

Cancer Stem Cell Literatures

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

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Literatures

Abdouh, M., S. Facchino, et al. (2009). "BMI1 sustains human glioblastoma multiforme stem cell renewal." *J Neurosci* **29**(28): 8884-96.

Glioblastoma multiforme (GBM) is one of the most common and aggressive types of brain tumors. In GBM, a subpopulation of CD133-positive cancer initiating cells displays stem cell characteristics. The Polycomb group (PcG) and oncogene BMI1 is part of the Polycomb repressive complex 1 (PRC1) that regulates gene expression by modifying chromatin organization. Here we show that BMI1 is expressed in human GBM tumors and highly enriched in CD133-positive cells. Stable BMI1 knockdown using short hairpin RNA-expressing lentiviruses resulted in inhibition of clonogenic potential in vitro and of brain tumor formation in vivo. Cell biology studies support the notion that BMI1 prevents CD133-positive cell apoptosis and/or differentiation into neurons and astrocytes, depending on the cellular context. Gene expression analyses suggest that BMI1 represses alternate tumor suppressor pathways that attempt to compensate for INK4A/ARF/P53 deletion and PI(3)K/AKT hyperactivity. Inhibition of EZH2, the main component of the PRC2, also impaired GBM tumor growth. Our results reveal that PcG proteins are involved in GBM tumor growth and required to sustain cancer initiating stem cell renewal.

Aboody, K. S., J. Najbauer, et al. (2008). "Stem and progenitor cell-mediated tumor selective gene therapy." *Gene Ther* **15**(10): 739-52.

The poor prognosis for patients with aggressive or metastatic tumors and the toxic side effects of currently available treatments necessitate the development of more effective tumor-selective

therapies. Stem/progenitor cells display inherent tumor-tropic properties that can be exploited for targeted delivery of anticancer genes to invasive and metastatic tumors. Therapeutic genes that have been inserted into stem cells and delivered to tumors with high selectivity include prodrug-activating enzymes (cytosine deaminase, carboxylesterase, thymidine kinase), interleukins (IL-2, IL-4, IL-12, IL-23), interferon-beta, apoptosis-promoting genes (tumor necrosis factor-related apoptosis-inducing ligand) and metalloproteinases (PEX). We and others have demonstrated that neural and mesenchymal stem cells can deliver therapeutic genes to elicit a significant antitumor response in animal models of intracranial glioma, medulloblastoma, melanoma brain metastasis, disseminated neuroblastoma and breast cancer lung metastasis. Most studies reported reduction in tumor volume (up to 90%) and increased survival of tumor-bearing animals. Complete cures have also been achieved (90% disease-free survival for >1 year of mice bearing disseminated neuroblastoma tumors). As we learn more about the biology of stem cells and the molecular mechanisms that mediate their tumor-tropism and we identify efficacious gene products for specific tumor types, the clinical utility of cell-based delivery strategies becomes increasingly evident.

Adams, J. M., P. N. Kelly, et al. (2008). "Role of "cancer stem cells" and cell survival in tumor development and maintenance." *Cold Spring Harb Symp Quant Biol* **73**: 451-9.

One critical issue for cancer biology is the nature of the cells that drive the inexorable growth of malignant tumors. Reports that only rare cell populations within human leukemias seeded leukemia in mice stimulated the now widely embraced hypothesis that only such "cancer stem cells" maintain

all tumor growth. However, the mouse microenvironment might instead fail to support the dominant human tumor cell populations. Indeed, on syngeneic transplantation of mouse lymphomas and leukemias, we and other investigators have found that a substantial proportion (>10%) of their cells drive tumor growth. Thus, dominant clones rather than rare cancer stem cells appear to sustain many tumors. Another issue is the role of cell survival in tumorigenesis. Because tumor development can be promoted by the overexpression of prosurvival genes such as bcl-2, we are exploring the role of endogenous Bcl-2-like proteins in lymphomagenesis. The absence of endogenous Bcl-2 in mice expressing an Emu-myc transgene reduced mature B-cell numbers and enhanced their apoptosis, but unexpectedly, lymphoma development was undiminished or even delayed. This suggests that these tumors originate in an earlier cell type, such as the pro-B or pre-B cell, and that the nascent neoplastic clones do not require Bcl-2 but may instead be protected by a Bcl-2 relative.

Baguley, B. C. (2006). "Tumor stem cell niches: a new functional framework for the action of anticancer drugs." Recent Pat Anticancer Drug Discov **1**(1): 121-7.

Newer treatments of advanced human cancer increasingly rely on combinations of drugs that have quite different actions yet unexpectedly potentiate each other's effects. Recent research in stem cell biology suggests a model for tumors in which tumor growth is governed by the generation of cells from tumor cell niches rather than from the population as a whole. Each niche contains a population of tumor stem cells supported by a closely associated vascular bed comprising mesenchyme-derived cells and an extracellular matrix. Division of tumor stem cells is asymmetric in the sense that some daughter cells are always retained within the niche while others leave the niche to proliferate further and eventually die. One important potential difference between normal and tumor stem cell niches is that while most normal stem cells are in a non-proliferating or G(0)-state, tumor stem cells are continuously in cycle. Combinations of cytotoxic drugs and antagonists of survival factors to reduce the stem cell population may require the addition of vascular disrupting agents to compromise the function of the tumor cell niche. As well as providing opportunities for new drug discovery, this model of tumor growth also presents challenges as to how the contributions of individual drugs in a combination might be assessed in individual patients.

Baum, C. (2007). "Insertional mutagenesis in gene therapy and stem cell biology." Curr Opin Hematol **14**(4): 337-42.

PURPOSE OF REVIEW: Recent preclinical and clinical studies revealed that the semirandom insertion of transgenes into chromosomal DNA of hematopoietic cells may induce clonal competition, which potentially may even trigger leukemia or sarcoma. Insertional mutagenesis caused by gene vectors has thus led to major uncertainty among those developing advanced hematopoietic cell therapies. This review summarizes novel studies of underlying mechanisms; these studies have demonstrated the possibility of improved gene vector biosafety and generated new insights into stem cell biology. **RECENT FINDINGS:** The characteristic insertion pattern of various retroviral gene vector systems may be explained by properties of the viral integrase and associated cellular cofactors. Cell culture assays and animal models, including disease-specific and cancer-prone mouse models, are emerging that reveal the contributions of vector features and systemic factors to induction of clonal imbalance. Databases summarizing vector insertion sites in dominant hematopoietic clones are evolving as new tools to identify genes that regulate clonal homeostasis. **SUMMARY:** Mechanistic studies of insertional mutagenesis by random gene vector insertion will lead to improved tools for advanced hematopoietic cell therapy. Simultaneously, fascinating insights into gene networks that regulate cell fitness will be generated, with important consequences for the fields of hematology, oncology and regenerative medicine.

Bidaut, G. and C. J. Stoeckert, Jr. (2009). "Characterization of unknown adult stem cell samples by large scale data integration and artificial neural networks." Pac Symp Biocomput: 356-67.

Stem cells represent not only a potential source of treatment for degenerative diseases but can also shed light on developmental biology and cancer. It is believed that stem cells differentiation and fate is triggered by a common genetic program that endows those cells with the ability to differentiate into specialized progenitors and fully differentiated cells. To extract the stemness signature of several cells types at the transcription level, we integrated heterogeneous datasets (microarray experiments) performed in different adult and embryonic tissues (liver, blood, bone, prostate and stomach in Homo sapiens and Mus musculus). Data were integrated by generalization of the hematopoietic stem cell hierarchy and by homology between mouse and human. The variation-filtered and integrated gene expression dataset was fed to a single-layered neural network to create a classifier to (i) extract the stemness signature and (ii) characterize unknown stem cell tissue samples by attribution of a stem cell differentiation stage. We were able to characterize mouse stomach progenitor

and human prostate progenitor samples and isolate gene signatures playing a fundamental role for every level of the generalized stem cell hierarchy.

Blagosklonny, M. V. (2007). "Cancer stem cell and cancer stemoids: from biology to therapy." *Cancer Biol Ther* **6**(11): 1684-90.

It has become a cliché that cancer therapy fails because it does not target rare cancer stem cells (CSCs). Here we are discuss that this is not how therapy fails and not any cancer cell with stem-like properties is CSC. Paradoxically, CSCs must be resting to explain their resistance to therapy yet must be cycling to explain their persistence in cell culture. To solve contradictions, this article introduces the term cancer stemoids (or stem cell-like cells) to describe proliferating self-renewing cells. The stem cell hierarchy (stem--proliferating--terminal cells) exists exactly to separate self-renewal (immortality) from proliferation. Cancer stemoids break the stem cell hierarchy and eventually may replace other cells. While CSC is shielded from any selective pressure and therefore unable to drive tumor progression, cancer stemoids undergo clonal selection, accumulate mutations, thus determining tumor progression and therapeutic failures. Unlike CSC, cancer stemoids are a crucial target for cancer therapy, exactly because they proliferate. Furthermore, two normally mutually-exclusive properties (proliferation and stemness) provide a means to design therapy to kill cancer stemoids selectively without killing normal stem and non-stem cells. In contrast, true CSCs are not only a difficult, but also an insufficient and perhaps even an unnecessary therapeutic target, especially in advanced malignancies.

Braydich-Stolle, L., C. Nolan, et al. (2005). "Role of glial cell line-derived neurotrophic factor in germ-line stem cell fate." *Ann N Y Acad Sci* **1061**: 94-9.

The overall goal of this study is to unravel the role(s) played by glial cell line-derived neurotrophic factor (GDNF) in the fate of spermatogonial stem cells. There is great interest in the biology of spermatogonial stem cells, or A(single) spermatogonia, because of their importance in the treatment of infertility, the development of contraceptives, and the understanding of the etiology of testicular cancer, particularly seminoma. In the mouse, spermatogonial stem cells express GFRalpha-1, the receptor for GDNF, and respond to this growth factor in vivo and in vitro. GDNF is produced by the adjacent Sertoli cells, which are part of the germ-line stem cell niche in vertebrates. We specifically isolated GFRalpha-1-positive spermatogonia using an immunomagnetic bead technique. We then stimulated the cells with 100 ng/mL of rGDNF for 10 hours;

unstimulated cells served as negative controls. Microarray analysis, immunocytochemistry, and Western blotting revealed that Numb, a regulator of the Notch pathway, is upregulated by GDNF in spermatogonial stem cells. There are indications that in rats, mice, and humans, the Notch pathway promotes spermatogonial differentiation. We observed that an increase in Numb expression is concomitant with Notch degradation in these cells. Thus, through Numb, GDNF might inhibit differentiation and allows the maintenance of the stem cell pool in the mouse seminiferous epithelium.

Cabanillas, R. and J. L. Llorente (2009). "The Stem Cell Network model: clinical implications in cancer." *Eur Arch Otorhinolaryngol* **266**(2): 161-70.

This review will discuss the aspects of stem cell biology that can contribute to explain tumor development and why standard oncology treatments sometimes fail. We also propose an integrated model of tumor progression based on the putative occurrence of Stem Cell Networks (SCNs). In a SCN, the somatic stem cells are derived from a common embryonic stem cell, share a specific molecular profile and maintain a high degree of cell-cycle synchronization. In the study of cancer, the SCN model introduces an additional conceptual frame to the interpretation of both the cancer stem cell (CSC) hypothesis and the field cancerization concept. The CSC model may explain how the cancer fields develop, justifies their sizes and shapes, contribute to explain the local recurrences in patients with free margins, the second primary tumors, the success of organ preserving surgical approaches or the trend of different tumors to metastasize to certain locations. We propose that the SCN model of cancer provides some clues for further understanding tumor progression and raises promising experimental and clinical implications.

Camper, S. A., T. L. Saunders, et al. (1995). "Implementing transgenic and embryonic stem cell technology to study gene expression, cell-cell interactions and gene function." *Biol Reprod* **52**(2): 246-57.

This review highlights the use of transgenic mice and gene targeting in the study of reproduction, pituitary gene expression, and cell lineage. Since 1980 numerous applications of transgenic animal technology have been reported. Altered phenotypes resulting from transgene expression demonstrated that introduced genes can exert profound effects on animal physiology. Transgenic mice have been important for the study of hormonal and developmental control of gene expression because gene expression in whole animals often requires more DNA sequence information than is necessary for expression in cell

cultures. This point is illustrated by studies of pituitary glycoprotein hormone alpha- and beta-subunit gene expression (Kendall et al., *Mol Endocrinol* 1994; in press [1]). Transgenic mice have also been invaluable for producing animal models of cancer and other diseases and testing the efficacy of gene therapy. In addition, cell-cell interactions and cell lineage relationships have been explored by cell-specific expression of toxin genes in transgenic mice. Recent studies suggest that attenuated and inducible toxins hold promise for future transgene ablation experiments. Since 1987, embryonic stem (ES) cell technology has been used to create numerous mouse strains with targeted gene alterations, contributing enormously to our understanding of the functional importance of individual genes. For example, the unexpected development of gonadal tumors in mice with a targeted disruption of the inhibin gene revealed a potential role for inhibin as a tumor suppressor (Matzuk et al., *Nature* 1992;360: 313-319 [2]). The transgenic and ES cell technologies will undoubtedly continue to expand our understanding and challenge our paradigms in reproductive biology.

Chang, S. J., T. Y. Wang, et al. (2009). "Increased epithelial stem cell traits in advanced endometrial endometrioid carcinoma." *BMC Genomics* **10**: 613.

BACKGROUND: It has been recognized cancer cells acquire characters reminiscent of those of normal stem cells, and the degree of stem cell gene expression correlates with patient prognosis. Lgr5(+) or CD133(+) epithelial stem cells (EpiSCs) have recently been identified and these cells are susceptible to neoplastic transformation. It is unclear, however, whether genes enriched in EpiSCs also contribute in tumor malignancy. Endometrial endometrioid carcinoma (EEC) is a dominant type of the endometrial cancers and is still among the most common female cancers. Clinically endometrial carcinoma is classified into 4 FIGO stages by the degree of tumor invasion and metastasis, and the survival rate is low in patients with higher stages of tumors. Identifying genes shared between advanced tumors and stem cells will not only unmask the mechanisms of tumor malignancy but also provide novel therapeutic targets. **RESULTS:** To identify EpiSC genes in late (stages III-IV) EECs, a molecular signature distinguishing early (stages I-II) and late EECs was first identified to delineate late EECs at the genomics level. ERBB2 and CCR1 were genes activated in late EECs, while APBA2 (MINT2) and CDK inhibitor p16 tumor suppressors in early EECs. MAPK pathway was significantly up in late EECs, indicating drugs targeting this canonical pathway might be useful for treating advanced EECs. A six-gene mini-signature was further identified to

differentiate early from advanced EECs in both the training and testing datasets. Advanced, invasive EECs possessed a clear EpiSC gene expression pattern, explaining partly why these tumors are more malignant. **CONCLUSIONS:** Our work provides new insights into the pathogenesis of EECs and reveals a previously unknown link between adult stem cells and the histopathological traits of EECs. Shared EpiSC genes in late EECs may contribute to the stem cell-like phenotypes shown by advanced tumors and hold the potential of being candidate therapeutic targets and novel prognosis biomarkers.

Chen, Z. G. (2009). "The cancer stem cell concept in progression of head and neck cancer." *J Oncol* **2009**: 894064.

Human head and neck cancer (HNC) is a highly heterogeneous disease. Understanding the biology of HNC progression is necessary for the development of novel approaches to its prevention, early detection, and treatment. A current evolutionary progression model has limitations in explaining the heterogeneity observed in a single tumor nest. Accumulating evidence supports the existence of cancer stem cells (CSCs) as small subpopulations in solid tumors, including HNC. These CSCs can be selected by appropriate cell surface markers, which are cancer type specific and have been confirmed by unique in vitro and in vivo assays. Selected CSC populations maintain a self-renewal capability and show aggressive behaviors, such as chemoresistance and metastasis. In addition to introducing the CSC concept in solid tumors, this short review summarizes current publications in HNC CSC and the prospective development and application of the CSC concept to HNC in the clinic.

Chiba, T., K. Kita, et al. (2006). "Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties." *Hepatology* **44**(1): 240-51.

Recent advances in stem cell biology enable us to identify cancer stem cells in solid tumors as well as putative stem cells in normal solid organs. In this study, we applied side population (SP) cell analysis and sorting to established hepatocellular carcinoma (HCC) cell lines to detect subpopulations that function as cancer stem cells and to elucidate their roles in tumorigenesis. Among four cell lines analyzed, SP cells were detected in Huh7 (0.25%) and PLC/PRF/5 cells (0.80%), but not in HepG2 and Huh6 cells. SP cells demonstrated high proliferative potential and anti-apoptotic properties compared with those of non-SP cells. Immunocytochemistry examination showed that SP fractions contain a large number of cells presenting characteristics of both hepatocyte and

cholangiocyte lineages. Non-obese diabetic/severe combined immunodeficiency (NOD/SCID) xenograft transplant experiments showed that only 1×10^3 SP cells were sufficient for tumor formation, whereas an injection of 1×10^6 non-SP cells did not initiate tumors. Re-analysis of SP cell-derived tumors showed that SP cells generated both SP and non-SP cells and tumor-initiating potential was maintained only in SP cells in serial transplantation. Microarray analysis discriminated a differential gene expression profile between SP and non-SP cells, and several so-called "stemness genes" were upregulated in SP cells in HCC cells. In conclusion, we propose that a minority population, detected as SP cells in HCC cells, possess extreme tumorigenic potential and provide heterogeneity to the cancer stem cell system characterized by distinct hierarchy.

Chu, P., D. J. Clanton, et al. (2009). "Characterization of a subpopulation of colon cancer cells with stem cell-like properties." *Int J Cancer* **124**(6): 1312-21.

The biology of the normal colonic mucosa suggests that colon cancer originates from normal colon stem cells. CD44 cancer stem cells have been identified in breast and prostate cancer, and we therefore examined whether CD44 similarly identified colon cancer stem cells. Initial assays found CD44(hi) colon tumor cells to have enhanced soft agar colony-forming ability. Subsequently, CD44(hi) cells isolated from 4 primary colon adenocarcinoma xenografts were found to be highly tumorigenic in immune deficient mice. CD44(hi) cells consistently formed tumors with 1,000 cells, and in multiple experiments, as few as 10 and 100 CD44(hi) cells formed tumors in 7/10 and 21/28 mice, respectively. In contrast, CD44(-) colon tumor cells were either nontumorigenic or 10-50-fold less tumorigenic. CD44(hi) cells could be serially passaged up to 4 times in vivo, suggesting self-renewal capacity, and formed tumors that recapitulated the heterogeneity of the original patient tumor. CD44(hi) cells were significantly enriched for nuclear activated beta-catenin, a key element in normal stem/progenitor cells and in early colon tumor progression. Bromodeoxyuridine (BrdU) labeling studies indicated that CD44(hi) cells divide slowly relative to the CD44(-) cells, suggesting their tumorigenicity is not simply due to faster proliferation. Aldehyde dehydrogenase (ALDH) sort further increased the tumorigenicity of CD44(hi) cells from 2/2 patient tumors, but CD133 tumor cells in our hands did not have increased tumorigenicity. Our observations indicate that CD44 is a marker of stem-like cells in colon cancer, and support the use of additional markers to further purify colon cancer stem cells.

Chumsri, S. and A. M. Burger (2008). "Cancer stem cell targeted agents: therapeutic approaches and consequences." *Curr Opin Mol Ther* **10**(4): 323-33.

An increased understanding of stem-cell biology at the molecular level, as well as the isolation and characterization of a rare subset of cells with tumor-initiating properties from several human tumor types, have renewed interest in the exploitation of cancer stem cells (CSCs) as therapeutic targets. Although CSCs share various characteristics with normal stem cells, including self-renewal, asymmetric cell division, indefinite proliferative capacity, and self-protection mechanisms, they also contain unique and disease-specific features suitable for exploitation as therapeutic targets. Several existing anticancer agents and experimental therapeutics can inhibit pathways critical to CSC maintenance, and could, therefore, be utilized to eradicate CSCs. In this review, general and tumor-type specific cancer-stem-cell targets are highlighted. In addition, known inhibitors of these targets that could be utilized in the design of clinical protocols together with conventional cytotoxics as debulking agents are described.

Chumsri, S., W. Matsui, et al. (2007). "Therapeutic implications of leukemic stem cell pathways." *Clin Cancer Res* **13**(22 Pt 1): 6549-54.

An emerging concept in cancer biology is that a rare population of cancer stem cells exists among the heterogeneous cell mass that constitutes a tumor. This concept is best understood in human myeloid leukemia. Normal and malignant hematopoietic stem cell functions are defined by a common set of critical stemness genes that regulate self-renewal and developmental pathways. Several stemness factors, such as Notch or telomerase, show differential activation in normal hematopoietic versus leukemia stem cells. These differences could be exploited therapeutically even with drugs that are already in clinical use for the treatment of leukemia. The translation of novel and existing leukemic stem cell-directed therapies into clinical practice, however, will require changes in clinical trial design and the inclusion of stem cell biomarkers as correlative end points.

Clark, A. T. (2007). "The stem cell identity of testicular cancer." *Stem Cell Rev* **3**(1): 49-59.

Testicular germ cell tumors account for 1% of all cancers, and are the most common malignancies to affect males between the ages of 15 and 34. Understanding the pathogenesis of testis cancer has been challenging because the molecular and cellular events that result in the formation of germ cell tumors are hypothesized to occur during human fetal development. In this review, the molecular pathways

involved in human testis cancer will be presented based on our research in human embryonic stem cells (hESCs), and also research using animal models. Testis germ cell tumors are unique in that the normal germ cell from which the tumor is derived has distinct stem cell characteristics that are shared with pluripotent hESCs. In particular, normal fetal germ cells express the core pluripotent transcription factors NANOG, SOX2 and OCT4. In contrast to hESCs, the germ line is not pluripotent. As a result, germ cell tumorigenesis may arise from loss of germ line-specific inhibitors which in normal germ cells prevent overt pluripotency and self-renewal and when absent in abnormal germ cells, result in the conversion to germ line cancer stem cells. At the conclusion of this review, a model for the molecular events involved in germ cell tumor formation and the relationship between germ cell tumorigenesis and stem cell biology will be presented.

Clarke, R. B., K. Spence, et al. (2005). "A putative human breast stem cell population is enriched for steroid receptor-positive cells." *Dev Biol* **277**(2): 443-56.

Breast epithelial stem cells are thought to be the primary targets in the etiology of breast cancer. Since breast cancers mostly express estrogen and progesterone receptor (ERalpha and PR), we examined the biology of these ERalpha/PR-positive cells and their relationship to stem cells in normal human breast epithelium. We employed several complementary approaches to identify putative stem cell markers, to characterise an isolated stem cell population and to relate these to cells expressing the steroid receptors ERalpha and PR. Using DNA radiolabelling in human tissue implanted into athymic nude mice, a population of label-retaining cells were shown to be enriched for the putative stem cell markers p21(CIP1) and Msi-1, the human homolog of *Drosophila* Musashi. Steroid receptor-positive cells were found to co-express these stem cell markers together with cytokeratin 19, another putative stem cell marker in the breast. Human breast epithelial cells with Hoechst dye-effluxing "side population" (SP) properties characteristic of mammary stem cells in mice were demonstrated to be undifferentiated "intermediate" cells by lack of expression of myoepithelial and luminal apical membrane markers. These SP cells were 6-fold enriched for ERalpha-positive cells and expressed several fold higher levels of the ERalpha, p21(CIP1) and Msi1 genes than non-SP cells. In contrast to non-SP cells, SP cells formed branching structures in matrigel which included cells of both luminal and myoepithelial lineages. The data suggest a model where scattered steroid receptor-positive cells are stem cells that self-renew through

asymmetric cell division and generate patches of transit amplifying and differentiated cells.

Clement, C. A., L. A. Larsen, et al. (2009). "Using nucleofection of siRNA constructs for knockdown of primary cilia in P19.CL6 cancer stem cell differentiation into cardiomyocytes." *Methods Cell Biol* **94**: 181-97.

Primary cilia assemble as solitary organelles in most mammalian cells during growth arrest and are thought to coordinate a series of signal transduction pathways required for cell cycle control, cell migration, and cell differentiation during development and in tissue homeostasis. Recently, primary cilia were suggested to control pluripotency, proliferation, and/or differentiation of stem cells, which may comprise an important source in regenerative biology. We here provide a method using a P19.CL6 embryonic carcinoma (EC) stem cell line to study the function of the primary cilium in early cardiogenesis. By knocking down the formation of the primary cilium by nucleofection of plasmid DNA with siRNA sequences against genes essential in ciliogenesis (IFT88 and IFT20) we block hedgehog (Hh) signaling in P19.CL6 cells as well as the differentiation of the cells into beating cardiomyocytes (Clement et al., 2009). Immunofluorescence microscopy, western blotting, and quantitative PCR analysis were employed to delineate the molecular and cellular events in cilia-dependent cardiogenesis. We optimized the nucleofection procedure to generate strong reduction in the frequency of ciliated cells in the P19.CL6 culture.

Cogle, C. R., S. M. Guthrie, et al. (2003). "An overview of stem cell research and regulatory issues." *Mayo Clin Proc* **78**(8): 993-1003.

Stem cells are noted for their ability to self-renew and differentiate into a variety of cell types. Some stem cells, described as totipotent cells, have tremendous capacity to self-renew and differentiate. Embryonic stem cells have pluripotent capacity, able to form tissues of all 3 germ layers but unable to form an entire live being. Research with embryonic stem cells has enabled investigators to make substantial gains in developmental biology, therapeutic tissue engineering, and reproductive cloning. However, with these remarkable opportunities many ethical challenges arise, which are largely based on concerns for safety, efficacy, resource allocation, and methods of harvesting stem cells. Discussing the moral and legal status of the human embryo is critical to the debate on stem cell ethics. Religious perspectives and political events leading to regulation of stem cell research are presented and discussed, with special attention directed toward the use of embryonic stem

cells for therapeutic and reproductive cloning. Adult stem cells were previously thought to have a restricted capacity to differentiate; however, several reports have described their plasticity potential. Furthermore, there have been close ties between the behavior of stem cells and cancer cells. True eradication of cancer will require a deeper understanding of stem cell biology. This article was written to inform medical scientists and practicing clinicians across the spectrum of medical education about the research and regulatory issues affecting the future of stem cell therapy.

Colleoni, F. and Y. Torrente (2008). "The new challenge of stem cell: brain tumour therapy." Cancer Lett **272**(1): 1-11.

The surprising similarity of much brain tumour behavior to the intrinsic properties of the neural stem/progenitor cell has triggered a recent interest in both arming stem cells to track and help eradicate tumours and in viewing stem cell biology as somehow integral to the emergence and/or production of the neoplasm itself. Moreover, based on the unique capacity of neural stem cells (NSCs) to migrate throughout the brain and to target invading tumour cells, the transplantation of NSCs offers a new potential therapeutic approach as a cell-based delivery system for gene therapy in brain tumours. On the one hand, both stem cells and cancer cells are thought to be capable of unlimited proliferation. While on the other, many tumours and cancer cell lines express stem cell markers, suggesting either that cancer cells resemble stem cells or that cancers contain stem-like cells. In this review we highlight the close relationship between normal neural stem cells and brain tumour stem cells and also suggest the possible clinical implications that these similarities could offer.

Couriel, D. R., K. Hicks, et al. (2000). "Role of tumor necrosis factor-alpha inhibition with infliximab in cancer therapy and hematopoietic stem cell transplantation." Curr Opin Oncol **12**(6): 582-7.

Tumor necrosis factor (TNF)-alpha is a central cytotoxic and proinflammatory cytokine. Research on the benefits of TNF-alpha inhibition as a form of therapy has focused almost exclusively on autoimmune, inflammatory disorders. Infliximab, a chimeric antibody to human TNF-alpha, was recently approved for the management of Crohn disease and rheumatoid arthritis. The potential applications of infliximab in the management of cancer are just beginning to be explored. This article reviews the biology, mechanism of action, pharmacology, and toxicity of infliximab. Existing clinical experience and infliximab's potential role as an

immunosuppressant and antitumor agent in the management of cancer are also discussed.

Crea, F., R. Danesi, et al. (2009). "Cancer stem cell epigenetics and chemoresistance." Epigenomics **1**(1): 63-79.

Cancer stem cells (CSCs) are thought to sustain cancer progression, metastasis and recurrence after therapy. There is in vitro and in vivo evidence supporting the idea that CSCs are highly chemoresistant. Epigenetic gene regulation is crucial for both stem cell biology and chemoresistance. In this review, we summarize current data on epigenetic mechanisms of chemoresistance in cancer stem cells. We propose a model integrating classical CSC pathways (Wnt, Hedgehog and Notch), epigenetic effectors (Polycomb) and drug resistance genes (ABCG2, CD44). Moreover, we analyze the potential of epigenetic drugs to reverse CSC chemoresistance. In the future, CSC epigenomic profiling could help to dissect specific chemoresistance pathways, and have a significant clinical impact for patient stratification and rational design of therapeutic regimens.

Daniels, J. T., G. A. Secker, et al. (2006). "Stem cell therapy delivery: treading the regulatory tightrope." Regen Med **1**(5): 715-9.

The concept of stem cell therapy has engaged the attention of the public and scientists alike. Intensive research effort is focused upon understanding the biology and therapeutic potential of embryonic and adult stem cells, with the eventual goal of treating such pathologies as Parkinson's disease, diabetes, neurological injury and degenerations and cancer. Ex vivo expansion and transplantation of limbal epithelial stem cells to the corneas to treat blinding ocular surface disease was one of the first stem cell therapies to successfully reach the clinic. However, limbal epithelial stem cell research and therapy delivery has remained largely within the noncommercial academic clinician-scientist environment from which it was originally pioneered. In our experience, gaining regulatory approval has been as great a hurdle as surmounting the scientific challenges of stem cell therapy. Based upon our model of delivering 'accredited' limbal epithelial stem cell therapy to patients in compliance with Good Manufacturing Practice and the new European Union Tissues and Cells Directive, we address the key regulatory questions. This may help colleagues who are developing innovative academic research-driven stem cell therapies regarding donor consent, raw materials, quality assurance, laboratory specification, indemnity and funding.

Ding, S. and P. G. Schultz (2004). "A role for chemistry in stem cell biology." *Nat Biotechnol* **22**(7): 833-40.

Although stem cells hold considerable promise for the treatment of numerous diseases including cardiovascular disease, neurodegenerative disease, musculoskeletal disease, diabetes and cancer, obstacles such as the control of stem cell fate, allogenic rejection and limited cell availability must be overcome before their therapeutic potential can be realized. This requires an improved understanding of the signaling pathways that affect stem cell fate. Cell-based phenotypic and pathway-specific screens of natural products and synthetic compounds have recently provided a number of small molecules that can be used to selectively control stem cell proliferation and differentiation. Examples include the selective induction of neurogenesis and cardiomyogenesis in murine embryonic stem cells, osteogenesis in mesenchymal stem cells and dedifferentiation in skeletal muscle cells. Such molecules will likely provide new insights into stem cell biology, and may ultimately contribute to effective medicines for tissue repair and regeneration.

Faustino, R. S., A. Behfar, et al. (2008). "Genomic chart guiding embryonic stem cell cardiopoiesis." *Genome Biol* **9**(1): R6.

BACKGROUND: Embryonic stem cells possess a pluripotent transcriptional background with the developmental capacity for distinct cell fates. Simultaneous expression of genetic elements for multiple outcomes obscures cascades relevant to specific cell phenotypes. To map molecular patterns critical to cardiogenesis, we interrogated gene expression in stem cells undergoing guided differentiation, and defined a genomic paradigm responsible for confinement of pluripotency. **RESULTS:** Functional annotation analysis of the transcriptome of differentiating embryonic stem cells exposed downregulated components of DNA replication, recombination and repair machinery, cell cycling, cancer mechanisms, and RNA post-translational modifications. Concomitantly, cardiovascular development, cell-to-cell signaling, cell development and cell movement were upregulated. These simultaneous gene ontology rearrangements engaged a repertoire switch that specified lineage development. Bioinformatic integration of genomic and gene ontology data further unmasked canonical signaling cascades prioritized within discrete phases of cardiopoiesis. Examination of gene relationships revealed a non-stochastic network anchored by integrin, WNT/beta-catenin, transforming growth factor beta and vascular endothelial growth factor pathways, validated by manipulation of selected

cascades that promoted or restrained cardiogenic yield. Moreover, candidate genes within anchor pathways acted as nodes that organized correlated expression profiles into functional clusters, which collectively orchestrated and secured an overall cardiogenic theme. **CONCLUSION:** The present systems biology approach reveals a dynamically integrated and tractable gene network fundamental to embryonic stem cell specification, and represents an initial step towards resolution of a genomic cardiopoietic atlas.

Flores, I., M. L. Cayuela, et al. (2005). "Effects of telomerase and telomere length on epidermal stem cell behavior." *Science* **309**(5738): 1253-6.

A key process in organ homeostasis is the mobilization of stem cells out of their niches. We show through analysis of mouse models that telomere length, as well as the catalytic component of telomerase, Tert, are critical determinants in the mobilization of epidermal stem cells. Telomere shortening inhibited mobilization of stem cells out of their niche, impaired hair growth, and resulted in suppression of stem cell proliferative capacity in vitro. In contrast, Tert overexpression in the absence of changes in telomere length promoted stem cell mobilization, hair growth, and stem cell proliferation in vitro. The effects of telomeres and telomerase on stem cell biology anticipate their role in cancer and aging.

Foulkes, W. D. (2004). "BRCA1 functions as a breast stem cell regulator." *J Med Genet* **41**(1): 1-5.

BRCA1 is an important susceptibility gene for breast cancer, which confers substantial lifetime risks of breast cancer, particularly in the pre-menopausal age group. Typically, carriers of BRCA1 mutations develop breast tumours that grow rapidly and are high grade and oestrogen receptor negative. They also possess a basal epithelial phenotype, as defined by cytokeratin expression, that is not present in most breast cancers. It has recently been proposed that the adult breast stem cell expresses only basal keratins. Others have indicated a CD44 positive, CD24 negative phenotype for breast cancer stem cells. In this paper, I argue that the biology of human BRCA1 and its rodent homologues and the clinicopathological features of breast cancer related to BRCA1 support the notion that one of the key functions of BRCA1 is to act as a stem cell regulator. This has implications for the management of carriers of mutations of BRCA1, in part because support for the role of BRCA1 as a stem cell regulator would emphasise the distinct nature of breast cancer related to BRCA1.

Gelain, F., D. Bottai, et al. (2006). "Designer self-assembling peptide nanofiber scaffolds for adult mouse neural stem cell 3-dimensional cultures." *PLoS One* **1**: e119.

Biomedical researchers have become increasingly aware of the limitations of conventional 2-dimensional tissue cell culture systems, including coated Petri dishes, multi-well plates and slides, to fully address many critical issues in cell biology, cancer biology and neurobiology, such as the 3-D microenvironment, 3-D gradient diffusion, 3-D cell migration and 3-D cell-cell contact interactions. In order to fully understand how cells behave in the 3-D body, it is important to develop a well-controlled 3-D cell culture system where every single ingredient is known. Here we report the development of a 3-D cell culture system using a designer peptide nanofiber scaffold with mouse adult neural stem cells. We attached several functional motifs, including cell adhesion, differentiation and bone marrow homing motifs, to a self-assembling peptide RADA16 (Ac-RADARADARADARADA-COHN2). These functionalized peptides undergo self-assembly into a nanofiber structure similar to Matrigel. During cell culture, the cells were fully embedded in the 3-D environment of the scaffold. Two of the peptide scaffolds containing bone marrow homing motifs significantly enhanced the neural cell survival without extra soluble growth and neurotrophic factors to the routine cell culture media. In these designer scaffolds, the cell populations with beta-Tubulin(+), GFAP(+) and Nestin(+) markers are similar to those found in cell populations cultured on Matrigel. The gene expression profiling array experiments showed selective gene expression, possibly involved in neural stem cell adhesion and differentiation. Because the synthetic peptides are intrinsically pure and a number of desired function cellular motifs are easy to incorporate, these designer peptide nanofiber scaffolds provide a promising controlled 3-D culture system for diverse tissue cells, and are useful as well for general molecular and cell biology.

Ghotra, V. P., J. C. Puigvert, et al. (2009). "The cancer stem cell microenvironment and anti-cancer therapy." *Int J Radiat Biol* **85**(11): 955-62.

PURPOSE: Tumours are composed of a heterogeneous cell population. Cancer stem cells, which make up a minor fraction of a tumour, may be the cells that initiate and sustain tumour growth. Cancer stem cells are believed to share many properties with normal stem cells that render them relatively insensitive to classical radio- and chemotherapy. **CONCLUSIONS:** We discuss what those (cancer) stem cell properties are and how the interactions with the microenvironment--'the niche'--

control those aspects of (cancer) stem cell biology. We also describe possible strategies to target cancer stem cells in order to prevent cancers from escaping therapy.

Ginestier, C., J. Wicinski, et al. (2009). "Retinoid signaling regulates breast cancer stem cell differentiation." *Cell Cycle* **8**(20): 3297-302.

The cancer stem cell (CSC) hypothesis implicates the development of new therapeutic approaches to target the CSC population. Characterization of the pathways that regulate CSCs activity will facilitate the development of targeted therapies. We recently reported that the enzymatic activity of ALDH1, as measured by the ALDELFUOR assay, can be utilized to isolate normal and malignant breast stem cells in both primary tumors and cell lines. In this study, utilizing a tumorsphere assay, we have demonstrated the role of retinoid signaling in the regulation of breast CSCs self-renewal and differentiation. Utilizing the gene set enrichment analysis (GSEA) algorithm we identified gene sets and pathways associated with retinoid signaling. These pathways regulate breast CSCs biology and their inhibition may provide novel therapeutic approaches to target breast CSCs.

Giordano, A., A. Fucito, et al. (2007). "Carcinogenesis and environment: the cancer stem cell hypothesis and implications for the development of novel therapeutics and diagnostics." *Front Biosci* **12**: 3475-82.

Stem cell research has greatly contributed to the field of oncology with the identification and isolation of cancer stem cells from a variety of tumors. The discovery of rare subpopulations of cancer stem cells has indeed entirely changed the focus of cancer research. Normal adult stem cells and cancer stem cells can both self-renew and undergo a differentiation program that, in turn, gives rise to a high number of differentiated cells. Adult stem cells and their malignant counterparts share almost all of the same intrinsic and extrinsic factors to regulate self-renewal, differentiation and proliferation pathways. Fractions of normal and cancer stem cells are naturally more resistant to toxic injuries than any other cell type. Overall, these observations lead to the conclusion that adult stem or progenitor cells can eventually become malignant by generating cancer stem cells, which are responsible for the development and maintenance of the tumor mass. In addition, chemo-resistant cancer stem cells may cause the relapse of the disease following an apparent beneficial treatment. Indeed, the study of the biology of cancer stem cells might lead to the improvement of preventive cancer diagnosis and to the development of novel therapeutics, which must

be designed to selectively target malignant stem cells without affecting normal adult stem cells.

Graziano, A., R. d'Aquino, et al. (2008). "The stem cell hypothesis in head and neck cancer." J Cell Biochem **103**(2): 408-12.

Cancer stem cells (CSCs) are tumoral cells which have stem features such as self-renewal, high migration capacity, drug resistance, high proliferation abilities. In the last 10 years the pathological meaning and the existence of CSCs have been matter of discussion and a large number of articles have been published about the role that these cells play in the development and maintenance of the tumors. Head and neck squamous-cell carcinoma (HNSCC) is the sixth most common cancer worldwide: early diagnosis of high-risk premalignant lesions are high priorities for reducing deaths due to head and neck cancer. In the last years the CSCs hypothesis has been faced also for head and neck cancer, with the aim of a better comprehension of the tumor biology and an early diagnosis. The evidence that the development of a tumor comes from a small number of cells with stem-like characteristic, could bring too to the identification of therapies against these cellular target, fundamental for maintenance and progression of the lesion. Here, a literature review has been reported about the detection of supposed CSCs in head and neck cancer.

Grichnik, J. M. (2008). "Melanoma, neovogenesis, and stem cell biology." J Invest Dermatol **128**(10): 2365-80.

It is now well established that a subpopulation of tumor stem cells (TSCs) are present within cancer tissues. This suggests that tumors evolve from stem cells; however, the exact cell of tumor origin, the potential role of dedifferentiation, and the role of plasticity in tumor development are largely unknown. A model cancer for the study of the oncologic process is melanoma. The developmental biology of melanocytes is relatively well understood, the cells pigment as they differentiate making them easy to identify, and benign and malignant tumors develop on the skin surface allowing direct observation of growth features, early detection, and removal. This ready access to early-stage tumors will facilitate study of the early oncologic processes and the role of tissue stem cells. Melanomas, like other cancers, include a subpopulation of TSCs. These TSCs have access to embryologic developmental programs, including the capacity to differentiate along multiple cell lineages. For example, melanomas can activate germ-cell pathways with major implications for TSC self-renewal through the activation of telomerase and genomic instability through the collision of meiotic and mitotic pathways

(meiomitosis). The TSC model is still evolving, but the existence of TSCs has significant ramifications for tumor development, diagnosis, prognosis, and treatment of melanoma and other cancers.

Grichnik, J. M., J. A. Burch, et al. (2006). "Melanoma, a tumor based on a mutant stem cell?" J Invest Dermatol **126**(1): 142-53.

Stem cells play a critical role in normal tissue maintenance, and mutations in these stem cells may give rise to cancer. We hypothesize that melanoma develops from a mutated stem cell and therefore residual stem cell characteristics should be able to be identified in melanoma cell lines. We studied three metastatic melanoma cell lines that exhibited multiple morphologic forms in culture and demonstrated the capacity to pigment. We used the ability to efflux Hoechst 33342 dye, a technique known to enrich for stem cells in many tissues, to segregate cell populations. The cells with the greatest ability to efflux the dye were (1) small in size, (2) had the capacity to give rise to larger cell forms, and (3) had the greatest ability to expand in culture. The small cells were found to have a decreased proliferative rate and were less melanized. Large dendritic cells that appeared to be nonproliferative were identified in cultures. Treatment with cytosine beta-D-arabinofuranoside hydrochloride (Ara-C) expanded the large cell population but the residual proliferative capacity, both in vitro and in vivo, remained concentrated in the smaller cell fraction. Antigenic staining patterns were variable and heterogeneous. Nestin (a neural stem cell marker) and gp100 (premelanosomal marker) favored the smaller cell population, while nerve growth factor receptor often labeled larger cells. Morphologic and antigenic heterogeneity remained intact after clonal purification. These findings are consistent with the behavior expected for a tumor based on stem cell biology; this finding has diagnostic and therapeutic implications for melanocytic neoplasias.

Grinstein, E. and C. Mahotka (2009). "Stem cell divisions controlled by the proto-oncogene BMI-1." J Stem Cells **4**(3): 141-6.

Divisions of somatic stem cells are required for the maintenance and regeneration of normal tissues, while divisions of cancerous stem cells likely underlie the existence of certain malignant diseases. Studies of recent years suggest that molecular mechanisms governing stem cell self-renewal can be subverted in tumorigenesis to maintain cancerous growth. This is exemplified by the proto-oncogene BMI-1 that is involved in the maintenance of somatic stem cells and in carcinogenesis within the same tissues. BMI-1 interferes with the central cellular

tumor suppressor pathways linked to retinoblastoma protein (Rb) and p53. These signaling pathways control the cell cycle, cell differentiation, cellular senescence and cell death. While the roles of the pathways associated with Rb and p53 in cancer are broadly established, further elucidation thereof in stem cells might have implications in cancer research, stem cell biology and regenerative medicine.

Gudjonsson, T. and M. K. Magnusson (2005). "Stem cell biology and the cellular pathways of carcinogenesis." *Apmis* **113**(11-12): 922-9.

Tissues in the body are maintained by somatic stem cells. This has been demonstrated both in organs with high cell turnover rate, such as the bone marrow, colon and skin, and in organs with low cell turnover rate, such as the brain. To maintain homeostasis in the body it is important to keep tight control over stem cell fate. Stem cells are under strict control from both intrinsic and extrinsic factors and loss of this control has been postulated to be a key step in the carcinogenic process. There is increasing evidence that cancer initiation results from accumulative oncogenic mutations (intrinsic loss of control) in long-lived stem cells or their immediate progenitor, followed by modification of the surrounding microenvironment (loss of extrinsic control). Decades ago, studies on teratocarcinoma led to the hypothesis that a small subset of self-renewing cancer stem cells with differentiation potential exists within tumors. These studies showed that teratocarcinomas contain undifferentiated embryonic carcinoma cells that are able to give rise to differentiated cells which belong to all three germ layers. More recent studies have confirmed cancer stem cells in such diverse cancers as leukemia, brain and breast cancer. It is, however, unclear whether cancer stem cells originate from resident stem cells or whether they arise as a result of an acquired gain of self-renewal capacity in tissue progenitor cells or even more differentiated cells. The characterization of a cancer stem cell profile within diverse cancer types may open up new avenues for cancer treatment. In this review we discuss the concept of cancer stem cells and focus on examples where these cells have been identified.

Gunaratne, P. H. (2009). "Embryonic stem cell microRNAs: defining factors in induced pluripotent (iPS) and cancer (CSC) stem cells?" *Curr Stem Cell Res Ther* **4**(3): 168-77.

The discovery of microRNAs (miRNAs - small non-coding RNAs of approximately 22 nt) heralded a new and exciting era in biology. During this period miRNAs have gone from ignominy due to their origin mainly in 'junk DNA' to notoriety where

they can be at once characterized as being all powerful (a single miRNA can target and potentially silence several hundred genes) and yet marginal (a given gene can be targeted by several miRNAs such that a given miRNA typically exerts a modest repression) [1-4]. The emerging paradox is exemplified by miRNAs that are prominently expressed in embryonic stem (ES) cells. The collective importance of miRNAs is firmly established by the fact that Dicer^{-/-} mouse embryos die on day 7.5 due to defects in differentiation [5]. However, oppositely correlated expression that is expected of conventional repressors is increasingly being defied in multiple systems in relation to miRNA-mRNA target pairs. This is most evident in ES cells where miR-290-295 and 302 clusters the most abundant ES cell miRNAs are found to be driven by pluripotency genes Oct4, Nanog and Sox2 and also target these genes in 'incoherent feed-forward loops' [7]. Here the miRNAs are co-expressed and positively correlated with these targets that they repress suggesting that one of their primary roles is to fine tune gene expression rather than act as ON/OFF switches. On the other hand, let-7 family members that are notably low in ES cells and rapidly induced upon differentiation exhibit more conventional anti-correlated expression patterns with their targets [7-8]. In an intricately designed auto-regulatory loop, LIN28, a key 'keeper' of the pluripotent state binds and represses the processing of let-7 (a key 'keeper' of the differentiated state) [9-11]. One of the let-7 family members, let-7g targets and represses LIN28 through four 3'-UTR binding sites [12]. We propose that LIN28/let-7 pair has the potential to act as a 'toggle switch' that balances the decision to maintain pluripotency vs. differentiation. We also propose that the c-Myc/E2F driven miR17-92 cluster that together controls the G1 to S transition is fundamental for ES self-renewal and cell proliferation [13-18]. In that context it is no surprise that LIN28 and c-Myc (and therefore let-7 and miR-17-92 by association) and more recently Oct4/Sox2 regulated miR-302 has been shown to be among a handful of factors shown to be necessary and sufficient to convert differentiated cells to induced pluripotent stem (iPS) cells [19-29]. It is also no surprise that activation of miR-17-92 (OncomiRs) and down-regulation of let-7 (tumor suppressors) is a recurring theme in relation to cancers from multiple systems [30-48]. We speculate that the LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 networks are fundamental to properties of pluripotency and self-renewal associated with embryonic stem cells. We also speculate that ES cell miRNA-mRNA associations may also regulate tissue homeostasis and regeneration in the fully developed adult. Consequently, the appropriate regulation of LIN28/let-

7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 gene networks will be critical for the success of regenerative strategies that involve iPS cells. Any perturbation in key ES cell miRNA-mRNA networks during any of the above processes may be a hallmark of (CSCs).

Guo, W., J. L. Lasky, et al. (2008). "Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation." *Nature* **453**(7194): 529-33.

Cancer stem cells, which share many common properties and regulatory machineries with normal stem cells, have recently been proposed to be responsible for tumorigenesis and to contribute to cancer resistance. The main challenges in cancer biology are to identify cancer stem cells and to define the molecular events required for transforming normal cells to cancer stem cells. Here we show that Pten deletion in mouse haematopoietic stem cells leads to a myeloproliferative disorder, followed by acute T-lymphoblastic leukaemia (T-ALL). Self-renewable leukaemia stem cells (LSCs) are enriched in the c-Kit(mid)CD3(+)/Lin(-) compartment, where unphosphorylated beta-catenin is significantly increased. Conditional ablation of one allele of the beta-catenin gene substantially decreases the incidence and delays the occurrence of T-ALL caused by Pten loss, indicating that activation of the beta-catenin pathway may contribute to the formation or expansion of the LSC population. Moreover, a recurring chromosomal translocation, T(14;15), results in aberrant overexpression of the c-myc oncogene in c-Kit(mid)CD3(+)/Lin(-) LSCs and CD3(+) leukaemic blasts, recapitulating a subset of human T-ALL. No alterations in Notch1 signalling are detected in this model, suggesting that Pten inactivation and c-myc overexpression may substitute functionally for Notch1 abnormalities, leading to T-ALL development. Our study indicates that multiple genetic or molecular alterations contribute cooperatively to LSC transformation.

Hatfield, S. and H. Ruohola-Baker (2008). "microRNA and stem cell function." *Cell Tissue Res* **331**(1): 57-66.

The identification and characterization of stem cells for various tissues has led to a greater understanding of development, tissue maintenance, and cancer pathology. Stem cells possess the ability to divide throughout their life and to produce differentiated daughter cells while maintaining a population of undifferentiated cells that remain in the stem cell niche and that retain stem cell identity. Many cancers also have small populations of cells with stem cell characteristics. These cells have been called

cancer stem cells and are a likely cause of relapse in cancer patients. Understanding the biology of stem cells and cancer stem cells offers great promise in the fields of regenerative medicine and cancer treatment. microRNAs (miRNAs) are emerging as important regulators of post-transcriptional gene expression and are considered crucial for proper stem cell maintenance and function. miRNAs have also been strongly implicated in the development and pathology of cancer. In this review, we discuss the characteristics of various stem cell types, including cancer stem cells, and the importance of miRNAs therein.

Heddleston, J. M., Z. Li, et al. (2009). "The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype." *Cell Cycle* **8**(20): 3274-84.

Glioblastomas are highly lethal cancers that contain cellular hierarchies with self-renewing cancer stem cells that can propagate tumors in secondary transplant assays. The potential significance of cancer stem cells in cancer biology has been demonstrated by studies showing contributions to therapeutic resistance, angiogenesis and tumor dispersal. We recently reported that physiologic oxygen levels differentially induce hypoxia inducible factor-2alpha (HIF2alpha) levels in cancer stem cells. HIF1alpha functioned in proliferation and survival of all cancer cells but also was activated in normal neural progenitors suggesting a potentially restricted therapeutic index while HIF2alpha was essential in only in cancer stem cells and was not expressed by normal neural progenitors demonstrating HIF2alpha is a cancer stem cell specific target. We now extend these studies to examine the role of hypoxia in regulating tumor cell plasticity. We find that hypoxia promotes the self-renewal capability of the stem and non-stem population as well as promoting a more stem-like phenotype in the non-stem population with increased neurosphere formation as well as upregulation of important stem cell factors, such as OCT4, NANOG and c-MYC. The importance of HIF2alpha was further supported as forced expression of non-degradable HIF2alpha induced a cancer stem cell marker and augmented the tumorigenic potential of the non-stem population. This novel finding may indicate a specific role of HIF2alpha in promoting glioma tumorigenesis. The unexpected plasticity of the non-stem glioma population and the stem-like phenotype emphasizes the importance of developing therapeutic strategies targeting the microenvironmental influence on the tumor in addition to cancer stem cells.

Hines, S. J., J. S. Litz, et al. (1999). "Coexpression of c-kit and stem cell factor in breast cancer results in

enhanced sensitivity to members of the EGF family of growth factors." *Breast Cancer Res Treat* **58**(1): 1-10.

Kit, a tyrosine kinase growth factor receptor, and its ligand, stem cell factor (SCF), are commonly coexpressed in breast cancer. We have previously shown that MCF7 cells (that naturally express SCF) transfected with a c-kit expression vector exhibit enhanced growth in serum-free medium supplemented with IGF-1. Consequently, we wished to examine the interaction of Kit/SCF with additional growth factors important in the biology of breast cancer. MCF7 transfectants expressing Kit, cultured in serum-free medium supplemented with EGF, displayed more than twice the growth of controls at identical EGF concentrations. Similar responses were seen in the presence of heregulin alpha. The specificity of the Kit-mediated response was illustrated by a reduction in heregulin-stimulated growth in the presence of a monoclonal antibody directed against the Kit receptor. In addition, EGF- and heregulin-stimulated growth of the ZR75-1 cell line that naturally coexpresses Kit and SCF was also inhibited by the Kit blocking antibody. Preliminary investigations into the signal transduction pathways activated by these growth factors revealed that SCF activated both the Ras-MAP kinase and phosphatidylinositol-3-kinase (PI3 kinase) pathway. Both EGF and heregulin activated MAPK but to a lesser degree than SCF, and combination of SCF with these growth factors resulted in enhanced MAPK activation. Assessment of PI3K pathway activation using antiphospho-Akt antibodies revealed that EGF was a poor activator of Akt; activation of this pathway was markedly enhanced by the addition of SCF. Heregulin activated Akt and addition of SCF provided no further activation. Taken together these results suggest that coexpression of SCF and Kit may enhance responsiveness to erbB ligands by enhancing activation of the MAPK and PI3K pathways.

Hoffman, J. A. and B. J. Merrill (2007). "New and renewed perspectives on embryonic stem cell pluripotency." *Front Biosci* **12**: 3321-32.

Although "stem cell biology" is frequently described as a young field, the examination of pluripotency and its effects on embryonic cells has had an interesting and somewhat unusual history. After decades of research into the pluripotency of mammalian embryonic cells, the use of pluripotent cells came into prominence as mouse embryonic stem cells (ESC) provided the foundation of knockout mouse technology; however, the basic biology of pluripotency in embryonic cells was not extensively examined for roughly another twenty years until the creation of human embryonic stem cell lines. With the burgeoning potential of cell based therapies and roles of cancer stem cells in disease, understanding basic

biological mechanisms regulating stem cell characteristics now presents great new opportunities. Therefore, it is not surprising that the underlying genetic and epigenetic forces allowing ESC to maintain pluripotency have been the focus of intense scientific scrutiny in recent years. In order to fully appreciate the importance of new discoveries regarding pluripotency in ESC, it is necessary to understand the role of pluripotency in normal embryonic development. The main purpose of this review is to highlight recent discoveries in the context of what was known about pluripotency and lineage commitment in the embryo prior to the bioinformatics and genomics age. In doing so we attempt to elucidate the importance and limitations of recent discoveries and identify important avenues for future research.

Jalkut, M. W. and R. E. Reiter (2002). "Role of prostate stem cell antigen in prostate cancer research." *Curr Opin Urol* **12**(5): 401-6.

PURPOSE OF REVIEW: The identification of cell surface antigens is critical to the development of future prognostic and therapeutic modalities for the treatment of prostate cancer. Several prostate-specific proteins have been identified and are under investigation. This review reports on prostate stem cell antigen (PSCA), a protein with restricted expression that may have prognostic and therapeutic utility. **RECENT FINDINGS:** PSCA is a glycosylphosphatidylinositol-anchored cell-surface protein belonging to the Ly-6/Thy-1 family of cell surface antigens, and a murine homologue has been described. It is expressed in the normal human prostate and overexpressed in human prostate cancers. Its overexpression has been correlated with increased Gleason score, advanced stage and bone metastasis. PSCA is co-amplified with MYC, an independent predictor of progression and death. PSCA may therefore be a useful predictor of tumor biology and a useful target of immunotherapy against prostate cancer. Evidence suggests a potential role in strategies employing cytotoxic T cell lymphocytes. Anti-tumor activity has been demonstrated with monoclonal antibodies in tumor take and established tumor xenograft models. Conjugated antibody has recently been reported to have anti-tumor activity in preclinical models. **SUMMARY:** PSCA may serve as a tool in refining the prognosis of an individual cancer and may be a useful therapeutic target for immunotherapy. Future studies will be required to confirm its clinical utility as a prognostic factor. Future animal and clinical studies will be required to test various immunotherapy strategies for safety and efficacy. The study of PSCA regulation and expression may provide information on normal prostate development and prostate carcinogenesis.

Jeong, Y. and D. J. Mangelsdorf (2009). "Nuclear receptor regulation of stemness and stem cell differentiation." *Exp Mol Med* **41**(8): 525-37.

Stem cells include a diverse number of toti-, pluri-, and multi-potent cells that play important roles in cellular genesis and differentiation, tissue development, and organogenesis. Genetic regulation involving various transcription factors results in the self-renewal and differentiation properties of stem cells. The nuclear receptor (NR) superfamily is composed of 48 ligand-activated transcription factors involved in diverse physiological functions such as metabolism, development, and reproduction. Increasing evidence shows that certain NRs function in regulating stemness or differentiation of embryonic stem (ES) cells and tissue-specific adult stem cells. Here, we review the role of the NR superfamily in various aspects of stem cell biology, including their regulation of stemness, forward- and trans-differentiation events; reprogramming of terminally differentiated cells; and interspecies differences. These studies provide insights into the therapeutic potential of the NR superfamily in stem cell therapy and in treating stem cell-associated diseases (e.g., cancer stem cell).

Jordan, C. T. (2004). "Cancer stem cell biology: from leukemia to solid tumors." *Curr Opin Cell Biol* **16**(6): 708-12.

The biology of stem cells and their intrinsic properties are now recognized as integral to tumor pathogenesis in several types of cancer. This observation has broad ramifications in the cancer research field and is likely to impact our understanding of the basic mechanisms of tumor formation and the strategies we use to treat cancers. A role for stem cells has been demonstrated for cancers of the hematopoietic system, breast and brain. Going forward it is likely that stem cells will also be implicated in other malignancies. Hence, a detailed understanding of stem cells and how they mediate tumor pathogenesis will be critical in developing more effective cancer therapies.

Kakarala, M. and M. S. Wicha (2008). "Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy." *J Clin Oncol* **26**(17): 2813-20.

Recent research in breast biology has provided support for the cancer stem-cell hypothesis. Two important components of this hypothesis are that tumors originate in mammary stem or progenitor cells as a result of dysregulation of the normally tightly regulated process of self-renewal. As a result, tumors contain and are driven by a cellular subcomponent that

retains key stem-cell properties including self-renewal, which drives tumorigenesis and differentiation that contributes to cellular heterogeneity. Advances in stem-cell technology have led to the identification of stem cells in normal and malignant breast tissue. The study of these stem cells has helped to elucidate the origin of the molecular complexity of human breast cancer. The cancer stem-cell hypothesis has important implications for early detection, prevention, and treatment of breast cancer. Both hereditary and sporadic breast cancers may develop through dysregulation of stem-cell self-renewal pathways. These aberrant stem cells may provide targets for the development of cancer prevention strategies. Furthermore, because breast cancer stem cells may be highly resistant to radiation and chemotherapy, the development of more effective therapies for this disease may require the effective targeting of this cell population.

Kiatpongsan, S., A. Mutirangura, et al. (2006). "Future cancer management with stem cell knowledge and technology." *J Med Assoc Thai* **89**(8): 1322-32.

Cancer has been proposed as a result of abnormal control of growth and development of stem cells for more than century. This is the "cancer stem cell hypothesis". Both cancer and stem cells share many common especial properties. They are immortal and have good differentiation potential. In addition, organogenesis and carcinogenesis are very similar processes. Recently, more evidence and convincing data from stem cell biology research are supporting this concept. Furthermore, the research provides new promising approaches for cancer diagnosis and treatment based on stem cell knowledge and technology. Upcoming data and evidence may revolutionize cancer management, making it more effective and safer.

Kim, C. F. (2007). "Paving the road for lung stem cell biology: bronchioalveolar stem cells and other putative distal lung stem cells." *Am J Physiol Lung Cell Mol Physiol* **293**(5): L1092-8.

New discoveries in stem cell biology are making the biology of solid tissues increasingly complex. Important seminal studies demonstrating the presence of damage-resistant cell populations together with new isolation and characterization techniques suggest that stem cells exist in the adult lung. More detailed in vivo molecular and cellular characterization of bronchioalveolar stem cells (BASCs), other putative lung stem and progenitor cells, and differentiated cells is needed to determine the lineage relationships in adult lung. Lung diseases such as cystic fibrosis or chronic obstructive pulmonary disease, as well as the most common form

of lung cancer in the United States, all involve apparent bronchiolar and alveolar cell defects. It is likely that the delicate balance of stem, progenitor, and differentiated cell functions in the lung is critically affected in patients with these devastating diseases. Thus the discovery of BASCs and other putative lung stem cells will lay the foundation for new inroads to understanding lung biology related to lung disease.

Kitamura, H., K. Okudela, et al. (2009). "Cancer stem cell: implications in cancer biology and therapy with special reference to lung cancer." *Lung Cancer* **66**(3): 275-81.

The cancer stem cell (CSC) theory is currently central to the field of cancer research, because it is not only a matter of academic interest but also crucial in cancer therapy. CSCs share a variety of biological properties with normal somatic stem cells in terms of self-renewal, the propagation of differentiated progeny, the expression of specific cell markers and stem cell genes, and the utilization of common signaling pathways and the stem cell niche. However, CSCs differ from normal stem cells in their tumorigenic activity. Thus, CSCs are also termed cancer initiating cells. In this paper, we briefly review hitherto described study results and refer to some excellent review articles to understand the basic properties of CSCs. In addition, we focus upon CSCs of lung cancers, since lung cancer is still increasing in incidence worldwide and remains the leading cause of cancer deaths. Understanding the properties of, and exploring cell markers and signaling pathways specific to, CSCs of lung cancers, will lead to progress in therapy, intervention, and improvement of the prognosis of patients with lung cancer. In the near future, the evaluation of CSCs may be a routine part of practical diagnostic pathology.

Knizetova, P., J. L. Darling, et al. (2008). "Vascular endothelial growth factor in astrogloma stem cell biology and response to therapy." *J Cell Mol Med* **12**(1): 111-25.

Malignant astroglomas are among the most aggressive, highly vascular and infiltrating tumours bearing a dismal prognosis, mainly due to their resistance to current radiation treatment and chemotherapy. Efforts to identify and target the mechanisms that underlie astrogloma resistance have recently focused on candidate cancer stem cells, their biological properties, interplay with their local microenvironment or 'niche', and their role in tumour progression and recurrence. Both paracrine and autocrine regulation of astrogloma cell behaviour by locally produced cytokines such as the vascular endothelial growth factor (VEGF) are emerging as key

factors that determine astrogloma cell fate. Here, we review these recent rapid advances in astrogloma research, with emphasis on the significance of VEGF in astrogloma stem-like cell biology. Furthermore, we highlight the unique DNA damage checkpoint properties of the CD133-marker-positive astrogloma stem-like cells, discuss their potential involvement in astrogloma radioresistance, and consider the implications of this new knowledge for designing combinatorial, more efficient therapeutic strategies.

Kraft, A. (2009). "Manhattan transfer: lethal radiation, bone marrow transplantation, and the birth of stem cell biology, ca. 1942-1961." *Hist Stud Nat Sci* **39**(2): 171-218.

This study investigates how, in the late 1940s and 1950s, fears of nuclear accidents and nuclear warfare shaped postwar radiobiology. The new and intense forms of radiation generated by nuclear reactor technology, and which would be released in the event of a nuclear war, created concerns about a public-health hazard unprecedented in form and scale. Fears of inadvertent exposure to acute and potentially lethal radiation launched a search for anti-radiation therapies, out of which emerged the new technique of bone marrow transplantation (BMT). This study analyzes the use of BMT first as a research tool to explore the biological effects of ionizing radiation, and then as an adjunct to radiotherapy for the treatment of cancer. In highlighting how BMT became the province of different research and clinical constituencies, this study develops an understanding of the forces and contingencies that shaped its development. Exploring the emergence of BMT and the uses to which it was put, it reveals that BMT remained a technique in the making -- unstable and far from standardized, even as it became both a widely used research tool and rapidly made its way into the clinic. More broadly, it casts new light on one route through which the Manhattan Project influenced postwar radiobiology; it also affords new insights into one means by which radiobiology came to serve the interests of the Cold War state. In its focus on BMT this paper provides a new perspective on the evolving relationship between radiobiology and biomedicine in the postwar period.

Krizhanovsky, V., W. Xue, et al. (2008). "Implications of cellular senescence in tissue damage response, tumor suppression, and stem cell biology." *Cold Spring Harb Symp Quant Biol* **73**: 513-22.

Cellular senescence is characterized by an irreversible cell cycle arrest that, when bypassed by mutation, contributes to cellular immortalization. Activated oncogenes induce a hyperproliferative response, which might be one of the senescence cues.

We have found that expression of such an oncogene, Akt, causes senescence in primary mouse hepatoblasts *in vitro*. Additionally, AKT-driven tumors undergo senescence *in vivo* following p53 reactivation and show signs of differentiation. In another *in vivo* system, i.e., liver fibrosis, hyperproliferative signaling through AKT might be a driving force of the senescence in activated hepatic stellate cells. Senescent cells up-regulate and secrete molecules that, on the one hand, can reinforce the arrest and, on the other hand, can signal to an innate immune system to clear the senescent cells. The mechanisms governing senescence and immortalization are overlapping with those regulating self-renewal and differentiation. These respective control mechanisms, or their dysregulation, are involved in multiple pathological conditions including fibrosis, wound healing, and cancer. Understanding extracellular cues that regulate these processes may enable new therapies for these conditions.

Mangel, M. and M. B. Bonsall (2008). "Phenotypic evolutionary models in stem cell biology: replacement, quiescence, and variability." *PLoS One* **3**(2): e1591.

Phenotypic evolutionary models have been used with great success in many areas of biology, but thus far have not been applied to the study of stem cells except for investigations of cancer. We develop a framework that allows such modeling techniques to be applied to stem cells more generally. The fundamental modeling structure is the stochastic kinetics of stem cells in their niche and of transit amplifying and fully differentiated cells elsewhere in the organism, with positive and negative feedback. This formulation allows graded signals to be turned into all or nothing responses, and shows the importance of looking beyond the niche for understanding how stem cells behave. Using the deterministic version of this framework, we show how competition between different stem cell lines can be analyzed, and under what circumstances stem cells in a niche will be replaced by other stem cells with different phenotypic characteristics. Using the stochastic version of our framework and state dependent life history theory, we show that the optimal behavior of a focal stem cell will involve long periods of quiescence and that a population of identical stem cells will show great variability in the times at which activity occurs; we compare our results with classic ones on quiescence and variability in the hematopoietic system.

Martens, J. E., J. Arends, et al. (2004). "Cytokeratin 17 and p63 are markers of the HPV target cell, the cervical stem cell." *Anticancer Res* **24**(2B): 771-5.

BACKGROUND: Basic research on HPV has focused on identifying the genetic changes that lead to cervical carcinoma. However, while focusing on the molecular biology of the cancer, understanding of its cellular biology has lagged: the target cell of the HPV infection is unknown. **MATERIALS AND METHODS:** In this study we identified the stem cell population of the cervical epithelium by monoclonal antibodies against p63, a homologue of the tumor suppressor gene p53 and cytokeratin 17 (CK17). **RESULTS:** We noted p63 expression consistently in the nuclei of reserve cells, hyperplasia of the reserve cells and the basal layer of the ectocervical epithelium, while CK17 only stained endocervical reserve cells and reserve cell hyperplasia. **CONCLUSION:** We conclude that both p63 and CK17 are suitable markers for cervical stem cell identification. Both markers, therefore, qualify for the identification of the HPV target cell.

McCulloch, E. A., M. D. Minden, et al. (1988). "Stem cell renewal and differentiation in acute myeloblastic leukaemia." *J Cell Sci Suppl* **10**: 267-81.

The defining properties of stem cells are capacities for self-renewal and, after determination, a limited number of terminal divisions. The blast cells of acute myeloblastic leukaemia (AML) are maintained by stem cells with these two properties. Since renewal and differentiation can be assessed separately in cultures of AML blasts, these cancer cells provide a useful model for examining stem regulation; such studies have practical importance for future developments in the treatment of AML. This paper considers three aspects of blast cell biology. First, evidence is presented that self-renewal and differentiation are regulated by specific genes; further, the DNA encoding these genes has structural features that affect the chemosensitivity of self-renewal. This sensitivity varies from patient-to-patient and is an important attribute contributing to variation in treatment efficacy. Second, the effects of myelopoietic growth factors on blast stem cells are presented and discussed, as these bear on the regulation of the balance between renewal and differentiation. Finally, models of leukaemic haemopoiesis are considered in light of the experimental findings. The suggestion is advanced that leukaemia can be explained better by abnormalities of gene expression than by blocked differentiation.

Melone, M. A., M. Giuliano, et al. (2009). "Genes involved in regulation of stem cell properties: studies on their expression in a small cohort of neuroblastoma patients." *Cancer Biol Ther* **8**(13): 1300-6.

Cancer stem cells have been isolated from many tumors. Several evidences prove that

neuroblastoma contains its own stem cell-like cancer cells. We chose to analyze 20 neuroblastoma tumor samples in the expression of 13 genes involved in the regulation of stem cell properties to evaluate if their misregulation could have a clinical relevance. In several specimens we detected the expression of genes belonging to the OCT3/SOX2/NANOG/KLF4 core circuitry that acts at the highest level in regulating stem cell biology. This result is in agreement with studies showing the existence of malignant stem cells in neuroblastoma. We also observed differences in the expression of some stemness-related genes that may be useful for developing new prognostic analyses. In fact, preliminary data suggests that the presence/absence of UTF1 along with differences in BMI1 mRNA levels could distinguish low grade neuroblastomas from IV stage tumors.

Merok, J. R., J. A. Lansita, et al. (2002). "Cosegregation of chromosomes containing immortal DNA strands in cells that cycle with asymmetric stem cell kinetics." *Cancer Res* **62**(23): 6791-5.

A long-standing intriguing hypothesis in cancer biology is that adult stem cells avoid mutations from DNA replication errors by a unique pattern of chromosome segregation. At each asymmetric cell division, adult stem cells have been postulated to selectively retain a set of chromosomes that contain old template DNA strands (i.e., "immortal DNA strands"). Using cultured cells that cycle with asymmetric cell kinetics, we confirmed both the existence of immortal DNA strands and the cosegregation of chromosomes that bear them. Our findings also lead us to propose a role for immortal DNA strands in tissue aging as well as cancer.

Mimeault, M. and S. K. Batra (2008). "Recent progress on tissue-resident adult stem cell biology and their therapeutic implications." *Stem Cell Rev* **4**(1): 27-49.

Recent progress in the field of the stem cell research has given new hopes to treat and even cure diverse degenerative disorders and incurable diseases in human. Particularly, the identification of a rare population of adult stem cells in the most tissues/organs in human has emerged as an attractive source of multipotent stem/progenitor cells for cell replacement-based therapies and tissue engineering in regenerative medicine. The tissue-resident adult stem/progenitor cells offer the possibility to stimulate their in vivo differentiation or to use their ex vivo expanded progenies for cell replacement-based therapies with multiple applications in human. Among the human diseases that could be treated by the stem cell-based therapies, there are hematopoietic and immune disorders, multiple degenerative disorders,

such as Parkinson's and Alzheimer's diseases, type 1 or 2 diabetes mellitus as well as eye, liver, lung, skin and cardiovascular disorders and aggressive and metastatic cancers. In addition, the genetically-modified adult stem/progenitor cells could also be used as delivery system for expressing the therapeutic molecules in specific damaged areas of different tissues. Recent advances in cancer stem/progenitor cell research also offer the possibility to targeting these undifferentiated and malignant cells that provide critical functions in cancer initiation and progression and disease relapse for treating the patients diagnosed with the advanced and metastatic cancers which remain incurable in the clinics with the current therapies.

Mimeault, M., R. Hauke, et al. (2007). "Stem cells: a revolution in therapeutics-recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies." *Clin Pharmacol Ther* **82**(3): 252-64.

Basic and clinical research accomplished during the last few years on embryonic, fetal, amniotic, umbilical cord blood, and adult stem cells has constituted a revolution in regenerative medicine and cancer therapies by providing the possibility of generating multiple therapeutically useful cell types. These new cells could be used for treating numerous genetic and degenerative disorders. Among them, age-related functional defects, hematopoietic and immune system disorders, heart failures, chronic liver injuries, diabetes, Parkinson's and Alzheimer's diseases, arthritis, and muscular, skin, lung, eye, and digestive disorders as well as aggressive and recurrent cancers could be successfully treated by stem cell-based therapies. This review focuses on the recent advancements in adult stem cell biology in normal and pathological conditions. We describe how these results have improved our understanding on critical and unique functions of these rare sub-populations of multipotent and undifferentiated cells with an unlimited self-renewal capacity and high plasticity. Finally, we discuss some major advances to translate the experimental models on ex vivo and in vivo expanded and/or differentiated stem cells into clinical applications for the development of novel cellular therapies aimed at repairing genetically altered or damaged tissues/organs in humans. A particular emphasis is made on the therapeutic potential of different tissue-resident adult stem cell types and their in vivo modulation for treating and curing specific pathological disorders.

Mroczko, B., M. Szmitkowski, et al. (2004). "Stem cell factor and macrophage-colony stimulating factor

in patients with pancreatic cancer." Clin Chem Lab Med **42**(3): 256-60.

Stem cell factor (SCF) and macrophage-colony stimulating factor (M-CSF) have assumed an increasing importance in cancer biology. In the present study we investigated the serum levels of these cytokines in pancreatic cancer patients in relation to controls and to patients with benign lesions of the pancreas (chronic pancreatitis group). The classical tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) were also tested. We compared the serum levels of cytokines with tumor stage. We also defined the receiver-operating characteristics (ROC) curve for cytokines and classical tumor markers. The cytokines were measured in 47 patients with pancreatic cancer, in 27 patients with chronic pancreatitis and in 35 healthy subjects. SCF and M-CSF were determined using enzyme-linked immunosorbent assay (ELISA). CEA and CA 19-9 were measured by microparticle enzyme immunoassay. There were significant differences in the levels of circulating SCF, M-CSF, CEA and CA 19-9 in the pancreatic cancer patients compared to the control group, but only the serum levels of M-CSF, CEA and CA 19-9 were significantly higher in pancreatic cancer patients compared to the pancreatitis group. The levels of cytokines and tumor markers were higher in patients with a more advanced tumor stage. The M-CSF serum levels correlated positively with the tested tumor markers. The M-CSF area under the ROC curve was higher than the SCF area. These results suggest that M-CSF is a better candidate for a pancreatic cancer tumor marker than SCF.

Murray, P. E. and F. Garcia-Godoy (2004). "Stem cell responses in tooth regeneration." Stem Cells Dev **13**(3): 255-62.

Scientific advances in the creation of restorative biomaterials, in vitro cell culture technology, tissue grafting, tissue engineering, molecular biology, and the human genome project provide the basis for the introduction of new technologies into dentistry. This review is intended to facilitate the development of stem cell therapy for use with established therapeutic modalities to restore and regenerate oral tissues. Teeth have been shown to mineralize in response to injury for many decades, but only in recent years has the position of the stem cells been localized around blood vessels. The cells have been identified as myofibroblastoid pericytes. The ability to control the differentiation and proliferation of these cells is being examined to create stem cell therapies that can solve dental problems more effectively than current treatment regimes. Although the problems of introducing these technologies are

substantial, the potential benefits to patients and the profession are equally promising - a cure for caries and diseases, a cure for oral cancer, correction of congenital defects, and the regeneration of teeth and tissues to restore oral functions. The purpose of this review is to describe how these new technologies can most usefully be employed in dentistry to enable clinicians to satisfy patient demand for a nondefective dentition.

Park, J. S., D. Y. Noh, et al. (2004). "Gene expression analysis in SV40-immortalized human breast luminal epithelial cells with stem cell characteristics using a cDNA microarray." Int J Oncol **24**(6): 1545-58.

The epithelial compartment of the human breast comprises two distinct cell types. Type I human breast epithelial cells (HBECs) are expressing luminal epithelial cell markers and stem cell characteristics, whereas Type II HBECs show basal epithelial cell phenotypes. When defined in terms of markers for normal cell lineages, most invasive breast cancer cells correspond to the phenotype of the common luminal epithelial cell. We had developed simian virus 40-immortalized cell lines from normal HBECs with luminal and stem cell characteristics. To identify molecular changes involved in immortalization, we analyzed the differential gene expression profiles of normal and non-tumorigenic immortalized Type I HBECs using cDNA microarray with 7,448 sequence-verified clones. Out of the 7,448 genes screened, consistent gene expression changes among biological replicates included 67 in Type I HBECs and 86 in Type II HBECs for 4-fold change criteria. Surprisingly, we identified 148 genes (>2.0-fold) as being either up- or down-regulated related to immortalization: 67 genes (MYBL2, UCHL1 et al) were up-regulated, and 81 genes (IGFBP3, CDKN1A et al) were down-regulated significantly. The altered expression levels of the selected genes were subsequently confirmed by semiquantitative RT-PCR. Our studies suggest that the immortalization of Type I HBECs might be an early step in the initiation of a subset of breast cancer. Furthermore, these results will open up an avenue for more detailed understanding of breast stem cell and tumor biology.

Parrott, J. A., G. Kim, et al. (2000). "Expression and action of kit ligand/stem cell factor in normal human and bovine ovarian surface epithelium and ovarian cancer." Biol Reprod **62**(6): 1600-9.

Greater than 95% of ovarian cancers originate from the epithelial cells on the surface of the ovary termed ovarian surface epithelium (OSE). A normal aspect of OSE function is repeated proliferation after ovulation, and this is postulated to be involved in part in the onset of ovarian cancer. The

hypothesis tested is that locally produced growth factors have an important role in controlling OSE proliferation. The current study investigates the potential role of the growth factor kit ligand (KL)/stem cell growth factor and its receptor c-kit in normal OSE biology and ovarian cancer. Human tumors from borderline, stage I, and stage III cases of ovarian cancer were found to express KL and c-kit protein in the epithelial cell component by ICC analysis. The stromal cell component of human ovarian tumors contained little immunostaining. Bovine ovarian physiology and endocrinology are similar to the human such that cow ovaries were used as a model system to investigate normal OSE functions. KL and c-kit proteins were detected in the OSE from both normal human and bovine ovaries. Adjacent ovarian stromal tissue contained less intense but positive KL and c-kit immunostaining. To extend the ICC results, RNA was collected from normal bovine OSE and ovarian stromal cells to examine KL gene expression. KL transcripts were detected in cultured OSE and stromal cells by Northern blot analysis. KL gene expression was found to be high in freshly isolated OSE but low in freshly isolated stroma using a quantitative polymerase chain reaction procedure. Levels of KL gene expression in cultured OSE and stroma increased to high levels. Observations indicate that normal OSE expresses high levels of KL in vivo and in vitro. The actions of KL on the growth of both normal OSE cells and ovarian cancer cells was investigated. KL was found to stimulate the growth of normal OSE cells in a similar manner to epidermal growth factor. Observations demonstrate the production and action of KL by normal OSE cells and ovarian cancer cells. Coexpression of KL and c-kit by normal OSE suggests that KL can act as an autocrine factor for OSE. The local production and action of KL on OSE provides insight into normal OSE biology, and a factor that may be involved in the onset and progression of ovarian cancer.

Patel, S. A., A. C. Heinrich, et al. (2009). "Inflammatory mediators: Parallels between cancer biology and stem cell therapy." *J Inflamm Res* 2: 13-19.

Inflammation encompasses diverse molecular pathways, and it is intertwined with a wide array of biological processes. Recently, there has been an upsurge of interest in the interactions between mediators of inflammation and other cells such as stem cells and cancer cells. Since tissue injuries are associated with the release of inflammatory mediators, it would be difficult to address this subject without considering the implications of their systemic effects. In this review, we discuss the effects of inflammatory

reactions on stem cells and extrapolate on information pertaining to cancer biology. The discussion focuses on integrins and cytokines, and identifies the transcription factor, nuclear factor-kappa B (NFkappaB) as central to the inflammatory response. Since stem cell therapy has been proposed for type II diabetes mellitus, metabolic syndrome, pulmonary edema, these disorders are used as examples to discuss the roles of inflammatory mediators. We propose prospects for future research on targeting the NFkappaB signaling pathway. Finally, we explore the bridge between inflammation and stem cells, including neural stem cells and adult stem cells from the bone marrow. The implications of mesenchymal stem cells in regenerative medicine as pertaining to inflammation are vast based on their anti-inflammatory and immunosuppressive effects. Such features of stem cells offer great potential for therapy in graft-versus-host disease, conditions with a significant inflammatory component, and tissue regeneration.

Perez-Caro, M., C. Cobaleda, et al. (2009). "Cancer induction by restriction of oncogene expression to the stem cell compartment." *Embo J* 28(1): 8-20.

In human cancers, all cancerous cells carry the oncogenic genetic lesions. However, to elucidate whether cancer is a stem cell-driven tissue, we have developed a strategy to limit oncogene expression to the stem cell compartment in a transgenic mouse setting. Here, we focus on the effects of the BCR-ABLp210 oncogene, associated with chronic myeloid leukaemia (CML) in humans. We show that CML phenotype and biology can be established in mice by restricting BCR-ABLp210 expression to stem cell antigen 1 (Sca1)(+) cells. The course of the disease in Sca1-BCR-ABLp210 mice was not modified on STI571 treatment. However, BCR-ABLp210-induced CML is reversible through the unique elimination of the cancer stem cells (CSCs). Overall, our data show that oncogene expression in Sca1(+) cells is all that is required to fully reprogramme it, giving rise to a full-blown, oncogene-specified tumour with all its mature cellular diversity, and that elimination of the CSCs is enough to eradicate the whole tumour.

Piazza, F., J. A. Costoya, et al. (2004). "Disruption of PLZF in mice leads to increased T-lymphocyte proliferation, cytokine production, and altered hematopoietic stem cell homeostasis." *Mol Cell Biol* 24(23): 10456-69.

Deregulated function of members of the POK (POZ and Kruppel) family of transcriptional repressors, such as promyelocytic leukemia zinc finger (PLZF) and B-cell lymphoma 6 (BCL-6), plays a critical role in the pathogenesis of acute

promyelocytic leukemia (APL) and non-Hodgkin's lymphoma, respectively. PLZP, also known as TZFP, FAZF, or ROG, is a novel POK protein that displays strong homology with PLZF and has been implicated in the pathogenesis of the cancer-predisposing syndrome, Fanconi's anemia, and of APL, in view of its ability to heterodimerize with the FANC-C and PLZF proteins, respectively. Here we report the generation and characterization of mice in which we have specifically inactivated the PLZP gene through in-frame insertion of a lacZ reporter and without perturbing the expression of the neighboring MLL2 gene. We show that PLZP-deficient mice display defects in cell cycle control and cytokine production in the T-cell compartment. Importantly, PLZP inactivation perturbs the homeostasis of the hematopoietic stem and/or progenitor cell. On the basis of our data, a deregulation of PLZP function in Fanconi's anemia and APL may affect the biology of the hematopoietic stem cell, in turn contributing to the pathogenesis of these disorders.

Pollard, S. M., K. Yoshikawa, et al. (2009). "Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens." *Cell Stem Cell* 4(6): 568-80.

Human brain tumors appear to have a hierarchical cellular organization suggestive of a stem cell foundation. In vitro expansion of the putative cancer stem cells as stable cell lines would provide a powerful model system to study their biology. Here, we demonstrate routine and efficient derivation of adherent cell lines from malignant glioma that display stem cell properties and initiate high-grade gliomas following xenotransplantation. Significantly, glioma neural stem (GNS) cell lines from different tumors exhibit divergent gene expression signatures and differentiation behavior that correlate with specific neural progenitor subtypes. The diversity of gliomas may, therefore, reflect distinct cancer stem cell phenotypes. The purity and stability of adherent GNS cell lines offer significant advantages compared to "sphere" cultures, enabling refined studies of cancer stem cell behavior. A proof-of-principle live cell imaging-based chemical screen (450 FDA-approved drugs) identifies both differential sensitivities of GNS cells and a common susceptibility to perturbation of serotonin signaling.

Pontier, S. M. and W. J. Muller (2009). "Integrins in mammary-stem-cell biology and breast-cancer progression--a role in cancer stem cells?" *J Cell Sci* 122(Pt 2): 207-14.

Cancer cells with stem cell-like properties (cancer stem cells) are believed to drive cancer and are

associated with poor prognosis. Data from mouse models have demonstrated that integrins, the major cellular receptors for extracellular-matrix components, have essential roles both during cancer initiation and progression, and during cell differentiation in normal development. By presenting an overview of the role of integrins in stem-cell biology and in cancer progression, this Commentary aims to present evidence for a role of integrins in the biology of cancer stem cells. Given the recent interest in the role of integrins in breast-cancer initiation and progression, we focus on the role of the members of the integrin family and their coupled signaling pathways in mammary-gland development and tumorigenesis.

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

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

Qian, J., G. Yehia, et al. (2001). "Cloning of human preprotachykinin-I promoter and the role of cyclic adenosine 5'-monophosphate response elements in its expression by IL-1 and stem cell factor." *J Immunol* 166(4): 2553-61.

Preprotachykinin-I gene (PPT-I) encodes several peptides with organ-specific functions that link the neuroendocrine-immune-hemopoietic axis. We cloned upstream of the initiation site of human PPT-I promoter and identified consensus sequences for two cAMP response elements (CRE). PPT-I is induced by cytokines including those that signal through the cAMP pathway. Therefore, we studied the role of the two CRE in IL-1 α and stem cell factor (SCF) stimulation of bone marrow stroma because both cytokines induce endogenous PPT-I in these cells and activate the cAMP pathway. Furthermore, bone marrow stroma expresses the transcription factors regulated by the cAMP pathways such as the repressor (ICERII γ) and activator (CREM τ). Mutagenesis of the two CRE and/or cotransfection with vectors that express ICERII γ or CREM τ indicated that the two CRE have major roles in PPT-I expression. The two CRE are also required for optimal promoter activity by SCF and IL-1 α . A particular cytokine could concomitantly induce PPT-I and the high affinity G protein-coupled receptor for PPT-I peptides, NK-1R. We showed that SCF, a representative cytokine, induced PPT-I and NK-1R leading to autocrine and/or paracrine cell activation. Because NK-1R activates cAMP through the G protein, the results suggest that the presence of CRE sequences within PPT-I promoter could be important in the regulation of PPT-I expression by cytokines, irrespective of their ability to signal through cAMP. As PPT-I is implicated in hemopoietic regulation, immune responses, breast cancer, and other neural functions, these studies add to the basic biology of these processes and could provide targets for drug development.

Rahman, R., R. Heath, et al. (2009). "Cellular immortality in brain tumours: an integration of the cancer stem cell paradigm." *Biochim Biophys Acta* **1792**(4): 280-8.

Brain tumours are a diverse group of neoplasms that continue to present a formidable challenge in our attempt to achieve curable intervention. Our conceptual framework of human brain cancer has been redrawn in the current decade. There is a gathering acceptance that brain tumour formation is a phenotypic outcome of dysregulated neurogenesis, with tumours viewed as abnormally differentiated neural tissue. In relation, there is accumulating evidence that brain tumours, similar to leukaemia and many solid tumours, are organized as a developmental hierarchy which is maintained by a small fraction of cells endowed with many shared properties of tissue stem cells. Proof that neurogenesis persists throughout adult life, compliments this concept. Although the cancer cell of origin is unclear,

the proliferative zones that harbour stem cells in the embryonic, post-natal and adult brain are attractive candidates within which tumour-initiation may ensue. Dysregulated, unlimited proliferation and an ability to bypass senescence are acquired capabilities of cancerous cells. These abilities in part require the establishment of a telomere maintenance mechanism for counteracting the shortening of chromosomal termini. A strategy based upon the synthesis of telomeric repeat sequences by the ribonucleoprotein telomerase, is prevalent in approximately 90% of human tumours studied, including the majority of brain tumours. This review will provide a developmental perspective with respect to normal (neurogenesis) and aberrant (tumourigenesis) cellular turnover, differentiation and function. Within this context our current knowledge of brain tumour telomere/telomerase biology will be discussed with respect to both its developmental and therapeutic relevance to the hierarchical model of brain tumourigenesis presented by the cancer stem cell paradigm.

Raiser, D. M., S. J. Zacharek, et al. (2008). "Stem cell biology in the lung and lung cancers: using pulmonary context and classic approaches." *Cold Spring Harb Symp Quant Biol* **73**: 479-90.

Classic stem cell biology approaches tailored specifically with lung biology in mind are needed to bring the field of lung stem cell biology up to speed with that in other tissues. The infrequent cellular turnover, the diversity of cell types, and the necessity of daily cell function in this organ must be considered in stem cell studies. Previous work has created a base from which to explore transplantation, label retention, and more sophisticated lineage-tracing schemes to identify and characterize stem cell populations in the normal lung. These approaches are also imperative for building on precedents set in other tissues in the exploration of the cancer stem cell hypothesis in lung cancers. Additionally, recent studies provide key leads to further explore the molecular mechanisms that regulate lung homeostasis. Here, we discuss strategies to advance the field of lung stem cell biology with an emphasis on developing new, lung-specific tools.

Rajasekhar, V. K., P. Dalerba, et al. (2008). "The 5th International Society for Stem Cell Research (ISSCR) Annual Meeting, June 2007." *Stem Cells* **26**(1): 292-8.

This report presents highlights of discussions that focused on the biology of cancer stem cells as conducted at the fifth Annual Meeting of the International Society for Stem Cell Research, held in Cairns, Australia, June 17-20, 2007. The function of adult stem cells is believed to depend on their niches, that is, the microenvironment in which these stem

cells reside. A similar concept applies to understanding the development of cancer, as it is becoming increasingly clear that only a small subset of cancer cell populations is capable of initiating/sustaining tumor formation. These tumorigenic cells, commonly referred to as cancer stem cells, also appear to reside in particular niches, and they bear the known, albeit dysfunctional, stem cell characteristics of self-renewal and differentiation. Dysregulation of stem cell niches is thought to contribute to tumorigenesis by affecting the complex network of signaling interactions that occur between stem cells and their neighboring cells, thus imbalancing the physiological controls on self-renewal and differentiation processes. This hypothesis was widely explored at the conference to shed new light on the mechanisms of tumor origin and progression and to unveil novel antitumor therapeutic approaches.

Ray, P., N. Krishnamoorthy, et al. (2008). "Emerging functions of c-kit and its ligand stem cell factor in dendritic cells: regulators of T cell differentiation." *Cell Cycle* 7(18): 2826-32.

The receptor tyrosine kinase, c-kit, and its ligand, stem cell factor (SCF), function in a diverse range of biological functions. The role of c-kit in the maintenance and survival of hematopoietic stem cells and of mast cells is well recognized. c-kit also plays an important role in melanogenesis, erythropoiesis and spermatogenesis. Recent work from our laboratory highlights an important role of c-kit in the regulation of expression of two molecules in dendritic cells (DCs), interleukin-6 (IL-6) and Jagged-2 (a ligand of Notch), which are known to regulate T helper cell differentiation. Our study shows that induction of c-kit expression and its signaling in DCs promotes Th2 and Th17 responses but not Th1 response. c-kit inhibition by imatinib mesylate (Gleevec) in DCs was previously shown to promote natural killer cell activation which may be due to dampening of IL-6 production by the DCs. Since dysregulation of c-kit function has been associated with various disease states including cancer, in this perspective we have focused on known and novel functions of c-kit to include molecules such as IL-6 and Notch that were not previously recognized to be within the purview of c-kit biology. We have also reviewed the differential expression pattern of SCF and c-kit on various cell types and its variation during development or pathology. The recognition of previously unappreciated roles for c-kit will provide better insights into its function within and beyond the immune system and pave the way for developing better therapeutic strategies.

Rhee, W. J. and G. Bao (2009). "Simultaneous detection of mRNA and protein stem cell markers in live cells." *BMC Biotechnol* 9: 30.

BACKGROUND: Biological studies and medical application of stem cells often require the isolation of stem cells from a mixed cell population, including the detection of cancer stem cells in tumor tissue, and isolation of induced pluripotent stem cells after eliciting the expression of specific genes in adult cells. Here we report the detection of Oct-4 mRNA and SSEA-1 protein in live carcinoma stem cells using respectively molecular beacon and dye-labeled antibody, aiming to establish a new method for stem cells detection and isolation. **RESULTS:** Quantification of Oct-4 mRNA and protein in P19 mouse carcinoma stem cells using respectively RT-PCR and immunocytochemistry confirmed that their levels drastically decreased after differentiation. To visualize Oct-4 mRNA in live stem cells, molecular beacons were designed, synthesized and validated, and the detection specificity was confirmed using control studies. We found that the fluorescence signal from Oct-4-targeting molecular beacons provides a clear discrimination between undifferentiated and retinoic acid-induced differentiated cells. Using deconvolution fluorescence microscopy, Oct-4 mRNAs were found to reside on one side of the cytosol. We demonstrated that, using a combination of Oct-4 mRNA-targeting molecular beacon with SSEA-1 antibody in flow cytometric analysis, undifferentiated stem cells can be clearly distinguished from differentiated cells. We revealed that Oct-4 targeting molecular beacons do not seem to affect stem cell biology. **CONCLUSION:** Molecular beacons have the potential to provide a powerful tool for highly specific detection and isolation of stem cells, including cancer stem cells and induced pluripotent stem (iPS) cells without disturbing cell physiology. It is advantageous to perform simultaneous detection of intracellular (mRNA) and cell-surface (protein) stem cell markers in flow cytometric analysis, which may lead to high detection sensitivity and efficiency.

Saba, N., R. Abraham, et al. (2000). "Overview of autologous stem cell transplantation." *Crit Rev Oncol Hematol* 36(1): 27-48.

There has been a dramatic increase in the number of autologous peripheral blood stem cell transplants over the last decade. Faster recovery of cell counts, lesser transplant morbidity, shorter hospital stay and reduced cost compared with marrow autografts have been the main advantages of autologous peripheral blood cell over marrow transplants. In this paper we attempt to review the advances in the biology and mobilization of stem cells, and focus on clinical results of autologous

peripheral stem cell and marrow transplants for disease specific sites such as breast cancer, myeloma, autoimmune diseases, germ cell tumors, the acute and chronic leukemias, the non-Hodgkin's lymphomas and Hodgkin's disease. We also discuss transplant related complications, gene therapy and the different methods of purging. This review was intended for autologous peripheral stem cell transplants, however, unavoidably, it also discusses autologous marrow transplantation and aspects common to both procedures.

Sanchez-Garcia, I., C. Vicente-Duenas, et al. (2007). "The theoretical basis of cancer-stem-cell-based therapeutics of cancer: can it be put into practice?" *Bioessays* **29**(12): 1269-80.

In spite of the advances in our knowledge of cancer biology, most cancers remain not curable with present therapies. Current treatments consider cancer as resulting from uncontrolled proliferation and are non-specific. Although they can reduce tumour burden, relapse occurs in most cases. This was long attributed to incomplete tumour elimination, but recent developments indicate that different types of cells contribute to the tumour structure, and that the tumour's cellular organization would be analogous to that of a normal tissue, with a main mass of differentiating cells sensitive to anti proliferative agents, together with a small percentage of quiescent, resistant stem cells responsible for replenishing the tumour: the Cancer Stem Cells (CSCs). Anti-CSCs targeted therapeutic agents would prevent tumour regeneration. New mouse models tailored to exploit this novel concept will be critical to develop CSC-based anti-cancer therapies. Here we review the biological basis and the therapeutic implications of the stem-cell model of cancer.

Schalken, J. A. and G. van Leenders (2003). "Cellular and molecular biology of the prostate: stem cell biology." *Urology* **62**(5 Suppl 1): 11-20.

The normal prostate shows a high degree of cellular organization. The basal layer is populated by prostate epithelial stem cells and a population of transiently proliferating/amplifying (TP/A) cells intermediate to the stem cells and fully differentiated cells. The luminal layer is composed of fully differentiated prostate epithelial cells. Neuroendocrine cells are scattered throughout the gland. This organization is also seen in prostate cancer, where the tumor cell origin (cancer stem cells) can be traced to a normal cell type by characteristic keratin expression patterns. Basal cells showed strong expression of K-[keratin]5, but they were only weakly positive for K18. Luminal cells strongly expressed K18. A subpopulation of basal cells coexpressed K5 and K14.

These keratin expression patterns changed with the degree of cell differentiation as well as location. The least differentiated stem cells in the basal layer were positive for K5 and K14, with weak expression for K18. Intermediate stages of differentiation were identified by expression of K5 and K18. Neuroendocrine cells also expressed K5 as well as typical neuroendocrine cell markers (eg, chromogranin A). Evidence supporting the hypothesis that prostate cancer arises from malignant transformation of intermediate stem cells included the presence in prostate cancers of keratin patterns associated with the intermediate stages of differentiation, androgen independence of both prostate cancers and intermediate stem cells, and expression of c-met by both the TP/A intermediate stem cells and tumor cells.

Scott, C. T. and R. A. Reijo Pera (2008). "The road to pluripotency: the research response to the embryonic stem cell debate." *Hum Mol Genet* **17**(R1): R3-9.

The controversies surrounding embryonic stem cell research have prompted scientists to invent beyond restrictive national policy and moral concerns. The impetus behind these reports comes from different sources, including individually held moral beliefs, societal pressures and resource constraints, both biological and financial. Along with other contributions to public policy such as advocacy or public testimony, experimentation and scientific curiosity are perhaps more natural responses scientists use to surmount impediments to research. In a research context, we review the history of the first stem cell discoveries, and describe scientific efforts leading up to recent reports of pluripotent lines made without the use of human embryos and eggs. We argue that despite the promise of these new lines, we must not lose sight of fundamental questions remaining at the frontiers of embryology and early human development. The answers to these questions will impact studies of genetics, cell biology and diseases such as cancer, autoimmunity and disorders of development. Human embryonic stem cell research is barely a decade old. The recent pace of discovery--in spite of federal restrictions--is testament to the potential of these cells to uncover some of biology's most intractable mysteries.

Serakinci, N. and C. Erzik (2007). "Road for understanding cancer stem cells: model cell lines." *Regen Med* **2**(6): 957-65.

There is increasing evidence suggesting that stem cells are susceptible to carcinogenesis and, consequently, can be the origin of many cancers. Recently, the neoplastic potential of stem cells has been supported by many groups showing the existence

of subpopulations with stem cell characteristics in tumor biopsies such as brain and breast. Evidence supporting the cancer stem cell hypothesis has gained impact due to progress in stem cell biology and development of new models to validate the self-renewal potential of stem cells. Recent evidence on the possible identification of cancer stem cells may offer an opportunity to use these cells as future therapeutic targets. Therefore, model systems in this field have become very important and useful. This review will focus on the state of knowledge on cancer stem cell research, including cell line models for cancer stem cells. The latter will, as models, help us both in the identification and characterization of cancer stem cells and in the further development of therapeutic strategies including tissue engineering.

Shcherbata, H. R., S. Hatfield, et al. (2006). "The MicroRNA pathway plays a regulatory role in stem cell division." *Cell Cycle* 5(2): 172-5.

One of the key characteristics of stem cells is their capacity for self-renewal for long periods of time. In this respect, stem cells are similar to cancer cells, which also have the ability to escape cell cycle stop signals. Therefore, a critical question in stem cell and cancer biology is how cell division is regulated in these cell types. In this review, we summarize recent progress and describe future challenges to understanding the role the microRNA pathway plays in regulating mechanisms controlling stem cell division.

Shupe, T. and B. E. Petersen (2005). "Evidence regarding a stem cell origin of hepatocellular carcinoma." *Stem Cell Rev* 1(3): 261-4.

The cellular origin of tumors remains as one of the unanswered, fundamental questions of cancer biology. The notion that tumors may arise from tissue stem cells is supported by phenotypic similarities between these two cell types, such as proliferative potential and expression of onco-fetal proteins. Liver stem cells, or oval cells, have been put forth as a possible target for hepatocarcinogens. Genetically modified and in vitro transformed oval cells have been shown to form tumors in transplantation to animals. Chemical carcinogenesis models in the liver demonstrate varying degrees of oval cell proliferation. There is also preliminary evidence that hepatocellular carcinoma may maintain a bipotential phenotype consistent with an oval cell origin. Whereas definitive proof of an oval cell origin of hepatocellular has yet to be presented, the current circumstantial evidence justifies continued research on this subject.

Siegmund, K. D., P. Marjoram, et al. (2009). "Inferring clonal expansion and cancer stem cell

dynamics from DNA methylation patterns in colorectal cancers." *Proc Natl Acad Sci U S A* 106(12): 4828-33.

Cancers are clonal expansions, but how a single, transformed human cell grows into a billion-cell tumor is uncertain because serial observations are impractical. Potentially, this history is surreptitiously recorded within genomes that become increasingly numerous, polymorphic, and physically separated after transformation. To correlate physical with epigenetic pairwise distances, small 2,000- to 10,000-cell gland fragments were sampled from left and right sides of 12 primary colorectal cancers, and passenger methylation at 2 CpG-rich regions was measured by bisulfite sequencing. Methylation patterns were polymorphic but differences were similar between different parts of the same tumor, consistent with relatively isotropic or "flat" clonal expansions that could be simulated by rapid initial population expansions. Methylation patterns were too diverse to be consistent with very rare cancer stem cells but were more consistent with multiple (approximately 4 to 1,000) long-lived cancer stem cell lineages per cancer gland. Our study illustrates the potential to reconstruct the unperturbed biology of human cancers from epigenetic passenger variations in their present-day genomes.

Silvan, U., A. Diez-Torre, et al. (2009). "Hypoxia and pluripotency in embryonic and embryonal carcinoma stem cell biology." *Differentiation* 78(2-3): 159-68.

Low oxygen availability (hypoxia) is a hallmark of rapidly proliferating tumors and has been suggested to be a characteristic of the embryonic and adult stem cell niche. The idea of relating cancer to stem cells is increasingly popular due to the identification of specific cancer stem cells sharing the typical plasticity and motility of pluripotent stem cells. Hypoxia plays a critical role in early embryonic development and in tumor progression, participating in processes such as angiogenesis, apoptosis, cell migration, invasion and metastasis. Some of the molecular pathways that have been shown to mediate these hypoxia-induced responses, such as the hypoxia inducible factor (HIF)-1 α and Notch signaling, appear to be active in both embryonic and neoplastic pluripotent stem cells. Nevertheless, the mechanisms underlying these regulatory processes are not yet fully understood. In this review, we attempt to shed some light on the mechanisms involved in hypoxia-dependent processes related to stem cell features and tumor progression, such as the maintenance of the undifferentiated state, cell proliferation, tumor neovascularization, extra-cellular matrix degradation and motility factor up-regulation. With this purpose in mind, we summarize recent observations in

embryonic, adult and cancer stem cells that demonstrate the parallelism existing in their hypoxia responses. Finally, based on the observations of our own laboratory and others, we suggest that the comparative analysis of the response to low oxygen levels of embryonic stem cells and cancer stem cells (such as embryonal carcinoma cells), may throw fresh light on our understanding of the mechanisms underlying hypoxia-induced invasiveness and the resistance to anticancer treatments, thereby stimulating the development of novel therapeutic strategies.

Singec, I., R. Knoth, et al. (2006). "Defining the actual sensitivity and specificity of the neurosphere assay in stem cell biology." *Nat Methods* **3**(10): 801-6.

For more than a decade the 'neurosphere assay' has been used to define and measure neural stem cell (NSC) behavior, with similar assays now used in other organ systems and in cancer. We asked whether neurospheres are clonal structures whose diameter, number and composition accurately reflect the proliferation, self-renewal and multipotency of a single founding NSC. Using time-lapse video microscopy, coculture experiments with genetically labeled cells, and analysis of the volume of spheres, we observed that neurospheres are highly motile structures prone to fuse even under ostensibly 'clonal' culture conditions. Chimeric neurospheres were prevalent independent of ages, species and neural structures. Thus, the intrinsic dynamic of neurospheres, as conventionally assayed, introduces confounders. More accurate conditions (for example, plating a single cell per miniwell) will be crucial for assessing clonality, number and fate of stem cells. These cautions probably have implications for the use of 'cytospheres' as an assay in other organ systems and with other cell types, both normal and neoplastic.

Steindler, D. A. (2006). "Redefining cellular phenotype based on embryonic, adult, and cancer stem cell biology." *Brain Pathol* **16**(2): 169-80.

Stem cell biology has provided constant alteration if not reversal of dogma related to the understanding of the behaviors of primitive and dynamic cells. This review summarizes recent findings on dynamic changes of phenotype that accompany the in vitro growth and differentiation of not only stem and progenitor cells, but also differentiated cells derived from a variety of normal and pathological tissues. As there are examples of apparent dedifferentiation and transdifferentiation of neural cells that appear to be terminally differentiated, there is a need to reconsider elements of cellular fate choice that have relevance to neurooncology and neural repair. Recent findings of dynamic behaviors

and mixed phenotype of both normal and cancer stem cells suggest that some of the diverse lineage attributes of different solid tumors may owe their existence to dynamic cellular phenotype gone awry.

Stevenson, M., W. Mostertz, et al. (2009). "Characterizing the clinical relevance of an embryonic stem cell phenotype in lung adenocarcinoma." *Clin Cancer Res* **15**(24): 7553-61.

PURPOSE: Cancer cells possess traits reminiscent of those ascribed to normal stem cells. It is unclear whether these phenotypic similarities are the result of a common biological phenotype, such as regulatory pathways. **EXPERIMENTAL DESIGN:** Lung cancer cell lines with corresponding gene expression data and genes associated with an embryonic stem cell identity were used to develop a signature of embryonic stemness (ES) activity specific to lung adenocarcinoma. Biological characteristics were elucidated as a function of cancer biology/oncogenic pathway dysregulation. The ES signature was applied to three independent early-stage (I-IIIa) lung adenocarcinoma data sets with clinically annotated gene expression data. The relationship between the ES phenotype and cisplatin (current standard of care) sensitivity was evaluated. **RESULTS:** Pathway analysis identified specific regulatory networks [Ras ($P = 0.0005$), Myc ($P = 0.0224$), wound healing ($P < 0.0001$), chromosomal instability ($P < 0.0001$), and invasiveness ($P < 0.0001$)] associated with the ES phenotype. The prognostic relevance of the ES signature, as related to patient survival, was characterized in three cohorts [CALGB 9761 ($n = 82$; $P = 0.0001$), National Cancer Institute Director's Challenge Consortium ($n = 442$; $P = 0.0002$), and Duke ($n = 45$; $P = 0.06$)]. The ES signature was not prognostic in prostate, breast, or ovarian adenocarcinomas. Lung tumors ($n = 569$) and adenocarcinoma cell lines ($n = 31$) expressing the ES phenotype were more likely to be resistant to cisplatin ($P < 0.0001$ and $P = 0.006$, respectively). **CONCLUSIONS:** Lung adenocarcinomas that share a common gene expression pattern with normal human embryonic stem cells were associated with decreased survival, increased biological complexity, and increased likelihood of resistance to cisplatin. This indicates the aggressiveness of these tumors.

Tan, B. T., C. Y. Park, et al. (2006). "The cancer stem cell hypothesis: a work in progress." *Lab Invest* **86**(12): 1203-7.

There is a growing body of evidence that supports the idea that malignant tumors are initiated and maintained by a population of tumor cells that share similar biologic properties to normal adult stem cells. This model, the cancer stem cell (CSC)

hypothesis, is based on the observation that tumors, like adult tissues, arise from cells that exhibit the ability to self-renew as well as give rise to differentiated tissue cells. Although the concept of the CSC is not entirely new, advances made over the past two decades in our understanding of normal stem cell biology in conjunction with the recent application of these concepts to experimentally define CSCs have resulted in the identification of CSCs in several human malignancies.

Tang, C., B. T. Ang, et al. (2007). "Cancer stem cell: target for anti-cancer therapy." *Faseb J* **21**(14): 3777-85.

Cancer has long been viewed as a heterogeneous population of cells. While the great majority of cells that make up tumors are destined to differentiate, albeit aberrantly, and eventually stop dividing, only a minority population of cells, termed cancer stem cells, possess extensive self-renewal capability and can recapitulate tumor pathophysiology in an immune-compromised animal model. Tumor-initiating cells have been identified and isolated in a variety of cancers of the blood, breast, central nervous system, pancreas, skin, head and neck, colon, and prostate. In this review we present scientific evidence supporting the cancer stem cell model of tumor progression, and discuss the experimental and therapeutic implications. The concept of cancer stem cells may have profound implications for our understanding of tumor biology and for the design of novel treatments targeted toward these cells. Current therapeutic strategies include targeting the cancer stem cell as well as its microenvironmental niche. We present an interesting, novel strategy that takes into account the reactive oxygen species status in cancer stem cells and how it might serve as a method for eradicating these cells in tumor growth.

Tilly, J. L. and B. R. Rueda (2008). "Minireview: stem cell contribution to ovarian development, function, and disease." *Endocrinology* **149**(9): 4307-11.

By virtue of the fact that oocytes not only serve to produce embryos after fertilization but also can effectively reprogram adult somatic cell nuclei to a pluripotent state, much of the interest in the role of stem cells in ovarian biology has been focused on the germline. However, very recent studies have revealed that somatic stem cells may also be of considerable relevance to the study of normal ovarian function. Furthermore, stem cell dysfunction may underlie or contribute to disease states such as ovarian cancer and polycystic ovary syndrome. Our objective is to explore these concepts in greater detail, with the hope of stimulating further research efforts into understanding what role stem cells may play in the

physiology and pathology of the mammalian female gonads.

Todaro, M., M. P. Alea, et al. (2007). "Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4." *Cell Stem Cell* **1**(4): 389-402.

A novel paradigm in tumor biology suggests that cancer growth is driven by stem-like cells within a tumor. Here, we describe the identification and characterization of such cells from colon carcinomas using the stem cell marker CD133 that accounts around 2% of the cells in human colon cancer. The CD133(+) cells grow in vitro as undifferentiated tumor spheroids, and they are both necessary and sufficient to initiate tumor growth in immunodeficient mice. Xenografts resemble the original human tumor maintaining the rare subpopulation of tumorigenic CD133(+) cells. Further analysis revealed that the CD133(+) cells produce and utilize IL-4 to protect themselves from apoptosis. Consistently, treatment with IL-4Ralpha antagonist or anti-IL-4 neutralizing antibody strongly enhances the antitumor efficacy of standard chemotherapeutic drugs through selective sensitization of CD133(+) cells. Our data suggest that colon tumor growth is dictated by stem-like cells that are treatment resistant due to the autocrine production of IL-4.

Trosko, J. E. (2007). "Gap junctional intercellular communication as a biological "Rosetta stone" in understanding, in a systems biological manner, stem cell behavior, mechanisms of epigenetic toxicology, chemoprevention and chemotherapy." *J Membr Biol* **218**(1-3): 93-100.

In spite of the early speculation by Loewenstein that one of the critical distinguishing phenotypes of cancers from normal cells was the dysfunction of gap junctional intercellular communication (GJIC), this hypothesis has not captured the attention of most birth defects and cancer researchers. Moreover, even with later demonstrations that factors that influence normal development and carcinogenesis by modulating GJIC, such as chemical teratogens and tumor-promoting chemicals, inflammatory factors, hormones and growth factors, antisense connexin genes, knockout mouse models, human inherited mutated connexin genes, si-connexin RNA, chemopreventive and chemotherapeutic chemicals, it is rare that one sees any reference to these studies by the mainstream investigators in these fields. Based on the assumption that the evolutionarily conserved connexin genes found in metazoans are needed for normal development and the maintenance of health and T. Dobzhansky's statement "Nothing in biology makes sense except in the light of evolution,"

a short review of the roles of endogenous and exogenous modulators of GJIC will be made in the context of the multistage, multimechanism process of carcinogenesis, the stem cell theory of carcinogenesis, the discovery and characterization of normal adult stem "cancer stem" cells and the observation that two distinct classes of GJIC-deficient cancer cells are known. The implications of these observations to a "systems biological" view of the role of gap junctions and the nutritional prevention and treatment of several chronic diseases and cancer will be discussed.

Tsai, R. Y. (2004). "A molecular view of stem cell and cancer cell self-renewal." *Int J Biochem Cell Biol* **36**(4): 684-94.

With the recent advances in cell biology and molecular genetics, scientists were able to isolate and culture tissue-specific stem cells from various sources and define their properties. The challenge has now shifted to understanding the genetic programs controlling the stem cell state, i.e. self-renewal and multipotential. Cracking the molecular codes that govern the stem cell state turns out to be a difficult task. This is in part because a single gene may exhibit distinct activities when expressed in different cell types. Comprehending the cell-context dependent readout of any given gene requires an integrated knowledge of the complex cellular machinery, a platform which can be provided by the research on stem cells. This review is an attempt to formulate a model for the self-renewal machinery operating in stem cells and cancer cells. Insight into this issue at the molecular and cellular levels will no doubt facilitate the realization of the stem cell potential in both regenerative medicine and anticancer therapy.

Varga, A. C. and J. L. Wrana (2005). "The disparate role of BMP in stem cell biology." *Oncogene* **24**(37): 5713-21.

Stem cells share several characteristics of cancer cells including loss of contact inhibition and immortality. Therefore, stem cells represent an excellent model system in which to define the molecular mechanisms underlying cancer development and progression. Several signal transduction pathways including leukemia inhibitory factor, Wnt and FGF have been demonstrated to function in stem cell self-renewal and differentiation. However, more recently bone morphogenetic proteins (BMPs) have emerged as key regulators of stem cell fate commitment. Intriguingly, BMPs have disparate roles in regulating the biology of embryonic stem (ES) cells compared with neural crest stem cells (NCSCs). Furthermore, although BMPs block neural differentiation of ES cells from both mouse and

human, they contribute to self-renewal specifically in mouse ES cells.

Veeravagu, A., S. R. Bababeygy, et al. (2008). "The cancer stem cell-vascular niche complex in brain tumor formation." *Stem Cells Dev* **17**(5): 859-67.

The cancer stem cell (CSC) theory hypothesizes that a small subpopulation of cells within a tumor mass is responsible for the initiation and maintenance of the tumor. The idea that brain tumors arise from this specific subset of self-renewing, multipotent cells that serve as the locus for tumor formation, has gained great support as evidenced by recent advancements in the biology of breast and colon cancer. It is well established that recruitment of bone marrow-derived proangiogenic progenitor cells and angiogenesis are key events in the process of brain tumor formation; however, the orchestration of these events by the CSC population has only recently been unveiled. In this review, we first introduce the CSC theory and examine the functional development of the vascular niche, its purpose, constituents, and contribution to the development of the CSC-vascular niche complex. Through this discussion, we aim to shed light on the events that may be targeted for therapeutic intervention.

Vezzoni, L. and G. Parmiani (2008). "Limitations of the cancer stem cell theory." *Cytotechnology* **58**(1): 3-9.

Cancer stem cells (CSCs) can be operationally defined as a subset of neoplastic cells which are responsible for the growth and re-growth of primary and metastatic tumors. Although the existence of perpetually dividing cells is a logical necessity to explain the malignant properties of human tumors, experimental data supporting their existence have only recently been obtained. New knowledge in basic stem cell biology and the availability of several cell surface markers for the definition and isolation of small subsets of immature cells coupled to the use of the classical model of xenotransplantation in immune deficient mice has identified putative CSCs in several solid tumors such as mammary, colon, brain, pancreas, prostate, melanoma and others. However, the theory must be considered as still in its infancy, since tumors grown in mice only partially recapitulate the biology of human cells. In addition, whether the "transformed" cell is the neoplastic counterpart of a normal stem cell or whether complete malignant behaviour can occur in a more differentiated cell has still to be demonstrated. In spite of these difficulties, the CSC hypothesis could be of clinical relevance, especially in the definition of new ways to assay drug sensitivity of primary human tumors.

Villadsen, R. (2005). "In search of a stem cell hierarchy in the human breast and its relevance to breast cancer evolution." *Apmis* **113**(11-12): 903-21.

By deliberate analogy with the well-established concept of hematopoiesis, the term "mammapoiesis" is occasionally used to describe the development of the different cellular lineages and functional units in the mammary gland. The use of this term signifies a strong bias towards the idea that tissue homeostasis during mammary development, pregnancy, lactation and involution is brought about by the action of somatic stem cells characterized by longevity and multipotency. The progenies hereof eventually differentiate into structurally and functionally well-defined ductal-lobular units. During the past two decades evidence of such a notion in the mouse has developed from being largely circumstantial based on non-clonal *in vivo* experiments to a quite elaborate characterization of individual candidate stem cells by a number of different properties. Within tumor biology this has led to a renaissance of the concept of tumors as caricatures of tissue renewal. Thus, recent molecular classification of breast cancer based on genome wide expression analysis operates with different subtypes with specific reference to the normal luminal epithelial and myoepithelial/basal lineages in the breast. Apparently some tumors are lineage restricted and others differentiate more broadly as if they have preserved some stem-like properties. This holds promise for the existence of a stem cell hierarchy, the understanding of which may prove to be instrumental in further dissecting the histogenesis of breast cancer evolution. Most attention has been devoted to the question of different cellular origins of cancer subtypes and different susceptibilities of possible stem cells to gain or loss of oncogenes and tumor suppressor genes, respectively. Invaluable progress has been made over the past two decades in culture technology not only in terms of population doubling and clonal growth, but also the availability of lineage specific markers, cell sorting, and three-dimensional functional assays for tissue specific morphogenesis. Transcriptional profiling of stem cell zones has unraveled a hitherto unknown preservation of signaling pathways for maintenance of stem cell properties across tissue boundaries and species. Somatic stem cells have therefore been narrowed down to specific anatomic locations not only in rapidly renewing tissues such as skin and skin derivatives, but also in tissues with slower turnover times, such as lung, kidney and prostate. It is therefore now possible to integrate this information in a search for similar cells within the breast. Even if cell turnover after birth is provided exclusively by dividing lineage-restricted cells, more information about the robustness

of breast differentiation programs during tumor progression is still very much required. Complete knowledge of the primary cell of origin of breast cancer and the mechanisms that influence differentiation programs during tumor initiation, promotion and progression may be crucial for the development of novel non-toxic therapies that influence tumor cell behaviour.

Walton, J. D., D. R. Kattan, et al. (2004). "Characteristics of stem cells from human neuroblastoma cell lines and in tumors." *Neoplasia* **6**(6): 838-45.

Cellular heterogeneity is a hallmark of human neuroblastoma tumors and cell lines. Within a single neuroblastoma are cells from distinct neural crest lineages whose relative abundance is significant for prognosis. We postulate that a self-renewing multipotent tumor stem cell, which gives rise to diverse cell lineages, is the malignant progenitor of this cancer. To test this hypothesis, we have established 22 cloned, phenotypically homogeneous populations of the three major cell types from 17 neuroblastoma cell lines. *In vitro*, malignant neuroblastoma stem cells, termed I-type (intermediate type), have distinct morphologic, biochemical, differentiative, and tumorigenic properties. I-type cells express features of both neuroblastic (N) cells (scant cytoplasm, neuritic processes, neurofilaments, pseudoganglia, and granin and neurotransmitter enzyme expression) and substrate-adherent (S) cells (extensive cytoplasm and vimentin and CD44 expression). Moreover, they show bidirectional differentiation to either N or S cells when induced by specific agents. I-type cells are significantly more malignant than N- or S-type cells, with four- to five-fold greater plating efficiencies in soft agar and six-fold higher tumorigenicity in athymic mice. Differences in malignant potential are unrelated to N-myc amplification/overexpression or the ability to digest and migrate through the extracellular matrix. Immunocytochemical analyses of a small series of tumors reveal that frequency of cells coexpressing N and S cell markers correlates with poor prognosis. Thus, I-type stem cells may be instrumental in the genesis and growth of tumors in the patient. Their unique biology deserves attention and further investigation.

Wang, H. and X. Chen (2008). "Imaging mesenchymal stem cell migration and the implications for stem cell-based cancer therapies." *Future Oncol* **4**(5): 623-8.

Mesenchymal stem cells are promising cellular vehicles for the delivery of therapeutic proteins to sites of cancer growth upon

transplantation. To better understand the physiology and biology of the transplanted stem cells, it is necessary and desirable to track the fate of stem cells noninvasively and longitudinally. Reporter gene imaging is a powerful tool to monitor live stem cells in vivo. In this special report, we review currently investigated reporter genes used for tracking stem cells in vivo by optical, radionuclide, magnetic resonance and multimodality imaging techniques. We also discuss the possibility and feasibility of applying reporter gene imaging to monitor stem-cell-based therapeutic gene delivery efficiency and treatment efficacy.

Wu, P. S., B. Egger, et al. (2008). "Asymmetric stem cell division: lessons from *Drosophila*." Semin Cell Dev Biol **19**(3): 283-93.

Asymmetric cell division is an important and conserved strategy in the generation of cellular diversity during animal development. Many of our insights into the underlying mechanisms of asymmetric cell division have been gained from *Drosophila*, including the establishment of polarity, orientation of mitotic spindles and segregation of cell fate determinants. Recent studies are also beginning to reveal the connection between the misregulation of asymmetric cell division and cancer. What we are learning from *Drosophila* as a model system has implication both for stem cell biology and also cancer research.

Yang, Y. M. and J. W. Chang (2008). "Current status and issues in cancer stem cell study." Cancer Invest **26**(7): 741-55.

Cancer stem cells (CSCs) have been positively identified and successfully isolated from some but not all cancers. The studies on CSCs to date suggest that these cells are rare among the tumor cell population, and they are capable of self-renewing and maintaining tumor growth and heterogeneity. Therapies aimed at CSCs have shown some promise, but their further development will require a more thorough understanding of the biology of CSCs and methods for identifying and isolating this cell subpopulation. This review examines what is known to date regarding the similarities and differences between cancer and somatic stem cells: CSC surface marker development and cell isolation (including a model isolation from our lab), the frequency, potential origin, and signal transduction of CSCs, and the current state of CSC-targeting therapeutic strategies.

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