought to confer therapy-resistance. However, whether MM cell has any stem cell characteristics is not known. To understand the molecular basis of MM, we first performed serial transplantation of surgical samples into NOD/SCID mice and established new cell lines. Next, we performed marker analysis of the MM cell lines and found that many of them contain SP cells and expressed several putative CSC markers such as CD9, CD24, and CD26. Interestingly, expression of CD26 closely correlated with that of CD24 in some cases. Sorting and culture assay revealed that SP and CD24(+) cells proliferated by asymmetric cell division-like manner. In addition, CD9(+) and CD24(+) cells have higher potential to generate spheroid colony than negative cells in the stem cell medium. Moreover, these marker-positive cells have clear tendency to generate larger tumors in mouse transplantation assay. Taken together, our data suggest that SP, CD9, CD24, and CD26 are CSC markers of MM and could be used as novel therapeutic targets.

Gisina, A. M., A. Y. Lupatov, et al. "Detection of minor subpopulations of colorectal adenocarcinoma cells expressing cancer stem cell markers." Bull Exp Biol Med **151**(2): 234-8.
The expression of puitative surface molecular markers of cancer stem cells on human colorectal adenocarcinoma cells was analyzed by flow cytofluorometry. Cell subpopulations expressing markers of epithelial and malignant cells and stem cell markers were identified. Four minor subpopulations with CD24(+)/CD133(+), CD44(+)/CD133(+), CD90(+)/CD71(+), or CD90(+)/CD24(+) phenotypes

meeting this requirement were detected; presumably, those were cancer stem cell subpopulations. These results extend our knowledge on heterogeneity of human colorectal adenocarcinoma cell population and outline new trends of research of cancer stem cell phenotype in these tumors.

Goidts, V., J. Bageritz, et al. "RNAi screening in glioma stem-like cells identifies PFKFB4 as a key molecule important for cancer cell survival." Oncogene **31**(27): 3235-43.
The concept of cancer stem-like cells (CSCs) has gained considerable attention in various solid tumors including glioblastoma, the most common primary brain tumor. This sub-population of tumor cells has been intensively investigated and their role in therapy resistance as well as tumor recurrence has been demonstrated. In that respect, development of therapeutic strategies that target CSCs (and possibly also the tumor bulk) appears a promising approach in patients suffering from primary brain tumors. In the present study, we utilized RNA interference (RNAi) to screen the complete human kinome and phosphatome (682 and 180 targets, respectively) in order to identify genes and pathways relevant for the survival of brain CSCs and thereby potential therapeutical targets for glioblastoma. We report of 46 putative candidates including known survival-related kinases and phosphatases. Interestingly, a number of genes identified are involved in metabolism, especially glycolysis, such as PDK1 and PKM2 and, most prominently PFKFB4. In vitro studies confirmed an essential role of PFKFB4 in the maintenance of brain CSCs. Furthermore, high PFKFB4 expression was associated with shorter survival of primary glioblastoma patients. Our findings support the importance of the glycolytic pathway in the maintenance of malignant glioma cells and brain CSCs and imply tumor metabolism as a promising therapeutic target in glioblastoma.

Grulke, N., C. Albani, et al. "Quality of life in patients before and after haematopoietic stem cell transplantation measured with the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Core Questionnaire QLQ-C30." Bone Marrow Transplant **47**(4): 473-82.
The EORTC Quality of Life Core Questionnaire QLQ-C30 is widely used, but no reference values are available for patients receiving HSCT. We retrieved data for 38 samples from 33 papers in English and German that provided evaluable information on QLQ-C30 scores (mean, s.d.) covering about 2800 patients. Results are presented as a table that provides reference data that allow QLQ-C30 scores at different points during the disease trajectory to be put in context. With respect to their central tendency and their variance, scores vary over time. Quality of life is lowest during inpatient time. About 1 year after HSCT, the pre-transplant level is reached. Physical functioning is the scale reaching the highest level of all scales. Fatigue, dyspnoea and insomnia are symptoms that remain at an elevated level and should thus be considered as persisting problems after HSCT. For the interpretation of differences between scores, a very conservative recommendation would be to set the s.d. at 30 points. Doing so, one could be quite sure of having found a clinically significant change if the difference of two scores exceeds 15 points. Differences below 5 points should be interpreted with caution.

Gu, W., E. Yeo, et al. "Silencing oncogene expression in cervical cancer stem-like cells inhibits their cell growth and self-renewal ability." Cancer Gene Ther **18**(12): 897-905.
Accumulating evidence supports the concept that cancer stem cells (CSCs) are responsible for tumor initiation and maintenance. They are also considered as an attractive target for advanced cancer therapy. Using a sphere culture method that favors the growth of self-renewal cells, we have isolated sphere-forming cells (SFCs) from cervical cancer cell lines HeLa and SiHa. HeLa-SFCs were resistant to multiple chemotherapeutic drugs and were more tumorigenic, as evidenced by the growth of tumors following injection of immunodeficient mice with 1 x 10(4) cells, compared with 1 x 10(6) parental HeLa cells required to grow tumors of similar size in the same time frame. These cells showed an expression pattern of CD44(high)/CD24(low) that resembles the CSC surface biomarker of breast cancer. We further demonstrated that HeLa-SFCs expressed a higher level (6.9-fold) of the human papillomavirus oncogene E6, compared with that of parental HeLa cells. Gene silencing of E6 with a lentiviral-short-hairpin RNA (shRNA) profoundly inhibited HeLa-SFC sphere formation and cell growth. The inhibition of cell growth was even greater than that for sphere formation after E6 silence, suggesting that the loss of self-renewing ability may be more important. We then measured the expression of self-renewal genes, transformation growth factor-beta (TGF-beta) and leukemia-inhibitory factor (LIF), in shRNA-transduced HeLa-SFCs and found that expression of all three TGF-beta isoforms was significantly downregulated while LIF remained unchanged. Expression of the Ras gene (a downstream component of TGF-beta) was also markedly decreased, suggesting that the growth-inhibitory effect could be via the TGF-beta pathway. The above data indicate RNA interference-based therapy may offer a new approach for CSC-targeted cancer therapy.

Gunn, E. J., J. T. Williams, et al. "The natural products parthenolide and andrographolide exhibit anti-cancer stem cell activity in multiple myeloma." Leuk Lymphoma **52**(6): 1085-97.
Multiple myeloma (MM) is an incurable plasma cell malignancy where nearly all patients succumb to a relapse. The current preclinical models of MM target the plasma cells, constituting the bulk of the tumor, leaving the cancer stem cells to trigger a relapse. Utilizing a three-dimensional tissue culture system where cells were grown in extracellular matrix designed to reconstruct human bone marrow, we tested the anti-multiple myeloma cancer stem cell (MM-CSC) potential of two natural product inhibitors of nuclear factor kappaB (NFkappaB). Here we show that parthenolide and andrographolide are potent anti-MM-CSC agents. Both natural products demonstrated preferential toxicity toward MM-CSCs over non-tumorigenic MM cells. Addition of the bone marrow stromal compartment abrogated andrographolide activity while having no effect on parthenolide cytoxicity. This is the first report of a natural product with anti-CSC activity in myeloma, suggesting that it has the potential to improve the survival of patients with MM by eliminating the relapse-causing MM-CSCs.

Hagiwara, S., M. Kudo, et al. "The cancer stem cell marker CD133 is a predictor of the effectiveness of S1+ pegylated interferon alpha-2b therapy against advanced hepatocellular carcinoma." J Gastroenterol **46**(2): 212-21.
BACKGROUND: Combination therapy with the oral fluoropyrimidine anticancer drug S1 and interferon is reportedly effective for the treatment of advanced hepatocellular carcinoma (HCC), but selection criteria for this therapy have not been clarified. In this study, we attempted to identify factors predicting the effectiveness of this combination therapy. METHODS: Pathological specimens of HCC were collected before treatment from 31 patients with advanced HCC who underwent S1+ pegylated-interferon (PEG-IFN) alpha-2b therapy between January 2007 and January 2009. In these pathological specimens, the expression levels of CD133, thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and interferon-receptor 2 (IFNR2) proteins were determined by Western blot assay. The presence or absence of p53 gene mutations was determined by direct sequencing. The relationships between these protein expression levels and the response rate (RR), progression-free survival (PFS), and overall survival (OS) were evaluated. RESULTS: The CD133 protein expression level was significantly lower in the responder group than in the nonresponder group. Comparing the PFS and OS between high- and low-level CD133 expression groups (n = 13 and 18, respectively) revealed that both parameters were significantly prolonged in the latter group. The expression levels of TS, DPD, and IFNR2 protein and the presence of p53 gene mutations did not correlate with the RR. CONCLUSIONS: CD133 was identified as a predictor of the therapeutic effect of S1+ PEG-IFN alpha-2b therapy against advanced HCC.

Halpern, J. L., A. Kilbarger, et al. "Mesenchymal stem cells promote mammary cancer cell migration in vitro via the CXCR2 receptor." Cancer Lett **308**(1): 91-9.
Bone metastasis is a common event during breast cancer progression. Recently, mesenchymal stem cells (MSCs) have been implicated in the metastasis of primary mammary cancer. Given that bone is the native environment for MSCs, we hypothesized MSCs facilitate the homing of circulating mammary cancer cells to the bone. To test this hypothesis, we examined in vitro whether bone derived MSCs from FVB mice could influence the migration of syngeneic murine mammary cancer cell lines derived from the polyoma virus middle-T (PyMT) model of mammary gland tumorigenesis. Our data show that conditioned media derived from MSCs significantly enhanced the migration of PyMT mammary cancer cell lines. Analysis of conditioned media using a cytokine array revealed the presence of numerous cytokines in the MSC conditioned media, most notably, the murine orthologs of CXCL1 and CXCL5 that are cognate ligands of the CXCR2 receptor. Further investigation identified that: (1) CXCL1, CXCL5 and CXCR2 mRNA and protein were expressed by the MSCs and PyMT cell lines and; (2) neutralizing antibodies to CXCL1, CXCL5 and CXCR2 or a CXCR2 small molecule inhibitor (SB265610) significantly abrogated the migratory effect of the MSC conditioned media on the PyMT cells. Therefore, in vitro evidence demonstrates that bone derived MSCs play a role in the migration of mammary cancer cells, a conclusion that has potential implications for breast to bone metastasis in vivo.

Han, M., M. Liu, et al. "Re-expression of miR-21 contributes to migration and invasion by inducing epithelial-mesenchymal transition consistent with cancer stem cell characteristics in MCF-7 cells." Mol Cell Biochem **363**(1-2): 427-36.
MiR-21 is known to play an important role in the development and progression, including migration and invasion, of many malignancies including breast cancer. Accumulating evidence suggest that the induction of epithelial-mesenchymal transition (EMT) phenotype and acquisition of cancer stem cell (CSC)

characteristics are highly interrelated, and contribute to tumorigenesis, tumor progression, metastasis, and relapse. The molecular mechanisms underlying EMT and CSC characteristics during miR-21 contributes to cell migration and invasion of breast cancer are poorly understood. Therefore, we established miR-21 re-expressing breast cancer MCF-7 (MCF-7/miR-21) cells, which showed increasing cell growth, migration and invasion, self-renewal and clonogenicity. Our data showed that re-expression of miR-21 induced the acquisition of EMT phenotype by activation of mesenchymal cell markers (N-cadherin, Vimentin, alpha-SMA) and inhibition of epithelial cell marker (E-cadherin) in MCF-7/miR-21 cells, which consistent with increased cell subpopulation expressing CSC surface markers (ALDH1(+) and CD44(+)/CD24(-/low)) and the capacity of sphereforming (mammospheres). Our results demonstrated that re-expression of miR-21 is responsible for migration and invasion by activating the EMT process and enhancing the characteristics of CSCs in MCF-7 cells.

Han, M. E., T. Y. Jeon, et al. "Cancer spheres from gastric cancer patients provide an ideal model system for cancer stem cell research." Cell Mol Life Sci **68**(21): 3589-605.
Cancer stem cells have been hypothesized to drive the growth and metastasis of tumors. Because they need to be targeted for cancer treatment, they have been isolated from many solid cancers. However, cancer stem cells from primary human gastric cancer tissues have not been isolated as yet. For the isolation, we used two cell surface markers: the epithelial cell adhesion molecule (EpCAM) and CD44. When analyzed by flow cytometry, the EpCAM(+)/CD44(+) population accounts for 4.5% of tumor cells. EpCAM(+)/CD44(+) gastric cancer cells formed tumors in immunocompromised mice; however, EpCAM(-)/CD44(-), EpCAM(+)/CD44(-) and EpCAM(-)/CD44(+) cells failed to do so. Xenografts of EpCAM(+)/CD44(+) gastric cancer cells maintained a differentiated phenotype and reproduced the morphological and phenotypical heterogeneity of the original gastric tumor tissues. The tumorigenic subpopulation was serially passaged for several generations without significant phenotypic alterations. Moreover, EpCAM(+)/CD44(+), but not EpCAM(-)/CD44(-), EpCAM(+)/CD44(-) or EpCAM(-)/CD44(+) cells grew exponentially in vitro as cancer spheres in serum-free medium, maintaining the tumorigenicity. Interestingly, a single cancer stem cell generated a cancer sphere that contained various differentiated cells, supporting multi-potency and self-renewal of a cancer stem cell. EpCAM(+)/CD44(+) cells had greater resistance to anti-cancer drugs than other subpopulation cells. The above in vivo and in vitro results suggest that cancer stem cells, which are enriched in the EpCAM(+)/CD44(+) subpopulation of gastric cancer cells, provide an ideal model system for cancer stem cell research.

Haugnes, H. S., A. Laurell, et al. "High-dose chemotherapy with autologous stem cell support in patients with metastatic non-seminomatous testicular cancer - a report from the Swedish Norwegian Testicular Cancer Group (SWENOTECA)." Acta Oncol **51**(2): 168-76.
BACKGROUND: The SWENOTECA IV protocol from 1995 is a prospective population-based study in metastatic non-seminomatous germ cell testicular cancer (NSGCT), designed for early identification of patients with poor response to standard cisplatin-based chemotherapy. A slow tumor marker decline (HCG T((1/2)) > 3 days, AFP T((1/2)) > 7 days) after BEP or BEP plus ifosfamide was regarded as poor response. The aim of this study was to present survival and toxicity data for patients treated with high-dose chemotherapy (HDCT) within the SWENOTECA IV cancer care program. MATERIAL AND METHODS: In total 882 adult men diagnosed with metastatic NSGCT between July 1995 and June 2007 in Sweden and Norway (except one center) were included in SWENOTECA IV and treated accordingly. Among these, 55 men (6.2%) were treated with HDCT according to three different indications in the protocol: A) poor response to standard-dose intensified chemotherapy (BEP plus ifosfamide); B) vital cancer at surgery after intensified chemotherapy; and C) selected relapses after previous chemotherapy. In situation A and C two HDCT cycles and in situation B one HDCT cycle was recommended. Situation A was the reason for HDCT in 36 patients, B in seven and C in 12 patients. The first HDCT cycle consisted of carboplatin 28 x (GFR + 25) mg, cyclofosfamide 6000 mg/m(2) and etoposide 1750 mg/m(2), administered over four days. In cycle two, etoposide was replaced by tiotepa 480 mg/m(2). RESULTS: After a median follow-up of 7.5 years, overall survival was 72%, 100% and 58%, while failure-free survival was 64%, 71% and 42% in situation A, B and C, respectively. Three patients (5.5%) died during HDCT (renal failure or intracerebral hemorrhage). Nephrotoxicity was the most common non-hematological grade 4 toxicity (n = 5, 9%). CONCLUSION: The population-based SWENOTECA strategy, selecting patients who do not respond adequately to primary standard-dose chemotherapy for immediate treatment intensification with HDCT, is feasible and might be advantageous.

Hayashi, S., K. Fujita, et al. "Isolation and identification of cancer stem cells from a side

population of a human hepatoblastoma cell line, HuH-6 clone-5." Pediatr Surg Int **27**(1): 9-16.
PURPOSE: It has been thought that the persistence of even a small number of tumor cells in the body may increase each tumor cell in a similar manner and may allow the disease to proceed. However, only a few percent of such tumor cells exist in cancerous tissue. They are called "cancer stem cells (CSCs)". If an alternative method of annihilating CSCs is found, it will greatly deter relapse and metastasis. We attempted to identify and separate CSCs in hepatoblastoma aiming to develop a new therapy for hepatoblastoma. METHODS: The side population (SP) method was used as an indicator when extracting the CSC candidate group from the hepatoblastoma cells. The SP cells and non-SP cells were studied for tumourigenesis. RESULTS: Although tumors were formed when SP fraction cells were inoculated into mice, tumor formation was not observed in non-SP cells. SP cells had higher tumor formation ability compared to non-SP cells. CONCLUSION: Cancer stem-like cells were separated by the SP fraction method from hepatoblastoma cells. The in vivo experiment proved that SP fraction cells inoculated into mice were self-replicated, and the existence of cancer stem-like cells was identified.

Heerma van Voss, M. R., P. van der Groep, et al. "Expression of the stem cell marker ALDH1 in BRCA1 related breast cancer." Cell Oncol (Dordr) **34**(1): 3-10.
INTRODUCTION: The BRCA1 protein makes mammary stem cells differentiate into mature luminal and myoepithelial cells. If a BRCA1 mutation results in a differentiation block, an enlarged stem cell component might be present in the benign tissue of BRCA1 mutation carriers, and these mammary stem cells could be the origin of BRCA1 related breast cancer. Since ALDH1 is a marker of both mammary stem cells and breast cancer stem cells, we compared ALDH1 expression in malignant tissue of BRCA1 mutation carriers to non-carriers. METHODS: Forty-one BRCA1 related breast cancers and 41 age-matched sporadic breast cancers were immunohistochemically stained for ALDH1. Expression in epithelium and stroma was scored and compared. RESULTS: Epithelial (P = 0.001) and peritumoral (P = 0.001) ALDH1 expression was significantly higher in invasive BRCA1 related carcinomas compared to sporadic carcinomas. Intratumoral stromal ALDH1 expression was similarly high in both groups. ALDH1 tumor cell expression was an independent predictor of BRCA1 mutation status. CONCLUSION: BRCA1 related breast cancers showed significantly more frequent epithelial ALDH1 expression, indicating that these hereditary tumors have an enlarged cancer stem cell component. Besides, (peritumoral) stromal ALDH1 expression was also more frequent in BRCA1 mutation carriers. ALDH1 may therefore be a diagnostic marker and a therapeutic target of BRCA1 related breast cancer.

Hellsten, R., M. Johansson, et al. "Galiellalactone inhibits stem cell-like ALDH-positive prostate cancer cells." PLoS One **6**(7): e22118.
Galiellalactone is a potent and specific inhibitor of STAT3 signaling which has been shown to possess growth inhibitory effects on prostate cancer cells expressing active STAT3. In this study we aimed to investigate the effect of galiellalactone on prostate cancer stem cell-like cells. We explored the expression of aldehyde dehydrogenase (ALDH) as a marker for cancer stem cell-like cells in different human prostate cancer cell lines and the effects of galiellalactone on ALDH expressing (ALDH+) prostate cancer cells. ALDH+ subpopulations were detected and isolated from the human prostate cancer cell lines DU145 and long-term IL-6 stimulated LNCaP cells using ALDEFLUOR(R) assay and flow cytometry. In contrast to ALDH- cells, ALDH+ prostate cancer cells showed cancer stem cell-like characteristics such as increased self-renewing and colony forming capacity and tumorigenicity. In addition, ALDH+ cells showed an increased expression of putative prostate cancer stem cell markers (CD44 and integrin alpha2beta1). Furthermore, ALDH+ cells expressed phosphorylated STAT3. Galiellalactone treatment decreased the proportion of ALDH+ prostate cancer cells and induced apoptosis of ALDH+ cells. The gene expression of ALDH1A1 was downregulated in vivo in galiellalactone treated DU145 xenografts. These findings emphasize that targeting the STAT3 pathway in prostate cancer cells, including prostate cancer stem cell-like cells, is a promising therapeutic approach and that galiellalactone is an interesting compound for the development of future prostate cancer drugs.

Herpel, E., K. Jensen, et al. "The cancer stem cell antigens CD133, BCRP1/ABCG2 and CD117/c-KIT are not associated with prognosis in resected early-stage non-small cell lung cancer." Anticancer Res **31**(12): 4491-500.
BACKGROUND: In various tumor entities, expression of cancer stem cell (CSC) antigens has been proven to be prognostically unfavorable. However, for lung cancer, the data are scant and conflicting. PATIENTS AND METHODS: The CSC antigens CD117/c-KIT, CD133 and breast cancer resistance protein-1 (BCRP1/ABCG2) were immunohistochemically analyzed in tissues from a

total of 133 completely resected stage I/II non-small cell lung cancer (NSCLC) patients with a median follow-up time of 53.8 months. Their expression was related to clinicopathological characteristics, angiogenic features and prognosis. RESULTS: Cox proportional hazards regression analysis revealed no association between CSC antigens, disease-free survival or overall survival (OS). However, in the subgroup of patients with relapse and tumors >3 cm, there was a trend towards worse OS upon expression of CD117 (hazard ratio=2.6, 95%, confidence interval=0.8-8.3, p=0.080). Except for CD133, which was overrepresented in T1 tumors (p=0.001), the CSC antigens were not linked to clinico-pathological characteristics or angiogenic features. CONCLUSION: In resected early-stage NSCLC, CSC antigens show no association with prognosis. However, in patients with relapse and tumors >3 cm, expression of CD117 might predict worse OS.

Hiraga, T., S. Ito, et al. "Side population in MDA-MB-231 human breast cancer cells exhibits cancer stem cell-like properties without higher bone-metastatic potential." Oncol Rep **25**(1): 289-96.
An increasing body of evidence suggests that cancers contain a small subset of their own stem-like cells called cancer stem cells (CSCs), which play critical roles in the initiation, maintenance and relapse of tumors. However, the role of CSCs in cancer metastasis, especially in metastasis to bone, has not been extensively studied. Side population (SP) has been shown to enrich CSCs in several types of cancer, including breast cancer. In the present study, we characterized the SP cells isolated from the human breast cancer cell line MDA-MB-231 in comparison to non-SP (NSP). Fluorescence-activated cell sorter analysis demonstrated the existence of SP in MDA-MB-231 cells, which was markedly reduced in the presence of fumitremorgin C, a specific inhibitor of ATP-binding cassette sub-family G member 2 (ABCG2). Quantitative RT-PCR analysis showed that ABCG2 mRNA expression was significantly higher in SP cells than in NSP cells. SP cells formed increased numbers of tumor-spheres in suspension culture. Furthermore, the tumor growth in the orthotopic mammary fat pad in nude mice was significantly accelerated in SP cells. On the other hand, the development of bone metastases determined by intracardiac injection into nude mice showed no difference between SP and NSP cells. SP abundance in the tumor cells isolated from the bone metastases was not increased either compared with that from the mammary tumors. These results suggest that the SP in MDA-MB-231 cells possesses some of the CSC-like properties but does not have higher metastatic potential to bone.

Hiroishi, K., M. Inomata, et al. "Cancer stem cell-related factors are associated with the efficacy of pre-operative chemoradiotherapy for locally advanced rectal cancer." Exp Ther Med **2**(3): 465-470.
Pre-operative chemoradiotherapy (CRT) is an important neoadjuvant therapy for locally advanced rectal cancer. In the present study, we investigated the factors that influence the efficacy of pre-operative CRT in locally advanced rectal cancer. We divided 50 patients with locally advanced rectal carcinoma treated with pre-operative CRT into two groups according to the grade of tumor response to pre-operative CRT: low-sensitivity group and high-sensitivity group. As candidates for the prediction of sensitivity to pre-operative CRT, clinicopathological factors and 12 biomarkers, including factors related to tumor growth, cell cycle, apoptosis, tumor stroma and cancer stem cells, were examined immunohistochemically in 48 resected specimens. Thirty-one tumors showed high sensitivity and 19 showed low sensitivity to pre-operative CRT. The status of stem cell-related factors, CD133 and CD24, was significantly associated respectively with sensitivity to pre-operative CRT (P=0.003, P=0.029). In 10 tumors positive for both CD133 and CD24, low sensitivity to CRT was found in 9 (90%), whereas in 16 tumors negative for both CD133 and CD24, low sensitivity was found in 3 (19%). Other pathological parameters were not associated with tumor response to pre-operative CRT. In conclusion, overexpression of cancer stem cell-related factors, CD133 and CD24, is associated with the sensitivity of locally advanced rectal cancer to pre-operative CRT.

Ho Sui, S. J., K. Begley, et al. "The Stem Cell Discovery Engine: an integrated repository and analysis system for cancer stem cell comparisons." Nucleic Acids Res **40**(Database issue): D984-91.
Mounting evidence suggests that malignant tumors are initiated and maintained by a subpopulation of cancerous cells with biological properties similar to those of normal stem cells. However, descriptions of stem-like gene and pathway signatures in cancers are inconsistent across experimental systems. Driven by a need to improve our understanding of molecular processes that are common and unique across cancer stem cells (CSCs), we have developed the Stem Cell Discovery Engine (SCDE)-an online database of curated CSC experiments coupled to the Galaxy analytical framework. The SCDE allows users to consistently describe, share and compare CSC data at the gene and pathway level. Our initial focus has been on carefully curating tissue and cancer stem cell-related experiments from blood, intestine and brain to create a high quality resource containing 53 public

studies and 1098 assays. The experimental information is captured and stored in the multi-omics Investigation/Study/Assay (ISA-Tab) format and can be queried in the data repository. A linked Galaxy framework provides a comprehensive, flexible environment populated with novel tools for gene list comparisons against molecular signatures in GeneSigDB and MSigDB, curated experiments in the SCDE and pathways in WikiPathways. The SCDE is available at http://discovery.hsci.harvard.edu.

Hu, G., F. Li, et al. "Intrinsic gemcitabine resistance in a novel pancreatic cancer cell line is associated with cancer stem cell-like phenotype." Int J Oncol **40**(3): 798-806.
Pancreatic ductal adenocarcinoma (PDA) remains one of the most lethal malignancies in the world, often diagnosed at an advanced stage, resistant to conventional chemotherapy and having high invasive and metastatic potential. The mechanism of drug resistance of PDA is still not clear. In the present study, we established two novel pancreatic cancer cell lines PAXC-002 and PAXC-003 from human primary xenograft models. In this study, we present two novel pancreatic cancer cell lines which could be used for gemcitabine resistance investigation, mechanism identification of pancreatic cancer and anticancer drug screening. The preliminary data indicate that the drug resistance of pancreatic carcinoma cells is associated with a cancer stem cell-like phenotype.

Hu, W., J. Wang, et al. "Human umbilical blood mononuclear cell-derived mesenchymal stem cells serve as interleukin-21 gene delivery vehicles for epithelial ovarian cancer therapy in nude mice." Biotechnol Appl Biochem **58**(6): 397-404.
Ovarian cancer causes more deaths than any other cancer of the female reproductive system, and its overall cure rate remains low. The present study investigated human umbilical blood mononuclear cell (UBMC)-derived mesenchymal stem cells (UBMC-MSCs) as interleukin-21 (IL-21) gene delivery vehicles for ovarian cancer therapy in nude mice. MSCs were isolated from UBMCs and the expanded cells were phenotyped by flow cytometry. Cultured UBMCs were differentiated into osteocytes and adipocytes using appropriate media and then the UBMC-MSCs were transfected with recombinant pIRES2-IL-21-enhancement green fluorescent protein. UBMC-MSCs expressing IL-21 were named as UBMC-MSC-IL-21. Mice with A2780 ovarian cancer were treated with UBMC-MSC-IL-21 intravenously, and the therapeutic efficacy was evaluated by the tumor volume and mouse survival. To address the mechanism of UBMC-MSC-IL-21 against ovarian cancer, the expression of IL-21, natural killer glucoprotein 2 domain and major histocompatibility complex class I chain-related molecules A/B were detected in UBMC-MSC-IL-21 and in the tumor sites. Interferon-gamma-secreting splenocyte numbers and natural killer cytotoxicity were significantly increased in the UBMC-MSC-IL-21-treated mice as compared with the UBMC-MSCs or the UBMC-MSC-mock plasmid-treated mice. Most notably, tumor growth was delayed and survival was prolonged in ovarian-cancer-bearing mice treated with UBMC-MSC-IL-21. Our data provide important evidence that UBMC-MSCs can serve as vehicles for IL-21 gene delivery and inhibit the established tumor.

Huang, Y., B. Agrawal, et al. "Evaluation of cancer stem cell migration using compartmentalizing microfluidic devices and live cell imaging." J Vis Exp(58): e3297.
In the last 40 years, the United States invested over 200 billion dollars on cancer research, resulting in only a 5% decrease in death rate. A major obstacle for improving patient outcomes is the poor understanding of mechanisms underlying cellular migration associated with aggressive cancer cell invasion, metastasis and therapeutic resistance. Glioblastoma Multiforme (GBM), the most prevalent primary malignant adult brain tumor, exemplifies this difficulty. These PDMS-made devices cast the tissue culture environment into three connected compartments: seeding chamber, receiving chamber and bridging microchannels. We tailored the device such that both chambers hold sufficient media to support viable BTSC for 4-5 days without media exchange. Highly mobile BTSCs initially introduced into the seeding chamber are isolated after migration though bridging microchannels to the parallel receiving chamber. This migration simulates cancer cellular spread through the interstitial spaces of the brain. The phase live images of cell morphology during migration are recorded over several days. Highly migratory BTSC can therefore be isolated, recultured, and analyzed further. Compartmentalizing microfluidics can be a versatile platform to study the migratory behavior of BTSCs and other cancer stem cells. By combining gradient generators, fluid handling, micro-electrodes and other microfluidic modules, these devices can also be used for drug screening and disease diagnosis. Isolation of an aggressive subpopulation of migratory cells will enable studies of underlying molecular mechanisms.

Huang, Y., B. Agrawal, et al. "Microfluidics-based devices: New tools for studying cancer and cancer stem cell migration." Biomicrofluidics **5**(1): 13412.
Cell movement is highly sensitive to stimuli from the extracellular matrix and media. Receptors on the

plasma membrane in cells can activate signal transduction pathways that change the mechanical behavior of a cell by reorganizing motion-related organelles. Cancer cells change their migration mechanisms in response to different environments more robustly than noncancer cells. Therefore, therapeutic approaches to immobilize cancer cells via inhibition of the related signal transduction pathways rely on a better understanding of cell migration mechanisms. In recent years, engineers have been working with biologists to apply microfluidics technology to study cell migration. As opposed to conventional cultures on dishes, microfluidics deals with the manipulation of fluids that are geometrically constrained to a submillimeter scale. Such small scales offer a number of advantages including cost effectiveness, low consumption of reagents, high sensitivity, high spatiotemporal resolution, and laminar flow. Therefore, microfluidics has a potential as a new platform to study cell migration. In this review, we summarized recent progress on the application of microfluidics in cancer and other cell migration researches. These studies have enhanced our understanding of cell migration and cancer invasion as well as their responses to subtle variations in their microenvironment. We hope that this review will serve as an interdisciplinary guidance for both biologists and engineers as they further develop the microfluidic toolbox toward applications in cancer research.

Hussein, D., W. Punjaruk, et al. "Pediatric brain tumor cancer stem cells: cell cycle dynamics, DNA repair, and etoposide extrusion." Neuro Oncol **13**(1): 70-83.
Reliable model systems are needed to elucidate the role cancer stem cells (CSCs) play in pediatric brain tumor drug resistance. The majority of studies to date have focused on clinically distinct adult tumors and restricted tumor types. Here, the CSC component of 7 newly established primary pediatric cell lines (2 ependymomas, 2 medulloblastomas, 2 gliomas, and a CNS primitive neuroectodermal tumor) was thoroughly characterized. Comparison of DNA copy number with the original corresponding tumor demonstrated that genomic changes present in the original tumor, typical of that particular tumor type, were retained in culture. In each case, the CSC component was approximately 3-4-fold enriched in neurosphere culture compared with monolayer culture, and a higher capacity for multilineage differentiation was observed for neurosphere-derived cells. DNA content profiles of neurosphere-derived cells expressing the CSC marker nestin demonstrated the presence of cells in all phases of the cell cycle, indicating that not all CSCs are quiescent. Furthermore, neurosphere-derived cells demonstrated an increased resistance to etoposide compared with monolayer-derived cells, having lower initial DNA damage, potentially due to a combination of increased drug extrusion by ATP-binding cassette multidrug transporters and enhanced rates of DNA repair. Finally, orthotopic xenograft models reflecting the tumor of origin were established from these cell lines. In summary, these cell lines and the approach taken provide a robust model system that can be used to develop our understanding of the biology of CSCs in pediatric brain tumors and other cancer types and to preclinically test therapeutic agents.

Jalmsell, L., E. Onelov, et al. "Hematopoietic stem cell transplantation in children with cancer and the risk of long-term psychological morbidity in the bereaved parents." Bone Marrow Transplant **46**(8): 1063-70.
We have investigated whether hematopoietic stem cell transplantation (HSCT) before the death of children with cancer has a long-term effect on the physical and psychological well-being of the parents. A nationwide questionnaire was sent out to all bereaved parents in Sweden who had lost a child due to a malignancy from 1992 to 1997. Self-reported levels of anxiety, depression and quality of life as well as overall psychological and physical well-being in bereaved parents of children who underwent HSCT were compared with bereaved parents whose children did not receive a transplant. The risks of these consequences were further augmented in case of multiple HSCT. We suggest that bereaved parents of children undergoing HSCT may be at greater risk of decreased psychological well-being than other bereaved parents of children with cancer.

Jeter, C. R., B. Liu, et al. "NANOG promotes cancer stem cell characteristics and prostate cancer resistance to androgen deprivation." Oncogene **30**(36): 3833-45.
Cancer cell molecular mimicry of stem cells (SC) imbues neoplastic cells with enhanced proliferative and renewal capacities. In support, numerous mediators of SC self-renewal have been evinced to show oncogenic potential. We have recently reported that short-hairpin RNA-mediated knockdown of the embryonic stem cell (ESC) self-renewal gene NANOG significantly reduced the clonogenic and tumorigenic capabilities of various cancer cells. In this study, we sought to test the potential pro-tumorigenic functions of NANOG, particularly, in prostate cancer (PCa). Using qRT-PCR, we first confirmed that PCa cells expressed NANOG mRNA primarily from the NANOGP8 locus on chromosome 15q14. We then constructed a lentiviral promoter reporter in which the -3.8-kb NANOGP8 genomic fragment was used to drive the expression of green fluorescence protein

(GFP). We observed that NANOGP8-GFP(+) PCa cells showed cancer stem cell (CSC) characteristics such as enhanced clonal growth and tumor regenerative capacity. To further investigate the functions and mechanisms of NANOG in tumorigenesis, we established tetracycline-inducible NANOG-overexpressing cancer cell lines, including both PCa (Du145 and LNCaP) and breast (MCF-7) cancer cells. NANOG induction promoted drug resistance in MCF-7 cells, tumor regeneration in Du145 cells and, most importantly, castration-resistant tumor development in LNCaP cells. These pro-tumorigenic effects of NANOG were associated with key molecular changes, including an upregulation of molecules such as CXCR4, IGFBP5, CD133 and ALDH1. The present gain-of-function studies, coupled with our recent loss-of-function work, establish the integral role for NANOG in neoplastic processes and shed light on its mechanisms of action.

Jewett, A. and H. C. Tseng "Tumor induced inactivation of natural killer cell cytotoxic function; implication in growth, expansion and differentiation of cancer stem cells." J Cancer **2**: 443-57.
Accumulated evidence indicates that cytotoxic function of immune effectors is largely suppressed in the tumor microenvironment by a number of distinct effectors and their secreted factors. Total population of monocytes and those depleted of CD16(+) subsets were able to substantially prevent NK cell mediated lysis of OSCSCs, MSCs and DPSCs. Taken together, our results suggest that stem cells are significant targets of the NK cell cytotoxicity. The concept of split anergy in NK cells and its contribution to tissue repair and regeneration and in tumor resistance and progression will be discussed in this review.

Joshua, B., M. J. Kaplan, et al. "Frequency of cells expressing CD44, a head and neck cancer stem cell marker: correlation with tumor aggressiveness." Head Neck **34**(1): 42-9.
BACKGROUND: We previously identified by flow cytometry a Lineage-CD44+ (Lin-CD44+) subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma (HNSCC). We now correlate clinical and histologic factors with Lin-CD44+ cell frequency. METHODS: The study included 31 patients with HNSCC, of whom 87% had stage IV disease. The frequency of Lin-CD44+ cells and the success of xenografting patient tumors in mice were correlated with clinical and pathologic data. RESULTS: The mean frequency of Lin-CD44+ cells was 25% (0.4%-81%). It was 36% in patients who had recurrence versus 15% for those without recurrence (p = .04). Successful xenograft implantation occurred in 53%. Seventy-five percent of patients with successful xenografts had recurrence versus 21% of patients with unsuccessful xenografts (p = .003). CONCLUSIONS: Successful xenograft implantation and a high frequency of Lin-CD44+ cells correlate with known poor prognostic factors such as advanced T classification and recurrence. These findings may support the stem cell concept in HNSCC.

Joung, J. Y., Y. S. Lee, et al. "Haplotype analysis of prostate stem cell antigen and association with prostate cancer risk." J Urol **185**(6): 2112-8.
PURPOSE: Prostate stem cell antigen has become a promising target as a potential biomarker for prostate cancer, but to our knowledge there are no reports of a genetic variation of the PSCA gene associated with prostate cancer risk. We determined the potential association between specific variations of the PSCA gene and prostate cancer in Korean men. MATERIALS AND METHODS: In this hospital based, case-control study 194 patients newly diagnosed with histologically confirmed prostate cancer were enrolled. Visitors for cancer screening served as healthy controls. We genotyped 12 PSCA gene single nucleotide polymorphisms in 194 cases and 169 healthy controls. RESULTS: Men with the rs1045531 AA genotype were at higher risk for prostate cancer than those with the CC genotype. Individuals with the CCCAGGTACGG haplotype were at significantly increased risk for prostate cancer. When considering clinical factors, rs3736001, which is a nonsynonymous cDNA single nucleotide polymorphism (Glu39Lys), showed an association with prostate specific antigen 10 ng/ml or greater and prostate cancer risk. CONCLUSIONS: Men with the rs1045531 AA genotype of PSCA were at higher risk for prostate cancer. On haplotype analysis CCCAGGTACGG and CGA haplotype carriers showed a significant association with prostate cancer risk. To our knowledge this is the first report of PSCA genetic variation associated with prostate cancer risk.

Kaneko, K. "Characterization of stem cells and cancer cells on the basis of gene expression profile stability, plasticity, and robustness: dynamical systems theory of gene expressions under cell-cell interaction explains mutational robustness of differentiated cells and suggests how cancer cells emerge." Bioessays **33**(6): 403-13.
Here I present and discuss a model that, among other things, appears able to describe the dynamics of cancer cell origin from the perspective of stable and unstable gene expression profiles. In identifying such aberrant gene expression profiles as lying outside the normal stable states attracted through development and normal cell differentiation, the hypothesis

explains why cancer cells accumulate mutations, to which they are not robust, and why these mutations create a new stable state far from the normal gene expression profile space. Such cells are in strong contrast with normal cell types that appeared as an attractor state in the gene expression dynamical system under cell-cell interaction and achieved robustness to noise through evolution, which in turn also conferred robustness to mutation. In complex gene regulation networks, other aberrant cellular states lacking such high robustness are expected to remain, which would correspond to cancer cells.

Kawasaki, Y., Y. Omori, et al. "Cytoplasmic accumulation of connexin32 expands cancer stem cell population in human HuH7 hepatoma cells by enhancing its self-renewal." Int J Cancer **128**(1): 51-62.

Although the connexin32 (Cx32)-mediated gap junction is abolished in hepatocellular carcinoma (HCC), the expression of cytoplasmic Cx32 tends to increase in correspondence with the grade of malignancy. Establishing a Tet-off expression system in human nonmetastatic HuH7 HCC cells where cytoplasmic Cx32 was overexpressed by doxycycline (Dox) withdrawal, we previously demonstrated that overexpression of cytoplasmic Cx32 made HuH7 cells metastatic in mice. In our study, hypothesizing that the cytoplasmic Cx32-induced metastasis may involve expansion of the cancer stem cell (CSC) population, we examined whether cytoplasmic Cx32 controlled the size of the side population (SP) in HuH7 Tet-off Cx32 cells. Fluorescence-activated cell sorting revealed that SP was expanded in a Dox-free medium compared with a Dox-supplemented one. Although cytoplasmic Cx32 did not block maturation from SP to non-SP, purified SP reconstituted a larger SP fraction in the Dox-free medium than in the Dox-supplemented one. Furthermore, although SP from HuH7 Tet-off mock cells formed a similar number of CSC spheres of a similar size whether with or without Dox, SP from HuH7 Tet-off Cx32 cells developed a greater number of larger CSC spheres in the Dox-free medium than in the Dox-supplemented one. Taken together, these results suggest that accumulation of cytoplasmic Cx32 should enhance self-renewal of CSC to expand the CSC population in HCC.

Kim, B. S., K. S. Kang, et al. "Knockdown of the potential cancer stem-like cell marker Rex-1 improves chemotherapeutic effects in gliomas." Hum Gene Ther **22**(12): 1551-62.

In the present study, we show that Rex-1 mRNA and protein are found at high levels in both 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)-resistant glioma cell subpopulations and malignant glioblastoma multiforme (GBM) tissue. We used a combination therapy of small interfering RNA (siRNA) against Rex-1 (siRex-1) and BCNU to target GBM cells. Rex-1 siRNA/BCNU treatment resulted in growth inhibition and a diminished S phase. The treatment efficiently induced P38/JNK and Akt/PI3K/GSK3beta signaling and led to apoptosis both in vitro and in vivo. We also show that Rex-1/ABCG2 (ATP binding cassette transporter G2)-coexpressing subpopulations were chemoresistant; however, BCNU was not a substrate for ABCG2. siRex-1 treatment led to cell death in GBM subpopulations by promoting apoptosis. Moreover, siRex-1/BCNU combination therapy targeted both the major population and cancer stem cell-like subpopulations. Our findings are important for the development of clinical applications to treat GBM.

Kim, J., J. Jung, et al. "Cancer stem-like cells persist in established cell lines through autocrine activation of EGFR signaling." Oncol Lett **3**(3): 607-612.

Recent studies have shown that a small subpopulation of stem-like cancer cells within most solid tumors are responsible for the malignancy of aggressive cancer cells, including tumorigenicity and relapse of solid tumors. These tumor cells may be enriched and maintained in vitro in the presence of growth factors (GFs), including epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), without serum in almost all solid tumor cell lines. In the present study, however, we found that the enrichment of cancer stem-like cells (CSCs) from established cell lines using sphere cultures was achieved efficiently without GFs. Enhanced sphere-forming activity was detected in GF-free cultures compared with GF-containing cultures in MCF7, A549 and U87 cells. Analysis of conditioned media (CM) demonstrated an elevated level of EGF secretion in GF-free CM compared with GF-containing CM in MCF7 cells. By contrast, cells in GF-free CM exhibited almost no secretion of bFGF, whereas cells in GF-containing CM secreted a high level of bFGF. The addition of EGF enhanced sphere formation, whereas bFGF suppressed sphere formation under GF-free conditions in MCF7 cells. Conversely, the addition of bFGF suppressed sphere formation in these cells. Notably, bFGF markedly suppressed EGF receptor (EGFR) expression and EGF secretion in MCF7 and A549 cells. Consistent with this result, EGFR blockade with pharmacological inhibitors significantly suppressed sphere formation in MCF7 and A549 cells under GF-free conditions. Furthermore, the neutralization of EGF also inhibited sphere formation, whereas bFGF neutralization still enhanced sphere formation under these conditions. Together, CSCs may be maintained in a serum-free culture condition without GFs,

possibly through autocrine secretion of GFs such as EGF, and the addition of bFGF may not be sufficient for the enrichment of stem-like cancer cells.

Kim, J. and S. H. Orkin "Embryonic stem cell-specific signatures in cancer: insights into genomic regulatory networks and implications for medicine." Genome Med **3**(11): 75.
Embryonic stem (ES) cells are of great interest as a model system for studying early developmental processes and because of their potential therapeutic applications in regenerative medicine. Obtaining a systematic understanding of the mechanisms that control the 'stemness' - self-renewal and pluripotency - of ES cells relies on high-throughput tools to define gene expression and regulatory networks at the genome level. Such recently developed systems biology approaches have revealed highly interconnected networks in which multiple regulatory factors act in combination. Interestingly, stem cells and cancer cells share some properties, notably self-renewal and a block in differentiation. Recently, several groups reported that expression signatures that are specific to ES cells are also found in many human cancers and in mouse cancer models, suggesting that these shared features might inform new approaches for cancer therapy. Here, we briefly summarize the key transcriptional regulators that contribute to the pluripotency of ES cells, the factors that account for the common gene expression patterns of ES and cancer cells, and the implications of these observations for future clinical applications.

Kim, M. O., S. H. Kim, et al. "Embryonic stem-cell-preconditioned microenvironment induces loss of cancer cell properties in human melanoma cells." Pigment Cell Melanoma Res **24**(5): 922-31.
The cancer microenvironment affects cancer cell proliferation and growth. Embryonic stem (ES)-preconditioned 3-dimensional (3-D) culture of cancer cells induces cancer cell reprogramming and results in a change in cancer cell properties such as differentiation and migration in skin melanoma. However, the mechanism has not yet been clarified. Using the ES-preconditioned 3-D microenvironment model, we provide evidence showing that the ES microenvironment inhibits proliferation and anchorage-independent growth of SK-MEL-28 melanoma cells. We also found that the ES microenvironment suppresses telomerase activity and thereby induces senescence in SK-MEL-28 cells. Furthermore, we observed that gremlin, an antagonist of BMP4, is secreted from ES cells and plays an important role in cellular senescence. Knocking down gremlin in the ES microenvironment increases proliferation and anchorage-independent growth of SK-MEL-28 melanoma cells. Taken together, these results demonstrated that gremlin is a crucial factor responsible for abrogating melanoma properties in the ES-preconditioned 3-D microenvironment.

Kleist, B., L. Xu, et al. "Expression of the adult intestinal stem cell marker Lgr5 in the metastatic cascade of colorectal cancer." Int J Clin Exp Pathol **4**(4): 327-35.
The recently advanced cancer stem cell model postulates that progression and metastasis of cancer are mainly driven by tumor cells with stem cell properties. Intestinal cancer stem cells are difficult to study due to the lack of reliable markers, but expression of the Wnt target gene Lgr5 is promising to define at least stem cell like cells in intestinal and colorectal cancer. The aim of this study was to find a possible link of stem cell like cancer cells to the metastatic process of colorectal cancer. To this end, we evaluated immunohistochemical Lgr5 expression in 31 distant metastases and in primary tumor compartments with relevance for metastasizing, comprising 89 colorectal carcinomas. Lgr5 expression was seen in 51.6% of distant metastases. 12.9%, 14.8% and 26.7% of primary tumors with histologically confirmed tumor buds, angioinvasion and perineural infiltrates, respectively, showed evidence of Lgr5 expression in these tumor compartments. However, distant metastases, which were derived from carcinomas with such Lgr5 positive tumor compartments, showed 6- to 11.5-fold higher median value of Lgr5 expression compared to those metastases derived from tumors without Lgr5 expressing cells in these compartments. These differences between the metastases were statistically significant, if being related to tumor buds (all tumors; $p = 0.047$) and to vascular infiltrates (stage IV tumors; $p = 0.007$). In conclusion, our results point to rare evidence of Lgr5 positive stem cell like cells in the metastatic cascade of colorectal cancer, but these few cells might be biologically powerful in the metastatic process of cancer subsets. Clonal analysis is necessary to proof this hypothesis.

Knoop, K., M. Kolokythas, et al. "Image-guided, tumor stroma-targeted 131I therapy of hepatocellular cancer after systemic mesenchymal stem cell-mediated NIS gene delivery." Mol Ther **19**(9): 1704-13.
Due to its dual role as reporter and therapy gene, the sodium iodide symporter (NIS) allows noninvasive imaging of functional NIS expression by (123)I-scintigraphy or (124)I-PET imaging before the application of a therapeutic dose of (131)I. NIS expression provides a novel mechanism for the evaluation of mesenchymal stem cells (MSCs) as gene

delivery vehicles for tumor therapy. In the current study, we stably transfected bone marrow-derived CD34(-) MSCs with NIS cDNA (NIS-MSC), which revealed high levels of functional NIS protein expression. In mixed populations of NIS-MSCs and hepatocellular cancer (HCC) cells, clonogenic assays showed a 55% reduction of HCC cell survival after (131)I application. We then investigated body distribution of NIS-MSCs by (123)I-scintigraphy and (124)I-PET imaging following intravenous (i.v.) injection of NIS-MSCs in a HCC xenograft mouse model demonstrating active MSC recruitment into the tumor stroma which was confirmed by immunohistochemistry and ex vivo gamma-counter analysis. Three cycles of systemic MSC-mediated NIS gene delivery followed by (131)I application resulted in a significant delay in tumor growth. Our results demonstrate tumor-specific accumulation and therapeutic efficacy of radioiodine after MSC-mediated NIS gene delivery in HCC tumors, opening the prospect of NIS-mediated radionuclide therapy of metastatic cancer using MSCs as gene delivery vehicles.

Kokko, L. L., S. Hurme, et al. "Significance of site-specific prognosis of cancer stem cell marker CD44 in head and neck squamous-cell carcinoma." Oral Oncol **47**(6): 510-6.
In several recent studies, CD44 expression has been associated with aggressive behavior in cancers of different types. CD44 expression is also linked to cancer stem cells, which have been shown to play a significant role in tumor progression and poor prognosis in head and neck squamous cell carcinoma (HNSCC), as well as in other cancers. Although CD44 is a potential prognostic marker, it has not been adopted to wider clinical use as a part of treatment planning in HNSCC patients. The aim of this research was to study whether CD44 overexpression is associated with 5year overall survival in HNSCC. We also studied site-specific associations between increased CD44 expression and 5year overall survival. Associations between relative tumor CD44 expressions and smoking, heavy alcohol consumption, histological grade of cancer, TNM staging and HNSCC staging were also studied. In total, 135 paraffin-embedded blocks from HNSCC patients were stained immunohistochemically with a CD44 antibody and were classified by the anatomic location of the tumor. CD44 overexpression had statistically significant association with decreased 5year survival rates when all HNSCC samples were studied ($p<0.001$). Significant association between intense CD44 expression and poor 5year survival rates was found in the patients with SCC of the oro- and hypopharynx ($p<0.001$) and the larynx ($p=0.042$). In patients suffering from HNSCC in the oral cavity, CD44 overexpression did not have a significant effect on overall 5year survival rates. Heavy smoking of over 10 pack years had a significant association with tumor CD44 overexpression ($p=0.009$). Only pharyngeal ($p=0.046$) and laryngeal ($p=0.047$) SCC, but not oral-cavity SCC, had statistically significant associations between heavy smoking and CD44 overexpression when HNSCC was studied in regional groups. Alcohol consumption and tumor grade did not have a significant association with the tumor's CD44 expression. Our results suggest that CD44 overexpression could be used as a sign of aggressiveness, in addition to the HNSCC staging, as a prognostic factor in pharyngeal and laryngeal HNSCC and to assist in treatment selection.

Kondo, S., N. Wakisaka, et al. "Epstein-Barr virus latent membrane protein 1 induces cancer stem/progenitor-like cells in nasopharyngeal epithelial cell lines." J Virol **85**(21): 11255-64.
Recent studies suggest the existence of cancer stem cells (CSC) and cancer progenitor cells (CPC), although strict definitions of neither CSC nor CPC have been developed. We have produced evidence that the principal oncoprotein of Epstein-Barr virus (EBV), latent membrane protein 1 (LMP1), which is associated with human malignancies, especially nasopharyngeal carcinoma (NPC), promotes tumor cell invasion and metastasis, as well as the epithelial-mesenchymal transition (EMT). However, whether LMP1 is involved in the development of CSC/CPC is still unclear. This study investigates whether the expression of EBV-LMP1 is related to the development of CSC/CPC. Analysis of cancer stem cell markers reveals that LMP1 induces the CD44(high) CD24(low) CSC/CPC-like phenotype as well as self-renewal abilities in LMP1-expressing epithelial cell lines. In addition, we show here that LMP1 induction in epithelial cells causes high tumorigenicity and rapid cellular proliferation. Furthermore, we found that LMP1 expression increased the expression of several CPC markers as well as producing increased levels of EMT markers. Our findings indicate that LMP1 can induce a CPC-like rather than a CSC-like phenotype in epithelial cells and suggest that LMP1-induced phenotypic changes contribute to the development of NPC.

Kuranda, K., C. Berthon, et al. "Expression of CD34 in hematopoietic cancer cell lines reflects tightly regulated stem/progenitor-like state." J Cell Biochem **112**(5): 1277-85.
Hematopoietic cancer stem cells preserve cellular hierarchy in a manner similar to normal stem cells, yet

the underlying regulatory mechanisms are poorly understood. It is known that both normal and malignant stem/progenitor cells express CD34. Here, we demonstrate that several cell lines (HL-60, U266) derived from hematopoietic malignancies contain not only CD34(-) but also CD34(+) subpopulations. The CD34(+) cells displayed a stem/progenitor-like phenotype since, in contrast to CD34(-) cells, they frequently underwent cellular division and rapidly formed colonies in methylcellulose-based medium. Strikingly, a constant fraction of the CD34(+) and CD34(-) cell subpopulations, when separated, rapidly switched their phenotype. Consequently, both separated fractions could generate tumors in immunocompromised NOD/LtSz-scid/scid mice. Cultures in vitro showed that the proportion of CD34(+) stem/progenitor-like cells in the population was decreased by cell-cell contact and increased by soluble factors secreted by the cells. Using cytokine arrays, we identified some of these factors, notably thymopoietin that was able to increase the proportion of CD34(+) cells and overall colony-forming capacity in tested cell lines. This action of thymopoietin was conserved in mononuclear cells from bone marrow. Therefore, we propose that hematopoietic cancer cell lines containing subpopulations of CD34(+) cells can provide an in vitro model for studies of cancer stem/progenitor cells.

Leal, J. A., A. Feliciano, et al. "Stem cell microRNAs in senescence and immortalization: novel players in cancer therapy." Med Res Rev **33**(1): 112-38.
The molecular etiology of malignancy remains one of the most challenging disease processes under scientific investigation; therefore, improved approaches for their treatment are urgently needed. MicroRNAs are highly conserved nonprotein-coding RNAs that regulate gene expression. They are involved in important homeostatic processes, such as cellular proliferation, cell death and development, and affect many diseases, including cancer. High-throughput screenings based on microRNAs related to senescence/immortalization are potential tools for identifying novel proliferative microRNAs that might be involved in carcinogenesis. Recently, a subgroup of highly proliferative microRNAs, which belong to a cluster expressed exclusively in embryonic stem cells and their malignant derivatives (embryonic carcinoma cells), was revealed to play a role in senescence bypass, thereby providing immortalization to human cells. This finding supports the cancer stem cell theory and the relevance of microRNAs in human tumors. This article recapitulates the role of microRNAs that are associated with stem cell properties and their possible link in common pathways related to immortalization and cancer. Ultimately, cancer therapy that is based on the induction of a senescence response is proposed to be highly associated with the loss of stemness properties. Thus, it would be possible to "kill two birds with one stone": along with the inhibition of stemness properties in cancer stem cells, the senescence response could be induced to destroy the cancer stem cell population within a tumor.

Ledur, P. F., E. S. Villodre, et al. "Extracellular ATP reduces tumor sphere growth and cancer stem cell population in glioblastoma cells." Purinergic Signal **8**(1): 39-48.
Glioblastoma is the most aggressive tumor in the CNS and is characterized by having a cancer stem cell (CSC) subpopulation essential for tumor survival. The purinergic system plays an important role in glioma growth, since adenosine triphosphate (ATP) can induce proliferation of glioma cells, and alteration in extracellular ATP degradation by the use of exogenous nucleotidases dramatically alters the size of gliomas in rats. The aim of this work was to characterize the effect of the purinergic system on glioma CSCs. Human U87 glioma cultures presented tumor spheres that express the markers of glioma cancer stem cells CD133, Oct-4, and Nanog. Messenger RNA of several purinergic receptors were differently expressed in spheres when compared to a cell monolayer not containing spheres. Treatment of human gliomas U87 or U343 as well as rat C6 gliomas with 100 muM of ATP reduced the number of tumor spheres when grown in neural stem cell medium supplemented with epidermal growth factor and basic fibroblast growth factor. Moreover, ATP caused a decline in the number of spheres observed in culture in a dose-dependent manner. ATP also reduces the expression of Nanog, as determined by flow cytometry, as well as CD133 and Oct-4, as analyzed by flow cytometry and RT-PCR in U87 cells. The differential expression of purinergic receptor in tumor spheres when compared to adherent cells and the effect of ATP in reducing tumor spheres suggest that the purinergic system affects CSC biology and that ATP may be a potential agonist for differentiation therapy.

Lee, D. G., J. H. Lee, et al. "H(+)-myo-inositol transporter SLC2A13 as a potential marker for cancer stem cells in an oral squamous cell carcinoma." Curr Cancer Drug Targets **11**(8): 966-75.
Cancer Stem Cells (CSCs) from tumors of different phenotypes possess a marked capacity for proliferation, self-renewal, and differentiation. They also play a critical role in cancer recurrence. Although CSC has been regarded as a new target for cancer therapy, the fundamental questions in the CSC study have not been resolved mainly due to the lack of

proper CSC markers. To find new CSC markers for oral squamous cell carcinoma (OSCC), we cultured the primary tumor cells from OSCC patients the regular culture condition and the sphere-forming culture condition to enrich primary tumor cells and potential CSCs. We compared gene expression profiles between sphere-forming and non-forming cells, thus identifying that 23 membrane protein-coding genes were over-expressed in the sphere-forming cells. Among them, 8 belonged to the solute carrier (SLC) protein family. H(+)-myo-inositol transporter SLC2A13 and monocarbohydrate transporter SLC16A6 genes that were consistently increased in the sphere-forming cells in the primary cultures of OSCC samples. Confocal microscopy revealed that SLC2A13-expressing cells were embedded in the limited areas of tumor tissue as a cluster, while SLC16A6 was uniformly detected in hyperplastic epithelium. Moreover, SLC2A13 an expression was induced in human breast adenocarcinoma MCF7 cells after serum starvation. Taken together, our results suggest that SLC2A13 can be a potential markers for CSC in various tumors.

Lee, H. E., J. H. Kim, et al. "An increase in cancer stem cell population after primary systemic therapy is a poor prognostic factor in breast cancer." Br J Cancer **104**(11): 1730-8.
BACKGROUND: The cancer stem cell (CSC) hypothesis has important clinical implications for cancer therapeutics because of the proposed role of CSCs in chemoresistance. The aim of this study was to investigate changes in the CSC populations before and after primary systemic therapy (PST) and their prognostic role in human breast cancer. METHODS: Paired samples (before and after PST) of breast cancer tissue were obtained from clinical stage II or III patients (n=92) undergoing PST with the regimen of doxorubicin plus docetaxel (AD) (n=50) or doxorubicin plus cyclophosphamide (AC) (n=42) and subsequent breast resection. The proportions of putative CSCs with CD44+/CD24- or aldehyde dehydrogenase 1+ (ALDH1+) phenotypes were determined by immunohistochemistry. RESULTS: A higher proportion of CD44+/CD24- tumour cells and ALDH1 positivity in pre-chemotherapy tissue was correlated with higher histologic grade, oestrogen receptor (ER) negativity, high Ki-67 proliferation index and basal-like subtype of breast cancer. Aldehyde dehydrogenase 1 positivity in pre-chemotherapy biopsy was also associated with a higher rate of pathologic complete response following PST. In comparisons of putative CSC populations before and after PST, the proportions of CD44+/CD24- and ALDH1+ tumour cells were significantly increased after PST. The cases with increased CD44+/CD24- tumour cell populations after PST showed high Ki-67 proliferation index in post-chemotherapy specimens and those with increased ALDH1+ tumour cell population after PST were associated with ER negativity and p53 overexpression. Furthermore, cases showing such an increase had significantly shorter disease-free survival time than those with no change or a reduced number of CSCs, and the survival difference was most notable with regard to the changes of ALDH1+ tumour cell population in the patients who received AC regimen. CONCLUSION: The present study provides the clinical evidence that the putative CSCs in breast cancer are chemoresistant and are associated with tumour progression, emphasising the need for targeting of CSCs in the breast cancer therapeutics.

Lee, H. J., D. D. You, et al. "Significance of CD133 as a cancer stem cell markers focusing on the tumorigenicity of pancreatic cancer cell lines." J Korean Surg Soc **81**(4): 263-70.
PURPOSE: The cancer stem cell hypothesis states that the capacity of a cancer to grow and propagate is dependent on a small subset of cells. To determine the significances of the cancer stem cell markers CD133, CD44, and CD24 using a comparative analysis with a focus on tumorigenicity. METHODS: Four pancreatic cancer cell lines, Capan-1, Mia-PACA-2, Panc-1, and SNU-410 were analyzed for the expressions of CD133, CD44, and CD24 by flow cytometry. The tumorigenicity was compared using tumor volumes and numbers of tumors formed/numbers of injection in nonobese diabetic severe combined deficiency mice. Fluorescence-activated cell sorting (FACS) analysis was used to confirm that xenograft explants originated from human pancreatic cancer cells. RESULTS: CD133 was positive in only Capan-1, CD44 positive in all, CD24 partially positive in Panc-1. After injecting 2 x 10(6) cells, all mice administered Capan-1 or Mia-Paca-2 developed tumors, 3 of 5 administered Panc-1 developed tumors, but no mouse administered SNU-410 developed any tumors. The volumes of Capan-1 tumors were seven times larger than those of Mia-Paca-2 tumors. When 2 x 10(5) or 2 x 10(4) of Capan-1 or Mia-Paca-2 was injected, tumors developed in all Capan-1 treated mice, but not in Mia-Paca-2 treated mice. Furthermore, xenograft explants of Capan-1 expressed CD133+CD44+ and Capan-1 injected mice developed lung metastasis. FACS analysis showed that xenograft explants originated from human pancreatic cancer cell lines. CONCLUSION: CD133 positive cells have higher tumorigenic and metastatic potential than CD44 and CD24 positive cells, which suggests that CD133 might be a meaningful cell surface marker of pancreatic cancer stem cells.

Leushacke, M. and N. Barker "Lgr5 and Lgr6 as markers to study adult stem cell roles in self-renewal and cancer." Oncogene **31**(25): 3009-22.
The extended longevity of many mammals imposes the need for an effective tissue renewal capacity within the vital organs to maintain optimal function. Resident adult stem cells are instrumental in delivering this renewal capacity by virtue of their characteristic ability to maintain themselves long-term as a population (self-renewal), whilst also supplying all functional cell-lineages of the respective tissue (multipotency). The homeostatic activity of these adult stem cell reservoirs is tailored to meet the specific renewal requirements of individual tissues through a combination of intrinsic genetic programming and local cues delivered from the surrounding environment (the niche). Considerable phenotypic diversity therefore exists between adult stem cell populations in different organs, making it a considerable challenge to identify broadly applicable markers that facilitate their identification and characterization. However, the 7-transmembrane receptor, Lgr5 has recently gained prominence as a marker of Wnt-regulated adult stem cell populations in the hair-follicle, intestine and stomach. A closely-related protein, Lgr6 marks adult stem cells responsible for fueling the renewal of the sebaceous gland and skin. The discovery of these markers has already greatly improved our understanding of stem cell biology in these rapidly renewing tissues and has major implications for the identification and isolation of human adult stem cell populations for exploitation of their regenerative medicine potential in the clinic.

Li, J. and B. P. Zhou "Activation of beta-catenin and Akt pathways by Twist are critical for the maintenance of EMT associated cancer stem cell-like characters." BMC Cancer **11**: 49.
BACKGROUND: Epithelial-mesenchymal transition (EMT) not only confers tumor cells with a distinct advantage for metastatic dissemination, but also it provides those cells with cancer stem cell-like characters for proliferation and drug resistance. However, the molecular mechanism for maintenance of these stem cell-like traits remains unclear. METHODS: In this study, we induced EMT in breast cancer MCF7 and cervical cancer Hela cells with expression of Twist, a key transcriptional factor of EMT. The morphological changes associated with EMT were analyzed by immunofluorescent staining and Western blotting. The stem cell-like traits associated with EMT were determined by tumorsphere-formation and expression of ALDH1 and CD44 in these cells. The activation of beta-catenin and Akt pathways was examined by Western blotting and luciferase assays. RESULTS: We found that expression of Twist induced a morphological change associated with EMT. We also found that the cancer stem cell-like traits, such as tumorsphere formation, expression of ALDH1 and CD44, were significantly elevated in Twist-overexpressing cells. Interestingly, we showed that beta-catenin and Akt pathways were activated in these Twist-overexpressing cells. Activation of beta-catenin correlated with the expression of CD44. Knockdown of beta-catenin expression and inhibition of the Akt pathway greatly suppressed the expression of CD44. CONCLUSIONS: Our results indicate that activation of beta-catenin and Akt pathways are required for the sustention of EMT-associated stem cell-like traits.

Li, L., H. Tian, et al. "Inhibition of lung cancer cell proliferation mediated by human mesenchymal stem cells." Acta Biochim Biophys Sin (Shanghai) **43**(2): 143-8.
Human mesenchymal stem cells (hMSCs) are mostly studied for their potential clinical use. Recently, much attention in the field of cancer research has been paid to hMSCs. In this study, we investigated the influence of hMSCs on the proliferation of lung cancer cell lines SK-MES-1 and A549 in vitro and in vivo by using a co-culture system and the hMSCs-conditioned medium. Our results demonstrated that hMSCs could inhibit the proliferation of SK-MES-1 and A549 cells, and induce the apoptosis of tumor cells in vitro via some soluble factors. Animal study showed that these soluble factors from hMSCs could suppress tumorigenesis and tumor angiogenesis by treating preliminarily tumor cells with the hMSCs-conditioned medium. The downregulated expression of vascular endothelial growth factor in tumor cells might be the mechanism of interference in tumor angiogenesis, which was verified by western blot analysis and immunohistochemistry assay. Taken together, our results suggested that the hMSCs could inhibit tumor cell growth by secreting some soluble factors.

Li, S. C., K. L. Lee, et al. "Convergence of normal stem cell and cancer stem cell developmental stage: Implication for differential therapies." World J Stem Cells **3**(9): 83-8.
Increased evidence shows that normal stem cells may contribute to cancer development and progression by acting as cancer-initiating cells through their interactions with abnormal environmental elements. We postulate that normal stem cells and cancer stem cells (CSC) possess similar mechanisms of self-renewal and differentiation. CSC can be the key to the elaboration of anti-cancer-based therapy. In this article, we focus on a controversial new theme relating to CSC. Tumorigenesis may have a critical

stage characterized as a "therapeutic window", which can be identified by association of molecular, biochemical and biological events. Identifying such a stage can allow the production of more effective therapies (e.g. manipulated stem cells) to treat several cancers. More importantly, confirming the existence of a similar therapeutic window during the conversion of normal stem cells to malignant CSC may lead to targeted therapy specifically against CSC. This conversion information may be derived from investigating the biological behaviour of both normal stem cells and cancerous stem cells. Currently, there is little knowledge about the cellular and molecular mechanisms that govern the initiation and maintenance of CSC. Studies on co-evolution and interdependence of cancer with normal tissues may lead to a useful treatment paradigm of cancer. The crosstalk between normal stem cells and cancer formation may converge developmental stages of different types of stem cells (e.g. normal stem cells, CSC and embryonic stem cells). The differential studies of the convergence may result in novel therapies for treating cancers.

Li, Y., M. S. Wicha, et al. "Implications of cancer stem cell theory for cancer chemoprevention by natural dietary compounds." J Nutr Biochem **22**(9): 799-806.
The emergence of cancer stem cell theory has profound implications for cancer chemoprevention and therapy. Cancer stem cells give rise to the tumor bulk through continuous self-renewal and differentiation. Understanding the mechanisms that regulate self-renewal is of greatest importance for discovery of anticancer drugs targeting cancer stem cells. Naturally occurring dietary compounds have received increasing attention in cancer chemoprevention. The anticancer effects of many dietary components have been reported for both in vitro and in vivo studies. Recently, a number of studies have found that several dietary compounds can directly or indirectly affect cancer stem cell self-renewal pathways. Herein we review the current knowledge of most common natural dietary compounds for their impact on self-renewal pathways and potential effect against cancer stem cells. Three pathways (Wnt/beta-catenin, Hedgehog and Notch) are summarized for their functions in self-renewal of cancer stem cells. The dietary compounds, including curcumin, sulforaphane, soy isoflavone, epigallocatechin-3-gallate, resveratrol, lycopene, piperine and vitamin D(3), are discussed for their direct or indirect effect on these self-renewal pathways. Curcumin and piperine have been demonstrated to target breast cancer stem cells. Sulforaphane has been reported to inhibit pancreatic tumor-initiating cells and breast cancer stem cells. These studies provide a basis for preclinical and clinical evaluation of dietary compounds for chemoprevention of cancer stem cells. This may enable us to discover more preventive strategies for cancer management by reducing cancer resistance and recurrence and improving patient survival.

Lifantseva, N., A. Koltsova, et al. "Expression patterns of cancer-testis antigens in human embryonic stem cells and their cell derivatives indicate lineage tracks." Stem Cells Int **2011**: 795239.
Pluripotent stem cells can differentiate into various lineages but undergo genetic and epigenetic changes during long-term cultivation and, therefore, require regular monitoring. The expression patterns of cancer-testis antigens (CTAs) MAGE-A2, -A3, -A4, -A6, -A8, -B2, and GAGE were examined in undifferentiated human embryonic stem (hES) cells, their differentiated derivatives, teratocarcinoma (hEC) cells, and cancer cell lines of neuroectodermal and mesodermal origin. Undifferentiated hES cells and embryoid body cells expressed MAGE-A3, -A6, -A4, -A8, and GAGEs while later differentiated derivatives expressed only MAGE-A8 or MAGE-A4. Likewise, mouse pluripotent stem cells also express CTAs of Magea but not Mageb family. Despite similarity of the hES and hEC cell expression patterns, MAGE-A2 and MAGE-B2 were detected only in hEC cells but not in hES cells. Moreover, our analysis has shown that CTAs are aberrantly expressed in cancer cell lines and display low tissue specificity. The identification of CTA expression patterns in pluripotent stem cells and their derivatives may be useful for isolation of abnormally CTA-expressing cells to improve the safety of stem-cell based therapy.

Lim, Y. C., S. Y. Oh, et al. "Cancer stem cell traits in squamospheres derived from primary head and neck squamous cell carcinomas." Oral Oncol **47**(2): 83-91.
A subpopulation of cancer stem cells (CSCs), but not the majority of non-tumorigenic cancer cells, in a variety of human malignancies plays a critical role in cancer cell proliferation, invasion, metastasis, and tumor recurrence post-therapies. We report the isolation of sphere-forming cells (squamospheres) from primary head and neck squamous cell carcinomas (HNSCCs), and characterization of their CSC properties. Squamospheres appeared within 2 weeks after seeding as single-dissociated cells obtained from primary HNSCC specimens in serum-free culture conditions. Real-time RT-PCR and immunocytochemistry assays revealed that a number of stem cell markers, including CK5, OCT4, SOX2, and nestin, were up-regulated in HNSCC-driven squamospheres. Fluorescence-activated cell sorting

(FACS) analysis showed that squamospheres contain enriched side population cells compared to serum-induced differentiated squamosphere cells. Furthermore, HNSCC-driven squamospheres appeared to be chemoresistant to cisplatin, 5-fluorouracil (FU), paclitaxel and doxetaxel, and showed increased levels of ABCG2, one of the ATP-binding cassette (ABC) transporters. Of particular interest, in sharp contrast to subcutaneous injection of 1x10(6) differentiated squamosphere cells, as few as 100 squamosphere cells were able to give rise to tumors in nude mice. Altogether, we assert that primary HNSCC-driven squamospheres possess CSC properties, and its functional analysis may provide a novel tool for investigating the tumorigenic process of HNSCC.

Lin, L., J. Fuchs, et al. "STAT3 signaling pathway is necessary for cell survival and tumorsphere forming capacity in ALDH(+)/CD133(+) stem cell-like human colon cancer cells." Biochem Biophys Res Commun **416**(3-4): 246-51.
Persistent activation of Signal Transducers and Activators of Transcription 3 (STAT3) is frequently detected in colon cancer. Increasing evidence suggests the existence of a small population of colon cancer stem or cancer-initiating cells may be responsible for tumor initiation, metastasis, and resistance to chemotherapy and radiation. Whether STAT3 plays a role in colon cancer-initiating cells and the effect of STAT3 inhibition is still unknown. Flow cytometry was used to isolate colon cancer stem-like cells from three independent human colon cancer cell lines characterized by both aldehyde dehydrogenase (ALDH)-positive and CD133-positive subpopulation (ALDH(+)/CD133(+)). The effects of STAT3 inhibition in colon cancer stem-like cells were examined. The phosphorylated or activated form of STAT3 was expressed in colon cancer stem-like cells and was reduced by a STAT3-selective small molecular inhibitor, FLLL32. FLLL32 also inhibited the expression of potential STAT3 downstream target genes in colon cancer stem-like cells including survivin, Bcl-XL, as well as Notch-1, -3, and -4, which may be involved in stem cell function. Furthermore, FLLL32 inhibited cell viability and tumorsphere formation as well as induced cleaved caspase-3 in colon cancer stem-like cells. FLLL32 is more potent than curcumin as evidenced with lower IC50 in colon cancer stem-like cells. In summary, our results indicate that STAT3 is a novel therapeutic target in colon cancer stem-like cells and inhibition of STAT3 in cancer stem-like cells may offer a potential treatment for colorectal cancer.

Lin, Q., W. Mao, et al. "A cluster of specified microRNAs in peripheral blood as biomarkers for metastatic non-small-cell lung cancer by stem-loop RT-PCR." J Cancer Res Clin Oncol **138**(1): 85-93.
PURPOSE: To investigate the levels for some specified microRNAs in human's peripheral blood so as to determine whether they can serve as biomarkers for metastatic non-small-cell lung cancer. METHODS: Use a quantitative stem-loop RT-PCR method to examine the serum levels for certain microRNAs including has-miR-125a-5p, has-miR-126, has-miR-183, has-miR-200, has-miR-221, and has-miR-222 from the patients with Stage IV, Stage I/II non-small-cell lung cancer and the controls. RESULTS: There was statistical difference in the serum levels for hsa-miR-126, hsa-miR-183, and hsa-miR-222 between the controls and the Stage IV patients, but not for has-miR-125a-5p, has-miR-200 and has-miR-221. It also showed statistical difference for hsa-miR-126 and hsa-miR-183 between the Stage I/II patients and Stage IV patients, but not between the controls and Stage I/II patients. CONCLUSION: Hsa-miR-126 and hsa-miR-183 may serve as potential serum biomarkers for metastatic non-small-cell lung cancer.

Ling, P. M., S. W. Cheung, et al. "Using self-assembled nanomaterials to inhibit the formation of metastatic cancer stem cell colonies in vitro." Cell Transplant **20**(1): 127-31.
The isolation of cells with stem-like properties from prostate tumors suggests the presence of a cancer stem cell (CSC) population, which may account for the initiation, progression, and metastasis as well as drug resistance of the disease. We hypothesized that containing, or at least immobilizing, the CSCs in a nano-self-assembling material might help prevent prostate tumor progression or metastasis. CSCs were plated in three conditions: 1) placed in 1% concentration self-assembled peptide (SAP) preequilibrate with culture medium; 2) placed in 3% concentration SAP preequilibrate with culture medium; and 3) in nonadherent culture. All were grown for 14 days, after which the cells in both 1% and 3% concentrations were washed out of the SAP and grown for an additional 14 days. Here we report that CSCs from prostate cancer cell lines remained quiescent for more than 28 days when embedded in SAP. When the prostate CSCs were embedded in 1% and 3% SAP, most of the CSCs remained single cells 14 days after plating in a nonadherent plate; no prostaspheres could be detected 14 days after plating, suggesting that self-renewal was significantly suppressed. In the controls, prostate CSCs began to divide 1 day after plating in a nonadherent plate and prostaspheres were visible at day 10, indicating the

active self-renewal property of the prostate CSCs. Our findings suggest that SAP can completely inhibit a prostate CSC from self-renewal while preserving its viability and CSC property. Therefore, SAP may be an effective nanomaterial for inhibiting cancer progression and metastasis to stop the progression during treatment and removal.

Liu, C. G., Y. Lu, et al. "Clinical implications of stem cell gene Oct-4 expression in breast cancer." Ann Surg **253**(6): 1165-71.
PURPOSE: To explore the expression of stem cell genes in breast cancer and the relationship between stem cell gene expression and clinical and pathological characteristics and prognosis of breast cancer. BACKGROUND: By now, stem cell differentiation-related genes and the relationship between the genes and clinic-pathological characteristics and prognosis of breast cancer are still unclear. MATERIALS AND METHODS: CD44+/CD24- tumor cells were selected by Flow cytometry. The differential expression of genes between CD44+/CD24- tumor cells and non-CD44+/CD24- tumor cells were detected by RT(2) Profiler PCR Array. The expression of stem cell gene Octamer-4 (Oct-4) was analyzed by immunohistochemistry staining and the relationship between Oct-4 and clinicopathological parameters of breast cancer was determined. RESULTS: Seven different genes including stem cell differentiation-related factors (CD44, Oct-4, and nestin), cell cycle regulators (APC and CDC2), and growth factors (HGF and TGF) were detected as significantly differently expressed between CD44+/CD24- tumor cells and non-CD44+/CD24- tumor cells. Oct-4 protein expressed significantly higher in cancerous tissues than adjacent-tumor tissues (P = 0.001). Moreover, we observed that the expression of Oct-4 protein was related to histological type, lymph node status and molecular type of breast cancer (P = 0.001, 0.006, and 0.001, respectively). After survival analysis, the cases with highly expressed Oct-4 protein attained a significantly poorer postoperative disease-specific survival than those with none/low expressed Oct-4 protein (P = 0.001). In the Cox regression test, tumor size, histological type, disease stage, lymph node metastasis, Her-2 and Oct-4 were detected as the independent prognostic factors (P = 0.031, 0.012, 0.001, 0.002, 0.030, and 0.003, respectively). CONCLUSIONS: Oct-4 was highly expressed in CD44+/CD24- tumor cells, and may be a potential biomarker for the initiation, progression, and differentiation of breast cancer.

Liu, H. G., C. Chen, et al. "Cancer stem cell subsets and their relationships." J Transl Med **9**: 50.
Emerging evidence suggests that cancer stem cells account for the initiation and progression of cancer. While many types of cancer stem cells with specific markers have been isolated and identified, a variety of differences among them began to be appreciated. Cancer stem cells are hierarchical populations that consist of precancerous stem cells, primary cancer stem cells, migrating cancer stem cells and chemoradioresistant cancer stem cells, playing different roles in cancer initiation and progression. Here we propose a new concept "horizontal hierarchy of cancer stem cells" to distinguish them from vertical hierarchy cancer stem cells, cancer transient-amplifying cells and cancer differentiated cells, and summarize our current understanding of these subsets of cancer stem cells with the aim to open up novel therapeutic strategies for cancer based on this understanding.

Liu, L. L., D. Fu, et al. "The power and the promise of liver cancer stem cell markers." Stem Cells Dev **20**(12): 2023-30.
Recently, there has been growing support for the cancer stem cell (CSC) hypothesis, which states that primary tumors are initiated and maintained by a small subpopulation of cancer cells that possess "stem-like" characteristics. CSCs have been identified in many tumor types, including hepatocellular carcinoma (HCC). The dye, Hoechst 33342, has been used to enrich CSCs into a side population. Alternatively, liver CSCs (LCSCs) can be identified by several cell surface antigens, including CD133, CD90, CD44, EpCAM, and CD13. In this review, we summarized the recent evidence regarding LCSC markers and discussed the origin and function of these markers. LCSC markers are essential to identify and isolate these cells, to develop future therapies targeting CSCs, and to predict prognosis and efficacy of these therapies. However, definite LCSC markers are still controversial, because none of these markers is exclusively expressed by LCSCs in HCC. By combining several positive or negative markers, it may be possible to isolate and identify CSC fractions beyond the ability of each individual assay. By grouping LCSC markers according to their cellular origin, the properties of LCSC markers may be better studied and new markers may be found. Lastly, markers could be used to estimate the number of LCSCs and therefore predict outcomes. From our point of view, selecting HCC tissue samples from patients with different prognoses and detecting expression patterns of marker combinations may be a new method to identify new and unique markers.

Liu, W., J. Q. Feng, et al. "Two stem cell markers, ATP-binding cassette, G2 subfamily (ABCG2) and

BMI-1, predict the transformation of oral leukoplakia to cancer: a long-term follow-up study." Cancer **118**(6): 1693-700.
BACKGROUND: Although oral leukoplakia (OL) is the best-known potentially malignant disorder, the risk of OL malignant transformation is difficult to assess. ATP-binding cassette, G2 subfamily (ABCG2) and BMI-1 are stem cell markers that have been found to be associated with head and neck tumorigenesis. The objective of the current study was to evaluate the usefulness of ABCG2 and BMI-1 in predicting OL transformation. METHODS: In a retrospective cohort of 135 patients with OL from the study institution who had a mean follow-up of 5.5 years, 32 developed cancer between 1985 and 2008. The expression of ABCG2 and BMI-1 was determined using immunohistochemistry in samples from these patients, and included untransformed OL (n = 103) and malignant-transformed OL (n = 32). The association between protein expression and clinicopathological parameters and transformation was analyzed. RESULTS: Expression of ABCG2 and BMI-1 was observed in 58 (43.0%) and 44 (32.6%) of 135 patients, respectively. The correlation between ABCG2 and BMI-1 expression was significant (P = .024). Kaplan-Meier analysis revealed that 37.9% of patients with ABCG2 positivity developed cancer compared with 13.0% of patients with ABCG2 negativity (P = .014, log-rank test). Approximately 40.9% of patients with BMI-1 positivity developed cancer compared with 15.4% of patients with BMI-1 negativity (P = .029, log-rank test). Multivariate analysis revealed that ABCG2 and BMI-1 expression was associated with a 3.24-fold (95% confidence interval [95% CI], 1.31-7.98; P = .011) and 4.03-fold (95% CI, 1.59-10.26; P = .003) increased the risk of transformation, respectively. CONCLUSIONS: ABCG2 and BMI-1 expression was found to be associated with the development of oral cancer in a large cohort of patients with OL for whom long-term follow-up was available, which suggests that ABCG2 and BMI-1 may be used as predictors of OL transformation.

Liu, Y., C. Zhang, et al. "Comprehensive analysis of clinical significance of stem-cell related factors in renal cell cancer." World J Surg Oncol **9**: 121.
BACKGROUND: C-MYC, LIN28, OCT4, KLF4, NANOG and SOX2 are stem cell related factors. We detected whether these factors express in renal cell carcinoma (RCC) tissues to study their correlations with the clinical and pathological characteristics. METHODS: The expressions of c-MYC, LIN28, SOX2, KLF4, OCT4 and NANOG in 30 RCC patients and 5 non-RCC patients were detected with quantitative real-time reverse transcription-PCR (qRT-PCR). The data were analyzed with Wilcoxon signed rank sum test and x2 test. RESULTS: In RCC group, c-MYC expression was significantly higher in RCC tissues compared with normal tissues ($P < 0.05$). The expression levels of OCT4, KLF4, NANOG and SOX2 were significantly lower in RCC tissues compared with normal tissues ($P < 0.05$). LIN28 expression level was not significant. No difference was observed when it comes to clinical and pathological characteristics such as gender, age, tumor size, cTNM classification and differentiation status ($P > 0.05$). Also the expression levels of all above factors were not significantly changed in non-RCC group ($P > 0.05$). CONCLUSIONS: The present analysis strongly suggests that altered expression of several stem cell related factors may play different roles in RCC. C-MYC may function as an oncogene and OCT4, KLF4, NANOG and SOX2 as tumor suppressors.

Lochhead, P., B. Frank, et al. "Genetic variation in the prostate stem cell antigen gene and upper gastrointestinal cancer in white individuals." Gastroenterology **140**(2): 435-41.
BACKGROUND & AIMS: An association between gastric cancer and the rs2294008 (C>T) polymorphism in the prostate stem cell antigen (PSCA) gene has been reported for several Asian populations. We set out to determine whether such an association exists in white individuals. METHODS: We genotyped 166 relatives of gastric cancer patients, including 43 Helicobacter pylori-infected subjects with hypochlorhydria and gastric atrophy, 65 infected subjects without these abnormalities, 58 H pylori-negative relatives, and 100 population controls. Additionally, a population-based study of chronic atrophic gastritis provided 533 cases and 1054 controls. We then genotyped 2 population-based, case-control studies of upper gastrointestinal cancer: the first included 312 gastric cancer cases and 383 controls; the second included 309 gastric cancer cases, 159 esophageal cancer cases, and 211 controls. Odds ratios were computed from logistic models and adjusted for confounding variables. RESULTS: Carriage of the risk allele (T) of rs2294008 in PSCA was associated with chronic atrophic gastritis (adjusted odds ratio [OR], 1.5; 95% confidence interval [CI]: 1.1-1.9) and noncardia gastric cancer (OR, 1.9; 95% CI: 1.3-2.8). The association was strongest for the diffuse histologic type (OR, 3.2; 95% CI: 1.2-10.7). An inverse association was observed between carriage of the risk allele and gastric cardia cancer (OR, 0.5; 95% CI: 0.3-0.9), esophageal adenocarcinoma (OR, 0.5; 95% CI: 0.3-0.9), and esophageal squamous cell carcinoma (OR, 0.4; 95% CI: 0.2-0.9). CONCLUSIONS: The rs2294008

polymorphism in PSCA increases the risk of noncardia gastric cancer and its precursors in white individuals but protects against proximal cancers.

Low, J., W. Blosser, et al. "Knockdown of ubiquitin ligases in glioblastoma cancer stem cells leads to cell death and differentiation." J Biomol Screen **17**(2): 152-62.
The cancer stem cell (CSC) hypothesis proposes that a subpopulation of CSCs is frequently responsible for chemotherapy resistance and metastasis and is now a point of attack for research into the next generation of therapeutics. Although many of these agents are directed at inducing CSC apoptosis (as well as the bulk tumor), some agents may also decrease cell "stemness" possibly through induction of differentiation. Ubiquitin ligases, critical to virtually all cellular signaling systems, alter the degradation or trafficking of most proteins in the cell, and indeed broad perturbation of this system, through inhibition of the proteosome, is a successful cancer treatment. The authors examined several glioblastoma stem cell isolates pre- and postdifferentiation to elucidate the phenotypic effects following shRNA knockdown of ubiquitin ligases. The results were analyzed using high-content imaging (HCI) and identified ubiquitin ligases capable of inducing both CSC differentiation and apoptosis. Quite often these effects were specific to CSCs, as ubiquitin ligase knockdown in terminally differentiated progeny yielded markedly different results. The resolution of HCI at the subpopulation level makes it an excellent tool for the analysis of CSC phenotypic changes induced by shRNA knockdown and may suggest additional methods to target these cells for death or differentiation.

Lu, X., Q. Deng, et al. "Altered characteristics of cancer stem/initiating cells in a breast cancer cell line treated with persistent 5-FU chemotherapy." Exp Ther Med **2**(5): 821-826.
Drug resistance of cancer stem/initiating cells has been considered to be one of the main reasons for tumor relapse. However, knowledge concerning the changes in stem/ initiating cells during chemotherapy is limited. In the present study, the breast cancer cell line MDA-MB-468 was cultured with 5-fluorouracil and serially passaged. Six cell generations were collected. Semi-quantitative RT-PCR and flow cytometric techniques were used to evaluate the protein and mRNA expression of stem/initiating factors (CD44(+)/CD24(-), Oct 3/4, SOX2 and beta-catenin), drug-resistance genes (BCRP and MRP1) and an anti-apoptosis gene (survivin). The clone formation rate was also examined in every generation of cells. The results showed that, under conditions of persistent chemotherapy, the factors representing the quantity of stem/initiating cells (beta-catenin, Oct 3/4 and SOX2) followed a fluctuating trend of decrease-increase-further increase-decrease-increase-decrease, and factors representing the proportion of stem/initiating cells (proportion of CD44(+)/CD24(-) and the clone formation rate) demonstrated a fluctuating trend of increase-further increase-further increase-decrease. The drug-resistance genes (BCRP and MRP1) and the anti-apoptosis gene (survivin) demonstrated a wave of increase-further increase-further increase-decrease-increase (MRP1 decrease)-decrease. beta-catenin, Oct 3/4 and SOX2 showed a positive correlation ($r=1$, $p<0.01$). Our study confirmed that the drug resistance of cancer cells is mainly due to tumor stem/initiating cells, and that under conditions of persistent chemotherapy, the quantity or function of breast cancer stem/initiating cells increases and decreases alternately.

Lu, X. and Y. Kang "Cell fusion hypothesis of the cancer stem cell." Adv Exp Med Biol **714**: 129-40.
A major advance in recent cancer research is the identification of tumor cells with stem cell-like properties. Cancer stem cells (CSCs) often represent a rare population in the tumor mass and possess the exclusive ability to initiate the growth of a heterogeneous tumor. The origin of CSCs remains elusive and is likely to be cancer type specific. One possible but under-appreciated potential mechanism for the generation of CSCs is through fusion between stem cells and differentiated cells. The cell fusion hypothesis of CSCs adds an important functional underpinning to the potential multifaceted roles of cell fusion in the initiation and progression of cancer.

Luk, S. U., T. K. Lee, et al. "Chemopreventive effect of PSP through targeting of prostate cancer stem cell-like population." PLoS One **6**(5): e19804.
Recent evidence suggested that prostate cancer stem/progenitor cells (CSC) are responsible for cancer initiation as well as disease progression. Unfortunately, conventional therapies are only effective in targeting the more differentiated cancer cells and spare the CSCs. Here, we report that PSP, an active component extracted from the mushroom Turkey tail (also known as Coriolus versicolor), is effective in targeting prostate CSCs. We found that treatment of the prostate cancer cell line PC-3 with PSP led to the down-regulation of CSC markers (CD133 and CD44) in a time and dose-dependent manner. Meanwhile, PSP treatment not only suppressed the ability of PC-3 cells to form prostaspheres under non-adherent culture conditions, but also inhibited their tumorigenicity in vivo, further proving that PSP can suppress prostate CSC properties. To investigate if the anti-CSC effect of

PSP may lead to prostate cancer chemoprevention, transgenic mice (TgMAP) that spontaneously develop prostate tumors were orally fed with PSP for 20 weeks. Whereas 100% of the mice that fed with water only developed prostate tumors at the end of experiment, no tumors could be found in any of the mice fed with PSP, suggesting that PSP treatment can completely inhibit prostate tumor formation. Our results not only demonstrated the intriguing anti-CSC effect of PSP, but also revealed, for the first time, the surprising chemopreventive property of oral PSP consumption against prostate cancer.

Luk, S. U., W. N. Yap, et al. "Gamma-tocotrienol as an effective agent in targeting prostate cancer stem cell-like population." Int J Cancer **128**(9): 2182-91.
Emerging evidence supports that prostate cancer originates from a rare subpopulation of cells, namely prostate cancer stem cells (CSCs). Conventional therapies for prostate cancer are believed to mainly target the majority of differentiated tumor cells but spare CSCs, which may account for the subsequent disease relapse after treatment. Therefore, successful elimination of CSCs may be an effective strategy to achieve complete remission from this disease. Gamma-tocotrienols (gamma-T3) is one of the vitamin-E constituents, which have been shown to have anticancer effects against a wide range of human cancers. Recently, we have reported that gamma-T3 treatment not only inhibits prostate cancer cell invasion but also sensitizes the cells to docetaxel-induced apoptosis, suggesting that gamma-T3 may be an effective therapeutic agent against advanced stage prostate cancer. Here, we demonstrate for the first time that gamma-T3 can downregulate the expression of prostate CSC markers (CD133/CD44) in androgen-independent prostate cancer cell lines (PC-3 and DU145), as evident from Western blotting analysis. Meanwhile, the spheroid formation ability of the prostate cancer cells was significantly hampered by gamma-T3 treatment. In addition, pretreatment of PC-3 cells with gamma-T3 was found to suppress tumor initiation ability of the cells. More importantly, although CD133-enriched PC-3 cells were highly resistant to docetaxel treatment, these cells were as sensitive to gamma-T3 treatment as the CD133-depleted population. Our data suggest that gamma-T3 may be an effective agent in targeting prostate CSCs, which may account for its anticancer and chemosensitizing effects reported in previous studies.

Ma, B., X. Lei, et al. "Maintenance of retinal cancer stem cell-like properties through long-term serum-free culture from human retinoblastoma." Oncol Rep **26**(1): 135-43.
Previous studies have demonstrated that a small population of cancer stem cell-like cells exists in retinoblastoma. To provide a model for studying this population, we sought to establish a long-term culture from human retinoblastoma that have cancer stem cell-like properties. Fresh tumor tissue was digested and cultured in serum-free medium. Tumor spheres formed and were passaged continuously. Stem cell properties were examined through immunostaining, real-time quantitative RT-PCR and chemoresistance assay. Tumorigenicity of the tumor sphere-forming cells was confirmed by xenograft experiments. Furthermore, we examined the expression of cell surface markers CD44 and CD133. Tumor cells expanded as floating spheres for more than 30 passages. Sphere-forming cells overexpressed stem cell genes Oct4, Nestin and Pax6. Immunostaining of spheres showed positivity for Nestin, Pax6 and also ABCG2. In contrast, differentiated cells derived from these spheres expressed high levels of mature retinal cell markers MAP2, GFAP, recoverin, Opsin B and Nrl, and showed immunoreactivity for NF200, GFAP, recoverin and PKCalpha. Furthermore, both CD44 and CD133 were highly expressed in sphere-forming cells vs. differentiated cells. Sphere-forming cells displayed higher chemoresistance to carboplatin as opposed to differentiated cells. Moreover, intraocular injection of as few as 2x103 sphere-forming cells into NOD/SCID mice gave rise to new tumors similar to the original patient tumors. These results revealed that the sphere-forming cells preserved their stem cell properties and tumorigenicity, even after long-term culture. This would be a suitable in vitro model to study cancer stem-like cells in retinoblastoma and to develop chemotherapeutic drugs and strategies.

Ma, Y., D. Liang, et al. "Prostate cancer cell lines under hypoxia exhibit greater stem-like properties." PLoS One **6**(12): e29170.
Hypoxia is an important environmental change in many cancers. Hypoxic niches can be occupied by cancer stem/progenitor-like cells that are associated with tumor progression and resistance to radiotherapy and chemotherapy. However, it has not yet been fully elucidated how hypoxia influences the stem-like properties of prostate cancer cells. In this report, we investigated the effects of hypoxia on human prostate cancer cell lines, PC-3 and DU145. In comparison to normoxia (20% O(2)), 7% O(2) induced higher expressions of HIF-1alpha and HIF-2alpha, which were associated with upregulation of Oct3/4 and Nanog; 1% O(2) induced even greater levels of these factors. The upregulated NANOG mRNA expression in hypoxia was confirmed to be predominantly retrogene NANOGP8. Similar growth rates were observed for cells cultivated under hypoxic and

normoxic conditions for 48 hours; however, the colony formation assay revealed that 48 hours of hypoxic pretreatment resulted in the formation of more colonies. Treatment with 1% O(2) also extended the G(0)/G(1) stage, resulting in more side population cells, and induced CD44 and ABCG2 expressions. Hypoxia also increased the number of cells positive for ABCG2 expression, which were predominantly found to be CD44(bright) cells. Correspondingly, the sorted CD44(bright) cells expressed higher levels of ABCG2, Oct3/4, and Nanog than CD44(dim) cells, and hypoxic pretreatment significantly increased the expressions of these factors. CD44(bright) cells under normoxia formed significantly more colonies and spheres compared with the CD44(dim) cells, and hypoxic pretreatment even increased this effect. Our data indicate that prostate cancer cells under hypoxia possess greater stem-like properties.

Madka, V. and C. V. Rao "Cancer stem cell markers as potential targets for epithelial cancers." Indian J Exp Biol **49**(11): 826-35.
In recent years, the role of tumor-initiating cells (popularly known as cancer stem cells) in tumor development and availability of novel cancer stem cell/tumor initiating cell markers promises a new arena in understanding their role in developing novel targeted molecules. It is important to identify and understand the relevance of cancer stem cells (CSC)/tumor initiating cells (TIC) in tumor development and to design appropriate strategies for CSCs and TICs elimination, which is crucial to future cancer prevention and treatment. In this review, we attempt to define various potential markers of cancer stem cells and potential exploration as therapeutic targets for epithelial cancer prevention and treatment.

Marcato, P., C. A. Dean, et al. "Aldehyde dehydrogenase: its role as a cancer stem cell marker comes down to the specific isoform." Cell Cycle **10**(9): 1378-84.
Recent evidence suggests that enhanced aldehyde dehydrogenase (ALDH) activity is a hallmark of cancer stem cells (CSC) measurable by the aldefluor assay. ALDH1A1, one of 19 ALDH isoforms expressed in humans, was generally believed to be responsible for the ALDH activity of CSCs. More recently, experiments with murine hematopoietic stem cells, murine progenitor pancreatic cells, and human breast CSCs indicate that other ALDH isoforms, particularly ALDH1A3, significantly contribute to aldefluor positivity, which may be tissue and cancer specific. Therefore, potential prognostic application involving the use of CSC prevalence in tumor tissue to predict patient outcome requires the identification and quantification of specific ALDH isoforms. Herein we review the suggested roles of ALDH in CSC biology and the immunohistological studies testing the potential application of ALDH isoforms as novel cancer prognostic indicators.

Margaritescu, C., D. Pirici, et al. "The utility of CD44, CD117 and CD133 in identification of cancer stem cells (CSC) in oral squamous cell carcinomas (OSCC)." Rom J Morphol Embryol **52**(3 Suppl): 985-93.
One of the theories regarding oral carcinogenesis is that the tumor growth is dependent on cancer stem cells (CSCs) that have the capacity of self-renewal and of giving rise to more differentiated tumor cells, like the stem cells do in normal tissues. The most used methods of CSCs isolation are based on their identification based on the expression of different cell surface markers. The markers qualified for this purpose have been described originally in studies involving hematopoietic or embryonic stem cells. Thus, we were interested to study the expression of the most used CSCs surface markers for formalin fixed paraffin embedded tissue samples from oral squamous cell carcinoma (OSCC). We investigated by immunohistochemistry thirty tissue samples of OSCCs with different degrees of differentiation and different oral locations. We were interested to establish the tissular localization pattern for cells expressing CD44, CD133 and CD117 in tumoral samples. The results indicated that with the exception of CD44, the other two surface markers were expressed only in tumoral stromal cells. When we looked at their origin (by double immunohistochemistry for cytokeratins AE1-AE3, vimentin and CD34) we concluded that they are of mesenchymal nature. Also, we proved that some of these cells also co-expressed CD44 but were negative for CK5/6. Moreover, some of the stromal cells that were positive to CD133 and especially for CD117 also had reactivity to tryptase showing their mast cell nature. In conclusion, our study proved that CD44 has limited utility in identifying oral CSCs, while CD117 and CD133 expression appears to be limited more in identifying mesenchymal stem cells.

Marotta, L. L., V. Almendro, et al. "The JAK2/STAT3 signaling pathway is required for growth of CD44(+)CD24(-) stem cell-like breast cancer cells in human tumors." J Clin Invest **121**(7): 2723-35.
Intratumor heterogeneity is a major clinical problem because tumor cell subtypes display variable sensitivity to therapeutics and may play different roles in progression. We previously characterized 2 cell populations in human breast tumors with distinct properties: CD44+CD24- cells that have stem cell-like

characteristics, and CD44-CD24+ cells that resemble more differentiated breast cancer cells. Here we identified 15 genes required for cell growth or proliferation in CD44+CD24- human breast cancer cells in a large-scale loss-of-function screen and found that inhibition of several of these (IL6, PTGIS, HAS1, CXCL3, and PFKFB3) reduced Stat3 activation. We found that the IL-6/JAK2/Stat3 pathway was preferentially active in CD44+CD24- breast cancer cells compared with other tumor cell types, and inhibition of JAK2 decreased their number and blocked growth of xenografts. Our results highlight the differences between distinct breast cancer cell types and identify targets such as JAK2 and Stat3 that may lead to more specific and effective breast cancer therapies.

Martin-Belmonte, F. and M. Perez-Moreno "Epithelial cell polarity, stem cells and cancer." Nat Rev Cancer **12**(1): 23-38.
After years of extensive scientific discovery much has been learned about the networks that regulate epithelial homeostasis. Loss of expression or functional activity of cell adhesion and cell polarity proteins (including the PAR, crumbs (CRB) and scribble (SCRIB) complexes) is intricately related to advanced stages of tumour progression and invasiveness. But the key roles of these proteins in crosstalk with the Hippo and liver kinase B1 (LKB1)-AMPK pathways and in epithelial function and proliferation indicate that they may also be associated with the early stages of tumorigenesis. For example, deregulation of adhesion and polarity proteins can cause misoriented cell divisions and increased self-renewal of adult epithelial stem cells. In this Review, we highlight some advances in the understanding of how loss of epithelial cell polarity contributes to tumorigenesis.

Masetti, R., C. Biagi, et al. "Focal nodular hyperplasia of the liver after intensive treatment for pediatric cancer: is hematopoietic stem cell transplantation a risk factor?" Eur J Pediatr **170**(6): 807-12.
Focal nodular hyperplasia (FNH) is a benign hepatic lesion very rarely described in the pediatric population. It has been reported more frequently in patients treated for pediatric cancers with chemotherapy or hematopoietic stem cell transplantation. The use of high dosage of alkylating agents, the occurrence of venous occlusive disease, graft-versus-host disease, and other variables linked to the hematopoietic stem cell transplantation procedure can represent risk factors for the development of FNH in the pediatric age. The discovery of hepatic nodules in the follow-up of patients treated for malignancies suggests recurrence of disease and raises a diagnostic dilemma. Here we describe possible risk factors, clinical and radiological findings of eight pediatric patients who developed focal nodular hyperplasia after hematopoietic stem cell transplantation. The aim of this report is to provide useful diagnostic tools to facilitate accurate diagnosis of FNH and suggest a correct management of this benign lesion during postcancer follow-up.

Masters, G. A., X. Wang, et al. "A phase II trial of high dose carboplatin and paclitaxel with G-CSF and peripheral blood stem cell support followed by surgery and/or chest radiation in patients with stage III non-small cell lung cancer: CALGB 9531." Lung Cancer **74**(2): 258-63.
PURPOSE: We designed a phase II trial to evaluate the efficacy and tolerability of high dose induction chemotherapy with carboplatin and paclitaxel with G-CSF and stem cell support followed by surgical resection and/or chest radiotherapy in patients with stage III non-small cell lung cancer (NSCLC). PATIENTS AND METHODS: Patients had pathologically confirmed stage IIIA-IIIB NSCLC, adequate end-organ function, no prior chemotherapy or radiation, and performance status 0-1. Peripheral stem cells were mobilized with G-CSF stimulation on days 1-5 and collected prior to chemotherapy. Chemotherapy consisted of 2 cycles of paclitaxel 250 mg/m(2) over 3h and carboplatin at an AUC 18 on days 11 and 32, each followed by stem cell reinfusion. Stable and responding patients went on to surgical resection (in patients deemed resectable) followed by post-operative radiation, or to conventional chest radiotherapy to 66 Gy in unresectable patients. RESULTS: Twelve patients (11 eligible) were accrued from 1996 to 1999. The 11 patients were predominately male (64%), white (82%), of performance status 0 (64%), and with weight loss less than 5% (55%). The median age was 51 (range 31-63). Ten (10) patients (91%) experienced grade 4 toxicity. There were no lethal toxicities. Grade 3-4 toxicities most commonly reported included: platelets (100%), lymphocytopenia (91%), leukopenia (91%), neutropenia (73%), anemia (55%), pain (45%), and nausea (27%). Three patients (27%) had a partial response to induction chemotherapy. Of the 11 patients, 7 underwent surgical exploration, and 10 received radiation. Two patients were completely resected, 3 patients had incomplete resections, and 2 patients had no resection. There were 4 complete responses and 3 partial responses following surgery and/or radiation. The median overall survival time was 17.8 months. The median failure-free survival time was 8.3 months. One-year and 2-year overall survival are estimated at 64% and 27%, respectively. CONCLUSIONS: High dose induction chemotherapy

with carboplatin and paclitaxel and stem cell support in patients with stage IIIA-IIIB NSCLC produced response rates and survival similar to standard therapy. Excessive toxicity (and cost) suggests that this approach does not merit further investigation.

Mathieu, J., Z. Zhang, et al. "HIF induces human embryonic stem cell markers in cancer cells." Cancer Res **71**(13): 4640-52.
Low oxygen levels have been shown to promote self-renewal in many stem cells. In tumors, hypoxia is associated with aggressive disease course and poor clinical outcomes. Furthermore, many aggressive tumors have been shown to display gene expression signatures characteristic of human embryonic stem cells (hESC). We now tested whether hypoxia might be responsible for the hESC signature observed in aggressive tumors. We show that hypoxia, through hypoxia-inducible factor (HIF), can induce an hESC-like transcriptional program, including the induced pluripotent stem cell (iPSC) inducers, OCT4, NANOG, SOX2, KLF4, cMYC, and microRNA-302 in 11 cancer cell lines (from prostate, brain, kidney, cervix, lung, colon, liver, and breast tumors). Furthermore, nondegradable forms of HIFalpha, combined with the traditional iPSC inducers, are highly efficient in generating A549 iPSC-like colonies that have high tumorigenic capacity. To test potential correlation between iPSC inducers and HIF expression in primary tumors, we analyzed primary prostate tumors and found a significant correlation between NANOG-, OCT4-, and HIF1alpha-positive regions. Furthermore, NANOG and OCT4 expressions positively correlated with increased prostate tumor Gleason score. In primary glioma-derived CD133 negative cells, hypoxia was able to induce neurospheres and hESC markers. Together, these findings suggest that HIF targets may act as key inducers of a dynamic state of stemness in pathologic conditions.

Merlos-Suarez, A., F. M. Barriga, et al. "The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse." Cell Stem Cell **8**(5): 511-24.
A frequent complication in colorectal cancer (CRC) is regeneration of the tumor after therapy. Here, we report that a gene signature specific for adult intestinal stem cells (ISCs) predicts disease relapse in CRC patients. ISCs are marked by high expression of the EphB2 receptor, which becomes gradually silenced as cells differentiate. Using EphB2 and the ISC marker Lgr5, we have FACS-purified and profiled mouse ISCs, crypt proliferative progenitors, and late transient amplifying cells to define a gene program specific for normal ISCs. Furthermore, we discovered that ISC-specific genes identify a stem-like cell population positioned at the bottom of tumor structures reminiscent of crypts. EphB2 sorted ISC-like tumor cells display robust tumor-initiating capacity in immunodeficient mice as well as long-term self-renewal potential. Taken together, our data suggest that the ISC program defines a cancer stem cell niche within colorectal tumors and plays a central role in CRC relapse.

Moore, N. and S. Lyle "Quiescent, slow-cycling stem cell populations in cancer: a review of the evidence and discussion of significance." J Oncol **2011**.
Long-lived cancer stem cells (CSCs) with indefinite proliferative potential have been identified in multiple epithelial cancer types. These cells are likely derived from transformed adult stem cells and are thought to share many characteristics with their parental population, including a quiescent slow-cycling phenotype. Various label-retaining techniques have been used to identify normal slow cycling adult stem cell populations and offer a unique methodology to functionally identify and isolate cancer stem cells. The quiescent nature of CSCs represents an inherent mechanism that at least partially explains chemotherapy resistance and recurrence in posttherapy cancer patients. Isolating and understanding the cell cycle regulatory mechanisms of quiescent cancer cells will be a key component to creation of future therapies that better target CSCs and totally eradicate tumors. Here we review the evidence for quiescent CSC populations and explore potential cell cycle regulators that may serve as future targets for elimination of these cells.

Morrison, R., S. M. Schleicher, et al. "Targeting the mechanisms of resistance to chemotherapy and radiotherapy with the cancer stem cell hypothesis." J Oncol **2011**: 941876.
Despite advances in treatment, cancer remains the 2nd most common cause of death in the United States. Poor cure rates may result from the ability of cancer to recur and spread after initial therapies have seemingly eliminated detectable signs of disease. A growing body of evidence supports a role for cancer stem cells (CSCs) in tumor regrowth and spread after initial treatment. Thus, targeting CSCs in combination with traditional induction therapies may improve treatment outcomes and survival rates. Unfortunately, CSCs tend to be resistant to chemo- and radiation therapy, and a better understanding of the mechanisms underlying CSC resistance to treatment is necessary. This paper provides an update on evidence that supports a fundamental role for CSCs in cancer progression, summarizes potential mechanisms of CSC resistance to treatment, and discusses classes of

drugs currently in preclinical or clinical testing that show promise at targeting CSCs.

Morton, C. I., L. Hlatky, et al. "Non-stem cancer cell kinetics modulate solid tumor progression." Theor Biol Med Model **8**: 48.
BACKGROUND: Solid tumors are heterogeneous in composition. Cancer stem cells (CSCs) are believed to drive tumor progression, but the relative frequencies of CSCs versus non-stem cancer cells span wide ranges even within tumors arising from the same tissue type. Tumor growth kinetics and composition can be studied through an agent-based cellular automaton model using minimal sets of biological assumptions and parameters. Herein we describe a pivotal role for the generational life span of non-stem cancer cells in modulating solid tumor progression in silico. RESULTS: We demonstrate that although CSCs are necessary for progression, their expansion and consequently tumor growth kinetics are surprisingly modulated by the dynamics of the non-stem cancer cells. Simulations reveal that slight variations in non-stem cancer cell proliferative capacity can result in tumors with distinctly different growth kinetics. Longer generational life spans yield self-inhibited tumors, as the emerging population of non-stem cancer cells spatially impedes expansion of the CSC compartment. Conversely, shorter generational life spans yield persistence-limited tumors, with symmetric division frequency of CSCs determining tumor growth rate. We show that the CSC fraction of a tumor population can vary by multiple orders of magnitude as a function of the generational life span of the non-stem cancer cells. CONCLUSIONS: Our study suggests that variability in the growth rate and CSC content of solid tumors may be, in part, attributable to the proliferative capacity of the non-stem cancer cell population that arises during asymmetric division of CSCs. In our model, intermediate proliferative capacities give rise to the fastest-growing tumors, resulting in self-metastatic expansion driven by a balance between symmetric CSC division and expansion of the non-stem cancer population. Our results highlight the importance of non-stem cancer cell dynamics in the CSC hypothesis, and may offer a novel explanation for the large variations in CSC fractions reported in vivo.

Nagata, T., C. Sakakura, et al. "Expression of cancer stem cell markers CD133 and CD44 in locoregional recurrence of rectal cancer." Anticancer Res **31**(2): 495-500.
BACKGROUND: Recent findings suggest that cells with surface CD markers include cancer stem cells (CSCs) which can produce a cancer cluster, and that the presence of CSCs may be linked with prognosis. CD133 and CD44 are among the most useful markers for identification of colorectal CSCs. MATERIALS AND METHODS: An immunohistological analysis of CD133 and CD44 was performed using tissue from cases shown to be locoregionally recurrent or non-recurrent clinico-pathologically. RESULTS: The CD133-positive rates were 38.7% and 59.23% in non-recurrent and recurrent cases, respectively, and the CD44-positive rates were 35.5% and 44.4%, respectively. Expression of the CD markers had no correlation with other clinicopathological factors. The prognosis of patients who were positive for both markers was significantly worse than that of other patients. CONCLUSION: These results suggest that detection of CD133 and CD44 can provide useful information for selection of treatment and performance of intensive follow-up of colorectal cancer.

Nemoto, Y., T. Maruo, et al. "Identification of cancer stem cells derived from a canine lung adenocarcinoma cell line." Vet Pathol **48**(5): 1029-34.
Accumulating evidence supporting the cancer stem cell (CSC) hypothesis is based on the finding that tumors contain a small population of self-renewing cells that generate differentiated progeny and thereby contribute to tumor heterogeneity. CSCs are reported to exist in several human cancers, yet only a few reports demonstrate the existence of CSCs in primary lung cancer in dogs. In this study, the authors established a cancer cell line derived from a canine primary lung adenocarcinoma and identified a side population (SP) of cells that displayed drug-resistant features. To confirm the characteristics of these SP cells, the authors investigated the tumorigenicity of the cells in vivo by using a nude mouse xenograft model. Only 100 SP cells were able to give rise to new tumors, giving a 10-fold enrichment over the main population (MP) of cells, suggesting that these cells have the cancer-initiating ability of CSCs. Further studies characterizing CSCs in canine lung adenocarcinoma might contribute to the elucidation of the mechanisms of tumorigenesis and to the establishment of novel therapeutic strategies.

Nian, W. Q., F. L. Chen, et al. "CXCR4 positive cells from Lewis lung carcinoma cell line have cancer metastatic stem cell characteristics." Mol Cell Biochem **355**(1-2): 241-8.
There is increasing evidence that cancer stem cells contribute to the initiation and propagation of many tumor. Therefore, to find out and identify the metastatic tumor stem-like cells in Lewis lung cancer cell line (LLC), the expression of CXCR4 was measured in LLC by flow cytometry and observed by

laser scanning confocal microscope (LSCM). After the CXCR4(+) LLC cell was isolated from LLC by magnetic cell sorting, its properties were evaluated by their tumorigenic and metastatic potentials. CXCR4(+) cells were counted for 0.18% of the total number of LLC, and immunofluorescent staining cells were identified by LSCM. CXCR4(+) LLC suspension cultured in a serum-free medium, cell spheres expressed a high level of Sca-1. The chemotherapy sensitivity to cisplatin of CXCR4(+) LLC was lower than that of CXCR4(-) LLC. The expression of ABCG2 and IGF1R mRNA in CXCR4(+) LLC was higher than that in CXCR4(-) LLC ($P < 0.01$). Most of CXCR4(+) LLC cells were close to vascular endothelial cells, aberrant vasculature around it was forming. The expression of VEGF and MMP9 mRNA in CXCR4(+) LLC was higher than that in CXCR4(-) LLC ($P < 0.05$), the microvessel density (MVD) of CXCR4(+) subsets growing were higher than that of CXCR4(-) subsets growing tumor tissue ($P < 0.01$). The tumor size, volume, and metastatic foci in the lungs of CXCR4(+) LLC was significantly higher than that in CXCR4(-) LLC ($P < 0.001$). Similarly, elevated expression of MMP9 and VEGF was also positively associated with CXCR4(+) LLC. Our results demonstrated that CXCR4(+) cells from Lewis lung carcinoma cell line exhibit cancer metastatic stem cell characteristics.

Nomura, M., T. Fukuda, et al. "Preferential expression of potential markers for cancer stem cells in large cell neuroendocrine carcinoma of the lung. An FFPE proteomic study." J Clin Bioinforma **1**(1): 23.
BACKGROUND: Large cell neuroendocrine carcinoma (LCNEC) of the lung, a subtype of large cell carcinoma (LCC), is characterized by neuroendocrine differentiation that small cell lung carcinoma (SCLC) shares. Pre-therapeutic histological distinction between LCNEC and SCLC has so far been problematic, leading to adverse clinical outcome. We started a project establishing protein targets characteristic of LCNEC with a proteomic method using formalin fixed paraffin-embedded (FFPE) tissues, which will help make diagnosis convincing. METHODS: Cancer cells were collected by laser microdissection from cancer foci in FFPE tissues of LCNEC (n = 4), SCLC (n = 5), and LCC (n = 5) with definite histological diagnosis. Proteins were extracted from the harvested sections, trypsin-digested, and subjected to HPLC/mass spectrometry. Proteins identified by database search were semi-quantified by spectral counting and statistically sorted by pair-wise G-statistics. The results were immunohistochemically verified using a total of 10 cases for each group to confirm proteomic results. RESULTS: A total of 1981 proteins identified from the three cancer groups were subjected to pair-wise G-test under $p < 0.05$ and specificity of a protein's expression to LCNEC was checked using a 3D plot with the coordinates comprising G-statistic values for every two group comparisons. We identified four protein candidates preferentially expressed in LCNEC compared with SCLC with convincingly low p-values: aldehyde dehydrogenase 1 family member A1 (AL1A1) ($p = 6.1 \times 10\text{-}4$), aldo-keto reductase family 1 members C1 (AK1C1) ($p = 9.6\text{x}10\text{-}10$) and C3 (AK1C3) ($p = 3.9\text{x}10\text{-}10$) and CD44 antigen ($p = 0.021$). These p-values were confirmed by non-parametric exact inference tests. Interestingly, all these candidates would belong to cancer stem cell markers. Immunohistochmistry supported proteomic results. CONCLUSIONS: These results suggest that candidate biomarkers of LCNEC were related to cancer stem cells and this proteomic approach via FFPE samples was effective to detect them.

Norde, W. J., F. Maas, et al. "PD-1/PD-L1 interactions contribute to functional T-cell impairment in patients who relapse with cancer after allogeneic stem cell transplantation." Cancer Res **71**(15): 5111-22.
Tumor relapses remain a serious problem after allogeneic stem cell transplantation (alloSCT), despite the long-term persistence of minor histocompatibility antigen (MiHA)-specific memory CD8(+) T cells specific for the tumor. We hypothesized that these memory T cells may lose their function over time in transplanted patients. Here, we offer functional and mechanistic support for this hypothesis, based on immune inhibition by programmed death-1 (PD-1) expressed on MiHA-specific CD8(+) T cells and the associated role of the PD-1 ligand PD-L1 on myeloid leukemia cells, especially under inflammatory conditions. PD-L1 was highly upregulated on immature human leukemic progenitor cells, whereas costimulatory molecules such as CD80 and CD86 were not expressed. Thus, immature leukemic progenitor cells seemed to evade the immune system by inhibiting T-cell function via the PD-1/PD-L1 pathway. Blocking PD-1 signaling using human antibodies led to elevated proliferation and IFN-gamma production of MiHA-specific T cells cocultured with PD-L1-expressing leukemia cells. Moreover, patients with relapsed leukemia after initial MiHA-specific T-cell responses displayed high PD-L1 expression on CD34(+) leukemia cells and increased PD-1 levels on MiHA-specific CD8(+) T cells. Importantly, blocking PD-1/PD-L1 interactions augment proliferation of MiHA-specific CD8(+) memory T cells from relapsed patients. Taken together, our findings indicate that the PD-1/PD-L pathway can be hijacked as an immune escape

mechanism in hematological malignancies. Furthermore, they suggest that blocking the PD-1 immune checkpoint offers an appealing immunotherapeutic strategy following alloSCT in patients with recurrent or relapsed disease.

Oh, P. S., V. B. Patel, et al. "Schlafen-3 decreases cancer stem cell marker expression and autocrine/juxtacrine signaling in FOLFOX-resistant colon cancer cells." Am J Physiol Gastrointest Liver Physiol **301**(2): G347-55.
We have previously demonstrated that expression of the novel gene schlafen-3 (Slfn-3) correlates with intestinal epithelial cell differentiation (Patel VB, Yu Y, Das JK, Patel BB, Majumdar AP. Biochem Biophys Res Commun 388: 752-756, 2009). The present investigation was undertaken to examine whether Slfn-3 plays a role in regulating differentiation of FOLFOX-resistant (5-fluorouracil + oxaliplatin) colon cancer cells that are highly enriched in cancer stem cells (CSCs). Transfection of Slfn-3 in FOLFOX-resistant colon cancer HCT-116 cells resulted in increase of alkaline phosphatase activity, a marker of intestinal differentiation. Additionally, Slfn-3 transfection resulted in reduction of mRNA and protein levels of the CSC markers CD44, CD133, CD166, and aldehyde dehydrogenase 1 in both FOLFOX-resistant HCT-116 and HT-29 cells. This was accompanied by decreased formation of tumorosphere/colonosphere (an in vitro model of tumor growth) in stem cell medium and inhibition of expression of the chemotherapeutic drug transporter protein ABCG2. Additionally, Slfn-3 transfection of FOLFOX-resistant HCT-116 and HT-29 cells reduced Hoechst 33342 dye exclusion. Finally, Slfn-3 transfection inhibited the expression of transforming growth factor-alpha in both FOLFOX-resistant colon cancer cells, but stimulated apoptosis in response to additional FOLFOX treatment. In summary, our data demonstrate that Slfn-3 expression inhibits multiple characteristics of CSC-enriched, FOLFOX-resistant colon cancer cells, including induction of differentiation and reduction in tumorosphere/colonosphere formation, drug transporter activity, and autocrine stimulation of proliferation. Thus Slfn-3 expression may render colon CSCs more susceptible to cancer chemotherapeutics.

Oliveira, L. R., J. P. Oliveira-Costa, et al. "Cancer stem cell immunophenotypes in oral squamous cell carcinoma." J Oral Pathol Med **40**(2): 135-42.
BACKGROUND: The presence of cancer stem cell (CSC) antigens can be evidenced in some human tumors by phenotypic analysis through immunostaining. This study aims to identify a putative CSC immunophenotype in oral squamous cell carcinoma (OSCC) and determine its influence on prognosis. METHODS: The following data were retrieved from 157 patents: age, gender, primary anatomic site, smoking and alcohol intake, recurrence, metastases, histologic classification, treatment, disease-free survival (DFS), and overall survival (OS). An immunohistochemical study for CD44 and CD24 was performed in a tissue microarray of 157 paraffin blocks of OSCCs. RESULTS: In univariate analysis, the immunostaining pattern showed significant influences in relation to OS for alcohol intake and treatment, as well as for the CD44(+) and CD44(-) /CD24(-) immunophenotypes. The multivariate test confirmed these associations. CONCLUSIONS: Based on our results, the CD44 immunostaining and the absence of immunoexpression of these two investigated markers can be used in combination with other clinicopathologic information to improve the assessment of prognosis in OSCC.

Olsson, E., G. Honeth, et al. "CD44 isoforms are heterogeneously expressed in breast cancer and correlate with tumor subtypes and cancer stem cell markers." BMC Cancer **11**: 418.
BACKGROUND: The CD44 cell adhesion molecule is aberrantly expressed in many breast tumors and has been implicated in the metastatic process as well as in the putative cancer stem cell (CSC) compartment. We aimed to investigate potential associations between alternatively spliced isoforms of CD44 and CSCs as well as to various breast cancer biomarkers and molecular subtypes. METHODS: We used q-RT-PCR and exon-exon spanning assays to analyze the expression of four alternatively spliced CD44 isoforms as well as the total expression of CD44 in 187 breast tumors and 13 cell lines. ALDH1 protein expression was determined by IHC on TMA. RESULTS: Breast cancer cell lines showed a heterogeneous expression pattern of the CD44 isoforms, which shifted considerably when cells were grown as mammospheres. Tumors characterized as positive for the CD44+/CD24- phenotype by immunohistochemistry were associated to all isoforms except the CD44 standard (CD44S) isoform, which lacks all variant exons. Conversely, tumors with strong expression of the CSC marker ALDH1 had elevated expression of CD44S. A high expression of the CD44v2-v10 isoform, which retain all variant exons, was correlated to positive steroid receptor status, low proliferation and luminal A subtype. The CD44v3-v10 isoform showed similar correlations, while high expression of CD44v8-v10 was correlated to positive EGFR, negative/low HER2 status and basal-like subtype. High expression of CD44S was associated with strong HER2 staining and also a

subgroup of basal-like tumors. Unsupervised hierarchical cluster analysis of CD44 isoform expression data divided tumors into four main clusters, which showed significant correlations to molecular subtypes and differences in 10-year overall survival. CONCLUSIONS: We demonstrate that individual CD44 isoforms can be associated to different breast cancer subtypes and clinical markers such as HER2, ER and PgR, which suggests involvement of CD44 splice variants in specific oncogenic signaling pathways. Efforts to link CD44 to CSCs and tumor progression should consider the expression of various CD44 isoforms.

O'Neill, I. D. "Concise review: transmissible animal tumors as models of the cancer stem-cell process." Stem Cells **29**(12): 1909-14.
Tasmanian devil facial tumor disease (DFTD) and canine transmissible venereal tumor (CTVT) are highly unusual cancers capable of being transmitted between animals as an allograft. The concept that these tumors represent a cancer stem-cell process has never been formally evaluated. For each, evidence of self-renewal is found in the natural history of these tumors in the wild, tumor initiation in recipient animals, and serial transplantation studies. Additional data for stem-cell-specific genes and markers in DFTD also exist. Although both tumor types manifest as undifferentiated cancers, immunocytohistochemistry supports a histiocytic phenotype for CTVT and a neural crest origin, possibly a Schwann-cell phenotype, for DFTD. In these data, differential expression of lineage markers is seen which may suggest some capacity for differentiation toward a heterogeneous variety of cell types. It is proposed that DFTD and CTVT may represent and may serve as models of the cancer stem-cell process, but formal investigation is required to clarify this. Appreciation of any such role may act as a stimulus to ongoing research in the pathology of DFTD and CTVT, including further characterization of their origin and phenotype and possible therapeutic approaches. Additionally, they may provide valuable models for future studies of their analogous human cancers, including any putative CSC component.

Ottinger, S., A. Kloppel, et al. "Targeting of pancreatic and prostate cancer stem cell characteristics by Crambe crambe marine sponge extract." Int J Cancer **130**(7): 1671-81.
Cancer stem cells (CSCs) are suggested as reason for resistance of tumors toward conventional tumor therapy including pancreatic and advanced prostate cancer. New therapeutic agents are urgently needed for targeting of CSCs. Marine sponges harbor novel and undefined compounds with antineoplastic activity but their potential to eliminate CSC characteristics is not examined so far. We collected 10 marine sponges and one freshwater sponge by diving at the seaside and prepared crude methanolic extracts. The effect to established pancreatic and prostate CSC lines was evaluated by analysis of apoptosis, cell cycle, side population, colony and spheroid formation, migratory potential in vitro and tumorigenicity in vivo. While each sponge extract at a 1:10 dilution efficiently diminished viability, Crambe crambe marine sponge extract (CR) still strongly reduced viability of tumor cells at a dilution of 1:1,000 but was less toxic to normal fibroblasts and endothelial cells. CR inhibited self-renewal capacity, apoptosis resistance, and proliferation even in gemcitabine-selected pancreatic cancer cells with acquired therapy resistance and enhanced CSC characteristics. CR pretreatment of tumor cells diminished tumorigenicity of gemcitabine-resistant tumor cells in mice and totally abolished tumor take upon combination with gemcitabine. Our data suggest that CR contains substances, which render standard cancer therapy more effective by targeting of CSC characteristics. Isolation of bioactive metabolites from CR and evaluation in mice are required for development of new CSC-specific chemotherapeutic drugs from a marine sponge.

Paranjape, A. N., T. Mandal, et al. "Introduction of SV40ER and hTERT into mammospheres generates breast cancer cells with stem cell properties." Oncogene **31**(15): 1896-909.
Emerging evidence suggests that cancers arise in stem/progenitor cells. Yet, the requirements for transformation of these primitive cells remains poorly understood. In this study, we have exploited the 'mammosphere' system that selects for primitive mammary stem/progenitor cells to explore their potential and requirements for transformation. Introduction of Simian Virus 40 Early Region and hTERT into mammosphere-derived cells led to the generation of NBLE, an immortalized mammary epithelial cell line. The NBLEs largely comprised of bi-potent progenitors with long-term self-renewal and multi-lineage differentiation potential. Clonal and karyotype analyses revealed the existence of heterogeneous population within NBLEs with varied proliferation, differentiation and sphere-forming potential. Significantly, injection of NBLEs into immunocompromised mice resulted in the generation of invasive ductal adenocarcinomas. Further, these cells harbored a sub-population of CD44(+)/CD24(-) fraction that alone had sphere- and tumor-initiating potential and resembled the breast cancer stem cell gene signature. Interestingly, prolonged in vitro culturing led to their further enrichment. The NBLE cells also showed increased expression of stemness

and epithelial to mesenchymal transition markers, deregulated self-renewal pathways, activated DNA-damage response and cancer-associated chromosomal aberrations-all of which are likely to have contributed to their tumorigenic transformation. Thus, unlike previous in vitro transformation studies that used adherent, more differentiated human mammary epithelial cells our study demonstrates that the mammosphere-derived, less-differentiated cells undergo tumorigenic conversion with only two genetic elements, without requiring oncogenic Ras. Moreover, the striking phenotypic and molecular resemblance of the NBLE-generated tumors with naturally arising breast adenocarcinomas supports the notion of a primitive breast cell as the origin for this subtype of breast cancer. Finally, the NBLEs represent a heterogeneous population of cells with striking plasticity, capable of differentiation, self-renewal and tumorigenicity, thus offering a unique model system to study the molecular mechanisms involved with these processes.

Park, S. Y., H. J. Kwon, et al. "Distinct patterns of promoter CpG island methylation of breast cancer subtypes are associated with stem cell phenotypes." Mod Pathol **25**(2): 185-96.
Although DNA methylation profiles in breast cancer have been connected to breast cancer molecular subtype, there have been no studies of the association of DNA methylation with stem cell phenotype. This study was designed to evaluate the promoter CpG island methylation of 15 genes in relation to breast cancer subtype, and to investigate whether the patterns of CpG island methylation in each subtype are associated with their cancer stem cell phenotype represented by CD44+/CD24- and ALDH1 expression. We performed MethyLight analysis of the methylation status of 15 promoter CpG island loci involved in breast cancer progression (APC, DLEC1, GRIN2B, GSTP1, HOXA1, HOXA10, IGF2, MT1G, RARB, RASSF1A, RUNX3, SCGB3A1, SFRP1, SFRP4, and TMEFF2) and determined cancer stem cell phenotype by CD44/CD24 and ALDH1 immunohistochemistry in 36 luminal A, 33 luminal B, 30 luminal-HER2, 40 HER2 enriched, and 40 basal-like subtypes of breast cancer. The number of CpG island loci methylated differed significantly between subtypes, and was highest in the luminal-HER2 subtype and lowest in the basal-like subtype. Methylation frequencies and levels in 12 of the 15 genes differed significantly between subtypes, and the basal-like subtype had significantly lower methylation frequencies and levels in nine of the genes than the other subtypes. CD44+/CD24- and ALDH1+ putative stem cell populations were most enriched in the basal-like subtype. Methylation of promoter CpG islands was significantly lower in CD44+/CD24-cell (+) tumors than in CD44+/CD24-cell (-) tumors, even within the basal-like subtype. ALDH1 (+) tumors were also less methylated than ALDH1 (-) tumors. Our findings showed that promoter CpG island methylation was different in relation to breast cancer subtype and stem cell phenotype of tumor, suggesting that breast cancers have distinct patterns of CpG island methylation according to molecular subtypes and these are associated with different stem cell phenotypes of the tumor.

Pellacani, D., R. J. Packer, et al. "Regulation of the stem cell marker CD133 is independent of promoter hypermethylation in human epithelial differentiation and cancer." Mol Cancer **10**: 94.
BACKGROUND: Epigenetic control is essential for maintenance of tissue hierarchy and correct differentiation. In cancer, this hierarchical structure is altered and epigenetic control deregulated, but the relationship between these two phenomena is still unclear. CD133 is a marker for adult stem cells in various tissues and tumour types. Stem cell specificity is maintained by tight regulation of CD133 expression at both transcriptional and post-translational levels. In this study we investigated the role of epigenetic regulation of CD133 in epithelial differentiation and cancer. METHODS: DNA methylation analysis of the CD133 promoter was done by pyrosequencing and methylation specific PCR; qRT-PCR was used to measure CD133 expression and chromatin structure was determined by ChIP. Cells were treated with DNA demethylating agents and HDAC inhibitors. All the experiments were carried out in both cell lines and primary samples. RESULTS: We found that CD133 expression is repressed by DNA methylation in the majority of prostate epithelial cell lines examined, where the promoter is heavily CpG hypermethylated, whereas in primary prostate cancer and benign prostatic hyperplasia, low levels of DNA methylation, accompanied by low levels of mRNA, were found. Moreover, differential methylation of CD133 was absent from both benign or malignant CD133+/alpha2beta1integrinhi prostate (stem) cells, when compared to CD133-/alpha2beta1integrinhi (transit amplifying) cells or CD133-/alpha2beta1integrinlow (basal committed) cells, selected from primary epithelial cultures. Condensed chromatin was associated with CD133 downregulation in all of the cell lines, and treatment with HDAC inhibitors resulted in CD133 re-expression in both cell lines and primary samples. CONCLUSIONS: CD133 is tightly regulated by DNA methylation only in cell lines, where promoter methylation and gene expression inversely correlate. This highlights the crucial choice of cell model

systems when studying epigenetic control in cancer biology and stem cell biology. Significantly, in both benign and malignant prostate primary tissues, regulation of CD133 is independent of DNA methylation, but is under the dynamic control of chromatin condensation. This indicates that CD133 expression is not altered in prostate cancer and it is consistent with an important role for CD133 in the maintenance of the hierarchical cell differentiation patterns in cancer.

Perkins, J. B., S. C. Goldstein, et al. "Phase I Study of Topotecan, Ifosfamide, and Etoposide (TIME) with autologous stem cell transplant in refractory cancer: pharmacokinetic and pharmacodynamic correlates." Clin Cancer Res **17**(24): 7743-53.
PURPOSE: To determine the maximum tolerated dose (MTD) of topotecan in combination with ifosfamide, mesna, and etoposide (TIME), followed by autologous hematopoietic cell transplant (HCT), in patients with chemotherapy-refractory malignancies. EXPERIMENTAL DESIGN: Patients were treated with (in mg/m(2)/d) ifosfamide 3,333, mesna 3,333, and topotecan 3.3 to 28.3 during days -8 through -6 and etoposide 500 (days -5 through -3) followed by HCT on day 0. Once MTD was defined, we expanded this dosing cohort to include patients with high-risk lymphoma due to activity seen during dose escalation. Topotecan pharmacokinetic analyses were carried out, and topoisomerase I levels and activity were measured. RESULTS: The topotecan MTD in this regimen was 64 mg/m(2) (21.3 mg/m(2)/d). Mucositis was dose limiting and correlated with topotecan dose level and area under the curve (AUC). Dose level was also correlated with length of hospitalization, number of days of parenteral nutrition, and neutrophil and platelet engraftment. Topotecan AUC was significantly correlated with time to platelet recovery. The baseline peripheral blood mononuclear cell topoisomerase I level was found to be a significant positive predictor for overall and progression-free survival. Topotecan AUC was positively correlated with dose level, with a trend toward decreasing clearance with increasing dose. CONCLUSION: Topotecan can be a useful drug in the high-dose setting given its activity in some malignancies when given in standard dose. Pharmacokinetic monitoring may be a valuable tool for optimizing the use of topotecan and to avoid toxicity seen with high-systemic exposures. Baseline topoisomerase I levels may have an important role in predicting topotecan efficacy.

Perona, R., B. D. Lopez-Ayllon, et al. "A role for cancer stem cells in drug resistance and metastasis in non-small-cell lung cancer." Clin Transl Oncol **13**(5): 289-93.
The cancer stem cell (CSC) theory is currently a very important field in cancer research. This theory states that tumours are organised in a hierarchical manner with a subpopulation of limited number called CSCs with the ability to self-renew and undergo asymmetrical divisions, giving rise to a differentiated progeny that represents most of the tumour populations. CSCs are metastatic and chemoresistant, two features that very likely contribute to the poor response of locally advanced lung cancer. CSCs have been identified in non-small-cell lung cancer cell lines as well as those from patient primary samples. A correlation has been found in terms of chemoresistance and bad prognosis in patient-derived samples enriched with CSCs, indicating that these cells are an important target for future therapy combinations. Therefore, understanding the biology and exploring cell markers and signalling pathways specific for CSCs of lung cancer may help in achieving progress in the treatment of the disease.

Pfeiffer, M. J., F. P. Smit, et al. "Steroidogenic enzymes and stem cell markers are upregulated during androgen deprivation in prostate cancer." Mol Med **17**(7-8): 657-64.
Considerable levels of testosterone and dihydrotestosterone (DHT) are found in prostate cancer (PCa) tissue after androgen deprivation therapy. Treatment of surviving cancer-initiating cells and the ability to metabolize steroids from precursors may be the keystones for the appearance of recurrent tumors. To study this hypothesis, we assessed the expression of several steroidogenic enzymes and stem cell markers in clinical PCa samples and cell cultures during androgen depletion. Gene expression profiles were determined by microarray or qRT-PCR. In addition, we measured cell viability and analyzed stem cell marker expression in DuCaP cells by immunocytochemistry. Seventy patient samples from different stages of PCa, and the PCa cell line DuCaP were included in this study. The androgen receptor (AR) and enzymes (AKR1C3, HSD17B2, HSD17B3, UGT2B15 and UGT2B17) that are involved in the metabolism of adrenal steroids were upregulated in castration resistant prostate cancer (CRPC). In vitro, some DuCaP cells survived androgen depletion, and eventually gave rise to a culture adapted to these conditions. During and after this transition, most of the steroidogenic enzymes were upregulated. These cells also are enriched with stem/progenitor cell markers cytokeratin 5 (CK5) and ATP-binding cassette sub-family G member 2 (ABCG2). Similarly, putative stem/progenitor cell markers CK5, c-Kit, nestin, CD44, c-met, ALDH1A1, alpha2-integrin,

CD133, ABCG2, CXCR4 and POU5F1 were upregulated in clinical CRPC. The upregulation of steroidogenic enzymes and stem cell markers in recurrent tumors suggests that cancer initiating cells can expand by adaptation to their T/DHT deprived environment. Therapies targeting the metabolism of adrenal steroids by the tumor may prove effective in preventing tumor regrowth.

Piggott, L., N. Omidvar, et al. "Suppression of apoptosis inhibitor c-FLIP selectively eliminates breast cancer stem cell activity in response to the anti-cancer agent, TRAIL." Breast Cancer Res **13**(5): R88.
INTRODUCTION: It is postulated that breast cancer stem cells (bCSCs) mediate disease recurrence and drive formation of distant metastases - the principal cause of mortality in breast cancer patients. Therapeutic targeting of bCSCs, however, is hampered by their heterogeneity and resistance to existing therapeutics. In order to identify strategies to selectively remove bCSCs from breast cancers, irrespective of their clinical subtype, we sought an apoptosis mechanism that would target bCSCs yet would not kill normal cells. Suppression of the apoptosis inhibitor cellular FLICE-Like Inhibitory Protein (c-FLIP) partially sensitizes breast cancer cells to the anti-cancer agent Tumour Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL). Here we demonstrate in breast cancer cell lines that bCSCs are exquisitely sensitive to the de-repression of this pro-apoptotic pathway, resulting in a dramatic reduction in experimental metastases and the loss of bCSC self-renewal. METHODS: Suppression c-FLIP was performed by siRNA (FLIPi) in four breast cancer cell lines and by conditional gene-knockout in murine mammary glands. Sensitivity of these cells to TRAIL was determined by complementary cell apoptosis assays, including a novel heterotypic cell assay, while tumour-initiating potential of cancer stem cell subpopulations was determined by mammosphere cultures, aldefluor assay and in vivo transplantation. RESULTS: Genetic suppression of c-FLIP resulted in the partial sensitization of TRAIL-resistant cancer lines to the pro-apoptotic effects of TRAIL, irrespective of their cellular phenotype, yet normal mammary epithelial cells remained refractory to killing. While 10% to 30% of the cancer cell populations remained viable after TRAIL/FLIPi treatment, subsequent mammosphere and aldefluor assays demonstrated that this pro-apoptotic stimulus selectively targeted the functional bCSC pool, eliminating stem cell renewal. This culminated in an 80% reduction in primary tumours and a 98% reduction in metastases following transplantation. The recurrence of residual tumour initiating capacity was consistent with the observation that post-treated adherent cultures re-acquired bCSC-like properties in vitro. Importantly however this recurrent bCSC activity was attenuated following repeated TRAIL/FLIPi treatment. CONCLUSIONS: We describe an apoptotic mechanism that selectively and repeatedly removes bCSC activity from breast cancer cell lines and suggest that a combined TRAIL/FLIPi therapy could prevent metastatic disease progression in a broad range of breast cancer subtypes.

Ponnusamy, M. P., P. Seshacharyulu, et al. "MUC4 stabilizes HER2 expression and maintains the cancer stem cell population in ovarian cancer cells." J Ovarian Res **4**(1): 7.
BACKGROUND: Recent evidence has suggested that the capability of cancer to grow, propagate and relapse after therapy is dependent on a small subset of the cell population within the tumor, called cancer stem cells. Therefore, this subpopulation of cells needs to be targeted with different approaches by identification of unique stem-cell specific target antigens. One of the well known tumor antigens is the epithelial cell mucin MUC4, which is aberrantly expressed in ovarian cancer as compared to the normal ovary and plays a pivotal role in the aggressiveness and metastasis of ovarian cancer cells. In the present study, we aimed to analyze the cancer stem cell population in MUC4 overexpressed ovarian cancer cells. METHODS: MUC4 was ectopically overexpressed in SKOV3 ovarian cancer cells. Western blot analysis was performed for MUC4, HER2, CD133, ALDH1 and Shh expression in MUC4 overexpressed cells. Confocal analysis of MUC4, HER2 and CD133 was also done in the MUC4 overexpressed cells. CD133 and Hoechst33342 dye staining was used to analyze the cancer stem cell population via FACS method in SKOV3-MUC4 cells. RESULTS: MUC4 overexpressed SKOV3 cells showed an increased expression of HER2 compared to control cells. MUC4 overexpression leads to increased (0.1%) side population (SP) and CD133-positive cancer stem cells compared to the control cells. Interestingly, the tumor sphere type circular colony formation was observed only in the MUC4 overexpressed ovarian cancer cells. Furthermore, the cancer stem cell marker CD133 was expressed along with MUC4 in the isolated circular colonies as analyzed by both confocal and western blot analysis. HER2 and cancer stem cell specific marker ALDH1 along with Shh, a self-renewal marker, showed increased expression in the isolated circular colonies compared to MUC4-transfected cells. CONCLUSION: These studies demonstrate that MUC4 overexpression leads to an enriched ovarian cancer stem cell population either directly or indirectly through HER2. In future, this study would

be helpful for MUC4-directed therapy for the ovarian cancer stem cell population.

Qiao, L. and Y. Feng "Genetic variations of prostate stem cell antigen (PSCA) contribute to the risk of gastric cancer for Eastern Asians: a meta-analysis based on 16792 individuals." Gene **493**(1): 83-91.
The associations between polymorphisms of prostate stem cell antigen (PSCA-rs2294008C>T and -rs2976392G>A) and gastric cancer (GC) risk for Eastern Asians have been commonly studied, but the results were conflicting. The aim of the present study was to further assess the associations by the method of meta-analysis. The databases of Medline, Embase and CNKI (up to May 25th, 2011) were retrieved to identify eligible case-control studies. Odds ratio (OR) and 95% confidence interval (95%CI) were used to present the strength of the associations. In total, eight case-control studies in seven articles with 16792 individuals (9738 cases of GC and 7054 controls) were included in this meta-analysis. Through quantitative analyses, we found that T allele of rs2294008C>T and A allele of rs2976392G>A were significantly associated with increased GC risk [rs2294008C>T: OR (95%CI)=1.31 (1.22-1.42), P(z-test)<0.001, P(heterogeneity)=0.166 for TT vs. C carriers; rs2976392G>A: OR (95%CI)=1.36(1.24-1.50), P(z-test)=0.015, P(heterogeneity)=0.111 for AA vs. G carriers]. The results of subgroup analyses (according to histopathology, countries and sources of controls) indicated that T allele of rs2294008C>T and A allele rs2976392G>A were associated with increased risk of both intestinal- and diffuse-type GC, and associated with increased risk of GC for Chinese, Japanese, Koreans, PCC and HCC/PHCC. Furthermore, T allele of rs2294008C>T was also associated with increased risk of cardia and non-cardia GC, and associated with increased risk of GC for males and females. Besides those, this meta-analysis also indicated that the interactions between T allele of rs2294008C>T and A allele of rs2976392G>A was associated with increased risk of GC (A-T vs. G-T: OR=1.16, 95%CI=1.06-1.27, P(z-test)=0.001, P(heterogeneity)=0.835). Although modest limitations and potential bias cannot be eliminated, this meta-analysis suggests that PSCA -rs2294008C>T and -rs2976392G>A are potential factors of GC development for Eastern Asians, and future work may incorporate these findings and evaluate these variants as potential markers for screening and early diagnosis of GC.

Rahman, M., L. Deleyrolle, et al. "The cancer stem cell hypothesis: failures and pitfalls." Neurosurgery **68**(2): 531-45; discussion 545.
Based on the clonal evolution model and the assumption that the vast majority of tumor cells are able to propagate and drive tumor growth, the goal of cancer treatment has traditionally been to kill all cancerous cells. This theory has been challenged recently by the cancer stem cell (CSC) hypothesis, that a rare population of tumor cells, with stem cell characteristics, is responsible for tumor growth, resistance, and recurrence. Evidence for putative CSCs has been described in blood, breast, lung, prostate, colon, liver, pancreas, and brain. This new hypothesis would propose that indiscriminate killing of cancer cells would not be as effective as selective targeting of the cells that are driving long-term growth (ie, the CSCs) and that treatment failure is often the result of CSCs escaping traditional therapies.The CSC hypothesis has gained a great deal of attention because of the identification of a new target that may be responsible for poor outcomes of many aggressive cancers, including malignant glioma. As attractive as this hypothesis sounds, especially when applied to tumors that respond poorly to current treatments, we will argue in this article that the proposal of a stemlike cell that initiates and drives solid tissue cancer growth and is responsible for therapeutic failure is far from proven. We will present the point of view that for most advanced solid tissue cancers such as glioblastoma multiforme, targeting a putative rare CSC population will have little effect on patient outcomes. This review will cover problems with the CSC hypothesis, including applicability of the hierarchical model, inconsistencies with xenotransplantation data, and nonspecificity of CSC markers.

Raof, N. A., B. M. Mooney, et al. "Bioengineering embryonic stem cell microenvironments for the study of breast cancer." Int J Mol Sci **12**(11): 7662-91.
Breast cancer is the most prevalent disease amongst women worldwide and metastasis is the main cause of death due to breast cancer. Metastatic breast cancer cells and embryonic stem (ES) cells display similar characteristics. However, unlike metastatic breast cancer cells, ES cells are nonmalignant. Furthermore, embryonic microenvironments have the potential to convert metastatic breast cancer cells into a less invasive phenotype. The creation of in vitro embryonic microenvironments will enable better understanding of ES cell-breast cancer cell interactions, help elucidate tumorigenesis, and lead to the restriction of breast cancer metastasis. In this article, we will present the characteristics of breast cancer cells and ES cells as well as their microenvironments, importance of embryonic microenvironments in inhibiting tumorigenesis, convergence of tumorigenic and embryonic signaling

pathways, and state of the art in bioengineering embryonic microenvironments for breast cancer research. Additionally, the potential application of bioengineered embryonic microenvironments for the prevention and treatment of invasive breast cancer will be discussed.

Raof, N. A., W. K. Raja, et al. "Bioengineering embryonic stem cell microenvironments for exploring inhibitory effects on metastatic breast cancer cells." Biomaterials **32**(17): 4130-9.
The recreation of an in vitro microenvironment to understand and manipulate the proliferation and migration of invasive breast cancer cells may allow one to put a halt to their metastasis capacity. Invasive cancer cells have been linked to embryonic stem (ES) cells as they possess certain similar characteristics and gene signatures. Embryonic microenvironments have the potential to reprogram cancer cells into a less invasive phenotype and help elucidate tumorigenesis and metastasis. In this study, we explored the feasibility of reconstructing embryonic microenvironments using mouse ES cells cultured in alginate hydrogel and investigated the interactions of ES cells and highly invasive breast cancer cells in 2D, 2&1/2D, and 3D cultures. Results showed that mouse ES cells inhibited the growth and tumor spheroid formation of breast cancer cells. The mouse ES cell microenvironment was further constructed and optimized in 3D alginate hydrogel microbeads, and co-cultured with breast cancer cells. Migration analysis displayed a significant reduction in the average velocity and trajectory of breast cancer cell locomotion compared to control, suggesting that bioengineered mouse ES cell microenvironments inhibited the proliferation and migration of breast cancer cells. This study may act as a platform to open up new options to understand and harness tumor cell plasticity and develop therapeutics for metastatic breast cancer.

Regala, R. P., V. Justilien, et al. "Matrix metalloproteinase-10 promotes Kras-mediated bronchio-alveolar stem cell expansion and lung cancer formation." PLoS One **6**(10): e26439.
Matrix metalloproteinase 10 (MMP-10; stromelysin 2) is a member of a large family of structurally related matrix metalloproteinases, many of which have been implicated in tumor progression, invasion and metastasis. We recently identified Mmp10 as a gene that is highly induced in tumor-initiating lung bronchioalveolar stem cells (BASCs) upon activation of oncogenic Kras in a mouse model of lung adenocarcinoma. However, the potential role of Mmp10 in lung tumorigenesis has not been addressed. Here, we demonstrate that Mmp10 is overexpressed in lung tumors induced by either the smoke carcinogen urethane or oncogenic Kras. In addition, we report a significant reduction in lung tumor number and size after urethane exposure or genetic activation of oncogenic Kras in Mmp10 null (Mmp10(-/-)) mice. This inhibitory effect is reflected in a defect in the ability of Mmp10-deficient BASCs to expand and undergo transformation in response to urethane or oncogenic Kras in vivo and in vitro, demonstrating a role for Mmp10 in the tumor-initiating activity of Kras-transformed lung stem cells. To determine the potential relevance of MMP10 in human cancer we analyzed Mmp10 expression in publicly-available gene expression profiles of human cancers. Our analysis reveals that MMP10 is highly overexpressed in human lung tumors. Gene set enhancement analysis (GSEA) demonstrates that elevated MMP10 expression correlates with both cancer stem cell and tumor metastasis genomic signatures in human lung cancer. Finally, Mmp10 is elevated in many human tumor types suggesting a widespread role for Mmp10 in human malignancy. We conclude that Mmp10 plays an important role in lung tumor initiation via maintenance of a highly tumorigenic, cancer-initiating, stem-like cell population, and that Mmp10 expression is associated with stem-like, highly metastatic genotypes in human lung cancers. These results indicate that Mmp10 may represent a novel therapeutic approach to target lung cancer stem cells.

Reuben, J. M., B. N. Lee, et al. "Primary breast cancer patients with high risk clinicopathologic features have high percentages of bone marrow epithelial cells with ALDH activity and CD44(+)CD24lo cancer stem cell phenotype." Eur J Cancer **47**(10): 1527-36.
BACKGROUND: Cancer stem cells (CSCs) are purported to be epithelial tumour cells expressing CD44(+)CD24(lo) that exhibit aldehyde dehydrogenase activity (Aldefluor(+)). We hypothesised that if CSCs are responsible for tumour dissemination, disseminated cells in the bone marrow (BM) would be positive for putative breast CSC markers. Therefore, we assessed the presence of Aldefluor(+) epithelial (CD326(+)CD45(dim)) cells for the presence of the CD44(+)CD24(lo) phenotype in BM of patients with primary breast cancer (PBC). METHODS: BM aspirates were collected at the time of surgery from 66 patients with PBC. Thirty patients received neoadjuvant chemotherapy (NACT) prior to aspiration. BM was analysed for Aldefluor(+) epithelial cells with or without CD44(+)CD24(lo) expression by flow cytometry. BM aspirates from three healthy donors (HD) were subjected to identical processing and analyses and served as controls. RESULTS: Patients with triple-receptor-negative (TN) tumours had a significantly higher median

percentage of CD44(+)CD24(lo) CSC within Aldefluor(+) epithelial cell population than patients with other immunohistochemical subtypes (P=0.018). Patients with TN tumours or with pN2 or higher pathologic nodal status were more likely to have a proportion of CD44(+)CD24(lo) CSC within Aldefluor(+) epithelial cell population above the highest level of HD. Furthermore, patients who received NACT were more likely to have percentages of Aldefluor(+) epithelial cells than the highest level of HD (P=0.004). CONCLUSION: The percentage of CD44(+)CD24(lo) CSC in the BM is higher in PBC patients with high risk tumour features. The selection or enrichment of Aldefluor(+) epithelial cells by NACT may represent an opportunity to target these cells with novel therapies.

Rhim, J. S., H. Li, et al. "Novel human prostate epithelial cell culture models for the study of carcinogenesis and of normal stem cells and cancer stem cells." Adv Exp Med Biol **720**: 71-80.
Research into the mechanisms of prostate cancer progression has been limited by the lack of suitable in vitro systems. A hurdle in understanding the molecular genetic changes in prostate cancer has been the difficulty in establishing premalignant lesions and primary prostate tumors as in vitro cell cultures. Primary prostate epithelial cells grow for a finite life span and then senesce. Immortalization is defined by continuous growth of otherwise senescing cells and is believed to represent an early stage in tumor progression. To examine these early stages, we and others have developed in vitro models of prostate epithelial cell immortalization. Generation of primary human prostate epithelial (HPE) cells has been achieved using the serum-free condition. Retrovirus containing human telomerase reverse transcriptase (hTERT) was successfully used for the immortalization of primary HPE cells. Putative stem cell markers CD133 and CXCR4 were further identified in hTERT-immortalized primary nonmalignant and malignant tumor-derived HPE lines. In addition, an hTERT-immortalized nonmalignant HPE cell were found to retain the properties of multipotent stem cells. These in vitro prostate cell culture models should be useful for the study of carcinogenesis and of normal and cancer stem cells. Prostate cancer is the most common male cancer in the Western World and second leading cause of male cancer death in the United States [1]. The therapy most widely used against advanced disease is androgen ablation and, initially, it almost always produces objective clinical responses. However, most patients eventually relapse with ablation-resistant prostate cancer and develop metastatic disease; currently, there is no treatment that will cure progressive hormone-refractory metastatic prostate cancer. The mechanisms of progression of prostate cancer have been extensively studied, yet are poorly understood. One of the concepts that has been evolved is that cancer arises from the neoplastic transformation of normal prostate epithelial stem cells or transit amplifying cells. Understanding normal stem cells and cancer stem cells (CSCs) may provide insight into the origin of and new therapeutics for prostate cancer. However, research in this field is limited by the lack of suitable in vitro systems.

Ricardo, S., A. F. Vieira, et al. "Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype." J Clin Pathol **64**(11): 937-46.
BACKGROUND AND AIM: The study of CD44/CD24 and ALDH1 expression is the most accurate method to identify cancer stem cells (CSC) from breast cancer populations. However, the overlap between CD44(+)CD24(-/low) and ALDH1(high) CSC phenotypes in breast cancer seems to be very small, as well as their distribution among intrinsic breast cancer subtypes. Due to this discrepancy, it is imperative to improve the understanding of breast CSC marker distribution. METHODS: 466 invasive breast carcinomas and eight breast cancer cell lines were analysed for the expression of CD44, CD24 and ALDH1, to evaluate their distribution among the distinct molecular subtypes. RESULTS: Basal-like tumours (76.5%) contained the higher percentage of cells with the CSC phenotype CD44(+)CD24(-/low) ($p<0.0001$). From ALDH1-positive cases, 39.4% were also basal-like tumours ($p<0.0001$). The analysis of breast cancer cell lines indicated that luminal cell lines are mainly enriched in a CD44(-/low)CD24(+) cell population, basal/mesenchymal breast cancer cell lines are enriched in the CD44(+)CD24(-/low) phenotype, whereas the remaining basal/epithelial cell lines are mainly positive for both markers. ALDH1 activity was mainly found in HER-OE and basal/epithelial breast cancer cell. CONCLUSIONS: CD44(+)CD24(-/low) and ALDH1(+) phenotypes seem to identify CSC with distinct levels of differentiation. It seems that the paramount method and biomarkers that identify breast CSC within the distinct molecular subtypes need to be better explored, because it is pivotal to translate the CSC concept to clinical practice. In the future, the recognition of reliable markers to distinguish the CSC pool in each molecular subtype will be decisive for the development of specific target therapies.

Richardson, R. B. "Stem cell niches and other factors that influence the sensitivity of bone marrow to

radiation-induced bone cancer and leukaemia in children and adults." Int J Radiat Biol **87**(4): 343-59.
PURPOSE: This paper reviews and reassesses the internationally accepted niches or 'targets' in bone marrow that are sensitive to the induction of leukaemia and primary bone cancer by radiation. CONCLUSIONS: The hypoxic conditions of the 10 mum thick endosteal/osteoblastic niche where preleukemic stem cells and hematopoietic stem cells (HSC) reside provides a radioprotective microenvironment that is 2- to 3-fold less radiosensitive than vascular niches. This supports partitioning the whole marrow target between the low haematological cancer risk of irradiating HSC in the endosteum and the vascular niches within central marrow. There is a greater risk of induced bone cancer when irradiating a 50 mum thick peripheral marrow adjacent to the remodelling/reforming portion of the trabecular bone surface, rather than marrow next to the quiescent bone surface. This choice of partitioned bone cancer target is substantiated by the greater radiosensitivity of: (i) Bone with high remodelling rates, (ii) the young, (iii) individuals with hypermetabolic benign diseases of bone, and (iv) the epidemiology of alpha-emitting exposures. Evidence is given to show that the absence of excess bone-cancer in atomic-bomb survivors may be partially related to the extremely low prevalence among Japanese of Paget's disease of bone. Radiation-induced fibrosis and the wound healing response may be implicated in not only radiogenic bone cancers but also leukaemia. A novel biological mechanism for adaptive response, and possibility of dynamic targets, is advocated whereby stem cells migrate from vascular niches to stress-mitigated, hypoxic niches.

Riggi, N., M. L. Suva, et al. "The cancer stem cell paradigm in Ewing's sarcoma: what can we learn about these rare cells from a rare tumor?" Expert Rev Anticancer Ther **11**(2): 143-5.

Rivera, C., S. Rivera, et al. "Lung cancer stem cell: new insights on experimental models and preclinical data." J Oncol **2011**: 549181.
Lung cancer remains the leading cause of cancer death. Understanding lung tumors physiopathology should provide opportunity to prevent tumor development or/and improve their therapeutic management. Cancer stem cell (CSC) theory refers to a subpopulation of cancer cells, also named tumor-initiating cells, that can drive cancer development. Cells presenting these characteristics have been identified and isolated from lung cancer. Exploring cell markers and signaling pathways specific to lung CSCs may lead to progress in therapy and improve the prognosis of patients with lung cancer. Continuous efforts in developing in vitro and in vivo models may yield reliable tools to better understand CSC abilities and to test new therapeutic targets. Preclinical data on putative CSC targets are emerging by now. These preliminary studies are critical for the next generation of lung cancer therapies.

Rizzo, S., J. M. Hersey, et al. "Ovarian cancer stem cell-like side populations are enriched following chemotherapy and overexpress EZH2." Mol Cancer Ther **10**(2): 325-35.
Platinum-based chemotherapy, with cytoreductive surgery, is the cornerstone of treatment of advanced ovarian cancer; however, acquired drug resistance is a major clinical obstacle. It has been proposed that subpopulations of tumor cells with stem cell-like properties, such as so-called side populations (SP) that overexpress ABC drug transporters, can sustain the growth of drug-resistant tumor cells, leading to tumor recurrence following chemotherapy. The histone methyltransferase EZH2 is a key component of the polycomb-repressive complex 2 required for maintenance of a stem cell state, and overexpression has been implicated in drug resistance and shorter survival of ovarian cancer patients. We observed higher percentage SP in ascites from patients that have relapsed following chemotherapy compared with chemonaive patients, consistent with selection for this subpopulation during platinum-based chemotherapy. Furthermore, ABCB1 (P-glycoprotein) and EZH2 are consistently overexpressed in SP compared with non-SP from patients' tumor cells. The siRNA knockdown of EZH2 leads to loss of SP in ovarian tumor models, reduced anchorage-independent growth, and reduced tumor growth in vivo. Together, these data support a key role for EZH2 in the maintenance of a drug-resistant, tumor-sustaining subpopulation of cells in ovarian cancers undergoing chemotherapy. As such, EZH2 is an important target for anticancer drug development.

Rothschild, S. I., A. Kappeler, et al. "The stem cell gene "inhibitor of differentiation 1" (ID1) is frequently expressed in non-small cell lung cancer." Lung Cancer **71**(3): 306-11.
AIMS: Inhibitor of differentiation 1 (ID1) plays a role in cellular differentiation, proliferation, angiogenesis and tumor invasion. As shown recently, ID1 is positively regulated by the tyrosine kinase SRC in lung carcinoma cell lines and with that appears as a potential new therapeutic target in non-small cell carcinoma (NSCLC). To substantiate this hypothesis we examined ID1, SRC and matrix metalloproteinase-9 (MMP-9) immunohistochemically in human NSCLC specimens. METHODS: From 61 consecutive patient tissue samples of a tumor tissue bank a one

core tissue microarray (TMA) was produced and whole slide tissue samples of preinvasive lesions used. The staining of commercial antibodies was assessed by the H-score. Statistical analyses based on Spearman's rank correlation coefficient. RESULTS: ID1 was expressed in the nucleus in 70% of squamous cell carcinomas and 50% of non-squamous cell carcinomas and in vascular endothelium of non-tumor tissue. Cytoplasmic staining was found in all samples for SRC and in 93% for MMP-9. ID1-positive tissue samples co-expressed SRC and MMP-9 in 94%. In non-squamous cell carcinomas, H-scores of ID1 and SRC correlated with each other (p=0.04). H-score of MMP-9 correlated with tumor grade (p=0.04). The carcinoma findings were reflected in preinvasive lesions. CONCLUSIONS: We describe for the first time the immunohistochemical expression of ID1 in the majority of NSCLC samples. The almost general co-expression of ID1, SRC and MMP-9 supports their cooperation in vivo and warrants further investigation of ID1 as a therapeutic target.

Russo, J. and I. H. Russo "The role of the basal stem cell of the human breast in normal development and cancer." Adv Exp Med Biol **720**: 121-34.
MCF-10F, an ERalpha negative human breast epithelial cell line derived from normal breast tissue, is able to form ductal structures in a tridimensional collagen matrix system. MCF-10F cells that are estrogen transformed (trMCF cells) progressively express phenotypes of in vitro cell transformation, including colony formation in agar methocel and loss of the ductulogenic capacity. Selection of these trMCF cells for invasiveness identified cells (bcMCF) that formed tumors in severe combined immunodeficient mice. The cell lines derived from those tumors (caMCF) were poorly differentiated ER, PR, and ERBB2 negative adenocarcinomas. These characteristics are similar to the human basal cell-like carcinomas. This in vitro-in vivo model demonstrates the importance of the basal cell type as a stem cell that reconstitutes the branching pattern of the breast and that is also target of a carcinogenic insult leading to transformation and cancer.

Ryu, H. S., J. Park do, et al. "Combination of epithelial-mesenchymal transition and cancer stem cell-like phenotypes has independent prognostic value in gastric cancer." Hum Pathol **43**(4): 520-8.
Epithelial-mesenchymal transition-related proteins have been suggested to interact with each other in various cancers and be associated with the aggressive behavior of cancer. To demonstrate the clinical significance of epithelial-mesenchymal transition and stem cell-like phenotypes in gastric cancer, we performed immunohistochemistry for 5 epithelial-mesenchymal transition-related proteins, including Snail-1, ZEB-1, E-cadherin, vimentin, and beta-catenin, and the gastric cancer stem cell marker CD44 in 276 consecutive primary gastric cancers and 54 matched lymph node metastases. Loss of E-cadherin expression and aberrant expression of vimentin were significantly associated with aggressive clinicopathologic features. The expression of epithelial-mesenchymal transition-related proteins was closely related to each other in gastric cancer. The known gastric cancer stem cell maker, CD44, was significantly associated with the protein expression of Snail-1, ZEB-1, and E-cadherin ($P < .05$). Univariate survival analysis was performed for the 6 proteins included in this study to find the best combination for predicting patient outcome. Protein expression of Snail-1, vimentin, E-cadherin, and CD44 resulted in the lowest P value using the Kaplan-Meier method ($P < .001$). This combination of proteins was significantly associated with advanced pT stage, lymph node metastasis, vascular invasion, and undifferentiated histologic type in a high-risk group ($P < .001$) and predicted disease-free survival independent of pTNM stage and histologic differentiation ($P = .029$). However, the acquired mesenchymal phenotype of gastric cancer cells at the primary site was restored to an epithelial phenotype in lymph node metastases. A combination of epithelial-mesenchymal transition and stem cell-like phenotypes is an important predictor of aggressive biologic behavior and has an independent prognostic value in predicting outcomes of primary gastric cancer.

Saito, S., K. Morita, et al. "Use of BAC array CGH for evaluation of chromosomal stability of clinically used human mesenchymal stem cells and of cancer cell lines." Hum Cell **24**(1): 2-8.
Array-based comparative genomic hybridization (aCGH) using bacterial artificial chromosomes (BAC) is a powerful method to analyze DNA copy number aberrations of the entire human genome. In fact, CGH and aCGH have revealed various DNA copy number aberrations in numerous cancer cells and cancer cell lines examined so far. In this report, BAC aCGH was applied to evaluate the stability or instability of cell lines. Established cell lines have greatly contributed to advancements in not only biology but also medical science. However, cell lines have serious problems, such as alteration of biological properties during long-term cultivation. Firstly, we investigated two cancer cell lines, HeLa and Caco-2. HeLa cells, established from a cervical cancer, showed significantly increased DNA copy number alterations with passage time. Caco-2 cells, established from a colon cancer, showed no remarkable differences under various culture conditions. These results indicate that BAC aCGH can

be used for the evaluation and validation of genomic stability of cultured cells. Secondly, BAC aCGH was applied to evaluate and validate the genomic stabilities of three patient's mesenchymal stem cells (MSCs), which were already used for their treatments. These three MSCs showed no significant differences in DNA copy number aberrations over their entire chromosomal regions. Therefore, BAC aCGH is highly recommended for use for a quality check of various cells before using them for any kind of biological investigation or clinical application.

Sala, N., X. Munoz, et al. "Prostate stem-cell antigen gene is associated with diffuse and intestinal gastric cancer in Caucasians: results from the EPIC-EURGAST study." Int J Cancer **130**(10): 2417-27.
A genome-wide study performed in a Japanese population identified a strong association between SNP rs2294008 (Met1Thr) in the Prostate Stem Cell Antigen gene (PSCA) and diffuse-type gastric cancer (GC). This association was validated in different Asian populations, and, very recently, a study has been published in Caucasians. In this study, we analyzed the association between PSCA variation and GC risk in Caucasians from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Six tagSNPs covering the PSCA gene region were genotyped in 411 incident gastric adenocarcinoma cases and 1530 matched controls from a nested case-control study in the EPIC cohort. Associations were analyzed by unconditional logistic regression, adjusting for age, sex and country. The T allele of rs2294008 in PSCA was found to be a highly significant risk factor for GC (per allele OR = 1.42, 95% CI: 1.23-1.66, p-value = 6.5 x 10(-6)), particularly of the noncardia-type (per allele OR = 1.47, 95% CI: 1.19-1.81, p-value = 3 x 10(-4)). At contrast with previous studies, no significant differences were observed between the diffuse (per allele OR = 1.54, 95% CI: 1.20-1.96, p-value = 5 x 10(-4)) and the intestinal (per allele OR = 1.52, 95% CI: 1.20-1.93, p-value = 5 x 10(-4)) GC histological subtypes. Although rs12155758 and rs9297976 were also found associated with GC, this association appeared to be due to linkage disequilibrium with rs2294008. Haplotype analysis did not provide additional information. These results confirm the association between variation in the promoter region of PSCA and GC risk in Caucasians and also indicate that the rs2294008 variant is a similar risk factor for both the diffuse and intestinal-types of GC.

Sarkar, D., B. Shields, et al. "BRACHYURY confers cancer stem cell characteristics on colorectal cancer cells." Int J Cancer **130**(2): 328-37.
Cancer stem cells (CSCs) are initiating cells in colorectal cancer (CRC). Colorectal tumours undergo epithelial to mesenchymal transition (EMT)-like processes at the invasive front, enabling invasion and metastasis, and recent studies have linked this process to the acquisition of stem cell-like properties. It is of fundamental importance to understand the molecular events leading to the establishment of cancer initiating cells and how these mechanisms relate to cellular transitions during tumourigenesis. We use an in vitro system to recapitulate changes in CRC cells at the invasive front (mesenchymal-like cells) and central mass (epithelial-like cells) of tumours. We show that the mesoderm inducer BRACHYURY is expressed in a subpopulation of CRC cells that resemble invasive front mesenchymal-like cells, where it acts to impose characteristics of CSCs in a fully reversible manner, suggesting reversible formation and modulation of such cells. BRACHYURY, itself regulated by the oncogene beta-catenin, influences NANOG and other 'stemness' markers including a panel of markers defining CRC-CSC whose presence has been linked to poor patient prognosis. Similar regulation of NANOG through BRACHYURY was observed in other cells lines, suggesting this might be a pathway common to cancer cells undergoing mesenchymal transition. We suggest that BRACHYURY may regulate NANOG in mesenchymal-like CRC cells to impose a 'plastic-state', allowing competence of cells to respond to signals prompting invasion or metastasis.

Sasaki, N., T. Ishii, et al. "Alpha-fetoprotein-producing pancreatic cancer cells possess cancer stem cell characteristics." Cancer Lett **308**(2): 152-61.
We aimed to demonstrate the existence of cancer stem cells in human pancreatic cancer, and to clarify that they are alpha-fetoprotein (AFP) producing cells. Six cell lines derived from human pancreatic cancers were examined, and AsPC-1 and PANC-1 were noted to express AFP. Single cell culture assays and xenotransplantation revealed that the AFP-producing cells had the capacity for self-renewal and differentiation, and that these cells were tumorigenic. Furthermore, they were resistant to anti-cancer agents. The ABCA12 transporter was expressed in the AFP-producing cells at a level more than twice as high as that in the non-AFP-producing cells. The AFP-producing cells were shown to be putative pancreatic cancer stem cells. Furthermore, the expression of ABCA12 appears to be associated with drug resistance.

Sathi, G. A., R. Tamamura, et al. "Analysis of immunoexpression of common cancer stem cell markers in ameloblastoma." Exp Ther Med **3**(3): 397-402.

Recent studies have established that, in benign tumors, a large number of cancer stem cells are present, which have great implications in tumor development. However, in ameloblastoma, a highly aggressive, locally invasive tumor with a high recurrence rate, whether or not cancer stem-like cells are present remains undetermined. Therefore, in this study we analyzed the protein expression of three candidate stem cell markers in ameloblastoma. Immunohistochemical staining for cancer stem cell (CSC) markers (CD133, CD44 and ABCG2) and for the proliferation marker Ki-67 was performed using 23 ameloblastoma cases. In all 23 samples, CD133, CD44 and ABCG2 were expressed. Nine (39.13%) cases showed high expression and 14 cases (60.87%) showed low expression for CD133. Twelve of the 23 cases (52.17%) showed high expression and 11 cases (47.83%) showed low expression for both CD44 and ABCG2, respectively. Ki-67 was mainly expressed in peripheral ameloblast-like cells, suggesting that these cells have a higher degree of differentiation and, therefore, are less likely to contain cancer stem-like cells. On the other hand, cells positive for CSC markers situated at the close proximity to peripheral cells were devoid of Ki-67 and may have the potential to be cancer stem-like cells. After analyzing the correlation between expression of three CSC markers with clinicopathological factors and Ki-67 expression, only CD44 expression was correlated with tumor recurrence (P=0.0391). In conclusion, this study showed various expression patterns of different types of cancer stem cell markers and the presence of candidate CSC-like cells in ameloblastoma, which are possibly involved in cell proliferation, tumor progression and recurrence.

Sayed, S. I., R. C. Dwivedi, et al. "Implications of understanding cancer stem cell (CSC) biology in head and neck squamous cell cancer." Oral Oncol **47**(4): 237-43.
Head and neck squamous cell cancer (HNSCC) is the sixth most common cancer in the world. Effective therapeutic modalities such as surgery, radiation, chemotherapy and combinations of each are used in the management of this disease. Efforts are ongoing throughout the world to improve early detection and prevention of HNSCCs. Often, treatment fails to obtain total cancer cure and this is more likely with advanced stage disease. In recent years it appears that one of the key determinants of treatment failure may be the presence of cancer stem cells (CSC) that 'escape' currently available therapies. CSCs form a minute portion of the total tumour burden but may play a disproportionately important role in determining outcomes. Molecular mechanisms which underlie the genesis of CSCs are yet not fully understood and their detection within the total tumour bulk remains a challenge. Specific markers like Aldehyde dehydrogenase 1 (ALDH1), CD44 and Bmi-1 have shown early promising results both in CSC detection and in guiding treatment protocols. CSCs have been shown to be relatively resistant to standard treatment modalities. It is hoped that developing robust in vitro and in vivo experimental models of CSCs might provide a means of devising more effective therapeutic strategies.

Scaffidi, P. and T. Misteli "In vitro generation of human cells with cancer stem cell properties." Nat Cell Biol **13**(9): 1051-61.
Cancer stem cells (CSCs) have been implicated in the maintenance and progression of several types of cancer. The origin and cellular properties of human CSCs are poorly characterized. Here we show that CSC-like cells can be generated in vitro by oncogenic reprogramming of human somatic cells during neoplastic transformation. We find that in vitro transformation confers stem-cell properties to primary differentiated fibroblasts, including the ability to self-renew and to differentiate along multiple lineages. Tumours induced by transformed fibroblasts are hierarchically organized, and the cells that act as CSCs to initiate and maintain tumour growth are marked by the stage-specific embryonic antigen SSEA-1. Heterogeneous lineages of cancer cells in the bulk of the tumour arise through differentiation of SSEA-1(+) fibroblasts, and differentiation is associated with loss of tumorigenic potential. These findings establish an experimental system to characterize cellular and molecular properties of human CSCs and demonstrate that somatic cells have the potential to de-differentiate and acquire properties of CSCs.

Sehl, M., H. Zhou, et al. "Extinction models for cancer stem cell therapy." Math Biosci **234**(2): 132-46.
Cells with stem cell-like properties are now viewed as initiating and sustaining many cancers. This suggests that cancer can be cured by driving these cancer stem cells to extinction. The problem with this strategy is that ordinary stem cells are apt to be killed in the process. This paper sets bounds on the killing differential (difference between death rates of cancer stem cells and normal stem cells) that must exist for the survival of an adequate number of normal stem cells. Our main tools are birth-death Markov chains in continuous time. In this framework, we investigate the extinction times of cancer stem cells and normal stem cells. Application of extreme value theory from mathematical statistics yields an accurate asymptotic distribution and corresponding moments for both

extinction times. We compare these distributions for the two cell populations as a function of the killing rates. Perhaps a more telling comparison involves the number of normal stem cells NH at the extinction time of the cancer stem cells. Conditioning on the asymptotic time to extinction of the cancer stem cells allows us to calculate the asymptotic mean and variance of NH. The full distribution of NH can be retrieved by the finite Fourier transform and, in some parameter regimes, by an eigenfunction expansion. Finally, we discuss the impact of quiescence (the resting state) on stem cell dynamics. Quiescence can act as a sanctuary for cancer stem cells and imperils the proposed therapy. We approach the complication of quiescence via multitype branching process models and stochastic simulation. Improvements to the tau-leaping method of stochastic simulation make it a versatile tool in this context. We conclude that the proposed therapy must target quiescent cancer stem cells as well as actively dividing cancer stem cells. The current cancer models demonstrate the virtue of attacking the same quantitative questions from a variety of modeling, mathematical, and computational perspectives.

Sellheyer, K. "Basal cell carcinoma: cell of origin, cancer stem cell hypothesis and stem cell markers." Br J Dermatol **164**(4): 696-711.
Cancer stem cells have recently been described in several high-grade neoplasms. It is still unclear if they also occur in cutaneous malignancies. Cancer stem cells are not identical with somatic stem cells. The presence of tumour stem cells in a neoplasm does not in itself equal that the tumour derives from a somatic stem cell. A cell originally lacking stem cell characteristics could also acquire those features during the course of carcinogenesis and then becomes the clonal founder cell of a tumour. Basal cell carcinoma (BCC) is the most common cutaneous malignancy. A plethora of various stem cell markers has been applied to study its cellular origin. Intriguingly, the anatomical origin of BCC is still uncertain. This review will discuss the various stem cell markers used in BCC and the cellular origin of this tumour, and touches briefly on the possibility of cancer stem cells in BCC. If BCC or other skin cancers harbour tumour stem cells, these cells could be specifically targeted, making use of specific cell surface molecules such as receptor proteins. Novel drugs directed against those receptor proteins could replace currently available shotgun approaches including imiquimod.

Shankar, S., D. Nall, et al. "Resveratrol inhibits pancreatic cancer stem cell characteristics in human and KrasG12D transgenic mice by inhibiting pluripotency maintaining factors and epithelial-mesenchymal transition." PLoS One **6**(1): e16530.
BACKGROUND: Cancer stem cells (CSCs) can proliferate and self-renew extensively due to their ability to express anti-apoptotic and drug resistant proteins, thus sustaining tumor growth. Therefore, the strategy to eradicate CSCs might have significant clinical implications. The objectives of this study were to examine the molecular mechanisms by which resveratrol inhibits stem cell characteristics of pancreatic CSCs derived from human primary tumors and Kras(G12D) transgenic mice. METHODOLOGY/PRINCIPAL FINDINGS: Human pancreatic CSCs (CD133(+)CD44(+)CD24(+)ESA(+)) are highly tumorigenic and form subcutaneous tumors in NOD/SCID mice. Human pancreatic CSCs expressing high levels of CD133, CD24, CD44, ESA, and aldehyde dehydrogenase also express significantly more Nanog, Oct-4, Notch1, MDR1 and ABCG2 than normal pancreatic tissues and primary pancreatic cancer cells. Similarly, CSCs from Kras(G12D) mice express significantly higher levels of Nanog and Oct-4 than pancreatic tissues from Pdx-Cre mice. Resveratrol inhibits the growth (size and weight) and development (PanIN lesions) of pancreatic cancer in Kras(G12D) mice. Resveratrol inhibits the self-renewal capacity of pancreatic CSCs derived from human primary tumors and Kras(G12D) mice. Resveratrol induces apoptosis by activating capase-3/7 and inhibiting the expression of Bcl-2 and XIAP in human CSCs. Resveratrol inhibits pluripotency maintaining factors (Nanog, Sox-2, c-Myc and Oct-4) and drug resistance gene ABCG2 in CSCs. Inhibition of Nanog by shRNA enhances the inhibitory effects of resveratrol on self-renewal capacity of CSCs. Finally, resveratrol inhibits CSC's migration and invasion and markers of epithelial-mesenchymal transition (Zeb-1, Slug and Snail). CONCLUSIONS/SIGNIFICANCE: These data suggest that resveratrol inhibits pancreatic cancer stem cell characteristics in human and Kras(G12D) transgenic mice by inhibiting pluripotency maintaining factors and epithelial-mesenchymal transition. In conclusion, resveratrol can be used for the management of pancreatic cancer.

Sharif, T., C. Auger, et al. "Selective proapoptotic activity of polyphenols from red wine on teratocarcinoma cell, a model of cancer stem-like cell." Invest New Drugs **29**(2): 239-47.
Cancer stem cells are expected to be responsible for tumor initiation and metastasis. These cells are therefore potential targets for innovative anticancer therapies. However, the absence of bona fide cancer stem cell lines is a real problem for the development of such approaches. Since teratocarcinoma cells are

totipotent stem cells with a high degree of malignancy, we used them as a model of cancer stem cells in order to evaluate the anticancer chemopreventive activity of red wine polyphenols (RWPs) and to determine the underlying cellular and molecular mechanisms. We therefore investigated the effects of RWPs on the embryonal carcinoma (EC) cell line P19 which was grown in the same culture conditions as the most appropriate normal cell line counterpart, the pluripotent embryonic fibroblast cell line NIH/3T3. The present study indicates that RWPs selectively inhibited the proliferation of P19 EC cells and induced G1 cell cycle arrest in a dose-dependent manner. Moreover, RWPs treatment specifically triggered apoptosis of P19 EC cells in association with a dramatic upregulation of the tumor suppressor gene p53 and caspase-3 activation. Our findings suggest that the chemopreventive activity of RWPs on tumor initiation and development is related to a growth inhibition and a p53-dependent induction of apoptosis in teratocarcinoma cells. In addition, this study also shows that the EC cell line is a convenient source for studying the responses of cancer stem cells to new potential anticancer agents.

Sharma, B. and R. K. Singh "Emerging candidates in breast cancer stem cell maintenance, therapy resistance and relapse." J Carcinog **10**: 36.
Therapy resistance is a major concern while treating breast cancer. Various mechanisms have been proposed, but so far nothing has been able to effectively address this problem. Accumulating evidences suggest that a subset of cancer cells provides survival benefits to the tumor and are responsible for therapy resistance and relapse of cancer. These so called the cancer stem cells, are known to be regulated by several pathways. Evidences shows that the tumor microenvironment plays a crucial role in maintaining the cancer stem cell pool. Signaling within the tumor is modulated by surrounding cells which secrete signals favoring tumor growth and metastasis. In breast cancer, the cancer stem cells have recently been reported to be influenced by tumor microenvironment via cytokines which act as chemoattractants for leukocytes. This review elucidates the emerging role of chemokine receptor and receptor activator of NFkappaB (RANK) ligand/RANK signaling pathways in mediating therapy resistance of breast cancer by maintaining the cancer stem cell pool.

Shats, I., M. L. Gatza, et al. "Using a stem cell-based signature to guide therapeutic selection in cancer." Cancer Res **71**(5): 1772-80.
Given the very substantial heterogeneity of most human cancers, it is likely that most cancer therapeutics will be active in only a small fraction of any population of patients. As such, the development of new therapeutics, coupled with methods to match a therapy with the individual patient, will be critical to achieving significant gains in disease outcome. One such opportunity is the use of expression signatures to identify key oncogenic phenotypes that can serve not only as biomarkers but also as a means of identifying therapeutic compounds that might specifically target these phenotypes. Given the potential importance of targeting tumors exhibiting a stem-like phenotype, we have developed an expression signature that reflects common biological aspects of various stem-like characteristics. The consensus stemness ranking (CSR) signature is upregulated in cancer stem cell-enriched samples at advanced tumor stages and is associated with poor prognosis in multiple cancer types. Using two independent computational approaches we utilized the CSR signature to identify clinically useful compounds that could target the CSR phenotype. In vitro assays confirmed selectivity of several predicted compounds including topoisomerase inhibitors and resveratrol towards breast cancer cell lines that exhibit a high-CSR phenotype. Importantly, the CSR signature could predict clinical response of breast cancer patients to a neoadjuvant regimen that included a CSR-specific agent. Collectively, these results suggest therapeutic opportunities to target the CSR phenotype in a relevant cohort of cancer patients.

Shigdar, S., J. Lin, et al. "RNA aptamer against a cancer stem cell marker epithelial cell adhesion molecule." Cancer Sci **102**(5): 991-8.
The lack of a specific targeting strategy against cancer stem cells in current cancer treatment regimens is at least partly responsible for life-threatening cytotoxicity for patients undergoing traditional chemotherapy. An effective cancer stem cell targeting system is urgently required for the next generation of cancer medicine. Epithelial cell adhesion molecule (EpCAM) is overexpressed in most solid cancers and it has recently been identified as a cancer stem cell marker. In this study, we isolated a 40-base RNA aptamer that binds to EpCAM from a random oligonucleotide library using systematic evolution of ligands by exponential enrichment. The aptamer was further truncated to 19 bases. This 19-nt RNA aptamer interacts specifically with a number of live human cancer cells derived from breast, colorectal, and gastric cancers that express EpCAM, but not with those not expressing EpCAM, as analyzed using flow cytometry and confocal microscopy. The binding affinity of the EpCAM RNA aptamer to human cancer cells is approximately 55 nM. Importantly, this EpCAM RNA aptamer is efficiently internalized after binding to cell surface EpCAM. To our knowledge,

this is the first RNA aptamer against a cancer stem cell surface marker being developed. Such cancer stem cell aptamers will greatly facilitate the development of novel targeted nanomedicine and molecular imaging agents for cancer theranostics.

Shiozawa, Y., E. A. Pedersen, et al. "Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow." J Clin Invest **121**(4): 1298-312.
HSC homing, quiescence, and self-renewal depend on the bone marrow HSC niche. A large proportion of solid tumor metastases are bone metastases, known to usurp HSC homing pathways to establish footholds in the bone marrow. However, it is not clear whether tumors target the HSC niche during metastasis. Here we have shown in a mouse model of metastasis that human prostate cancer (PCa) cells directly compete with HSCs for occupancy of the mouse HSC niche. Importantly, increasing the niche size promoted metastasis, whereas decreasing the niche size compromised dissemination. Furthermore, disseminated PCa cells could be mobilized out of the niche and back into the circulation using HSC mobilization protocols. Finally, once in the niche, tumor cells reduced HSC numbers by driving their terminal differentiation. These data provide what we believe to be the first evidence that the HSC niche serves as a direct target for PCa during dissemination and plays a central role in bone metastases. Our work may lead to better understanding of the molecular events involved in bone metastases and new therapeutic avenues for an incurable disease.

Singh, B. N., J. Fu, et al. "Hedgehog signaling antagonist GDC-0449 (Vismodegib) inhibits pancreatic cancer stem cell characteristics: molecular mechanisms." PLoS One **6**(11): e27306.
BACKGROUND: Recent evidence from in vitro and in vivo studies has demonstrated that aberrant reactivation of the Sonic Hedgehog (SHH) signaling pathway regulates genes that promote cellular proliferation in various human cancer stem cells (CSCs). Therefore, the chemotherapeutic agents that inhibit activation of Gli transcription factors have emerged as promising novel therapeutic drugs for pancreatic cancer. GDC-0449 (Vismodegib), orally administrable molecule belonging to the 2-arylpyridine class, inhibits SHH signaling pathway by blocking the activities of Smoothened. The objectives of this study were to examine the molecular mechanisms by which GDC-0449 regulates human pancreatic CSC characteristics in vitro. METHODOLOGY/PRINCIPAL FINDINGS: GDC-0499 inhibited cell viability and induced apoptosis in three pancreatic cancer cell lines and pancreatic CSCs. This inhibitor also suppressed cell viability, Gli-DNA binding and transcriptional activities, and induced apoptosis through caspase-3 activation and PARP cleavage in pancreatic CSCs. GDC-0449-induced apoptosis in CSCs showed increased Fas expression and decreased expression of PDGFRalpha. Furthermore, Bcl-2 was down-regulated whereas TRAIL-R1/DR4 and TRAIL-R2/DR5 expression was increased following the treatment of CSCs with GDC-0449. Suppression of both Gli1 plus Gli2 by shRNA mimicked the changes in cell viability, spheroid formation, apoptosis and gene expression observed in GDC-0449-treated pancreatic CSCs. Thus, activated Gli genes repress DRs and Fas expressions, up-regulate the expressions of Bcl-2 and PDGFRalpha and facilitate cell survival. CONCLUSIONS/SIGNIFICANCE: These data suggest that GDC-0499 can be used for the management of pancreatic cancer by targeting pancreatic CSCs.

Smith, B. H., L. S. Gazda, et al. "Three-dimensional culture of mouse renal carcinoma cells in agarose macrobeads selects for a subpopulation of cells with cancer stem cell or cancer progenitor properties." Cancer Res **71**(3): 716-24.
The culture of tumor cell lines in three-dimensional scaffolds is considered to more closely replicate the in vivo tumor microenvironment than the standard method of two-dimensional cell culture. We hypothesized that our method of encapsulating and maintaining viable and functional pancreatic islets in agarose-agarose macrobeads (diameter 6-8 mm) might provide a novel method for the culture of tumor cell lines. In this report we describe and characterize tumor colonies that form within macrobeads seeded with mouse renal adenocarcinoma cells. Approximately 1% of seeded tumor cells survive in the macrobead and over several months form discrete elliptical colonies appearing as tumor cell niches with increasing metabolic activity in parallel to colony size. The tumor colonies demonstrate ongoing cell turnover as shown by BrdU incorporation and activated caspase-3 and TUNEL staining. Genes upregulated in the tumor colonies of the macrobead are likely adaptations to this novel environment, as well as an amplification of G(1)/S cell-cycle checkpoints. The data presented, including SCA-1 and Oct4 positivity and the upregulation of stem cell-like genes such as those associated with the Wnt pathway, support the notion that the macrobead selects for a subpopulation of cells with cancer stem cell or cancer progenitor properties.

So, J. Y., H. J. Lee, et al. "A novel Gemini vitamin D analog represses the expression of a stem cell marker CD44 in breast cancer." Mol Pharmacol **79**(3): 360-7.
CD44 is a multifunctional transmembrane protein involved in cell proliferation, angiogenesis, invasion, and metastasis. CD44 is identified as a cancer stem cell marker, and the CD44-positive breast cancer cells are enriched in residual breast cancer cell populations after conventional therapies, suggesting that CD44 may be an important target for cancer prevention and therapy. Therefore, we investigated for the inhibitory effect of a novel Gemini vitamin D analog, 1alpha,25-dihydroxy-20R-21(3-hydroxy-3-deuteromethyl-4,4,4-trideuterobutyl)-23-yn e-26,27-hexafluoro-cholecalciferol (BXL0124), on mammary tumor growth and CD44 expression in MCF10DCIS.com human breast cancer in vitro and in vivo. MCF10DCIS.com cells were injected into mammary fat pads in immunodeficient mice, and BXL0124 was then administered intraperitoneally (0.1 mug/kg body weight) or orally (0.03 or 0.1 mug/kg body weight) 6 days a week for 5 weeks. At necropsy, mammary tumors and blood were collected for evaluating tumor growth, CD44 expression, and serum calcium level. BXL0124 suppressed mammary tumor growth and markedly decreased the expression of CD44 protein in MCF10DCIS xenograft tumors without causing hypercalcemic toxicity. BXL0124 also inhibited the expression of CD44 protein and mRNA as well as the transcriptional activity of the CD44 promoter in cultured MCF10DCIS.com cells. The repression of CD44 expression induced by BXL0124 was blocked by siRNA vitamin D receptor (VDR), indicating that the regulation of CD44 expression by BXL0124 is a VDR-dependent event. The novel Gemini vitamin D analog, BXL0124, represses CD44 expression in MCF10DCIS.com cells in vitro and in xenograft tumors, suggesting an inhibitory role of a Gemini vitamin D derivative on breast cancer stem cells.

Song, H. R., H. N. Kim, et al. "Association of a common genetic variant in prostate stem-cell antigen with gastric cancer susceptibility in a Korean population." Mol Carcinog **50**(11): 871-5.
A recent genome wide association study (GWAS) indentified a significant association between rs2294008 (C > T) polymorphism in prostate stem-cell antigen (PSCA) and increased risk of gastric cancer in Japanese and Korean populations. The aim of this study was to determine whether rs2294008 polymorphism is associated with risk of gastric cancer in a Korean population. We conducted a large-scale case-control study of 3,245 gastric cancer patients and 1,700 controls. The frequencies of the CC, CT, and TT genotypes of rs2294008 polymorphism were 17.8%, 49.9%, and 32.3% in the gastric cancer patients; and 24.4%, 48.1%, and 27.5% in the controls, respectively. We found that the CT and TT genotypes were associated with a significantly increased risk of gastric cancer (OR(CT) = 1.50, 95% confidence intervals, 95% CI: 1.28-1.76; OR(TT) = 1.71, 95% CI: 1.43-2.04), compared with the CC genotype. Further, stratified by tumor location and histological type, the effect of the rs2294008 T allele was larger in cardia (OR(TT) = 2.62, 95% CI = 1.42-4.85) than non-cardia (OR(TT) = 1.67, 95% CI = 1.40-2.00), in diffuse-type (OR(TT) = 2.00, 95% CI: 1.55-2.59) than in intestinal-type (OR(TT) = 1.51, 95% CI: 1.22-1.86). Our study showed that rs2294008 in the PSCA gene was associated with increased risks of gastric cancer in a Korean population, suggests that rs2294008 might play an important role in gastric carcinogenesis.

Spyra, M., L. Kluwe, et al. "Cancer stem cell-like cells derived from malignant peripheral nerve sheath tumors." PLoS One **6**(6): e21099.
This study aims to examine whether or not cancer stem cells exist in malignant peripheral nerve sheath tumors (MPNST). Cells of established lines, primary cultures and freshly dissected tumors were cultured in serum free conditions supplemented with epidermal and fibroblast growth factors. From one established human MPNST cell line, S462, cells meeting the criteria for cancer stem cells were isolated. Clonal spheres were obtained, which could be passaged multiple times. Enrichment of stem cell-like cells in these spheres was also supported by increased expression of stem cell markers such as CD133, Oct4, Nestin and NGFR, and decreased expression of mature cell markers such as CD90 and NCAM. Furthermore, cells of these clonal S462 spheres differentiated into Schwann cells, smooth muscle/fibroblast and neurons-like cells under specific differentiation-inducing cultural conditions. Finally, subcutaneous injection of the spheres into immunodeficient nude mice led to tumor formation at a higher rate compared to the parental adherent cells (66% versus 10% at 2.5 x 10(5)). These results provide evidence for the existence of cancer stem cell-like cells in malignant peripheral nerve sheath tumors.

Steg, A. D., K. S. Bevis, et al. "Stem cell pathways contribute to clinical chemoresistance in ovarian cancer." Clin Cancer Res **18**(3): 869-81.
PURPOSE: Within heterogeneous tumors, subpopulations often labeled cancer stem cells (CSC) have been identified that have enhanced tumorigenicity and chemoresistance in ex vivo models. However, whether these populations are more capable of surviving chemotherapy in de novo tumors is unknown. EXPERIMENTAL DESIGN: We

examined 45 matched primary/recurrent tumor pairs of high-grade ovarian adenocarcinomas for expression of CSC markers ALDH1A1, CD44, and CD133 using immunohistochemistry. Tumors collected immediately after completion of primary therapy were then laser capture microdissected and subjected to a quantitative PCR array examining stem cell biology pathways (Hedgehog, Notch, TGF-beta, and Wnt). Select genes of interest were validated as important targets using siRNA-mediated downregulation. RESULTS: Primary samples were composed of low densities of ALDH1A1, CD44, and CD133. Tumors collected immediately after primary therapy were more densely composed of each marker, whereas samples collected at first recurrence, before initiating secondary therapy, were composed of similar percentages of each marker as their primary tumor. In tumors collected from recurrent platinum-resistant patients, only CD133 was significantly increased. Of stem cell pathway members examined, 14% were significantly overexpressed in recurrent compared with matched primary tumors. Knockdown of genes of interest, including endoglin/CD105 and the hedgehog mediators Gli1 and Gli2, led to decreased ovarian cancer cell viability, with Gli2 showing a novel contribution to cisplatin resistance. CONCLUSIONS: These data indicate that ovarian tumors are enriched with CSCs and stem cell pathway mediators, especially at the completion of primary therapy. This suggests that stem cell subpopulations contribute to tumor chemoresistance and ultimately recurrent disease.

Stratford, E. W., R. Castro, et al. "Liposarcoma Cells with Aldefluor and CD133 Activity have a Cancer Stem Cell Potential." Clin Sarcoma Res **1**(1): 8.
Aldehyde dehydrogenase (ALDH) has recently been shown to be a marker of cancer stem-like cells (CSCs) across tumour types. The primary goals of this study were to investigate whether ALDH is expressed in liposarcomas, and whether CSCs can be identified in the ALDHhigh subpopulation. We have demonstrated that ALDH is indeed expressed in 10 out of 10 liposarcoma patient samples. Using a liposarcoma xenograft model, we have identified a small population of cells with an inducible stem cell potential, expressing both ALDH and CD133 following culturing in stem cell medium. This potential CSC population, which makes up for 0,1-1,7 % of the cells, displayed increased self-renewing abilities and increased tumourigenicity, giving tumours in vivo from as few as 100 injected cells.

Su, J., X. H. Xu, et al. "Identification of cancer stem-like CD44+ cells in human nasopharyngeal carcinoma cell line." Arch Med Res **42**(1): 15-21.
BACKGROUND AND AIMS: Recent studies suggest that cancer stem cells (CSC) may be responsible for tumorigenesis and contribute to some individuals' resistance to cancer therapy. Although research is rapidly advancing in this field, to our knowledge there are few published reports about the CSC in human nasopharyngeal carcinoma (NPC). We undertook this study to separate, expand, and explore the biological features of CD44+ stem-like cancer cells from the human NPC SUNE-1 5-8F cell line. METHODS: Immunocytochemistry and flow cytometry were used to detect the expression of CD44 in SUNE-1 5-8F. Fluorescence-activated cell sorting was applied to purify CD44+ cells. MTT assay or clone formation assay was used to detect the differences of CD44+ and CD44- cells in proliferation, differentiation, radiosensitivity and chemosensitivity in vitro. The expression of stem cell markers Oct-4 and Bmi-1 was examined by reverse transcriptase polymerase chain reaction (RT-PCR). RESULTS: CD44 was positively expressed in approximately 52.5% of NPC SUNE-1 5-8F cell line. Regardless of serum-free medium and serum medium culture conditions, freshly sorted CD44+ cells showed stronger proliferative capacity than CD44- and unsorted cells. The expression levels of Bmi-1 and Oct-4 mRNA in CD44+ cells were significantly higher than CD44- cells. After 2 Gy radiation, the average clone formation efficiency for CD44+ and CD44- cells was 22.17 +/- 6.65% and 11.50 +/- 5.00%, respectively ($p < 0.05$). After cisplatin and docetaxel treatment with the same drug concentration, CD44+ cells showed a higher survival rate compared with CD44- cells. CONCLUSIONS: CD44+ cells have the biological characteristics of tumor stem cell and may be assumed as one of the markers of NPC tumor stem cells.

Su, Y. J., H. M. Lai, et al. "Direct reprogramming of stem cell properties in colon cancer cells by CD44." Embo J **30**(15): 3186-99.
Cancer progression is commonly segregated into processes of primary tumour growth and secondary metastasis. Recent evidence suggests that a subpopulation of cancer cells, cancer stem cells (CSCs), is responsible for tumour growth in cancer. However, the role of CSCs in cancer metastasis is unclear. In this study, we found that the C terminus of CD44 contributes to sphere formation and survival in vitro via the CD44-SRC-integrin axis. In addition, nuclear CD44/acetylated-STAT3 is required for clonal formation in vitro and tumourigenicity in vivo. Nuclear CD44 binds to various promoters identified by chromatin immunoprecipitation-seq, including that of c-myc and Twist1, leading to cell fate change through transcriptional reprogramming. We propose that nuclear CD44/acetylated-STAT3 performs an

unexpected tumour-progressing function by enhancing cell outgrowth into structures where cells with properties of CSCs can be generated from differentiated somatic cells in suspension culture, and then exhibit attributes of cells that have undergone an epithelial-mesenchymal transition, leading to tumour metastasis, and a resulting worse prognosis.

Sun, S. and Z. Wang "Head neck squamous cell carcinoma c-Met(+) cells display cancer stem cell properties and are responsible for cisplatin-resistance and metastasis." Int J Cancer **129**(10): 2337-48.
c-Met, the tyrosine kinase receptor for hepatocyte growth factor, is overexpressed in a variety of tumors in which it plays a central role in malignant transformation. Although c-Met has also been determined to be a critical signaling molecule in normal stem cell function, the potential role of c-Met as a single marker for cancer stem cells (CSCs) has not been previously examined. In our study, we reported that human head neck squamous cell carcinoma (HNSCC) cells expressing c-Met were capable of self-renewal and of generating tumors that recapitulate the heterogeneity of the parental tumors, and isolation of HNSCC cells using a second marker CD44 could further enhance upon the in-vivo tumorigenicity. We also reported that c-Met(+) HNSCC cells could readily make spherical colonies in nonadherent culture conditions, in contrast, c-Met(-) population did not; these spherical colonies could be passaged multiple times without loss of colony-forming capability. Furthermore, we showed that c-Met(+) HNSCC cells have increased expression of self-renewal pathways are spared by cisplatin treatment and are responsible for mediating metastasis. These results indicated that c-Met could serve as a novel marker for CSCs at least in HNSCC, and the highly chemoresistant and metastatic capabilities of c-Met(+) HNSCC population make them an important cell type to better define and understand their function.

Sun, X. Y., J. Nong, et al. "Mesenchymal stem cell-mediated cancer therapy: A dual-targeted strategy of personalized medicine." World J Stem Cells **3**(11): 96-103.
Cancer remains one of the leading causes of mortality and morbidity throughout the world. To a significant extent, current conventional cancer therapies are symptomatic and passive in nature. The major obstacle to the development of effective cancer therapy is believed to be the absence of sufficient specificity. Since the discovery of the tumor-oriented homing capacity of mesenchymal stem cells (MSCs), the application of specific anticancer gene-engineered MSCs has held great potential for cancer therapies. The dual-targeted strategy is based on MSCs' capacity of tumor-directed migration and incorporation and in situ expression of tumor-specific anticancer genes. With the aim of translating bench work into meaningful clinical applications, we describe the tumor tropism of MSCs and their use as therapeutic vehicles, the dual-targeted anticancer potential of engineered MSCs and a putative personalized strategy with anticancer gene-engineered MSCs.

Syrjala, K. L., A. C. Stover, et al. "Development and implementation of an Internet-based survivorship care program for cancer survivors treated with hematopoietic stem cell transplantation." J Cancer Surviv **5**(3): 292-304.
INTRODUCTION: The Internet provides a widely accessible modality for meeting survivorship care needs of cancer survivors. In this paper, we describe the development and implementation of an Internet site designed as a base from which to conduct a randomized controlled trial to meet psycho-educational needs of hematopoietic stem cell transplantation (HSCT) survivors. METHODS: A cross-disciplinary team designed, wrote content, and programmed an Internet site for online study registration, consent, assessment, and study implementation. All survivors who were 3-18 years after HSCT for hematologic malignancy and treated at one transplant center were approached by mail for participation. All study activities could be conducted without study staff contact. However, participants had options for phone or email contact with study staff as desired. RESULTS: Of 1,775 participants approached for the study, 775 (58% of those eligible) consented and completed baseline assessment. Mean age was 51.7 (SD, 12.5; age range, 18-79 years), with 56% male. Fifty-seven percent required staff contact one or more times; a majority were for minor technical issues or delays in completion of enrollment or baseline assessment. DISCUSSIONS/CONCLUSIONS: This study demonstrated the potential for providing Internet-based survivorship care to long-term survivors of HSCT. Although building a survivorship Internet site requires a team with diverse expertise, once built, these resources can be implemented rapidly with large numbers of survivors. IMPLICATIONS FOR CANCER SURVIVORS: While Internet-based services will not meet all the needs of cancer survivors, this methodology represents an important modality for augmenting onsite clinical services as a method for meeting psycho-educational, information, and resource needs of cancer survivors.

Tang, A. L., S. J. Hauff, et al. "UM-SCC-104: a new human papillomavirus-16-positive cancer stem cell-

containing head and neck squamous cell carcinoma cell line." Head Neck **34**(10): 1480-91.
BACKGROUND: Few human papillomavirus (HPV)(+) head and neck squamous cell carcinoma (HNSCC) cell lines exist. We established University of Michigan-squamous cell carcinoma-104 (UM-SCC-104), a new HPV(+) HNSCC cell line from a recurrent oral cavity tumor, and characterized it for the presence of cancer stem cells (CSCs). METHODS: Tumor cells were tested for biomarker expression by immunohistology, and the presence of HPV was assessed by several methods. RESULTS: UM-SCC-104 has a unique genotype, contains HPV-16, and expresses E6/E7. Inoculation of aldehyde dehydrogenase (ALDH)(+) and ALDH(-) cells in an immunocompromised mouse resulted in tumor growth from the ALDH(+) cells after 6 weeks that recapitulated the histology of the primary, whereas ALDH(-) cells did not produce tumors. CONCLUSION: UM-SCC-104, a new HPV-16, CSC-containing HNSCC cell line will aid in studying recurrent HPV(+) tumors. The aggressive nature of this tumor is consistent with high uniform expression of epidermal growth factor receptor (EGFR) and a functionally significant proportion of ALDH(+) CSCs.

Tang, S. N., J. Fu, et al. "Inhibition of sonic hedgehog pathway and pluripotency maintaining factors regulate human pancreatic cancer stem cell characteristics." Int J Cancer **131**(1): 30-40.
Activation of the sonic hedgehog (SHh) pathway is required for the growth of numerous tissues and organs and recent evidence indicates that this pathway is often recruited to stimulate growth of cancer stem cells (CSCs) and to orchestrate the reprogramming of cancer cells via epithelial mesenchymal transition (EMT). The objectives of this study were to examine the molecular mechanisms by which (-)-epigallocatechin-3-gallate (EGCG), an active compound in green tea, inhibits self-renewal capacity of pancreatic CSCs and synergizes with quercetin, a major polyphenol and flavonoid commonly detected in many fruits and vegetables. Our data demonstrated that EGCG inhibited the expression of pluripotency maintaining transcription factors (Nanog, c-Myc and Oct-4) and self-renewal capacity of pancreatic CSCs. Inhibition of Nanog by shRNA enhanced the inhibitory effects of EGCG on self-renewal capacity of CSCs. EGCG inhibited cell proliferation and induced apoptosis by inhibiting the expression of Bcl-2 and XIAP and activating caspase-3. Interestingly, EGCG also inhibited the components of SHh pathway (smoothened, patched, Gli1 and Gli2) and Gli transcriptional activity. Furthermore, EGCG inhibited EMT by inhibiting the expression of Snail, Slug and ZEB1, and TCF/LEF transcriptional activity, which correlated with significantly reduced CSC's migration and invasion, suggesting the blockade of signaling involved in early metastasis. Furthermore, combination of quercetin with EGCG had synergistic inhibitory effects on self-renewal capacity of CSCs through attenuation of TCF/LEF and Gli activities. Since aberrant SHh signaling occurs in pancreatic tumorigenesis, therapeutics that target SHh pathway may improve the outcomes of patients with pancreatic cancer by targeting CSCs.

Tao, H. and Y. Zhu "Colorectal cancer stem cell: a potential therapeutic target." Clin Transl Oncol **13**(12): 833-8.
The discovery of cancer stem cells has improved our understanding of tumour occurrence and development. Colorectal cancer stem cells may be derived from mutations in normal intestinal epithelial stem cells. CD133+ and aldehyde dehydrogenase 1 (ALDH1)+ cells have strong tumorigenic capacities and may represent different subpopulations of colorectal cancer stem cells. Multiple signalling pathways, especially the Wnt pathway, are important in colorectal cancer occurrence and development, and maintaining the stemness of colorectal cancer stem cells. Identifying colorectal cancer stem cells and understanding the related signalling pathways are important for developing new targeted interventions for colorectal cancer.

Taylor, R. M., V. Severns, et al. "Prostate cancer targeting motifs: expression of alphanu beta3, neurotensin receptor 1, prostate specific membrane antigen, and prostate stem cell antigen in human prostate cancer cell lines and xenografts." Prostate **72**(5): 523-32.
BACKGROUND: Membrane receptors are frequent targets of cancer therapeutic and imaging agents. However, promising in vitro results often do not translate to in vivo clinical applications. To better understand this obstacle, we measured the expression differences in receptor signatures among several human prostate cancer cell lines and xenografts as a function of tumorigenicity. METHODS: Messenger RNA and protein expression levels for integrin alpha(nu) beta(3), neurotensin receptor 1 (NTSR1), prostate specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA) were measured in LNCaP, C4-2, and PC-3 human prostate cancer cell lines and in murine xenografts using quantitative reverse transcriptase polymerase chain reaction, flow cytometry, and immunohistochemistry. RESULTS: Stable expression patterns were observed for integrin alpha(nu) and PSMA in all cells and corresponding xenografts. Integrin beta(3) mRNA expression was greatly reduced in C4-2 xenografts and greatly

elevated in PC-3 xenografts compared with the corresponding cultured cells. NTSR1 mRNA expression was greatly elevated in LNCaP and PC-3 xenografts. PSCA mRNA expression was elevated in C4-2 xenografts when compared with C4-2 cells cultured in vitro. Furthermore, at the protein level, PSCA was re-expressed in all xenografts compared with cells in culture. CONCLUSIONS: The regulation of mRNA and protein expression of the cell-surface target proteins alpha(nu) beta(3), NTSR1, PSMA, and PSCA, in prostate cancer cells with different tumorigenic potential, was influenced by factors of the microenvironment, differing between cell cultures and murine xenotransplants. Integrin alpha(nu) beta(3), NTRS1 and PSCA mRNA expression increased with tumorigenic potential, but mRNA expression levels for these proteins do not translate directly to equivalent expression levels of membrane bound protein.

Tong, D. L., D. J. Boocock, et al. "A simpler method of preprocessing MALDI-TOF MS data for differential biomarker analysis: stem cell and melanoma cancer studies." Clin Proteomics **8**: 14.
INTRODUCTION: Raw spectral data from matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) with MS profiling techniques usually contains complex information not readily providing biological insight into disease. The association of identified features within raw data to a known peptide is extremely difficult. Data preprocessing to remove uncertainty characteristics in the data is normally required before performing any further analysis. This study proposes an alternative yet simple solution to preprocess raw MALDI-TOF-MS data for identification of candidate marker ions. Two in-house MALDI-TOF-MS data sets from two different sample sources (melanoma serum and cord blood plasma) are used in our study. METHOD: Raw MS spectral profiles were preprocessed using the proposed approach to identify peak regions in the spectra. The preprocessed data was then analysed using bespoke machine learning algorithms for data reduction and ion selection. Using the selected ions, an ANN-based predictive model was constructed to examine the predictive power of these ions for classification. RESULTS: Our model identified 10 candidate marker ions for both data sets. These ion panels achieved over 90% classification accuracy on blind validation data. Receiver operating characteristics analysis was performed and the area under the curve for melanoma and cord blood classifiers was 0.991 and 0.986, respectively. CONCLUSION: The results suggest that our data preprocessing technique removes unwanted characteristics of the raw data, while preserving the predictive components of the data. Ion identification analysis can be carried out using MALDI-TOF-MS data with the proposed data preprocessing technique coupled with bespoke algorithms for data reduction and ion selection.

Vainstein, V., O. U. Kirnasovsky, et al. "Strategies for cancer stem cell elimination: insights from mathematical modeling." J Theor Biol **298**: 32-41.
The cancer stem cell (CSC) hypothesis states that only a small fraction of a malignant cell population is responsible for tumor growth and relapse. Understanding the relationships between CSC dynamics and cancer progression may contribute to improvements in cancer treatment. Analysis of a simple discrete mathematical model has suggested that homeostasis in developing tissues is governed by a "quorum sensing" control mechanism, in which stem cells differentiate or proliferate according to feedback they receive from neighboring cell populations. Further analysis of the same model has indicated that excessive stem cell proliferation leading to malignant transformation mainly results from altered sensitivity to such micro-environmental signals. Our aim in this work is to expand the analysis to the dynamics of established populations of cancer cells and to examine possible therapeutic avenues for eliminating CSCs. The proposed model considers two populations of cells: CSCs, which can divide indefinitely, and differentiated cancer cells, which do not divide and have a limited lifespan. We assume that total cell density has negative feedback on CSC proliferation and that high CSC density activates CSC differentiation. We show that neither stimulation of CSC differentiation nor inhibition of CSC proliferation alone is sufficient for complete CSC elimination and cancer cure, since each of these two therapies affects a different subpopulation of CSCs. However, a combination of these two strategies can substantially reduce the population sizes and densities of all types of cancer cells. Therefore, we propose that in clinical trials, CSC differentiation therapy should only be examined in combination with chemotherapy. Our conclusions are corroborated by clinical experience with differentiating agents in acute promyelocytic leukemia and neuroblastoma.

van den Hoogen, C., G. van der Horst, et al. "Integrin alphav expression is required for the acquisition of a metastatic stem/progenitor cell phenotype in human prostate cancer." Am J Pathol **179**(5): 2559-68.
Integrins participate in multiple cellular processes, including cell adhesion, migration, proliferation, survival, and the activation of growth factor receptors. Recent studies have shown that expression of alphav integrins is elevated in the prostate cancer stem/progenitor cell subpopulation compared with

more differentiated, committed precursors. Here, we examine the functional role of alphav integrin receptor expression in the acquisition of a metastatic stem/progenitor phenotype in human prostate cancer. Stable knockdown of alphav integrins expression in PC-3M-Pro4 prostate cancer cells coincided with a significant decrease of prostate cancer stem/progenitor cell characteristics (alpha2 integrin, CD44, and ALDH(hi)) and decreased expression of invasion-associated genes Snail, Snail2, and Twist. Consistent with these observations, alphav-knockdown strongly inhibited the clonogenic and migratory potentials of human prostate cancer cells in vitro and significantly decreased tumorigenicity and metastatic ability in preclinical models of orthotopic growth and bone metastasis. Our data indicate that integrin alphav expression is functionally involved in the maintenance of a highly migratory, mesenchymal cellular phenotype as well as the acquisition of a stem/progenitor phenotype in human prostate cancer cells with metastasis-initiating capacity.

Vazquez-Martin, A., E. Lopez-Bonetc, et al. "Repositioning chloroquine and metformin to eliminate cancer stem cell traits in pre-malignant lesions." Drug Resist Updat **14**(4-5): 212-23.
Ideal oncology drugs would be curative after a short treatment course if they could eliminate epithelium-originated carcinomas at their non-invasive, pre-malignant stages. Such ideal molecules, which are expected to molecularly abrogate all the instrumental mechanisms acquired by migrating cancer stem cells (CSCs) to by-pass tumour suppressor barriers, might already exist. We here illustrate how system biology strategies for repositioning existing FDA-approved drugs may accelerate our therapeutic capacity to eliminate CSC traits in pre-invasive intraepithelial neoplasias. First, we describe a signalling network signature that overrides bioenergetics stress- and oncogene-induced senescence (OIS) phenomena in CSCs residing at pre-invasive lesions. Second, we functionally map the anti-malarial chloroquine and the anti-diabetic metformin ("old drugs") to their recently recognized CSC targets ("new uses") within the network. By discussing the preclinical efficacy of chloroquine and metformin to inhibiting the genesis and self-renewal of CSCs we finally underscore the expected translational impact of the "old drugs-new uses" repurposing strategy to open a new CSC-targeted chemoprevention era.

Venere, M., H. A. Fine, et al. "Cancer stem cells in gliomas: identifying and understanding the apex cell in cancer's hierarchy." Glia **59**(8): 1148-54.
Neuro-oncology research has rediscovered a complexity of nervous system cancers through the incorporation of cellular heterogeneity into tumor models with cellular subsets displaying stem-cell characteristics. Self-renewing cancer stem cells (CSCs) can propagate tumors and yield nontumorigenic tumor bulk cells that display a more differentiated phenotype. The ability to prospectively isolate and interrogate CSCs is defining molecular mechanisms responsible for the tumor maintenance and growth. The clinical relevance of CSCs has been supported by their resistance to cytotoxic therapies and their promotion of tumor angiogenesis. Although the field of CSC biology is relatively young, continued elucidation of the features of these cells holds promise for the development of novel patient therapies. (c) 2011 Wiley-Liss, Inc.

Vizio, B., F. A. Mauri, et al. "Comparative evaluation of cancer stem cell markers in normal pancreas and pancreatic ductal adenocarcinoma." Oncol Rep **27**(1): 69-76.
Chemoresistance and self-renewal of cancer stem cells (CSC), found in many tumors including pancreatic ductal adenocarcinoma (PDAC), are believed to underlie tumor mass regrowth. The distribution of cells carrying the putative stem-cell markers CD133, Nestin, Notch1-4, Jagged1 and 2, ABCG2 and aldehyde dehydrogenase (ALDH1) was assessed immunohistochemically using PDAC and normal pancreas tissue microarrays. The immunoreactivity was semi-quantitatively graded against the normal pancreas and was correlated with the differentiation grade and disease stage. No statistical significant differences were found between normal pancreas and PDAC in the expression of Nestin, Notch1, 3 and 4, ABCG2 or ALDH1. Notch2 and Jagged1 and 2 expression were increased in PDAC. CD133-positive cells were above-normal in PDAC, but the difference was not statistically significant. Nestin, Notch1-4, Jagged1, ABCG2 and ALDH1 immunostaining scores were not correlated with tumor grade or disease stage. CD133 and Notch2 expression was significantly inversely correlated with tumor grade, but not disease stage. Notch3 immunostaining positively correlated with tumor stage, but not with differentiation grade. Jagged2 protein expression correlated inversely with disease stage, but not with tumor grade. From the clinical standpoint, improved delineation of the tumor CSC signature, putatively responsible for tumor initiation and recurrence after initial response to chemotherapy, may offer novel therapeutic targets for this highly lethal cancer.

Vo, B. T. and S. A. Khan "Expression of nodal and nodal receptors in prostate stem cells and prostate cancer cells: autocrine effects on cell proliferation and migration." Prostate **71**(10): 1084-96.

BACKGROUND: Nodal, a TGFbeta like growth factor, functions as an embryonic morphogen that maintains the pluripotency of embryonic stem cells. Nodal has been implicated in cancer progression; however, there is no information on expression and functions of Nodal in prostate cancer. In this study, we have investigated the expression of Nodal, its receptors, and its effects on proliferation and migration of human prostate cells. METHODS: RT-PCR, qPCR, and Western blot analyses were performed to analyze expression of Nodal and Nodal receptors and its effects on phosphorylation of Smad2/3 in prostate cells. The effects on proliferation and migration were determined by (3) H-Thymidine incorporation and cell migration assays in the presence or absence of Nodal receptor inhibitor (SB431542). RESULTS: Nodal was highly expressed in WPE, DU145, LNCaP, and LNCaP-C81 cells with low expression in RWPE1 and RWPE2 cells, but not in PREC, PC3 and PC3M cells. Nodal receptors are expressed at varying levels in all prostate cells. Treatment with exogenous Nodal induced phosphorylation of Smad2/3 in WPE, DU145, and PC3 cells, which was blocked by SB431542. Nodal dose-dependently inhibited proliferation of WPE, RWPE1 and DU145 cells, but not LNCaP and PC3 cells. Nodal induced cell migration in PC3 cells, which was inhibited by SB431542; Nodal had no effect on cell migration in WPE and DU145 cells. The effects of Nodal on cell proliferation and migration are mediated via ALK4 and ActRII/ActRIIB receptors and Smad 2/3 phosphorylation. CONCLUSIONS: Nodal may function as an autocrine regulator of proliferation and migration of prostate cancer cells.

von Drygalski, A., T. B. Tran, et al. "Obesity is an independent predictor of poor survival in metastatic breast cancer: retrospective analysis of a patient cohort whose treatment included high-dose chemotherapy and autologous stem cell support." Int J Breast Cancer **2011**: 523276.
The purpose of the study was to identify predictors of long-term survival in metastatic breast cancer (MBC). A cohort of 96 patients, who received high-dose chemotherapy with autologous stem cell support (HD-ASCT) as part of their treatment, was analyzed. Percent long-term survival at 10 years was 24.5% (CI 17.2-34.9%) when metastasis was diagnosed and 14.4% (CI 8.7-23.9%) when MBC was diagnosed. Survival was impacted significantly by body mass index (BMI). Median overall survival from initial diagnosis or from time of metastasis for patients with BMIs </=30 and >30 (obese) was 7.1 (CI 4.4-8.7) and 3.2 years (2.41-6.75), respectively, or 3.2 or 2.3 years (all P = 0.02). Also, obesity was the only independent patient-related predictor of time to metastasis and of survival. While obesity is linked with poor outcomes in earlier stages of breast cancer, this has not been previously reported for MBC.

von Rahden, B. H., S. Kircher, et al. "LgR5 expression and cancer stem cell hypothesis: clue to define the true origin of esophageal adenocarcinomas with and without Barrett's esophagus?" J Exp Clin Cancer Res **30**: 23.
BACKGROUND: Investigation of the expression of an intestinal stem cell marker in esophageal adenocarcinomas (EAC) with and without Barrett's Esophagus (BE), with respect to a cancer stem cell (CSC) hypothesis. MATERIALS AND METHODS: Expression of a putative intestinal stem cell marker LgR5 was analyzed in esophageal cancer specimen (n = 70: 41 EAC with BE, 19 EAC without BE, and n = 10 esophageal squamous-cell carcinomas, ESCC) and in the adenocarcinoma cell line OE-33. Ki-67 and Cdx-2 were co-labelled with LgR5 in double staining experiments. Immunohistochemical expression results were confirmed by RT-PCR and correlated with tumor stage and five-year survival rates. RESULTS: LgR5was found expressed in 35 of 41 (85%) EAC with BE and in 16 of 19 (81%) EAC without BE. By contrast, LgR5 was not found to be expressed in ESCC. Quantification of immunolabeling showed 15% LgR5+ cells in EAC with BE, 32% LgR5+ cells in adjacent BE and 13% in EAC without BE. Immunofluorescence double staining experiments with LgR5 and Ki-67 revealed a subpopulation (~5%) of proliferating LgR+/Ki-67+ cells. On mRNA-level, expression of LgR5 was higher in BE in comparison to EAC (p = 0.0159). High levels of LgR5 expression in BE associated EAC were associated with poorer survival in univariate analysis. CONCLUSION: The stem cell marker LgR5 is expressed in EAC, irrespective of association with BE, and appears to have negative impact on survival. The subset of proliferating LgR5+ cells (<5%) might resemble rapidly cycling CSCs, which needs to be substantiated in further investigations.

Voss, M. H., D. R. Feldman, et al. "High-dose chemotherapy and stem cell transplantation for advanced testicular cancer." Expert Rev Anticancer Ther **11**(7): 1091-103.
High-dose chemotherapy (HDCT) with autologous stem cell support has been studied in both the salvage and first-line setting in advanced germ cell tumor (GCT) patients with poor-risk features. While early studies reported significant treatment-related mortality, introduction of peripheral blood stem cell transplantation, recombinant growth factors and better supportive care have decreased toxicity; and in more recent reports treatment-related deaths are observed in

<3% of patients. Two to three cycles of high-dose carboplatin and etoposide is the standard backbone for HDCT, given with or without additional agents including ifosfamide, cyclophosphamide and paclitaxel. Three large randomized Phase III trials have failed to show a benefit of HDCT over conventional-dose chemotherapy (CDCT) in the first-line treatment of patients with intermediate- or poor-risk advanced GCT, and to date the routine use of HDCT has been reserved for the salvage setting. Several prognostic models have been developed to help predict outcome of salvage HDCT, the most recent of which applies to both CDCT and HDCT in the initial salvage setting. Patients that relapse after HDCT are usually considered incurable, and additional therapy is provided with palliative intent.

Wakamatsu, Y., N. Sakamoto, et al. "Expression of cancer stem cell markers ALDH1, CD44 and CD133 in primary tumor and lymph node metastasis of gastric cancer." Pathol Int **62**(2): 112-9.
Gastric cancer (GC) is one of the most common malignancies worldwide. Recently, cancer stem cells (CSCs) in tumors were found to possess the ability to sustain tumor self-renewal, initiate tumor progression, and possibly also contribute to cancer metastasis. We immunohistochemically examined expression and distribution of representative CSC markers ALDH1, CD44, and CD133 in primary tumors and lymph node metastasis of GC. Among 190 GC primary tumors, 104 (55%) were positive for ALDH1, 117 (62%) were positive for CD44, and 18 (9%) were positive for CD133. Expression of these three CSC markers was significantly associated with advanced clinicopathologic factors. Patients with CD44- and CD133-positive GC had a poorer survival rate than patients with CD44- and CD133-negative GC (CD44: $P < 0.001$, CD133: $P= 0.006$). Univariate and multivariate Cox proportional hazards analysis revealed tumor node metastasis stage, CD44 expression, and CD133 expression to be independent predictors of survival in patients with GC. Comparison of CSC markers in primary and metastatic sites showed ALDH1 positivity to be significantly higher in diffuse-type lymph node metastasis than in the primary tumor ($P < 0.001$). These results indicate that these CSC markers are important in tumor invasion and metastasis and may be good markers indicating long-term survival in patients with GC.

Wang, H., J. Wu, et al. "Transforming growth factor beta-induced epithelial-mesenchymal transition increases cancer stem-like cells in the PANC-1 cell line." Oncol Lett **3**(1): 229-233.
The epithelial-mesenchymal transition plays a crucial role in the progression of pancreatic cancer. The aim of this study was to examine the possible association between the epithelial-mesenchymal transition and cancer stem-like cells in pancreatic cancer. We used transforming growth factor beta to induce an epithelial-mesenchymal transition. The proportion of pancreatic cancer stem-like cells was measured and sorted by flow cytometry. The expression of markers was measured by quantitative PCR and Western blot analysis. Cell cycle distribution was assessed by flow cytometry. We evaluated the migration and invasion activity by Transwell tests. The proportion of pancreatic cancer stem-like cells was significantly increased following transforming growth factor beta treatment. Cells were sorted in culture, the cancer stem-like cells exhibited a higher degree of epithelial-mesenchymal transition and demonstrated upregulation of vimentin, a mesenchymal phenotypic marker, compared to the CD44(-)CD24(-) cells. Pancreatic cancer stem-like cells exhibited greater invasion and migration activity in vitro compared to the CD44(-)CD24(-) cells. These results suggested a direct link between epithelial-mesenchymal transition and cancer stem-like cells in pancreatic cancer.

Wang, J., F. Lu, et al. "Novel histone demethylase LSD1 inhibitors selectively target cancer cells with pluripotent stem cell properties." Cancer Res **71**(23): 7238-49.
Histone modification determines epigenetic patterns of gene expression with methylation of histone H3 at lysine 4 (H3K4) often associated with active promoters. LSD1/KDM1 is a histone demethylase that suppresses gene expression by converting dimethylated H3K4 to mono- and unmethylated H3K4. LSD1 is essential for metazoan development, but its pathophysiologic functions in cancer remain mainly uncharacterized. In this study, we developed specific bioactive small inhibitors of LSD1 that enhance H3K4 methylation and derepress epigenetically suppressed genes in vivo. Strikingly, these compounds inhibited the proliferation of pluripotent cancer cells including teratocarcinoma, embryonic carcinoma, and seminoma or embryonic stem cells that express the stem cell markers Oct4 and Sox2 while displaying minimum growth-inhibitory effects on non-pluripotent cancer or normal somatic cells. RNA interference-mediated knockdown of LSD1 expression phenocopied these effects, confirming the specificity of small molecules and further establishing the high degree of sensitivity and selectivity of pluripotent cancer cells to LSD1 ablation. In support of these results, we found that LSD1 protein level is highly elevated in pluripotent cancer cells and in human testicular seminoma tissues

that express Oct4. Using these novel chemical inhibitors as probes, our findings establish LSD1 and histone H3K4 methylation as essential cancer-selective epigenetic targets in cancer cells that have pluripotent stem cell properties.

Wang, K., L. Liu, et al. "Oxaliplatin-incorporated micelles eliminate both cancer stem-like and bulk cell populations in colorectal cancer." Int J Nanomedicine **6**: 3207-18.
PURPOSE: The failure of cancer treatments is partly due to the enrichment of cancer stem-like cells (CSLCs) that are resistant to conventional chemotherapy. A novel micelle formulation of oxaliplatin (OXA) encapsulated in chitosan vesicle was developed. The authors postulate that micelle encapsulation of OXA would eliminate both CSLCs and bulk cancer cells in colorectal cancer (CRC). EXPERIMENTAL DESIGN: In this study, using stearic acid-g-chitosan oligosaccharide (CSO-SA) polymeric micelles as a drug-delivery system, OXA-loaded CSO-SA micelles (CSO-SA/OXA) were prepared. Intracellular uptake of CSO-SA/OXA micelles was assessed by confocal microscope. The effects of free OXA, the empty carrier, and CSO-SA/OXA micelles were tested using human CRC cell lines in vitro and in vivo. RESULTS: The micelles showed excellent internalization ability that increased OXA accumulation both in CRC cells and tissues. Furthermore, CSO-SA/OXA micelles could either increase the cytotoxicity of OXA against the bulk cancer cells or reverse chemoresistance of CSLC subpopulations in vitro. Intravenous administration of CSO-SA/OXA micelles effectively suppressed the tumor growth and reduced CD133+/CD24+ cell (putative CRC CSLC markers) compared with free OXA treatment, which caused CSLC enrichment in xenograft tumors ($P < 0.05$). CONCLUSION: The results of this study indicate that CSO-SA micelle as a drug-delivery carrier is effective for eradicating CSLCs and may act as a new option for CRC therapy.

Wang, L., R. Mezencev, et al. "Isolation and characterization of stem-like cells from a human ovarian cancer cell line." Mol Cell Biochem **363**(1-2): 257-68.
Increasing evidence supports the existence of a subpopulation of cancer cells capable of self-renewal and differentiation into diverse cell lineages. These cancer stem-like or cancer-initiating cells (CICs) also demonstrate resistance to chemo- and radiotherapy and may function as a primary source of cancer recurrence. We report here on the isolation and in vitro propagation of multicellular ovarian cancer spheroids from a well-established ovarian cancer cell line (OVCAR-3). The spheroid-derived cells (SDCs) display self-renewal potential, the ability to produce differentiated progeny, and increased expression of genes previously associated with CICs. SDCs also demonstrate higher invasiveness, migration potential, and enhanced resistance to standard anticancer agents relative to parental OVCAR-3 cells. Furthermore, SDCs display up-regulation of genes associated with epithelial-to-mesenchymal transition (EMT), anticancer drug resistance and/or decreased susceptibility to apoptosis, as well as, down-regulation of genes typically associated with the epithelial cell phenotype and pro-apoptotic genes. Pathway and biological process enrichment analyses indicate significant differences between the SDCs and precursor OVCAR-3 cells in TGF-beta-dependent induction of EMT, regulation of lipid metabolism, NOTCH and Hedgehog signaling. Collectively, our results indicate that these SDCs will be a useful model for the study of ovarian CICs and for the development of novel CIC-targeted therapies.

Wang, L., P. Park, et al. "BMP-2 inhibits the tumorigenicity of cancer stem cells in human osteosarcoma OS99-1 cell line." Cancer Biol Ther **11**(5): 457-63.
Previously, based on high ALDH activity, we showed that cancer stem cells (CSCs) could be identified as ALDH(br) cells from an aggressive human osteosarcoma OS99-1 cell line. In this study, we evaluate the impact of BMP-2 on CSCs.Three types of BMP receptors were expressed in freshly sorted ALDH(br) cells. In vitro, growth of the sorted ALDH(br) cells was inhibited by BMP-2. Using RT-PCR analysis, BMP-2 was found to down-regulate the expression of embryonic stem cell markers Oct3/4, Nanog, and Sox-2, and up-regulate the transcription of osteogenic markers Runx-2 and Collagen Type I. In vivo, all animals receiving ALDH(br) cells treated with BMP-2 did not form significant tumors, while untreated ALDH(br) cells developed large tumor masses in NOD/SCID mice. Immunostaining confirmed few Ki-67 positive cells were present in the sections of tumor containing ALDH(br) cells treated with BMP-2. These results suggest that BMP-2 suppresses tumor growth by reducing the gene expression of tumorigenic factors and inducing the differentiation of CSCs in osteosarcoma. BMP-2 or BMP-2-mimetic drugs, if properly delivered to tumor and combined with traditional therapies, may therefore provide a new therapeutic option for treatment of osteosarcoma.

Wang, S. J. "The stem cell patent landscape as relevant to cancer vaccines." Hum Vaccin **7**(10): 1100-8.

Cancer vaccine targeting cancer stem cells is proposed to serve as a potent immunotherapy. Thus, it would be useful to examine the main trends in stem cell patenting activity as a guide for those seeking to develop such cancer vaccines. We found that a substantial number of stem cell patents were granted up to the end of 2010, including ~2000 issued in the US. Many of these have been filed since 2001, including 7,551 applications in the US. Stem cell development, as evidenced by the numbers of PubMed articles, has matured steadily in recent years. However, the other metrics, such as the number of patent applications, the technology-science linkage and the number of patent assignees, have been stagnant. Moreover, the ownership of stem cell patents is still quiet fragmented across multiple organizations, and the number of stem cell patent assignees from the business sector has not increased significantly. Academic and nonprofit institutions not only account for a large share of stem cell patents but also apply for patents continually. Based on this analysis, the strength of stem cell resources seems to remain stagnant in recent years due to the ban on government funding of embryonic stem cell research. Furthermore, the patent prosecution or technical barriers in the field of stem cells would be another main reason that the number of US-issued stem cell patents for each application have been in gradual decline since 2000. Therefore, we consider stem cell technology to still be under development.

Wang, Y. "Effects of salinomycin on cancer stem cell in human lung adenocarcinoma A549 cells." Med Chem **7**(2): 106-11.
Lung cancer is a leading cause of death in human. Cancer stem cells have been regarded as basis for failure of current therapeutic options. Salinomycin was shown to kill these cancer stem cells in some types of cancer such as breast cancer and leukemia. The in vitro anticancer activities of salinomycin have been validated against the lung cancer cell line A549 via sulforhodamine B and colony formation assay. Salinomycin has been demonstrated to significantly rupture the in vitro lung cancer tumorospheres from ALDH positive A549 lung cells using flow cytometry. Expression of stem cell markers OCT-4, NANOG and SOX2 in ALDH positive A549 lung cells was decreased significantly by real-time RT-PCR analysis after 24 hour salinomycin treatment. Taken together, salinomycin may provide a promising approach for lung cancer chemotherapy.

Wang, Y., Y. Yu, et al. "Transforming growth factor-beta regulates the sphere-initiating stem cell-like feature in breast cancer through miRNA-181 and ATM." Oncogene **30**(12): 1470-80.
Recent studies indicate that a subset of cancer cells possessing stem cell properties, referred to as cancer-initiating or cancer stem cells (CSCs), have crucial roles in tumor initiation, metastasis and resistance to anticancer therapies. Transforming growth factor (TGF)-beta and their family members have been implicated in both normal (embryonic and somatic) stem cells and CSCs. In this study, we observed that exposure to TGF-beta increased the population of breast cancer (BC) cells that can form mammospheres in suspension, a feature endowed by stem cells. This was mediated by the micro (mi)RNA family miR-181, which was upregulated by TGF-beta at the post-transcriptional level. Levels of the miR-181 family members were elevated in mammospheres grown in undifferentiating conditions, compared with cells grown in two-dimensional conditions. Ataxia telangiectasia mutated (ATM), a target gene of miR-181, exhibited reduced expression in mammospheres and upon TGF-beta treatment. Overexpression of miR-181a/b, or depletion of ATM or its substrate CHK2, was sufficient to induce sphere formation in BC cells. Finally, knockdown of ATM enhanced in vivo tumorigenesis of the MDA361 BC cells. Our results elucidate a novel mechanism through which the TGF-beta pathway regulates the CSC property by interfering with the tumor suppressor ATM, providing insights into the cellular and environmental factors regulating CSCs, which may guide future studies on therapeutic strategies targeting these cells.

Wang, Y., H. Zhe, et al. "Cancer stem cell marker ALDH1 expression is associated with lymph node metastasis and poor survival in esophageal squamous cell carcinoma: a study from high incidence area of northern China." Dis Esophagus **25**(6): 560-5.
Tumor recurrence and metastasis is the leading cause of death in esophageal squamous cell carcinoma (ESCC). Cancer stem cell (CSC) may be responsible for tumor growth and maintenance of aggressive behavior. Aldehyde dehydrogenase 1 (ALDH1) has been proposed as one of the possible candidates for a CSC marker. The expression of ALDH1 may be correlated with the clinicopathologic factor and clinical outcome of patients with ESCC. The purpose of this study was to investigate the expression of ALDH1 protein in human ESCC tissues, and evaluated the clinical implication of ALDH1 expression for these patients. All 79 patients who underwent esophagectomy for ESCC between January 2005 and June 2006 were enrolled in this study. The expression of ALDH1 in ESCC and adjacent noncancerous tissues was analyzed by immunohistochemistry. ALDH1 was mainly expressed in ESCC cell nucleus. For the 79 ESCC patients, increased nuclear accumulation of ALDH1

was found in 12 (15.2%) specimens. ALDH1 expression was correlated with poor histological differentiation (P= 0.003), lymph node metastasis (P= 0.011), and late pathologic TNM classification (pTNM) staging (P= 0.003). Patients in ALDH1 positive group had a significantly poor 5-year overall survival than those in the negative group (8.3% vs. 52.2%, P= 0.025). We have demonstrated for the first time that the CSC marker, ALDH1, is expressed in human ESCC. The expression of ALDH1 protein in nucleus of the ESCC is significantly associated with lymph node metastasis and poor survival. Our results highly indicate the involvement of ALDH1 in the aggressive behavior of ESCC.

Wang, Z., Q. Shi, et al. "Clinicopathologic correlation of cancer stem cell markers CD44, CD24, VEGF and HIF-1alpha in ductal carcinoma in situ and invasive ductal carcinoma of breast: an immunohistochemistry-based pilot study." Pathol Res Pract **207**(8): 505-13.
CD24(-/low)CD44(+) cells have been identified as putative cancer stem cells (CSCs) in breast cancer. However, the expression of these markers, as well as their association with clinical parameters or tumor microenvironment of breast cancer, remains largely unknown. In the present study, we examined the expression of CD44, CD24, VEGF, and HIF-1alpha in human breast tumor tissues and assessed their clinicopathological correlations. We investigated tissue samples, including 117 cases of invasive ductal carcinoma (IDCa), 14 cases of ductal carcinoma in situ (DCIS), and 15 cases of intraductal hyperplasia (IDH) from breast tissues. The expression of CD44, CD24, HIF-1alpha, and VEGF was evaluated using immunohistochemical staining. CD24, CD44, HIF-1alpha, and VEGF were expressed in 49 (41.9%), 51 (43.6%), 32 (27.4%), and 97 cases (82.9%), respectively, in IDCa. CD24(-/low)CD44(+) cells were noted in 48 (41.3%) cases. The levels of CD24 and VEGF expression correlated positively with tumor malignancy ($P<0.05$). Meanwhile, the expression of CD24, CD44, and VEGF correlated significantly positively with increasing tumor grade ($P<0.05$). In addition, associations between CD44 and VEGF, CD24 and VEGF, HIF-1alpha and VEGF, CD24(-/low)CD44(+) and VEGF, CD24(-/low)CD44(+) and HIF-1alpha were also observed ($P<0.05$). The HIF-1alpha expression level was relatively higher in early stage breast cancer patients with CD24(-/low)CD44(+) cells. Taken together, our results suggest that CD24 and VEGF may play important roles in breast tumorigenesis and progression, while HIF-1alpha may play a role in the early stage of breast carcinogenesis.

Wijaya, L., D. Agustina, et al. "Reversing breast cancer stem cell into breast somatic stem cell." Curr Pharm Biotechnol **12**(2): 189-95.
Stem cells have an important role in cell biology, allowing tissues to be renewed by freshly created cells throughout their lifetime. The specific micro-environment of stem cells is called stem cell niche; this environment influences the development of stem cells from quiescence through stages of differentiation. Recent advance researches have improved the understanding of the cellular and molecular components of the micro-environment--or niche--that regulates stem cells. We point out an important trend to the study of niche activity in breast cancers. Breast cancer has long been known to conserve a heterogeneous population of cells. While the majority of cells that make up tumors are destined to differentiate and eventually stop dividing, only minority populations of cells, termed cancer stem cell, possess extensive self renewal capability. These cancer stem cells possess characteristics of both stem cells and cancer cells. Breast cancer stem cells reversal to breast somatic stem cells offer a new therapy, that not only can stop the spread of breast cancer cells, but also can differentiate breast cancer stem cells into normal breast somatic stem cells. These can replace damaged breast tissue. Nevertheless, the complexity of realizing this therapy approach needs further research.

Wu, K., X. Jiao, et al. "Cell fate determination factor Dachshund reprograms breast cancer stem cell function." J Biol Chem **286**(3): 2132-42.
The cell fate determination factor Dachshund was cloned as a dominant inhibitor of the hyperactive epidermal growth factor receptor ellipse. The expression of Dachshund is lost in human breast cancer associated with poor prognosis. Breast tumor-initiating cells (TIC) may contribute to tumor progression and therapy resistance. Here, endogenous DACH1 was reduced in breast cancer cell lines with high expression of TIC markers and in patient samples of the basal breast cancer phenotype. Re-expression of DACH1 reduced new tumor formation in serial transplantations in vivo, reduced mammosphere formation, and reduced the proportion of CD44(high)/CD24(low) breast tumor cells. Conversely, lentiviral shRNA to DACH1 increased the breast (B)TIC population. Genome-wide expression studies of mammary tumors demonstrated DACH1 repressed a molecular signature associated with stem cells (SOX2, Nanog, and KLF4) and genome-wide ChIP-seq analysis identified DACH1 binding to the promoter of the Nanog, KLF4, and Lin28 genes. KLF4/c-Myc and Oct4/Sox2 antagonized DACH1 repression of BTIC. Mechanistic

studies demonstrated DACH1 directly repressed the Nanog and Sox2 promoters via a conserved domain. Endogenous DACH1 regulates BTIC in vitro and in vivo.

Wu, Q., Z. Yang, et al. "Stem cell associated genes working with one miRNA cluster have different clinic pathologic values in gastric cancer." Pathol Oncol Res **17**(4): 939-46.
Cancer stem cells are nowadays considered to be the origin of cancer. Also, stem cell associated genes are emerging as predictors of cancer malignancy. We investigated the association of several stemness genes (c-Myc, PTEN, p57 and p21) with clinic pathological parameters and survival in stomach cancer by performing immunohistochemistry on paraffin sections of gastric cancer patients who underwent surgical staging with following-up statistics. We discovered that expression of c-Myc was significantly related to distant metastasis, the combined expression of PTEN and p21 correlated positively to overall survival, while p57 was less useful in overall survival prediction in gastric cancer. Additionally, there is a positive correlation between expressions of p57 and p21. In conclusion, our present study indicated that expression of stemness genes (c-Myc, PTEN, p57 and p21) performed different predictive potential in the evaluation of clinical malignancy levels in gastric cancer.

Xu, L., H. Xiao, et al. "Hypoxia facilitates cancer associated cell marker expression in stem cells." Cell Mol Biol (Noisy-le-grand) **57 Suppl**: OL1456-61.
How normal body cells differentiate to cancer cells is not clear. The present study aimed to investigate the mechanism by which hypoxia drives the expression of cancer associated cell markers in stem cells. In this study, mouse bone marrow mononuclear cells were prepared and cultured under hypoxic environment. Rate of cancer associated cell markers on stem cells was determined by flow cytometry. Inflammatory cytokine levels in culture supernatant were determined by enzyme-linked immunoassay. The results showed that after cultured under hypoxic environment for 48 h, the cancer associated cell markers increased significantly in stem cells. IL-1beta levels increased markedly after cultured in hypoxia. Macrophages were identified as the major source of IL-1beta. Blocking IL-1beta abolished the differentiation of cancer associated cell markers in stem cells. We conclude that hypoxia can increase aberrant expression of IL-1beta in macrophages that further facilitates the expression of cancer associated cell markers in stem cells.

Xu, Y., Y. D. Hu, et al. "Establishing a lung cancer stem cell culture using autologous intratumoral fibroblasts as feeder cells." Cell Biol Int **35**(5): 509-17.
Human LCSCs (lung cancer stem cells) were first isolated from lung cancer patients and cultured using serum-free culture methods. To recreate the intratumoural microenvironment to sustain LCSC growth, autologous intratumoral fibroblasts were used as feeder cells. In this study, we investigated the growth and maintenance of pluripotency in prolonged LCSCs culture on autologous intratumoural fibroblasts. LCSCs isolated from three clinical samples all showed vigorous growth on feeder cells for 16 weeks of continuous cultures with a doubling time of 41-47 h. The cells continued expressing stem cell marker CD133 and remained undifferentiated. Pluripotency was demonstrated by tumour formation in immunodeficient mice. In a feeder-free culture system, growth of LCSCs spheres was retarded and would cease when the diameter reached 100 mum if immediate passage was not performed. Moreover, spontaneous differentiation was more frequently seen in a serum-free culture system. In conclusion, we have successfully established a culture system using autologous intratumoural fibroblast cells as feeder cells for prolonged culture of undifferentiated LCSCs in vitro.

Yamazaki, H., C. W. Xu, et al. "Regulation of cancer stem cell properties by CD9 in human B-acute lymphoblastic leukemia." Biochem Biophys Res Commun **409**(1): 14-21.
Although the prognosis of acute lymphoblastic leukemia (ALL) has improved considerably in recent years, some of the cases still exhibit therapy-resistant. We have previously reported that CD9 was expressed heterogeneously in B-ALL cell lines and CD9(+) cells exhibited an asymmetric cell division with greater tumorigenic potential than CD9(-) cells. CD9(+) cells were also serially transplantable in immunodeficient mice, indicating that CD9(+) cell possess self-renewal capacity. In the current study, we performed more detailed analysis of CD9 function for the cancer stem cell (CSC) properties. In patient sample, CD9 was expressed in the most cases of B-ALL cells with significant correlation of CD34-expression. Gene expression analysis revealed that leukemogenic fusion proteins and Src family proteins were significantly regulated in the CD9(+) population. Moreover, CD9(+) cells exhibited drug-resistance, but proliferation of bulk cells was inhibited by anti-CD9 monoclonal antibody. Knockdown of CD9 remarkably reduced the leukemogenic potential. Furthermore, gene ablation of CD9 affected the expression and tyrosine-phosphorylation of Src family proteins and

reduced the expression of histone-deubiquitinase USP22. Taken together, our results suggest that CD9 links to several signaling pathways and epigenetic modification for regulating the CSC properties of B-ALL.

Yeung, T. M., S. C. Gandhi, et al. "Hypoxia and lineage specification of cell line-derived colorectal cancer stem cells." Proc Natl Acad Sci U S A **108**(11): 4382-7.
Hypoxia is an important regulator of normal and cancer stem cell (CSC) differentiation. Colorectal CSCs from SW1222, LS180, and CCK81 colorectal cancer-derived cell lines are able to differentiate into complex 3D lumen-containing structures in normoxia, whereas in hypoxia, they form undifferentiated dense colonies that have reduced expression of the enterocyte differentiation marker CDX1, lack goblet cell formation, and have increased expression of BMI1 and activated Notch1. Hypoxia increases the clonogenicity of CSCs, which is cumulative as each round of hypoxia enriches for more CSCs. The hypoxic phenotype is reversible, because cells from hypoxic-dense colonies are able to reform differentiated structures when regrown in normoxia. We show that CDX1 is able to stimulate the generation of lumens even in hypoxia and has a negative feedback on BMI1 expression. Knockdown of CDX1 reduces lumen formation but does not affect goblet cell formation, suggesting that enterocytes and goblet cells form from different progenitor cells. Notch inhibition by dibenzazepine (DBZ) allowed CSCs to form goblet cells in both normoxia and hypoxia. Finally, we show that Hif1alpha, but not CA9, is an important mediator of the effects of hypoxia on the clonogenicity and differentiation of CSCs. In summary, hypoxia maintains the stem-like phenotype of colorectal cell line-derived CSCs and prevents differentiation of enterocytes and goblet cells by regulating CDX1 and Notch1, suggesting that this regulation is an important component of how hypoxia controls the switch between stemness and differentiation in CSCs.

Yin, B. B., S. J. Wu, et al. "Preliminary screening and identification of stem cell-like sphere clones in a gallbladder cancer cell line GBC-SD." J Zhejiang Univ Sci B **12**(4): 256-63.
This paper aims to screen and identify sphere clone cells with characteristics similar to cancer stem cells in human gallbladder cancer cell line GBC-SD. GBC-SD cells were cultured in a serum-free culture medium with different concentrations of the chemotherapeutic drug cisplatin for generating sphere clones. The mRNA expressions of stem cell-related genes CD133, OCT-4, Nanog, and drug resistance genes ABCG2 and MDR-1 in sphere clones were detected by quantitative real-time polymerase chain reaction (PCR). Stem cell markers were also analyzed by flow cytometry and immunofluorescent staining. Different amounts of sphere clones were injected into nude mice to test their abilities to form tumors. Sphere clones were formed in serum-free culture medium containing cisplatin (30 mumol/L). Flow cytometry results demonstrated that the sphere clones expressed high levels of stem cell markers CD133(+) (97.6%) and CD44(+) (77.9%) and low levels of CD24(+) (2.3%). These clones also overexpressed the drug resistance genes ABCG2 and MDR-1. Quantitative real-time PCR showed that sphere clones expressed stem cell genes Nanog and OCT-4 284 and 266 times, respectively, more than those in the original GBC-SD cells. Immunofluorescent staining showed that sphere clones overexpressed OCT-4, Nanog, and SOX-2, and low expressed MUC1 and vimentin. Tumor formation experiments showed that 1x10(3) sphere clone cells could induce much larger tumors in nude mice than 1x10(5) GBC-SD cells. In conclusion, sphere clones of gallbladder cancer with stem cell-like characteristics can be obtained using suspension cultures of GBC-SD cells in serum-free culture medium containing cisplatin.

Yin, S., L. Xu, et al. "Cisplatin and TRAIL enhance breast cancer stem cell death." Int J Oncol **39**(4): 891-8.
Triple negative breast cancer (TNBC) has increased recurrence and poor survival, despite a high response rate to neoadjuvant chemotherapy. The aim of this study was to determine whether current drug treatment(s) eliminates bulk of tumor cells, but it has a minimal effect on cancer stem cells (CSCs) leading to tumor recurrence. We studied the effects of PARP inhibitors (AZD2281 and BSI-201), paclitaxel, docetaxel, cisplatin and cisplatin plus TRAIL on CSCs derived from CRL-2335 and MDA-MB-468 TNBC cells in vitro. The in vitro data indicate that cisplatin plus TRIAL treatment was most effective in eliminating CSCs compared to PARP inhibitors, cisplatin, paclitaxel and docetaxel. Treatment with cisplatin plus TRAIL also inhibits Wnt-1 signaling and its downstream target, beta-catenin, phospho beta-catenin, cyclin D1, increased apoptosis, reduced proliferation and mammosphere formation. Inhibition of Wnt-1 by siRNA significantly reduced the ability of CSCs to form mammospheres compared to control. However, maximum effect was seen in cisplatin plus TRAIL-treated cells. Taken together the data suggest that cisplatin plus TRAIL treatment has the potential of providing a new strategy for improving the therapeutic outcome in TNBC patients.

Yip, N. C., I. S. Fombon, et al. "Disulfiram modulated ROS-MAPK and NFkappaB pathways and targeted breast cancer cells with cancer stem cell-like properties." Br J Cancer **104**(10): 1564-74.
BACKGROUND: Previous studies indicate that disulfiram (DS), an anti-alcoholism drug, is cytotoxic to cancer cell lines and reverses anticancer drug resistance. Cancer stem cells (CSCs) are the major cause of chemoresistance leading to the failure of cancer chemotherapy. This study intended to examine the effect of DS on breast cancer stem cells (BCSCs). METHODS: The effect of DS on BC cell lines and BCSCs was determined by MTT, western blot, CSCs culture and CSCs marker analysis. RESULTS: Disulfiram was highly toxic to BC cell lines in vitro in a copper (Cu)-dependent manner. In Cu-containing medium (1 muM), the IC(50) concentrations of DS in BC cell lines were 200-500 nM. Disulfiram/copper significantly enhanced (3.7-15.5-fold) cytotoxicity of paclitaxel (PAC). Combination index isobologram analysis demonstrated a synergistic effect between DS/Cu and PAC. The increased Bax and Bcl2 protein expression ratio indicated that intrinsic apoptotic pathway may be involved in DS/Cu-induced apoptosis. Clonogenic assay showed DS/Cu-inhibited clonogenicity of BC cells. Mammosphere formation and the ALDH1(+VE) and CD24(Low)/CD44(High) CSCs population in mammospheres were significantly inhibited by exposure to DS/Cu for 24 h. Disulfiram/copper induced reactive oxygen species (ROS) generation and activated its downstream apoptosis-related cJun N-terminal kinase and p38 MAPK pathways. Meanwhile, the constitutive NFkappaB activity in BC cell lines was inhibited by DS/Cu. CONCLUSION: Disulfiram/copper inhibited BCSCs and enhanced cytotoxicity of PAC in BC cell lines. This may be caused by simultaneous induction of ROS and inhibition of NFkappaB.

Yu, C., Z. Yao, et al. "ALDH activity indicates increased tumorigenic cells, but not cancer stem cells, in prostate cancer cell lines." In Vivo **25**(1): 69-76.
BACKGROUND: Cancer stem cells (CSCs) have been shown to be a small stem cell-like cell population which appears to drive tumorigenesis, tumor recurrence and metastasis. Thus, identification and characterization of CSCs may be critical to defining effective anticancer therapies. In prostate cancer (PCa), the CD44(+) cell population appears to have stem cell-like properties including being tumorigenic. The enzyme aldehyde dehydrogenase (ALDH) has been found to identify hematopoietic stem cells and our aim was to determine the utility of ALDH activity and CD44 in identifying PCa stem cell-like cells in PCa cell lines. MATERIALS AND METHODS: LNCaP cells and PC-3 cells were sorted based on their expression of CD44 and ALDH activity. The cell populations were investigated using colony-forming assays, invasion assays, sphere formation experiments in a non-adherent environment and 3-D Matrigel matrix culture to observe the in vitro stem-cell like properties. Different sorted cell populations were injected subcutaneously into NOD/SCID mice to determine the corresponding tumorigenic capacities. RESULTS: ALDH(hi) CD44(+) cells exhibit a higher proliferative, clonogenic and metastatic capacity in vitro and demonstrate higher tumorigenicity capacity in vivo than did ALDH(lo) CD44(-) cells. The tumors recapitulated the population of the original cell line. However, ALDHlo CD44(-) cells were able to develop tumors, albeit with longer latency periods. CONCLUSION: ALDH activity and CD44 do not appear to identify PCa stem cells; however, they do indicate increased tumorigenic and metastatic potential, indicating their potential importance for further exploration.

Yu, F., J. Li, et al. "Kruppel-like factor 4 (KLF4) is required for maintenance of breast cancer stem cells and for cell migration and invasion." Oncogene **30**(18): 2161-72.
Kruppel-like factor 4 (KLF4) is highly expressed in more than 70% of breast cancers and functions as an oncogene. However, an exact mechanism by which KLF4 enhances tumorigenesis of breast cancer remains unknown. In this study, we show that KLF4 was highly expressed in cancer stem cell (CSC)-enriched populations in mouse primary mammary tumor and breast cancer cell lines. Knockdown of KLF4 in breast cancer cells (MCF-7 and MDA-MB-231) decreased the proportion of stem/progenitor cells as demonstrated by expression of stem cell surface markers such as aldehyde dehydrogenase 1, side population and by in vitro mammosphere assay. Consistently KLF4 overexpression led to an increase of the cancer stem cell population. KLF4 knockdown also suppressed cell migration and invasion in MCF-7 and MDA-MB-231 cells. Furthermore, knockdown of KLF4 reduced colony formation in vitro and inhibited tumorigenesis in immunocompromised non-obese diabetic/severe combined immunodeficiency mice, supporting an oncogenic role for KLF4 in breast cancer development. Further mechanistic studies revealed that the Notch signaling pathway was required for KLF4-mediated cell migration and invasion, but not for CSC maintenance. Taken together, our study provides evidence that KLF4 has a potent oncogenic role in mammary tumorigenesis likely by maintaining stem cell-like features and by promoting cell migration and invasion. Thus, targeting

KLF4 may provide an effective therapeutic approach to suppress tumorigenicity in breast cancer.

Zanichelli, F., S. Capasso, et al. "Dose-dependent effects of R-sulforaphane isothiocyanate on the biology of human mesenchymal stem cells, at dietary amounts, it promotes cell proliferation and reduces senescence and apoptosis, while at anti-cancer drug doses, it has a cytotoxic effect." Age (Dordr) **34**(2): 281-93.
Brassica vegetables are attracting a great deal of attention as healthy foods because of the fact that they contain substantial amounts of secondary metabolite glucosinolates that are converted into isothiocyanates, such as sulforaphane [(-)1-isothiocyanato-4R-(methylsulfinyl)-butane] (R-SFN), through the actions of chopping or chewing the vegetables. Several studies have analyzed the biological and molecular mechanisms of the anti-cancer activity of synthetic R,S-sulforaphane, which is thought to be a result of its antioxidant properties and its ability to inhibit histone deacetylase enzymes (HDAC). Few studies have addressed the possible antioxidant effects of R-SFN, which could protect cells from the free radical damage that strongly contribute to aging. Moreover, little is known about the effect of R-SFN on stem cells whose longevity is implicated in human aging. We evaluated the effects of R-SFN on the biology on human mesenchymal stem cells (MSCs), which, in addition to their ability to differentiate into mesenchymal tissues, support hematopoiesis, and contribute to the homeostatic maintenance of many organs and tissues. Our investigation found evidence that low doses of R-SFN promote MSCs proliferation and protect them from apoptosis and senescence, while higher doses have a cytotoxic effect, leading to the induction of cell cycle arrest, programmed cell death and senescence. The beneficial effects of R-SFN may be ascribed to its antioxidant properties, which were observed when MSC cultures were incubated with low doses of R-SFN. Its cytotoxic effects, which were observed after treating MSCs with high doses of R-SFN, could be attributed to its HDAC inhibitory activity. In summary, we found that R-SFN, like many other dietary supplements, exhibits a hormetic behavior; it is able to induce biologically opposite effects at different doses.

Zeng, Z., X. Wu, et al. "Polymorphisms in prostate stem cell antigen gene rs2294008 increase gastric cancer risk in Chinese." Mol Carcinog **50**(5): 353-8.
A recent genome-wide study identified a strong association between polymorphisms in the prostate stem cell antigen (PSCA) gene and the risk of diffuse-type of gastric cancer in Japanese and Korean population. In this case-control study, we aimed to investigate the possible association between PSCA rs2294008 C/T with clinicopathological features and the prognosis of gastric cancer in a Southern Chinese population. Genotypes of 460 gastric cancer patients and 549 controls were determined by PCR-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing. We found that individuals with at least one copy of the rs2294008T allele (CT or TT genotype) had an increased risk for gastric cancer compared with CC genotype (OR = 1.42, 95% CI = 1.10-1.82, P = 0.006). Further stratification analyses indicated that the effect of PSCA rs2294008T carriers was noteworthy in intestinal type (OR = 1.55, 95% CI = 1.18-2.04, P = 0.002), poorly differentiated (OR = 1.59, 95% CI = 1.19-2.13, P = 0.002), noncardia (OR = 1.55, 95% CI = 1.17-2.04, P = 0.002) subtypes of gastric cancer. Cox proportional hazards analyses demonstrated that TT genotype (HR = 2.12, 95% CI = 1.22-3.69, P = 0.008) as well as TNM staging were prognostic factors of gastric cancer patients. In conclusion, The T allele of PSCA rs2294008 is associated with increased risk of gastric cancer, especially intestinal type, poorly differentiated, early onset, and noncardia gastric cancer in Chinese population. TNM staging and TT genotype might be involved in the prognosis of gastric cancer patients.

Zhang, F., C. Song, et al. "Effect of fibroblasts on breast cancer cell mammosphere formation and regulation of stem cell-related gene expression." Int J Mol Med **28**(3): 365-71.
The purpose of this study was to investigate the regulatory effects of breast cancer fibroblasts (BCFs) vs. normal mammary fibroblasts (NMFs) on mammosphere formation and stem cell-related gene expression in breast cancer cells. Breast cancer cells (MCF-7) were cultured in suspension to generate primary and secondary mammospheres. The proportion of CD44+/CD24low/- cells was assessed by flow cytometry (FCM), and Wnt1, Notch1, beta-catenin, CXCR4, SOX2 and ALDH3A1 gene expression was detected by quantitative real-time PCR. The fibroblasts from either breast cancer tissue or normal mammary tissue were purified from tissue specimens and co-cultured with breast cancer cells. The mammosphere formation efficacy was approximately 180/10,000 MCF-7 cells. FCM analysis showed that, compared to the 2.1% positive expression in the MCF-7 monolayer culture cells, the expression of CD44+/CD24low/- in MCF-7 mammosphere cells was significantly elevated to 10.4% (P<0.01). The proportion of the CD44+/CD24low/- subpopulation of the cells in mammospheres was nearly 5-fold higher than that of general MCF-7 cells. Compared with MCF-7 monolayer culture cells, mammosphere cells showed

significantly (P<0.01) enhanced expression of Wnt1 [fold-change (FC), 2.25], Notch1 (FC, 2.45), beta-catenin (FC, 1.72), CXCR4 (FC, 4.68), SOX2 (FC, 4.25) and ALDH3A1 (FC, 5.38). When BCFs were co-cultured with MCF-7 cells under mammosphere culture conditions, the length of time of mammosphere formation decreased, the volume of the mammo-spheres increased and the mammosphere-forming efficiency (MFE) was higher than that of NMFs and the control group. Both the BCF and NMF groups showed enhanced gene expression for the following genes: Wnt1 (FC, 3.18 and 1.27, respectively), beta-catenin (FC, 1.75 and 1.22, respectively), Notch1 (FC, 2.09 and 1.31, respectively), CXCR4 (FC, 2.77 and 1.33, respectively), SOX2 (FC, 2.77 and 1.80, respectively) and ALDH3A1 (FC, 5.23 and 1.85, respectively). Cancer fibroblast cells can promote the MFE and up-regulate stem cell-related gene expression in breast cancer cells.

Zhang, H., W. Li, et al. "MicroRNA expression profile of colon cancer stem-like cells in HT29 adenocarcinoma cell line." Biochem Biophys Res Commun **404**(1): 273-8.
Increasing evidence has suggested cancer stem cells (CSCs) are considered to be responsible for cancer formation, recurrence, and metastasis. Recently, many studies have also revealed that microRNAs (miRNAs) strongly implicate in regulating self renewal and tumorigenicity of CSCs in human cancers. However, with respect to colon cancer, the role of miRNAs in stemness maintenance and tumorigenicity of CSCs still remains to be unknown. In the present study, we isolated a population of colon CSCs expressing a CD133 surface phenotype from human HT29 colonic adenocarcinoma cell line by Flow Cytometry Cell Sorting. The CD133(+) cells possess a greater tumor sphere-forming efficiency in vitro and higher tumorigenic potential in vivo. Furthermore, the CD133(+) cells are endowed with stem/progenitor cells-like property including expression of "stemness" genes involved in Wnt2, BMI1, Oct3/4, Notch1, C-myc and other genes as well as self-renewal and differentiation capacity. Moreover, we investigated the miRNA expression profile of colon CSCs using miRNA array. Consequently, we identified a colon CSCs miRNA signature comprising 11 overexpressed and 8 underexpressed miRNAs, such as miR-429, miR-155, and miR-320d, some of which may be involved in regulation of stem cell differentiation. Our results suggest that miRNAs might play important roles in stemness maintenance of colon CSCs, and analysis of specific miRNA expression signatures may contribute to potential cancer therapy.

Zhang, M., Q. Ma, et al. "Stem cell factor/c-kit signaling enhances invasion of pancreatic cancer cells via HIF-1alpha under normoxic condition." Cancer Lett **303**(2): 108-17.
The SCF/c-kit signaling plays an important role in invasion of c-kit-expressing tumor cells, however, the molecular mechanisms have not been studied yet. Using a pancreatic cancer model, we demonstrate that SCF/c-kit binding up-regulates the expression of invasion-related genes through the accumulation of HIF-1alpha. Furthermore, the expression of HIF-1alpha induced by SCF is not dependent on the oxygen level, but rather on both the PI3K/Akt and Ras/MEK/ERK signaling pathways. In conclusion, under normoxic conditions, SCF/c-kit binding increases expression of HIF-1alpha through the PI3K/Akt and Ras/MEK/ERK pathways, and the accumulation of HIF-1alpha up-regulates expression of invasion-related genes that augment the invasiveness of pancreatic cancer, a fatal cancer. Therefore, our results suggest that the inhibition of both c-kit and HIF-1alpha may be an effective strategy for pancreatic cancer therapy.

Zhao, Z., W. Ma, et al. "Small interference RNA-mediated silencing of prostate stem cell antigen attenuates growth, reduces migration and invasion of human prostate cancer PC-3M cells." Urol Oncol **31**(3): 343-51.
OBJECTIVES: Prostate stem cell antigen (PSCA), a glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein, is highly expressed in both local and metastatic prostate cancer (CaP). Elevated PSCA expression has been shown to correlate with malignant phenotype and clinical progression. The purpose of the current study is to investigate the therapeutic potential of small interference RNA (siRNA) targeting PSCA on human CaP cells. MATERIALS AND METHODS: A set of two siRNAs directed different regions of human PSCA (siRNA-PSCA) were designed and transfected into a human CaP PC-3M cell line. The silencing effect was screened by RT-PCR and Western blotting. The biological effects of siRNA-PSCA on PC-3M cells were investigated by examining the cell proliferation through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, cell cycle distribution through flow cytometry, and migration and invasion potencies through transwell invasion assay upon the PSCA silencing. RESULTS: PC-3M cells had positive PSCA expression on immunocytochemical assay. PSCA expression was depleted at 48 hours after transfection with siRNA-PSCA. Silencing of PSCA significantly suppressed cell proliferation. Cell cycle assay showed that the anti-proliferation effect of siRNA-PSCA was

mediated by arresting cells in the G0/G1 phase rather than apoptosis. Furthermore, PSCA knockdown resulted in a marked decrease of cell migration and invasion capabilities in PC-3M cells. CONCLUSIONS: The present study provides the first evidence that silencing PSCA using siRNA can inhibit the proliferation and invasiveness properties of human CaP cells, which may provide a promising therapeutic strategy for CaP and open a novel avenue toward the investigation of the role of PSCA overexpression in cancers.

Zhao, Z., G. Zeng, et al. "Peripheral blood reverse transcription PCR assay for prostate stem cell antigen correlates with androgen-independent progression in advanced prostate cancer." Int J Cancer **131**(4): 902-10.
Recent studies show that prostate stem cell antigen (PSCA) mRNA positivity in peripheral blood correlates with disease progression in prostate cancer (PCa). AIP developed in 59 (50.9%) patients during a median follow-up period of 35.4 months (range: 4-78 months). Patients with PSCA negativity experienced significantly longer remissions compared to those with PSCA positivity (log-rank test: $p < 0.001$). Multivariate Cox regression analysis further demonstrated that PSCA positivity had a significantly increased risk of AIP (HR = 4.303, 95% CI: 3.761-7.482, $p < 0.001$). Pretreatment RT-PCR PSCA positivity in peripheral blood independently signals the presence of AIP in patients with advanced PCa treated with ADT.

Zhau, H. E., H. He, et al. "Human prostate cancer harbors the stem cell properties of bone marrow mesenchymal stem cells." Clin Cancer Res **17**(8): 2159-69.
PURPOSE: Prostate tumor cells frequently show the features of osteoblasts, which are differentiated from bone marrow mesenchymal stem cells. We examined human prostate cancer cell lines and clinical prostate cancer specimens for additional bone marrow mesenchymal stem cell properties. EXPERIMENTAL DESIGN: Prostate cancer cell lines were induced for osteoblastogenic and adipogenic differentiation, detected by standard staining methods and confirmed by lineage-specific marker expression. Abnormal expression of the markers was then assessed in clinical prostate cancer specimens. RESULTS: After osteoblastogenic induction, cells of the LNCaP lineage, PC-3 lineage, and DU145 displayed osteoblastic features. Upon adipogenic induction, PC-3 lineage and DU145 cells differentiated into adipocyte-like cells. The adipocyte-like cancer cells expressed brown adipocyte-specific markers, suggesting differentiation along the brown adipocyte lineage. The adipogenic differentiation was accompanied by growth inhibition, and most of the adipocyte-like cancer cells were committed to apoptotic death. During cyclic treatments with adipogenic differentiation medium and then with control medium, the cancer cells could commit to repeated adipogenic differentiation and retrodifferentiation. In clinical prostate cancer specimens, the expression of uncoupling protein 1 (UCP1), a brown fat-specific marker, was enhanced with the level of expression correlated to disease progression from primary to bone metastatic cancers. CONCLUSIONS: This study thus revealed that prostate cancer cells harbor the stem cell properties of bone marrow mesenchymal stem cells. The abnormally expressed adipogenic UCP1 protein may serve as a unique marker, while adipogenic induction can be explored as a differentiation therapy for prostate cancer progression and bone metastasis.

Zhu, T. S., M. A. Costello, et al. "Endothelial cells create a stem cell niche in glioblastoma by providing NOTCH ligands that nurture self-renewal of cancer stem-like cells." Cancer Res **71**(18): 6061-72.
One important function of endothelial cells in glioblastoma multiforme (GBM) is to create a niche that helps promote self-renewal of cancer stem-like cells (CSLC). However, the underlying molecular mechanism for this endothelial function is not known. Since activation of NOTCH signaling has been found to be required for propagation of GBM CSLCs, we hypothesized that the GBM endothelium may provide the source of NOTCH ligands. Here, we report a corroboration of this concept with a demonstration that NOTCH ligands are expressed in endothelial cells adjacent to NESTIN and NOTCH receptor-positive cancer cells in primary GBMs. Coculturing human brain microvascular endothelial cells (hBMEC) or NOTCH ligand with GBM neurospheres promoted GBM cell growth and increased CSLC self-renewal. Notably, RNAi-mediated knockdown of NOTCH ligands in hBMECs abrogated their ability to induce CSLC self-renewal and GBM tumor growth, both in vitro and in vivo. Thus, our findings establish that NOTCH activation in GBM CSLCs is driven by juxtacrine signaling between tumor cells and their surrounding endothelial cells in the tumor microenvironment, suggesting that targeting both CSLCs and their niche may provide a novel strategy to deplete CSLCs and improve GBM treatment.

Zhu, X., X. Zhou, et al. "Cancer stem cell, niche and EGFR decide tumor development and treatment response: A bio-computational simulation study." J Theor Biol **269**(1): 138-49.

Recent research in cancer biology has suggested the hypothesis that tumors are initiated and driven by a small group of cancer stem cells (CSCs). Furthermore, cancer stem cell niches have been found to be essential in determining fates of CSCs, and several signaling pathways have been proven to play a crucial role in cellular behavior, which could be two important factors in cancer development. To better understand the progression, heterogeneity and treatment response of breast cancer, especially in the context of CSCs, we propose a mathematical model based on the cell compartment method. In this model, three compartments of cellular subpopulations are constructed: CSCs, progenitor cells (PCs), and terminal differentiated cells (TCs). Moreover, (1) the cancer stem cell niche is, considered by modeling its effect on division patterns (symmetric or asymmetric) of CSCs, and (2) the EGFR signaling pathway is integrated by modeling its role in cell proliferation, apoptosis. Our simulation results indicate that (1) a higher probability for symmetric division of CSC may result in a faster expansion of tumor population, and for a larger number of niches, the tumor grows at a slower rate, but the final tumor volume is larger; (2) higher EGFR expression correlates to tumors with larger volumes while a saturation function is observed, and (3) treatments that inhibit tyrosine kinase activity of EGFR may not only repress the tumor volume, but also decrease the CSCs percentages by shifting CSCs from symmetric divisions to asymmetric divisions. These findings suggest that therapies should be designed to effectively control or eliminate the symmetric division of CSCs and to reduce or destroy the CSC niches.

Zolochevska, O., G. Yu, et al. "Pigment epithelial-derived factor and melanoma differentiation associated gene-7 cytokine gene therapies delivered by adipose-derived stromal/mesenchymal stem cells are effective in reducing prostate cancer cell growth." Stem Cells Dev **21**(7): 1112-23.

Adipose-derived stromal/mesenchymal stem cells (ASC) have gained interest as promising tools for delivering cancer therapy. Adipose tissue can be obtained readily in amounts sufficient for ASC isolation, which can be expanded rapidly, allowing its use at low passage numbers, and can be transduced by viral and nonviral means. Our goal was to examine the potential of ASC to deliver cytokine gene therapies melanoma differentiation associated gene-7 (MDA-7) or pigment epithelial-derived factor (PEDF) to cancer cells. These novel cytokines are a potent proapoptotic and an antiangiogenesis mediator, respectively, with potential as antitumor agents. Expression of cytokine therapies did not adversely affect ASC biology, and these cells were still able to differentiate and retain normal viability. The ASC cytokine therapies were efficient in reducing tumor cell growth in coculture and also in suppressing in vitro angiogenesis phenotypes. We also observed that ASC retained their innate ability to migrate toward tumor cells in coculture, and this ability could be blocked by inhibition of CXCR4 signaling. The ASC were found to be nontumorigenic in vitro using a soft agar assay, as well as in vivo, utilizing 2 prostate cancer xenograft models. The ASC-MDA7 only reduced tumor growth in the TRAMP-C2-Ras (TC2Ras) prostate cancer model. The ASC-PEDF, however, reduced growth in both the TC2Ras and the PC3 highly aggressive prostate cancer models, and it was able to completely prevent prostate tumor establishment in vivo. In conclusion, ASC expressing PEDF and MDA7 could effectively reduce prostate tumor growth in vivo, suggesting ASC-cytokine therapies might have translational applications, especially the PEDF modality.

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