Cancer Stem Cell Research Literatures

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Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the cancer stem cell related studies.
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1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. There are many methods to deliver the transcription factors into target cells to generate iPSCs. The first method is retrovirus or lentivirus transduction. The problem of this technique is the genome integration of virus DNA which could possibly alter differentiation potential or other malignant transformation. The second method is adenoviral vectors to induce iPSC. The advantage of adenovirus vector based expression is that the transgenes will not integrate into the house genome, thus reduces the risk of tumorogenesis. The third one is a plasmid based transfection that can avoid the genome integration also. Recently, the Cre-recombinase excisable systems are used in iPSC induction and subsequent transgene removal making the iPSC technology closer to clinic applications.

The following introduces recent reports as references in the related studies.

Ansieau, S. "EMT in breast cancer stem cell generation." Cancer Lett. 2013 Sep 10;338(1):63-8. doi: 10.1016/j.canlet.2012.05.014. Epub 2012 May 22.

The concept of cancer stem cells (CSCs) has been proposed to explain the ability of single disseminated cancer cells to reconstitute tumours with heterogeneity similar to that of the primary tumour they arise from. Although this concept is now commonly accepted, the origin of these CSCs remains a source of debate. First proposed to arise through stem/progenitor cell transformation, CSCs might also or alternatively arise from differentiated cancer cells through epithelial to mesenchymal transition (EMT), an embryonic transdifferentiation process. Using breast carcinomas as a study model, I propose revisiting the role of EMT in generating CSCs and the debate on potential underlying mechanisms and biological significance.

Balch, C., K. P. Nephew, et al. "Epigenetic "bivalently marked" process of cancer stem cell-driven tumorigenesis." Bioessays. 2007 Sep;29(9):842-5.

Silencing of tumor suppressor genes (TSGs), by DNA methylation, is well known in adult cancers. However, based on the "stem cell" theory of tumorigenesis, the early epigenetic events arising in malignant precursors remain unknown. A recent report demonstrates that, while pluripotent embryonic stem cells lack DNA methylation and possess a "bivalent" pattern of activating and repressive histone marks in numerous TSGs, analogous multipotent malignant cells derived from germ cell tumors (embryonic carcinoma cells) gain additional silencing modifications to those same genes. These results suggest a possible mechanism by which aberrant differentiation, mediated by histone and DNA methylation, instigates tumor progression.

Balic, M., D. Schwarzenbacher, et al. "Genetic and epigenetic analysis of putative breast cancer stem cell models." BMC Cancer. 2013 Jul 24;13:358. doi: 10.1186/1471-2407-13-358.

BACKGROUND: Cancer stem cell model hypothesizes existence of a small proportion of tumor cells capable of sustaining tumor formation, self-renewal and differentiation. In breast cancer, these

cells were found to be associated with CD44(+)CD24-low and ALDH(+) phenotype. Our study was performed to evaluate the suitability of current approaches for breast cancer stem cell analyses to evaluate heterogeneity of breast cancer cells through their extensive genetic and epigenetic characterization. METHODS: Breast cancer cell lines MCF7 and SUM159 were cultured in adherent conditions and as mammospheres. Flow cytometry sorting for CD44, CD24 and ALDH was performed. Sorted and unsorted populations, mammospheres and adherent cell cultures were subjected to DNA profiling by array CGH and methylation profiling by Epitect Methyl qPCR array. Methylation status of selected genes was further evaluated by pyrosequencing. Functional impact of methylation was evaluated by mRNA analysis for selected genes. RESULTS: Array CGH did not reveal any genomic differences. In contrast, putative breast cancer stem cells showed altered methylation levels of several genes compared to parental tumor cells. CONCLUSIONS: Our results underpin the hypothesis that epigenetic mechanisms seem to play a major role in the regulation of CSCs. However, it is also clear that more efficient methods for CSC enrichment are needed. This work underscores requirement of additional approaches to reveal heterogeneity within breast cancer.

Bao, B., Z. Wang, et al. "Over-expression of FoxM1 leads to epithelial-mesenchymal transition and cancer stem cell phenotype in pancreatic cancer cells." J Cell Biochem. 2011 Sep;112(9):2296-306. doi: 10.1002/jcb.23150.

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

Bapat, S. A. "Epigenetic regulation of cancer stem cell gene expression." Subcell Biochem. 2013;61:419-34. doi: 10.1007/978-94-007-4525-4_18.

The concept of cancer as a stem cell disease has slowly gained ground over the last decade. A 'stem-like' state essentially necessitates that some cells in the developing tumor express the properties of remaining quiescent, self-renewing and regenerating tumors through establishment of aberrant cellular hierarchies. Alternatively, such capacities may also be reacquired through a de-differentiation process. The abnormal cellular differentiation patterns involved during either process during carcinogenesis are likely to be driven through a combination of genetic events and epigenetic regulation. The role(s) of the latter is increasingly being appreciated in acquiring the requisite genomic specificity and flexibility required for phenotypic plasticity, specifically in a context wherein genome sequences are not altered for differentiation to ensue. In this chapter, the recent advances in elucidating epigenetic mechanisms that govern the self-renewal, differentiation and regenerative potentials of cancer stem cells will be presented.

Beck, S., X. Jin, et al. "Telomerase activity-independent function of TERT allows glioma cells to attain cancer stem cell characteristics by inducing EGFR expression." Mol Cells. 2011 Jan;31(1):9-15. doi: 10.1007/s10059-011-0008-8. Epub 2010 Dec 24.

Telomerase reverse transcriptase (TERT), the catalytic subunit of the enzyme telomerase, is robustly expressed in cancer cells. TERT enables cells to avoid chromosome shortening during repeated replication by maintaining telomere length. However, several lines of evidence indicate that many cancer cells exhibit shorter telomere length than normal tissues, implying an additional function of TERT in tumor formation and progression. Here, we report a telomerase activity-independent function of TERT that induces cancer stemness in glioma cells. Overexpression of

TERT712, a telomerase activity-deficient form of TERT, in U87MG cells promoted cell self-renewal in vitro, and induced EGFR expression and formation of gliomas exhibiting cellular heterogeneity in vivo. In patients with glioblastoma multiforme, TERT expression showed a high correlation with EGFR expression, which is closely linked to the stemness gene signature. Induction of differentiation and TERT-knockdown in glioma stem cells led to a marked reduction in EGFR expression, cancer stemness, and anticancer drug resistance. Together, our findings indicate that TERT plays a crucial role in tumor progression by promoting cancer stemness through expression of EGFR.

Bjerkvig, R., B. B. Tysnes, et al. "Opinion: the origin of the cancer stem cell: current controversies and new insights." Nat Rev Cancer. 2005 Nov;5(11):899-904.

Most tumours are derived from a single cell that is transformed into a cancer-initiating cell (cancer stem cell) that has the capacity to proliferate and form tumours in vivo. However, the origin of the cancer stem cell remains elusive. Interestingly, during development and tissue repair the fusion of genetic and cytoplasmic material between cells of different origins is an important physiological process. Such cell fusion and horizontal gene-transfer events have also been linked to several fundamental features of cancer and could be important in the development of the cancer stem cell.

Brown, D. V. and T. Mantamadiotis "Insights into the next generation of cancer stem cell research." Front Biosci (Landmark Ed). 2014 Jun 1;19:1015-27.

The understanding of how cancer stem cells (CSCs) or tumor-initiating cells (TICs) behave is important in understanding how tumors are initiated and how they recur following initial treatment. More specifically to understand how CSCs behave, the different signaling mechanisms orchestrating their growth, cell cycle dynamics, differentiation, trans-differentiation and survival following cytotoxic challenges need to be deciphered. Ultimately this will advance the ability to predict how these cells will behave in individual patients and under different therapeutic conditions. Second or next-generation sequencing (NGS) capabilities have provided researchers a window into the molecular and genetic clockwork of CSCs at an unprecedented resolution and depth, with throughput capabilities allowing sequencing of hundreds of samples in relatively short timeframes and at relatively modest costs More specifically NGS gives us the ability to accurately determine the genomic and transcriptomic nature of CSCs. These technologies and the publicly available cancer genome databases, together with the ever increasing computing power available to researchers locally or via cloud-based servers are changing the way biomedical cancer research is approached.

Cabezas-Wallscheid, N., V. Eichwald, et al. "Instruction of haematopoietic lineage choices, evolution of transcriptional landscapes and cancer stem cell hierarchies derived from an AML1-ETO mouse model." EMBO Mol Med. 2013 Dec;5(12):1804-20. doi: 10.1002/emmm.201302661. Epub 2013 Oct 4.

The t(8;21) chromosomal translocation activates aberrant expression of the AML1-ETO (AE) fusion protein and is commonly associated with core binding factor acute myeloid leukaemia (CBF AML). Combining a conditional mouse model that closely resembles the slow evolution and the mosaic AE expression pattern of human t(8;21) CBF AML with global transcriptome sequencing, we find that disease progression was characterized by two principal pathogenic mechanisms. Initially, AE expression modified the lineage potential of haematopoietic stem cells (HSCs), resulting in the selective expansion of the myeloid compartment at the expense of normal erythro- and lymphopoiesis. This lineage skewing was followed by a second substantial rewiring of transcriptional networks occurring in the trajectory to manifest leukaemia. We also find that both HSC and lineage-restricted granulocyte macrophage progenitors (GMPs) acquired leukaemic stem cell (LSC) potential being capable of initiating and maintaining the disease. Finally, our data demonstrate that long-term expression of AE induces an indolent myeloproliferative disease (MPD)-like myeloid leukaemia phenotype with complete penetrance and that acute inactivation of AE function is a potential novel therapeutic option.

Chan, C. H., J. K. Morrow, et al. "Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression." Cell. 2013 Aug 1;154(3):556-68. doi: 10.1016/j.cell.2013.06.048.

Skp2 E3 ligase is overexpressed in numerous human cancers and plays a critical role in cell-cycle progression, senescence, metabolism, cancer progression, and metastasis. In the present study, we identified a specific Skp2 inhibitor using high-throughput in silico screening of large and diverse chemical libraries. This Skp2 inhibitor selectively suppresses Skp2 E3 ligase activity, but not activity of other SCF complexes. It also phenocopies the effects observed upon genetic Skp2 deficiency, such as suppressing survival and Akt-mediated glycolysis and triggering p53-independent cellular senescence. Strikingly, we discovered a critical function of Skp2 in

positively regulating cancer stem cell populations and self-renewal ability through genetic and pharmacological approaches. Notably, Skp2 inhibitor exhibits potent antitumor activities in multiple animal models and cooperates with chemotherapeutic agents to reduce cancer cell survival. Our study thus provides pharmacological evidence that Skp2 is a promising target for restricting cancer stem cell and cancer progression.

Clement, V., P. Sanchez, et al. "HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity." Curr Biol. 2007 Jan 23;17(2):165-72. Epub 2006 Dec 28.

Cancer stem cells are rare tumor cells characterized by their ability to self-renew and to induce tumorigenesis. They are present in gliomas and may be responsible for the lethality of these incurable brain tumors. In the most aggressive and invasive type, glioblastoma multiforme (GBM), an average of about one year spans the period between detection and death [1]. The resistence of gliomas to current therapies may be related to the existence of cancer stem cells [2-6]. We find that human gliomas display a stemness signature and demonstrate that HEDGEHOG (HH)-GLI signaling regulates the expression of stemness genes in and the self-renewal of CD133(+) glioma cancer stem cells. HH-GLI signaling is also required for sustained glioma growth and survival. It displays additive and synergistic effects with temozolomide (TMZ), the current chemotherapeutic agent of choice. TMZ, however, does not block glioma stem cell self-renewal. Finally, interference of HH-GLI signaling with cyclopamine or through lentiviral-mediated silencing demonstrates that the tumorigenicity of human gliomas in mice requires an active pathway. Our results reveal the essential role of HH-GLI signaling in controlling the behavior of human glioma cancer stem cells and offer new therapeutic possibilities.

Crea, F., R. Danesi, et al. "Cancer stem cell epigenetics and chemoresistance." Epigenomics. 2009 Oct;1(1):63-79. doi: 10.2217/epi.09.4.

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.

D'Andrea, F. P., A. Safwat, et al. "Cancer stem cell overexpression of nicotinamide N-methyltransferase enhances cellular radiation resistance." Radiother Oncol. 2011 Jun;99(3):373-8. doi: 10.1016/j.radonc.2011.05.086. Epub 2011 Jun 29.

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

Di Fiore, R., D. Fanale, et al. "Genetic and molecular characterization of the human osteosarcoma 3AB-OS cancer stem cell line: a possible model for studying osteosarcoma origin and stemness." J Cell Physiol. 2013 Jun;228(6):1189-201. doi: 10.1002/jcp.24272.

Finding new treatments targeting cancer stem cells (CSCs) within a tumor seems to be critical to halt cancer and improve patient survival. Osteosarcoma is an aggressive tumor affecting adolescents, for which there is no second-line chemotherapy. Uncovering new molecular mechanisms underlying the development of osteosarcoma and origin of CSCs is crucial to identify new possible therapeutic strategies. Here, we aimed to characterize genetically and molecularly the human osteosarcoma 3AB-OS CSC line, previously selected from MG63 cells and which proved to have both in vitro and in vivo features of CSCs. Classic cytogenetic

studies demonstrated that 3AB-OS cells have hypertriploid karyotype with 71-82 chromosomes. By comparing 3AB-OS CSCs to the parental cells, array CGH, Affymetrix microarray, and TaqMan(R) Human MicroRNA array analyses identified 49 copy number variations (CNV), 3,512 dysregulated genes and 189 differentially expressed miRNAs. Some of the chromosomal abnormalities and mRNA/miRNA expression profiles appeared to be congruent with those reported in human osteosarcomas. Bioinformatic analyses selected 196 genes and 46 anticorrelated miRNAs involved in carcinogenesis and stemness. For the first time, a predictive network is also described for two miRNA family (let-7/98 and miR-29a,b,c) and their anticorrelated mRNAs (MSTN, CCND2, Lin28B, MEST, HMGA2, and GHR), which may represent new biomarkers for osteosarcoma and may pave the way for the identification of new potential therapeutic targets.

D'Uva, G., S. Bertoni, et al. "Beta-catenin/HuR post-transcriptional machinery governs cancer stem cell features in response to hypoxia." PLoS One. 2013 Nov 15;8(11):e80742. doi: 10.1371/journal.pone.0080742. eCollection 2013.

Hypoxia has been long-time acknowledged as major cancer-promoting microenvironment. In such an energy-restrictive condition, post-transcriptional mechanisms gain importance over the energy-expensive gene transcription machinery. Here we show that the onset of hypoxia-induced cancer stem cell features requires the beta-catenin-dependent post-transcriptional up-regulation of CA9 and SNAI2 gene expression. In response to hypoxia, beta-catenin moves from the plasma membrane to the cytoplasm where it binds and stabilizes SNAI2 and CA9 mRNAs, in cooperation with the mRNA stabilizing protein HuR. We also provide evidence that the post-transcriptional activity of cytoplasmic beta-catenin operates under normoxia in basal-like/triple-negative breast cancer cells, where the beta-catenin knockdown suppresses the stem cell phenotype in vitro and tumor growth in vivo. In such cells, we unravel the generalized involvement of the beta-catenin-driven machinery in the stabilization of EGF-induced mRNAs, including the cancer stem cell regulator IL6. Our study highlights the crucial role of post-transcriptional mechanisms in the maintenance/acquisition of cancer stem cell features and suggests that the hindrance of cytoplasmic beta-catenin function may represent an unprecedented strategy for targeting breast cancer stem/basal-like cells.

Fan, H., X. Zhao, et al. "Function of focal adhesion kinase scaffolding to mediate endophilin A2 phosphorylation promotes epithelial-mesenchymal transition and mammary cancer stem cell activities in vivo." J Biol Chem. 2013 Feb 1;288(5):3322-33. doi: 10.1074/jbc.M112.420497. Epub 2012 Dec 19.

Tyrosine kinases have been shown to play critical roles in cancer development and progression, and their inhibitors hold the potential as effective targeted therapies for breast and other cancers. However, some of these kinases like focal adhesion kinase (FAK) also possess scaffolding functions in intracellular signaling, but such kinase-independent functions of FAK or other kinases have not been examined in cancer directly in vivo. Here, we report that disruption of the function of FAK scaffolding through its Pro-878/881 motif suppressed mammary tumor growth and metastasis in a well characterized murine model of human breast cancer. P878A/P881A mutation in the endogenous FAK gene decreased the expression of markers for epithelial-mesenchymal transition (EMT) and mammary cancer stem cell (MaCSC) activities in tumors derived from mutant mice. This mutation disrupted the function of FAK scaffolding to mediate endophilin A2 phosphorylation at Tyr-315 by Src, leading to the decreased surface expression of MT1-MMP, as observed previously in transformed fibroblasts in vitro. Inhibition of the downstream components of this FAK scaffolding function by Y315F endophilin A2 mutant or MT1-MMP knockdown reduced markers for EMT and MaCSC activities. Conversely, bypass of the scaffolding function using the phosphorylation mimic mutant Y315E endophilin A2 or endophilin A2 knockdown rescued the decreased markers for EMT and MaCSCs as well as surface expression of MT1-MMP in tumor cells harboring the P878A/P881A mutation. Together, these results identify a novel role of FAK scaffolding function in breast cancer, which could serve as a new target in combination with kinase inhibition for more effective treatment strategies.

Fredebohm, J., M. Boettcher, et al. "Establishment and characterization of a highly tumourigenic and cancer stem cell enriched pancreatic cancer cell line as a well defined model system." PLoS One. 2012;7(11):e48503. doi: 10.1371/journal.pone.0048503. Epub 2012 Nov 12.

Standard cancer cell lines do not model the intratumoural heterogeneity situation sufficiently. Clonal selection leads to a homogeneous population of cells by genetic drift. Heterogeneity of tumour cells, however, is particularly critical for therapeutically relevant studies, since it is a prerequisite for acquiring drug resistance and reoccurrence of tumours. Here, we report the isolation of a highly tumourigenic primary pancreatic cancer cell line, called JoPaca-1 and its detailed characterization at multiple levels.

Implantation of as few as 100 JoPaca-1 cells into immunodeficient mice gave rise to tumours that were histologically very similar to the primary tumour. The high heterogeneity of JoPaca-1 was reflected by diverse cell morphology and a substantial number of chromosomal aberrations. Comparative whole-genome sequencing of JoPaca-1 and BxPC-3 revealed mutations in genes frequently altered in pancreatic cancer. Exceptionally high expression of cancer stem cell markers and a high clonogenic potential in vitro and in vivo was observed. All of these attributes make this cell line an extremely valuable model to study the biology of and pharmaceutical effects on pancreatic cancer.

Gerger, A., W. Zhang, et al. "Common cancer stem cell gene variants predict colon cancer recurrence." Clin Cancer Res. 2011 Nov 1;17(21):6934-43. doi: 10.1158/1078-0432.CCR-11-1180. Epub 2011 Sep 14.

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

Ho Sui, S. J., K. Begley, et al. "The Stem Cell Discovery Engine: an integrated repository and analysis system for cancer stem cell comparisons." Nucleic Acids Res. 2012 Jan;40(Database issue):D984-91. doi: 10.1093/nar/gkr1051. Epub 2011 Nov 24.

Mounting evidence suggests that malignant tumors are initiated and maintained by a subpopulation of cancerous cells with biological properties similar to those of normal stem cells. However, descriptions of stem-like gene and pathway signatures in cancers are inconsistent across experimental systems. Driven by a need to improve our understanding of molecular processes that are common and unique across cancer stem cells (CSCs), we have developed the Stem Cell Discovery Engine (SCDE)-an online database of curated CSC experiments coupled to the Galaxy analytical framework. The SCDE allows users to consistently describe, share and compare CSC data at the gene and pathway level. Our initial focus has been on carefully curating tissue and cancer stem cell-related experiments from blood, intestine and brain to create a high quality resource containing 53 public studies and 1098 assays. The experimental information is captured and stored in the multi-omics Investigation/Study/Assay (ISA-Tab) format and can be queried in the data repository. A linked Galaxy framework provides a comprehensive, flexible environment populated with novel tools for gene list comparisons against molecular signatures in GeneSigDB and MSigDB, curated experiments in the SCDE and pathways in WikiPathways. The SCDE is available at http://discovery.hsci.harvard.edu.

Jeter, C. R., B. Liu, et al. "NANOG promotes cancer stem cell characteristics and prostate cancer resistance to androgen deprivation." Oncogene. 2011 Sep 8;30(36):3833-45. doi: 10.1038/onc.2011.114. Epub 2011 Apr 18.

Cancer cell molecular mimicry of stem cells (SC) imbues neoplastic cells with enhanced proliferative and renewal capacities. In support, numerous mediators of SC self-renewal have been evinced to show oncogenic potential. We have recently reported that short-hairpin RNA-mediated knockdown of the embryonic stem cell (ESC) self-renewal gene NANOG significantly reduced the clonogenic and tumorigenic capabilities of various cancer cells. In this study, we sought to test the potential pro-tumorigenic functions of NANOG, particularly, in prostate cancer (PCa). Using qRT-PCR, we first confirmed that PCa cells expressed NANOG mRNA primarily from the NANOGP8 locus on chromosome 15q14. We then constructed a lentiviral promoter reporter in which the -3.8-kb NANOGP8 genomic fragment was used to drive the

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

Jiang, R., Y. Li, et al. "The acquisition of cancer stem cell-like properties and neoplastic transformation of human keratinocytes induced by arsenite involves epigenetic silencing of let-7c via Ras/NF-kappaB." Toxicol Lett. 2014 Jun 5;227(2):91-8. doi: 10.1016/j.toxlet.2014.03.020. Epub 2014 Apr 2.

Exposure of humans to inorganic arsenic can cause skin cancer. The acquisition of cancer stem cell-like properties is involved in the initiation of some cancers, and there are changes in let-7 levels in some tumors. The mechanisms of action, however, remain obscure. Here, we report that there are decreased levels of let-7a, let-7b, and let-7c in human keratinocyte HaCaT cells during malignant transformation induced by a low concentration (1.0muM) of arsenite. The process by which arsenite reduces the level of let-7c apparently involves methylation, for 5-aza-2'-deoxycytidine, an inhibitor of methyltransferases, prevents arsenite-induced hypermethylation, decreases the level of let-7c, and thereby blocks arsenite-induced activation of the Ras/NF-kappaB signal pathway. Let-7c is an up-stream regulator of the Ras/NF-kappaB signal pathway and down-regulates activation of this pathway. In arsenite-transformed HaCaT cells, the acquisition of cancer stem cell-like properties is prevented by over-expression of let-7c, and over-expression of let-7c decreases the malignancy of transformed HaCaT cells. Thus, we conclude that epigenetic silencing of let-7c via Ras/NF-kappaB is involved in the acquisition of cancer stem cell-like properties and neoplastic transformation of HaCaT cells induced by arsenite, which contribute to the tumorigenesis of arsenite.

Kagara, N., K. T. Huynh, et al. "Epigenetic regulation of cancer stem cell genes in triple-negative breast cancer." Am J Pathol. 2012 Jul;181(1):257-67. doi: 10.1016/j.ajpath.2012.03.019. Epub 2012 May 21.

Expression of specific breast cancer stem cells (BCSCs) is seen in aggressive tumors, but their regulation is unclear. Epigenetic changes influence gene expression and are implicated in breast cancer progression. We hypothesized that promoter methylation regulates specific BCSC-related genes [CD44, CD133, CD24, MSH1 (alias, Musashi-1), and ALDH1] and that this epigenetic profile can identify aggressive subtypes, such as triple-negative breast cancer (TNBC). Methylation analysis was performed using MassARRAY EpiTYPER sequencing; CpG-rich sites were identified in the promoter regions of BCSC genes, except ALDH1. These sites were screened by treatment with 5-aza-2'-deoxycytidine in four TN and five non-TNBC cell lines. The specific regulatory CpG site demonstrating the most significant inverse correlation between CpG site methylation and mRNA expression was identified for CD44, CD133, and Musashi-1, but not for CD24. Methylation of CD44, CD133, and Musashi-1 was evaluated in 91 American Joint Committee on Cancer stage I to III primary breast cancer tumors, and these sites were significantly hypomethylated in TNBC versus non-TNBC. The IHC staining of primary tumors with the highest and lowest methylation levels revealed the strongest staining in hypomethylated specimens, suggesting that hypomethylation leads to gene activation. We demonstrate that methylation is a significant mechanism regulating CD44, CD133, and Musashi-1, and that gene hypomethylation correlates with TNBC. Assessment of epigenetic changes in BCSC genes may provide a more accurate classification of TNBC and could be developed as potential therapeutic targets.

Kanzawa, M., S. Semba, et al. "WNT5A is a key regulator of the epithelial-mesenchymal transition and cancer stem cell properties in human gastric carcinoma cells." Pathobiology. 2013;80(5):235-44. doi: 10.1159/000346843. Epub 2013 Apr 24.

OBJECTIVE: Direct interaction with cancer-associated fibroblasts triggers WNT5A expression in human gastric carcinoma (GC) cells. In this study, we performed gene transduction experiments to investigate the significance of WNT5A in the GC tumor microenvironment. METHODS: Gene transduction (pWNT5A and shWNT5A) was performed in human GC-derived MKN-7 cells. Altered gene expression was examined by RT-PCR and cDNA microarray analysis. Immunohistochemical examination was carried out in human GC tissues. RESULTS: Transduction of exogenous WNT5A expression into MKN-7 cells upregulated genes related

to the epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs), and the pWNT5A transfectant showed high tumorigenicity in vivo. These results were confirmed by knockdown experiments using a lentivirus expressing shWNT5A. A cDNA microarray analysis suggested that depletion of endogenous WNT5A downregulated genes involved in intracellular signaling, chemokine-cytokine interaction and focal adhesion. High levels of WNT5A expression were observed in 66% of GC cases, with significant correlation with histological type. Interestingly, in intestinal-type GCs, WNT5A expression was detected in the periphery of tumor nests. CONCLUSIONS: WNT5A regulates the induction of EMT and the maintenance of CSC properties in MKN-7 cells. WNT5A may play an important role in constructing an advantageous tumor microenvironment for the progression and development of human GC.

Kemper, K., M. R. Sprick, et al. "The AC133 epitope, but not the CD133 protein, is lost upon cancer stem cell differentiation." Cancer Res. 2010 Jan 15;70(2):719-29. doi: 10.1158/0008-5472.CAN-09-1820. Epub 2010 Jan 12.

Colon cancer stem cells (CSC) can be identified with AC133, an antibody that detects an epitope on CD133. However, recent evidence suggests that expression of CD133 is not restricted to CSCs, but is also expressed on differentiated tumor cells. Intriguingly, we observed that detection of the AC133 epitope on the cell surface decreased upon differentiation of CSC in a manner that correlated with loss of clonogenicity. However, this event did not coincide with a change in CD133 promoter activity, mRNA, splice variant, protein expression, or even cell surface expression of CD133. In contrast, we noted that with CSC differentiation, a change occured in CD133 glycosylation. Thus, AC133 may detect a glycosylated epitope, or differential glycosylation may cause CD133 to be retained inside the cell. We found that AC133 could effectively detect CD133 glycosylation mutants or bacterially expressed unglycosylated CD133. Moreover, cell surface biotinylation experiments revealed that differentially glycosylated CD133 could be detected on the membrane of differentiated tumor cells. Taken together, our results argue that CD133 is a cell surface molecule that is expressed on both CSC and differentiated tumor cells, but is probably differentially folded as a result of differential glycosylation to mask specific epitopes. In summary, we conclude that AC133 can be used to detect cancer stem cells, but that results from the use of this antibody should be interpreted with caution.

Kumar, S. M., S. Liu, et al. "Acquired cancer stem cell phenotypes through Oct4-mediated dedifferentiation." Oncogene. 2012 Nov 22;31(47):4898-911. doi: 10.1038/onc.2011.656. Epub 2012 Jan 30.

There is enormous interest to target cancer stem cells (CSCs) for clinical treatment because these cells are highly tumorigenic and resistant to chemotherapy. Oct4 is expressed by CSC-like cells in different types of cancer. However, function of Oct4 in tumor cells is unclear. In this study, we showed that expression of Oct4 gene or transmembrane delivery of Oct4 protein promoted dedifferentiation of melanoma cells to CSC-like cells. The dedifferentiated melanoma cells showed significantly decreased expression of melanocytic markers and acquired the ability to form tumor spheroids. They showed markedly increased resistance to chemotherapeutic agents and hypoxic injury. In the subcutaneous xenograft and tail vein injection assays, these cells had significantly increased tumorigenic capacity. The dedifferentiated melanoma cells acquired features associated with CSCs such as multipotent differentiation capacity and expression of melanoma CSC markers such as ABCB5 and CD271. Mechanistically, Oct4-induced dedifferentiation was associated with increased expression of endogenous Oct4, Nanog and Klf4, and global gene expression changes that enriched for transcription factors. RNAi-mediated knockdown of Oct4 in dedifferentiated cells led to diminished CSC phenotypes. Oct4 expression in melanoma was regulated by hypoxia and its expression was detected in a sub-population of melanoma cells in clinical samples. Our data indicate that Oct4 is a positive regulator of tumor dedifferentiation. The results suggest that CSC phenotype is dynamic and may be acquired through dedifferentiation. Oct4-mediated tumor cell dedifferentiation may have an important role during tumor progression.

Lehmann, C., G. Jobs, et al. "Established breast cancer stem cell markers do not correlate with in vivo tumorigenicity of tumor-initiating cells." Int J Oncol. 2012 Dec;41(6):1932-42. doi: 10.3892/ijo.2012.1654. Epub 2012 Oct 5.

The tumor-initiating capacity of primary human breast cancer cells is maintained in vitro by culturing these cells as spheres/aggregates. Inoculation of small cell numbers derived from these non-adherent cultures leads to rapid xenograft tumor formation in mice. Accordingly, injection of more differentiated monolayer cells derived from spheres results in significantly decelerated tumor growth. For our study, two breast cancer cell lines were generated from primary tumors and cultured as mammospheres or as their adherent counterparts. We examined the in vivo tumorigenicity of these cells by injecting serial dilutions into immunodeficient mice. Inoculation of

106 cells per mouse led to rapid tumor formation, irrespective of cell line or culture conditions. However, after injection of only 103 cells, solely sphere cells were highly tumorigenic. In vitro, we investigated differentiation markers, established breast CSC markers and conducted mRNA profiling. Cytokeratin 5 and 18 were increased in both monolayer cell types, indicating a more differentiated phenotype. All cell lines were CD24(-)/CD44(+) and did not express CD133, CD326 or E-cadherin. ALDH1 activity was not detectable in any cell line. A verapamilsensitive Hoechst side population was present in sphere cells, but there was no correlation with tumorigenicity in vivo. mRNA profiling did not reveal upregulation of relevant transcription factors. In vitro cell cycle kinetics and in vivo tumor doubling times displayed no difference between sphere and monolayer cultures. Our data indicate that intrinsic genetic and functional markers investigated are not indicative of the in vivo tumori-genicity of putative breast tumor-initiating cells.

Leth-Larsen, R., M. G. Terp, et al. "Functional heterogeneity within the CD44 high human breast cancer stem cell-like compartment reveals a gene signature predictive of distant metastasis." Mol Med. 2012 Sep 25;18:1109-21. doi: 10.2119/molmed.2012.00091.

The CD44(hi) compartment in human breast cancer is enriched in tumor-initiating cells; however, the functional heterogeneity within this subpopulation remains poorly defined. We used a triple-negative breast cancer cell line with a known bilineage phenotype to isolate and clone CD44(hi) single cells that exhibited mesenchymal/basal B and luminal/basal A features, respectively. Herein, we demonstrate in this and other triple-negative breast cancer cell lines that, rather than CD44(hi)/CD24(-) mesenchymal-like basal B cells, the CD44(hi)/CD24(lo) epithelioid basal A cells retained classic cancer stem cell features, such as tumor-initiating capacity in vivo, mammosphere formation and resistance to standard chemotherapy. These results complement previous findings using oncogene-transformed normal mammary cells showing that only cell clones with a mesenchymal phenotype exhibit cancer stem cell features. Further, we performed comparative quantitative proteomic and gene array analyses of these cells and identified potential novel markers of breast cancer cells with tumor-initiating features, such as lipolysis-stimulated lipoprotein receptor (LSR), RAB25, S100A14 and mucin 1 (MUC1), as well as a novel 31-gene signature capable of predicting distant metastasis in cohorts of estrogen receptor-negative human breast cancers. These findings strongly favor functional heterogeneity in the breast cancer cell compartment and hold promise for further refinements of prognostic marker profiling. Our work confirms that, in addition to cancer stem cells with mesenchymal-like morphology, those tumor-initiating cells with epithelial-like morphology should also be the focus of drug development.

Lim, Y. Y., J. A. Wright, et al. "Epigenetic modulation of the miR-200 family is associated with transition to a breast cancer stem-cell-like state." J Cell Sci. 2013 May 15;126(Pt 10):2256-66. doi: 10.1242/jcs.122275. Epub 2013 Mar 22.

The miR-200 family is a key regulator of the epithelial-mesenchymal transition, however, its role in controlling the transition between cancer stem-cell-like and non-stem-cell-like phenotypes is not well understood. We utilized immortalized human mammary epithelial (HMLE) cells to investigate the regulation of the miR-200 family during their conversion to a stem-like phenotype. HMLE cells were found to be capable of spontaneous conversion from a non-stem to a stem-like phenotype and this conversion was accompanied by the loss of miR-200 expression. Stem-like cell fractions isolated from metastatic breast cancers also displayed loss of miR-200 indicating similar molecular changes may occur during breast cancer progression. The phenotypic change observed in HMLE cells was directly controlled by miR-200 because restoration of its expression decreased stem-like properties while promoting a transition to an epithelial phenotype. Investigation of the mechanisms controlling miR-200 expression revealed both DNA methylation and histone modifications were significantly altered in the stem-like and non-stem phenotypes. In particular, in the stem-like phenotype, the miR-200b-200a-429 cluster was silenced primarily through polycomb group-mediated histone modifications whereas the miR-200c-141 cluster was repressed by DNA methylation. These results indicate that the miR-200 family plays a crucial role in the transition between stem-like and non-stem phenotypes and that distinct epigenetic-based mechanisms regulate each miR-200 gene in this process. Therapy targeted against miR-200 family members and epigenetic modifications might therefore be applicable to breast cancer.

Lottaz, C., D. Beier, et al. "Transcriptional profiles of CD133+ and CD133- glioblastoma-derived cancer stem cell lines suggest different cells of origin." Cancer Res. 2010 Mar 1;70(5):2030-40. doi: 10.1158/0008-5472.CAN-09-1707. Epub 2010 Feb 9.

Glioblastoma multiforme (GBM) is paradigmatic for the investigation of cancer stem cells (CSC) in solid tumors. Growing evidence suggests that different types of CSC lead to the formation of GBM.

This has prompted the present comparison of gene expression profiles between 17 GBM CSC lines and their different putative founder cells. Using a newly derived 24-gene signature, we can now distinguish two subgroups of GBM: Type I CSC lines display "proneural" signature genes and resemble fetal neural stem cell (fNSC) lines, whereas type II CSC lines show "mesenchymal" transcriptional profiles similar to adult NSC (aNSC) lines. Phenotypically, type I CSC lines are CD133 positive and grow as neurospheres. Type II CSC lines, in contrast, display (semi-)adherent growth and lack CD133 expression. Molecular differences between type I and type II CSC lines include the expression of extracellular matrix molecules and the transcriptional activity of the WNT and the transforming growth factor-beta/bone morphogenetic protein signaling pathways. Importantly, these characteristics were not affected by induced adherence on laminin. Comparing CSC lines with their putative cells of origin, we observed greatly increased proliferation and impaired differentiation capacity in both types of CSC lines but no cancer-associated activation of otherwise silent signaling pathways. Thus, our data suggest that the heterogeneous tumor entity GBM may derive from cells that have preserved or acquired properties of either fNSC or aNSC but lost the corresponding differentiation potential. Moreover, we propose a gene signature that enables the subclassification of GBM according to their putative cells of origin.

Maria, T., A. Panagiotis, et al. "How prostate-specific membrane antigen level may be correlated with stemness in prostate cancer stem cell-like cell populations?" J Cancer Res Ther. 2014 Jan-Mar;10(1):133-41. doi: 10.4103/0973-1482.131461.

BACKGROUND: Prostate-specific membrane antigen (PSMA) is a widely used targeted molecule in prostate patients. The present research, attempts to support the hypothesis that PSMA expression in prostate cancer stem cell-like (CSC) cell populations may be correlated with nanog and other transcription factors in different stages of prostate carcinomas. MATERIALS AND METHODS: To provide more accurate evidence of the above, a population of prostate CSCs was isolated and analyzed using different protocols. The first method was based in the ability of CSCs to form spherical colonies in semi-suspension of a culture. A qPCRbased protocol and a flow cytometric analysis protocol were chosen to test the presence of stemness markers and PSMA in the selected populations. RESULTS: The formation of micro-sphere in semi-suspension has been pointed out. In the other panels of the test, the linear correlation between PSMA and nanog in gene and protein level was shown. However, the statistical analysis including the coefficient of variationand standard deviation's values) has proved that there were differences in PSMA expression between cancer cells and CSCs. CONCLUSION: The previous analysis has pointed out that PSMA expression may be correlated with nanog's expression as well as with other confounders in a population of prostate CSCs.

Martin-Castillo, B., C. Oliveras-Ferraros, et al. "Basal/HER2 breast carcinomas: integrating molecular taxonomy with cancer stem cell dynamics to predict primary resistance to trastuzumab (Herceptin)." Cell Cycle. 2013 Jan 15;12(2):225-45. doi: 10.4161/cc.23274. Epub 2012 Jan 15.

High rates of inherent primary resistance to the humanized monoclonal antibody trastuzumab (Herceptin) are frequent among HER2 gene-amplified breast carcinomas in both metastatic and adjuvant settings. The clinical efficacy of trastuzumab is highly correlated with its ability to specifically and efficiently target HER2-driven populations of breast cancer stem cells (CSCs). Intriguingly, many of the possible mechanisms by which cancer cells escape trastuzumab involve many of the same biomarkers that have been implicated in the biology of CS-like tumor-initiating cells. In the traditional, one-way hierarchy of CSCs in which all cancer cells descend from special self-renewing CSCs, HER2-positive CSCs can occur solely by self-renewal. Therefore, by targeting CSC self-renewal and resistance, trastuzumab is expected to induce tumor shrinkage and further reduce breast cancer recurrence rates when used alongside traditional therapies. In a new, alternate model, more differentiated non-stem cancer cells can revert to trastuzumab-refractory, CS-like cells via the activation of intrinsic or microenvironmental paths-to-stemness, such as the epithelial-to-mesenchymal transition (EMT). Alternatively, stochastic transitions of trastuzumab-responsive CSCs might also give rise to non-CSC cellular states that lack major attributes of CSCs and, therefore, can remain "hidden" from trastuzumab activity. Here, we hypothesize that a better understanding of the CSC/non-CSC social structure within HER2-overexpressing breast carcinomas is critical for trastuzumab-based treatment decisions in the clinic.

Munoz, P., M. S. Iliou, et al. "Epigenetic alterations involved in cancer stem cell reprogramming." Mol Oncol. 2012 Dec;6(6):620-36. doi: 10.1016/j.molonc.2012.10.006. Epub 2012 Oct 26.

Current hypotheses suggest that tumors originate from cells that carry out a process of "malignant reprogramming" driven by genetic and epigenetic alterations. Multiples studies reported the existence of stem-cell-like cells that acquire the ability

to self-renew and are able to generate the bulk of more differentiated cells that form the tumor. This population of cancer cells, called cancer stem cells (CSC), is responsible for sustaining the tumor growth and, under determined conditions, can disseminate and migrate to give rise to secondary tumors or metastases to distant organs. Furthermore, CSCs have shown to be more resistant to anti-tumor treatments than the non-stem cancer cells, suggesting that surviving CSCs could be responsible for tumor relapse after therapy. These important properties have raised the interest in understanding the mechanisms that govern the generation and maintenance of this special population of cells, considered to lie behind the on/off switches of gene expression patterns. In this review, we summarize the most relevant epigenetic alterations, from DNA methylation and histone modifications to the recently discovered miRNAs that contribute to the regulation of cancer stem cell features in tumor progression, metastasis and response to chemotherapy.

Okamoto, O. K. "Cancer stem cell genomics: the quest for early markers of malignant progression." Expert Rev Mol Diagn. 2009 Sep;9(6):545-54. doi: 10.1586/erm.09.40.

Biologically distinct populations of neoplastic stem cells have been identified in a variety of human cancers, in which they are associated with the initial steps of tumorigenesis. The intrinsic properties of self-renewal, clonogenicity and multipotency, along with a longer half-life within the body, may render normal adult stem cells more prone to accumulate genetic mutations leading to neoplastic transformation, as predicted by the cancer stem cell hypothesis. Tumor formation is also associated with the pluripotency of embryonic stem cells and may be induced as a consequence of complete dedifferentiation of mature cells, as recently reported for induced pluripotent stem cells. The tumor-initiating cell phenotype may result from genetic alterations affecting the expression of critical genes regulating typical stem cell processes such as self-renewal and pluripotency, in addition to genes determining stem cell senescence or longevity. Detailed genome-wide analysis of cancer stem cells and respective normal counterparts will help elucidate the cellular and molecular nature of tumors, providing fundamental information about the initial steps toward malignant transformation. Devising ways of detecting such genetic and epigenetic alterations and cell populations displaying them would allow medical interventions at the early phases of cancer development, thereby improving the chances of favorable clinical outcomes.

O'Neill, I. D. "Concise review: transmissible animal tumors as models of the cancer stem-cell process." Stem Cells. 2011 Dec;29(12):1909-14. doi: 10.1002/stem.751.

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

Oshima, N., Y. Yamada, et al. "Induction of cancer stem cell properties in colon cancer cells by defined factors." PLoS One. 2014 Jul 9;9(7):e101735. doi: 10.1371/journal.pone.0101735. eCollection 2014.

Cancer stem cells (CSCs) are considered to be responsible for the dismal prognosis of cancer patients. However, little is known about the molecular mechanisms underlying the acquisition and maintenance of CSC properties in cancer cells because of their rarity in clinical samples. We herein induced CSC properties in cancer cells using defined factors. We retrovirally introduced a set of defined factors (OCT3/4, SOX2 and KLF4) into human colon cancer cells, followed by culture with conventional serum-containing medium, not human embryonic stem cell medium. We then evaluated the CSC properties in the cells. The colon cancer cells transduced with the three factors showed significantly enhanced CSC properties in terms of the marker gene expression, sphere formation, chemoresistance and tumorigenicity. We designated the cells with CSC properties induced by the factors, a subset of the transduced cells, as induced CSCs (iCSCs). Moreover, we established a novel technology to isolate and collect the iCSCs based on the differences in the degree of the dye-effluxing

activity enhancement. The xenografts derived from our iCSCs were not teratomas. Notably, in contrast to the tumors from the parental cancer cells, the iCSC-based tumors mimicked actual human colon cancer tissues in terms of their immunohistological findings, which showed colonic lineage differentiation. In addition, we confirmed that the phenotypes of our iCSCs were reproducible in serial transplantation experiments. By introducing defined factors, we generated iCSCs with lineage specificity directly from cancer cells, not via an induced pluripotent stem cell state. The novel method enables us to obtain abundant materials of CSCs that not only have enhanced tumorigenicity, but also the ability to differentiate to recapitulate a specific type of cancer tissues. Our method can be of great value to fully understand CSCs and develop new therapies targeting CSCs.

Piggott, L., N. Omidvar, et al. "Suppression of apoptosis inhibitor c-FLIP selectively eliminates breast cancer stem cell activity in response to the anti-cancer agent, TRAIL." Breast Cancer Res. 2011 Sep 14;13(5):R88. doi: 10.1186/bcr2945.

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

Polytarchou, C., D. Iliopoulos, et al. "An integrated transcriptional regulatory circuit that reinforces the breast cancer stem cell state." Proc Natl Acad Sci U S A. 2012 Sep 4;109(36):14470-5. doi: 10.1073/pnas.1212811109. Epub 2012 Aug 20.

Cancer stem-like cells (CSCs) are a highly tumorigenic cell type present as a minority population in developmentally diverse tumors and cell lines. Using a genetic screen in an inducible model of CSC formation in a breast cell line, we identify microRNAs (miRNAs) that inhibit CSC growth and are down-regulated in CSCs. Aside from the previously identified miR-200 family, these include the miR-15/16 (miR-16, miR-15b) and miR-103/107 (miR-103, miR-107) families as well as miR-145, miR-335, and miR-128b. Interestingly, these miRNAs affect common target genes that encode the Bmi1 and Suz12 components of the polycomb repressor complexes as well as the DNA-binding transcription factors Zeb1, Zeb2, and Klf4. Conversely, expression of the CSC-modulating miRNAs is inhibited by Zeb1 and Zeb2. There is an inverse relationship between the levels of CSC-regulating miRNAs and their respective targets in samples from triple-negative breast cancer patients, providing evidence for the relevance of these interactions in human cancer. In addition, combinatorial overexpression of these miRNAs progressively attenuates the growth of CSCs derived from triple-negative breast cancers. These observations suggest that CSC formation and function are reinforced by an integrated regulatory circuit of miRNAs, transcription factors, and chromatin-modifying activities that can act as a bistable switch to drive cells into either the CSC or the nonstem state within the population of cancer cells.

Quail, D. F., M. J. Taylor, et al. "Microenvironmental regulation of cancer stem cell phenotypes." Curr Stem Cell Res Ther. 2012 May;7(3):197-216.

Cancer is a complex set of diseases, driven by genomic instability overlaid with epigenetic modifications. Two prevailing concepts, the stochastic theory and the hierarchical theory, are traditionally used to understand tumor progression. These seemingly contradictory theories can be reconciled with the concept of cellular plasticity, such that certain genetic mutations enable epigenetic alterations in cell fate. A growing body of evidence suggests that cancer cells co-opt embryonic stem cell-associated regulatory networks in order to sustain tumor cell plasticity concomitant with growth and progression. The expression of these stem cell associated factors is regulated by dynamic niches, characterized by cellderived proteins as well as biophysical features such low oxygen tensions. In this review we describe specific embryo-associated proteins such as NODAL, NOTCH, and canonical WNT, which cooperate to maintain stem cell phenotypes in cancer. We also illustrate how biophysical factors, in particular oxygen, can orchestrate plasticity by modulating the expression of stem cell-associated proteins. As the microenvironment is known to play a key role in cellular regulation, it is essential to understand its role in cancer progression in order to improve and create new therapies.

Samadani, A. A. and H. Akhavan-Niaki "Interaction of sonic hedgehog (SHH) pathway with cancer stem cell genes in gastric cancer." Med Oncol. 2015 Mar;32(3):48. doi: 10.1007/s12032-015-0492-3. Epub 2015 Jan 31.

Gastric cancer may appear by frequent genetic or epigenetic changes in oncogenes, tumor suppressor or DNA mismatch repair genes. Molecular studies show the possibility of involvement of certain cancer pathways in gastric cancer. In this respect, DNA methylation is one of the most important epigenetic alterations in gastric cancer and identifying the signaling mechanism and also methylation of some genes that are involved in gastric cancer can help to improve treatment strategies. Relatively, there are many reported methylation alteration of genes in stem cells in all kinds of tumors with some of these genes having a key role in tumor development. Correspondingly, KLF5, CDX1/2, WNT1 and FEM1A are considerable genes in gastric cancer, although many researches and studies have illustrated that sonic hedgehog and expression of its signaling cascade proteins are related in gastric cancer. Relatively, modification in these genes causes many eclectic cancers such as rhabdomyosarcoma and diverse kinds of digestive system tumor development. Conspicuously, these master genes have a noticeable role in stem cell's growth regulation as well as other kinds of cancer such as breast cancer and leukemia. Hence, we concluded that research and studies on methylation and expression of these genes and also the investigation of molecular signaling in gastric cancer can acquire impressive conclusions in order to control and treat this common place and serious problem.

Sharma, K. L., A. Yadav, et al. "Association of genetic variants of cancer stem cell gene CD44 haplotypes with gallbladder cancer susceptibility in North Indian population." Tumour Biol. 2014 Mar;35(3):2583-9. doi: 10.1007/s13277-013-1340-8. Epub 2013 Nov 5.

CD44 is an important marker for cancer stem cells. Germline variants in CD44 gene have been associated with susceptibility to breast and nasopharyngeal carcinomas but no study in gallbladder cancer (GBC) has been done yet. The present study included 405 GBC patients and 200 healthy controls from North India. Tagger SNPs for CD44 were selected from the GIH population data. Genotyping was carried out by PCR-RFLP and Taqman probes. Statistical analysis was done by SPSS. Bonferroni correction was applied in subgroup analysis. Logistic regression analysis showed no individual association of CD44 polymorphisms with GBC risk. However, [CCAT] haplotype was associated with overall reduced risk of GBC [P = 0.04, odds ratios (OR) = 0.47]. Gender stratification revealed that [CCAT] and [TAGT] haplotypes were significantly associated with decreased risk in female GBC patients [P = 0.022, OR = 0.38; P = 0.011, OR = 0.17, respectively]. The CAAT haplotype was marginally associated with low GBC risk in patients with co-existing gallstones [P = 0.026, OR = 0.53]. The cancer risk was not further modified with tobacco usage or age of onset. In silico analysis showed change in transcriptional regulation of selected SNPs. This study reports an important role of CD44 haplotypes with reduced risk of GBC.

Shipitsin, M. and K. Polyak "The cancer stem cell hypothesis: in search of definitions, markers, and relevance." Lab Invest. 2008 May;88(5):459-63. doi: 10.1038/labinvest.2008.14. Epub 2008 Mar 31.

Cancer is a disease of genes. Inherited or somatic alterations in genes are what make a normal cell ignore growth-controlling signals and form a tumor that eventually leads to the destruction of the organism. Based on accumulated knowledge on the genetic composition of cancer cells, the clonal evolution model of tumorigenesis was established, which explains multiple aspects of human disease and clinical observations. However, the recently popularized cancer stem cell hypothesis questions that all or most tumor cells can participate in tumor

evolution and restricts this property to a subset of them defined as 'cancer stem cells' due to their stem cell-like characteristics. Enthusiasm surrounding this area of investigation and its presumed clinical implications led to a spurt of studies in various cancer types and model systems. Rigorous study design and critical data interpretation have to be employed to test the scientific and clinical relevance of the cancer stem cell hypothesis and its relationship to the clonal evolution model.

Taube, J. H., G. G. Malouf, et al. "Epigenetic silencing of microRNA-203 is required for EMT and cancer stem cell properties." Sci Rep. 2013;3:2687. doi: 10.1038/srep02687.

The epithelial-mesenchymal transition (EMT) imparts metastatic competence on otherwise non-metastatic cancer cells through decreased inter-cellular adhesions, increased migratory capacity, stem cell properties and anoikis and chemotherapy resistance. In this study, we profiled changes in microRNA expression during EMT in conjunction with changes in DNA methylation at microRNA promoters to discover essential mediators of EMT-imparted stemness properties. MicroRNA-203 (miR-203) expression is repressed following EMT induced by multiple different stimuli and in established claudin-low cell lines as well as the CD44hi/CD24lo stem cell-enriched fraction. Expression of miR-203 in mesenchymal cells compromises migratory and invasive capacity in vitro, and tumor initiation and metastasis in vivo. Unexpectedly, miR-203 expression affects the sphere-forming capacity of neighboring cells by indirectly enhancing expression of DKK1, a secreted inhibitor of Wnt signaling and stemness resulting in suppression of beta-catenin protein levels. Our data suggest that restoring miR-203 expression levels may inhibit metastasis and combat deregulated Wnt signaling.

Todorova, R. "Ewing's sarcoma cancer stem cell targeted therapy." Curr Stem Cell Res Ther. 2014 Jan;9(1):46-62.

Ewing`s sarcoma (ES) family of tumors (ESFTs) are round cell tumors of bone and soft tissues, afflicting children and young adults. This review summarizes the present findings about ES cancer stem cell (CSC) targeted therapy: prognostic factors, chromosomal translocations, initiation, epigenetic mechanisms, candidate cell of ES origin (Mesenchymal stem cells (MSCs) and Neural crest stem cells (NCSCs)). The ES CSC model, histopathogenesis, histogenesis, pathogenesis, ES mediated Hematopoietic stem progenitor cells (HSPCs) senescence are also discussed. ESFTs therapy is reviewed concerning CSCs, radiotherapy, risk of subsequent neoplasms, stem cell (SC) support, promising therapeutic targets for ES CSCs (CSC markers, immune targeting, RNAi phenotyping screens, proposed new drugs), candidate EWS-FLI1 target genes and further directions (including human embryonic stem cells (hESCs)). Bone marrow-derived human MSCs are permissive for EWS-FLI1 expression with transition to ESFT-like cellular phenotype. ESFTs are genetically related to NCSC, permissive for EWS-FLI1 expression and susceptible to oncogene-induced immortalization. Primitive neuroectodermal features and MSC origin of ESFTs provide a basis of immune targeting. The microRNAs profile of ES CSCs is shared by ESCs and CSCs from divergent tumor types. Successful reprogramming of differentiated human somatic cells into a pluripotent state allows creation of patient- and disease-specific SCs. The functional role of endogenous EWS at stem cell level on both senescence and tumorigenesis is a link between cancer and aging. The regulatory mechanisms of oncogenic activity of EWS fusions could provide new prognostic biomarkers, therapeutic opportunities and tumor-specific anticancer agents against ESFTs.

van Vlerken, L. E., C. M. Kiefer, et al. "EZH2 is required for breast and pancreatic cancer stem cell maintenance and can be used as a functional cancer stem cell reporter." Stem Cells Transl Med. 2013 Jan;2(1):43-52. doi: 10.5966/sctm.2012-0036. Epub 2012 Dec 27.

Although cancer is largely seen as a disease stemming from genetic mutations, evidence has implicated epigenetic regulation of gene expression as a driving force for tumorigenesis. Epigenetic regulation by histone modification, specifically through polycomb group (PcG) proteins such as EZH2 and BMI-1, is a major driver in stem cell biology and is found to be correlated with poor prognosis in many tumor types. This suggests a role for PcG proteins in cancer stem cells (CSCs). We hypothesized that epigenetic modification by EZH2, specifically, helps maintain the CSC phenotype and that in turn this epigenetic modifier can be used as a reporter for CSC activity in an in vitro high-throughput screening assay. CSCs isolated from pancreatic and breast cancer lines had elevated EZH2 levels over non-CSCs. Moreover, EZH2 knockdown by RNA interference significantly reduced the frequency of CSCs in all models tested, confirming the role of EZH2 in maintenance of the CSC population. Interestingly, genes affected by EZH2 loss, and therefore CSC loss, were inversely correlated with genes identified by CSC enrichment, further supporting the function of EZH2 CSC regulation. We translated these results into a novel assay whereby elevated EZH2 staining was used as a reporter for CSCs. Data confirmed that this assay

could effectively measure changes, both inhibition and enrichment, in the CSC population, providing a novel approach to look at CSC activity. This assay provides a unique, rapid way to facilitate CSC screening across several tumor types to aid in further CSC-related research.

Vedeld, H. M., R. I. Skotheim, et al. "The recently suggested intestinal cancer stem cell marker DCLK1 is an epigenetic biomarker for colorectal cancer." Epigenetics. 2014 Mar;9(3):346-50. doi: 10.4161/epi.27582. Epub 2014 Jan 2.

Recently, Dclk1 expression was identified to be an intestinal cancer stem cell specific biomarker in mouse models, implicating a potential role for targeting the DCLK1-postive cancer cells as a treatment for colorectal cancer. Using quantitative methylation specific PCR (qMSP) we here demonstrated that the DCLK1 promoter is hypermethylated in the vast majority of colorectal cancers (134/164; 82%), with no methylation in the normal mucosa samples (0/106). We further showed by Affymetrix exon arrays that DCLK1 is significantly downregulated in human colorectal cancer (n = 125) compared with normal colonic mucosa (n = 15), which was further confirmed by real-time RT-PCR of a subgroup of the samples. Additionally, a significant negative correlation was observed between methylation and DCLK1 expression in 74 cancer cell lines derived from 15 different tissues, and gene expression increased significantly after epigenetic drug treatment of initially methylated cancer cell lines. These findings underscore the potential of DCLK1 as a colorectal cancer biomarker for early detection, but may also have clinical implications regarding the previously proposed therapy toward DCLK1-positive cancer cells. This therapy would at best affect the cancer stem cell population, but will, based on the present results, not be efficient to treat the bulk of the tumor.

Vicente-Duenas, C., I. Romero-Camarero, et al. Understanding telomerase in cancer stem cell biology, Cell Cycle. 2012 Apr 15;11(8):1479-80. doi: 10.4161/cc.20108. Epub 2012 Apr 15.

Wang, G. G., M. P. Pasillas, et al. "Meis1 programs transcription of FLT3 and cancer stem cell character, using a mechanism that requires interaction with Pbx and a novel function of the Meis1 C-terminus." Blood. 2005 Jul 1;106(1):254-64. Epub 2005 Mar 8.

Meis1 is a homeodomain transcription factor coexpressed with Hoxa9 in most human acute myeloid leukemias (AMLs). In mouse models of leukemia produced by Hoxa9, Meis1 accelerates leukemogenesis. Because Hoxa9 immortalizes myeloid progenitors in the absence of Meis1 expression, the contribution of Meis1 toward leukemia remains unclear. Here, we describe a cultured progenitor model in which Meis1 programs leukemogenicity. Progenitors immortalized by Hoxa9 in culture are myeloid-lineage restricted and only infrequently caused leukemia after more than 250 days. Coexpressed Meis1 programmed rapid AML-initiating character, maintained multipotent progenitor potential, and induced expression of genes associated with short-term hematopoietic stem cells (HSCs), such as FLT3 and CD34, whose expression also characterizes the leukemia-initiating stem cells of human AML. Meis1 leukemogenesis functions required binding to Pbx, binding to DNA, and a conserved function of its C-terminal tail. We hypothesize that Meis1 is required for the homing and survival of leukemic progenitors within their hematopoietic niches, functions mediated by HSC-specific genes such as CD34 and Fms-like tyrosine kinase 3 (FLT3), respectively. This is the first example of a transcription factor oncoprotein (Meis1) that establishes expression of a tyrosine kinase oncoprotein (FLT3), and explains their coexpression in human leukemia. This cultured progenitor model will be useful to define the genetic basis of leukemogenesis involving Hoxa9 and Meis1.

Wang, X., J. F. Hu, et al. "Cancer stem cell marker Musashi-1 rs2522137 genotype is associated with an increased risk of lung cancer." PLoS One. 2014 May 2;9(5):e95915. doi: 10.1371/journal.pone.0095915. eCollection 2014.

Gene single nucleotide polymorphisms (SNPs) have been extensively studied in association with development and prognosis of various malignancies. However, the potential role of genetic polymorphisms of cancer stem cell (CSC) marker genes with respect to cancer risk has not been examined. We conducted a case-control study involving a total of 1000 subjects (500 lung cancer patients and 500 age-matched cancer-free controls) from northeastern China. Lung cancer risk was analyzed in a logistic regression model in association with genotypes of four lung CSC marker genes (CD133, ALDH1, Musashi-1, and EpCAM). Using univariate analysis, the Musashi-1 rs2522137 GG genotype was found to be associated with a higher incidence of lung cancer compared with the TT genotype. No significant associations were observed for gene variants of CD133, ALDH1, or EpCAM. In multivariate analysis, Musashi-1 rs2522137 was still significantly associated with lung cancer when environmental and lifestyle factors were incorporated in the model, including lower BMI; family history of cancer; prior diagnosis of chronic obstructive

pulmonary disease, pneumonia, or pulmonary tuberculosis; occupational exposure to pesticide; occupational exposure to gasoline or diesel fuel; heavier smoking; and exposure to heavy cooking emissions. The value of the area under the receiver-operating characteristic (ROC) curve (AUC) was 0.7686. To our knowledge, this is the first report to show an association between a Musashi-1 genotype and lung cancer risk. Further, the prediction model in this study may be useful in determining individuals with high risk of lung cancer.

Wang, Z., J. Liu, et al. "Dynamic modeling of genes controlling cancer stem cell proliferation." Front Genet. 2012 May 22;3:84. doi: 10.3389/fgene.2012.00084. eCollection 2012.

The growing evidence that cancer originates from stem cells (SC) holds a great promise to eliminate this disease by designing specific drug therapies for removing cancer SC. Translation of this knowledge into predictive tests for the clinic is hampered due to the lack of methods to discriminate cancer SC from non-cancer SC. Here, we address this issue by describing a conceptual strategy for identifying the genetic origins of cancer SC. The strategy incorporates a high-dimensional group of differential equations that characterizes the proliferation, differentiation, and reprogramming of cancer SC in a dynamic cellular and molecular system. The deployment of robust mathematical models will help uncover and explain many still unknown aspects of cell behavior, tissue function, and network organization related to the formation and division of cancer SC. The statistical method developed allows biologically meaningful hypotheses about the genetic control mechanisms of carcinogenesis and metastasis to be tested in a quantitative manner.

Won, H. Y., J. Y. Lee, et al. "Loss of Mel-18 enhances breast cancer stem cell activity and tumorigenicity through activating Notch signaling mediated by the Wnt/TCF pathway." FASEB J. 2012 Dec;26(12):5002-13. doi: 10.1096/fj.12-209247. Epub 2012 Sep 5.

Mel-18 has been proposed as a negative regulator of Bmi-1, a cancer stem cell (CSC) marker, but it is still unclear whether Mel-18 is involved in CSC regulation. Here, we examined the effect of Mel-18 on the stemness of human breast CSCs. In Mel-18 small hairpin RNA (shRNA)-transduced MCF-7 cells, side population (SP) cells and breast CSC surface marker (CD44(+)/CD24(-)/ESA(+))-expressing cells, which imply a CSC population, were enriched. Moreover, the self-renewal of CSCs was enhanced by Mel-18 knockdown, as measured by the ability for tumorsphere formation in vitro and tumor-initiating capacity in vivo. Similarly, Mel-18 overexpression inhibited the number and self-renewal activity of breast CSCs in SK-BR-3 cells. Furthermore, our data showed that Mel-18 blockade up-regulated the expression of the Wnt/TCF target Jagged-1, a Notch ligand, and consequently activated the Notch pathway. Pharmacologic inhibition of the Notch and Wnt pathways abrogated Mel-18 knockdown-mediated tumorsphere formation ability. Taken together, our findings suggest that Mel-18 is a novel negative regulator of breast CSCs that inhibits the stem cell population and in vitro and in vivo self-renewal through the inactivation of Wnt-mediated Notch signaling.

Wu, K., X. Jiao, et al. "Cell fate determination factor Dachshund reprograms breast cancer stem cell function." J Biol Chem. 2011 Jan 21;286(3):2132-42. doi: 10.1074/jbc.M110.148395. Epub 2010 Oct 11.

The cell fate determination factor Dachshund was cloned as a dominant inhibitor of the hyperactive epidermal growth factor receptor ellipse. The expression of Dachshund is lost in human breast cancer associated with poor prognosis. Breast tumor-initiating cells (TIC) may contribute to tumor progression and therapy resistance. Here, endogenous DACH1 was reduced in breast cancer cell lines with high expression of TIC markers and in patient samples of the basal breast cancer phenotype. Re-expression of DACH1 reduced new tumor formation in serial transplantations in vivo, reduced mammosphere formation, and reduced the proportion of CD44(high)/CD24(low) breast tumor cells. Conversely, lentiviral shRNA to DACH1 increased the breast (B)TIC population. Genome-wide expression studies of mammary tumors demonstrated DACH1 repressed a molecular signature associated with stem cells (SOX2, Nanog, and KLF4) and genome-wide ChIP-seq analysis identified DACH1 binding to the promoter of the Nanog, KLF4, and Lin28 genes. KLF4/c-Myc and Oct4/Sox2 antagonized DACH1 repression of BTIC. Mechanistic studies demonstrated DACH1 directly repressed the Nanog and Sox2 promoters via a conserved domain. Endogenous DACH1 regulates BTIC in vitro and in vivo.

Xiang, L., D. M. Gilkes, et al. "Hypoxia-inducible factor 1 mediates TAZ expression and nuclear localization to induce the breast cancer stem cell phenotype." Oncotarget. 2014 Dec 30;5(24):12509-27.

Intratumoral hypoxia, which is associated with breast cancer metastasis and patient mortality, increases the percentage of breast cancer stem cells (BCSCs) but the underlying molecular mechanisms have not been delineated. Here we report that hypoxia-inducible factor 1 (HIF-1) triggers the expression and activity of TAZ, a transcriptional co-activator that is

required for BCSC maintenance, through two discrete mechanisms. First, HIF-1 binds directly to the WWTR1 gene and activates transcription of TAZ mRNA. Second, HIF-1 activates transcription of the SIAH1 gene, which encodes a ubiquitin protein ligase that is required for the hypoxia-induced ubiquitination and proteasome-dependent degradation of LATS2, a kinase that inhibits the nuclear localization of TAZ. Inhibition of HIF-1alpha, TAZ, or SIAH1 expression by short hairpin RNA blocked the enrichment of BCSCs in response to hypoxia. Human breast cancer database analysis revealed that increased expression (greater than the median) of both TAZ and HIF-1 target genes, but neither one alone, is associated with significantly increased patient mortality. Taken together, these results establish a molecular mechanism for induction of the BCSC phenotype in response to hypoxia.

Yamazaki, H., C. W. Xu, et al. "Regulation of cancer stem cell properties by CD9 in human B-acute lymphoblastic leukemia." Biochem Biophys Res Commun. 2011 May 27;409(1):14-21. doi: 10.1016/j.bbrc.2011.04.098. Epub 2011 Apr 24.

Although the prognosis of acute lymphoblastic leukemia (ALL) has improved considerably in recent years, some of the cases still exhibit therapy-resistant. We have previously reported that CD9 was expressed heterogeneously in B-ALL cell lines and CD9(+) cells exhibited an asymmetric cell division with greater tumorigenic potential than CD9(-) cells. CD9(+) cells were also serially transplantable in immunodeficient mice, indicating that CD9(+) cell possess self-renewal capacity. In the current study, we performed more detailed analysis of CD9 function for the cancer stem cell (CSC) properties. In patient sample, CD9 was expressed in the most cases of B-ALL cells with significant correlation of CD34-expression. Gene expression analysis revealed that leukemogenic fusion proteins and Src family proteins were significantly regulated in the CD9(+) population. Moreover, CD9(+) cells exhibited drug-resistance, but proliferation of bulk cells was inhibited by anti-CD9 monoclonal antibody. Knockdown of CD9 remarkably reduced the leukemogenic potential. Furthermore, gene ablation of CD9 affected the expression and tyrosine-phosphorylation of Src family proteins and reduced the expression of histone-deubiquitinase USP22. Taken together, our results suggest that CD9 links to several signaling pathways and epigenetic modification for regulating the CSC properties of B-ALL.

Yao, Z. X., W. Jogunoori, et al. "Epigenetic silencing of beta-spectrin, a TGF-beta signaling/scaffolding protein in a human cancer stem cell disorder: Beckwith-Wiedemann syndrome." J Biol Chem. 2010 Nov 12;285(46):36112-20. doi: 10.1074/jbc.M110.162347. Epub 2010 Aug 25.

Hereditary cancer syndromes provide powerful insights into dysfunctional signaling pathways that lead to sporadic cancers. Beckwith-Wiedemann syndrome (BWS) is a hereditary human cancer stem cell syndrome currently linked to deregulated imprinting at chromosome 11p15 and uniparental disomy. However, causal molecular defects and genetic models have remained elusive to date in the majority of cases. The non-pleckstrin homology domain beta-spectrin (beta2SP) (the official name for human is Spectrin, beta, nonerythrocytic 1 (SPTBN1), isoform 2; the official name for mouse is Spectrin beta 2 (Spnb2), isoform 2), a scaffolding protein, functions as a potent TGF-beta signaling member adaptor in tumor suppression and development. Yet, the role of the beta2SP in human tumor syndromes remains unclear. Here, we report that beta2SP(+/-) mice are born with many phenotypic characteristics observed in BWS patients, suggesting that beta2SP mutant mice phenocopy BWS, and beta2SP loss could be one of the mechanisms associated with BWS. Our results also suggest that epigenetic silencing of beta2SP is a new potential causal factor in human BWS patients. Furthermore, beta2SP(+/-) mice provide an important animal model for BWS, as well as sporadic cancers associated with it, including lethal gastrointestinal and pancreatic cancer. Thus, these studies could lead to further insight into defects generated by dysfunctional stem cells and identification of new treatment strategies and functional markers for the early detection of these lethal cancers that otherwise cannot be detected at an early stage.

Yoo, B. H., S. D. Axlund, et al. "A high-content assay to identify small-molecule modulators of a cancer stem cell population in luminal breast cancer." J Biomol Screen. 2012 Oct;17(9):1211-20. Epub 2012 Jun 29.

Breast cancers expressing hormone receptors for estrogen (ER) and progesterone (PR) represent ~70% of all cases and are treated with both ER-targeted and chemotherapies, with near 40% becoming resistant. We have previously described that in some ER(+) tumors, the resistant cells express cytokeratin 5 (CK5), a putative marker of breast stem and progenitor cells. CK5(+) cells have lost expression of ER and PR, express the tumor-initiating cell surface marker CD44, and are relatively quiescent. In addition, progestins, which increase breast cancer incidence, expand the CK5(+) subpopulation in ER(+)PR(+) breast cancer cell lines. We have developed models to induce and quantitate CK5(+)ER(-)PR(-) cells, using CK5 promoter-driven luciferase (Fluc) or green fluorescent

protein (GFP) reporters stably transduced into T47D breast cancer cells (CK5Pro-GFP or CK5Pro-Luc). We validated the CK5Pro-GFP-T47D model for high-content screening in 96-well microplates and performed a pilot screen using a focused library of 280 compounds from the National Institutes of Health clinical collection. Four hits were obtained that significantly abrogated the progestin-induced CK5(+) cell population, three of which were members of the retinoid family. Hence, this approach will be useful in discovering small molecules that could potentially be developed as combination therapies, preventing the acquisition of a drug-resistant subpopulation.

Zakaria, N., N. M. Yusoff, et al. "Human non-small cell lung cancer expresses putative cancer stem cell markers and exhibits the transcriptomic profile of multipotent cells." BMC Cancer. 2015 Feb 25;15:84. doi: 10.1186/s12885-015-1086-3.

BACKGROUND: Despite significant advances in staging and therapies, lung cancer remains a major cause of cancer-related lethality due to its high incidence and recurrence. Clearly, a novel approach is required to develop new therapies to treat this devastating disease. Recent evidence indicates that tumours contain a small population of cells known as cancer stem cells (CSCs) that are responsible for tumour maintenance, spreading and resistant to chemotherapy. The genetic composition of CSCs so far is not fully understood, but manipulation of the specific genes that maintain their integrity would be beneficial for developing strategies to combat cancer. Therefore, the goal of this study isto identify the transcriptomic composition and biological functions of CSCs from non-small cell lung cancer (NSCLC). METHODS: We isolated putative lung CSCs from lung adenocarcinoma cells (A549 and H2170) and normal stem cells from normal bronchial epithelial cells (PHBEC) on the basis of positive expression of stem cell surface markers (CD166, CD44, and EpCAM) using fluorescence-activated cell sorting. The isolated cells were then characterised for their self-renewal characteristics, differentiation capabilities, expression of stem cell transcription factor and in vivo tumouregenicity. The transcriptomic profiles of putative lung CSCs then were obtained using microarray analysis. Significantly regulated genes ($p < 0.05$, fold change (FC) > 2.0) in putative CSCs were identified and further analysed for their biological functions using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). RESULTS: The putative lung CSCs phenotypes of CD166(+)/CD44(+) and CD166(+)/EpCAM(+) showed multipotent characteristics of stem cells, including the ability to differentiate into adipogenic and osteogenic cells, self-renewal, and expression of stem cell transcription factors such as Sox2 and Oct3/4. Moreover, the cells also shows the in vivo tumouregenicity characteristic when transplanted into nude mice. Microarray and bioinformatics data analyses revealed that the putative lung CSCs have molecular signatures of both normal and cancer stem cells and that the most prominent biological functions are associated with angiogenesis, migration, pro-apoptosis and anti-apoptosis, osteoblast differentiation, mesenchymal cell differentiation, and mesenchyme development. Additionally, self-renewal pathways such as the Wnt and hedgehog signalling pathways, cancer pathways, and extracellular matrix (ECM)-receptor interaction pathways are significantly associated with the putative lung CSCs. CONCLUSION: This study revealed that isolated lung CSCs exhibit the characteristics of multipotent stem cells and that their genetic composition might be valuable for future gene and stem cells therapy for lung cancer.

Zapperi, S. and C. A. La Porta "Do cancer cells undergo phenotypic switching? The case for imperfect cancer stem cell markers." Sci Rep. 2012;2:441. doi: 10.1038/srep00441. Epub 2012 Jun 7.

The identification of cancer stem cells in vivo and in vitro relies on specific surface markers that should allow to sort cancer cells in phenotypically distinct subpopulations. Experiments report that sorted cancer cell populations after some time tend to express again all the original markers, leading to the hypothesis of phenotypic switching, according to which cancer cells can transform stochastically into cancer stem cells. Here we explore an alternative explanation based on the hypothesis that markers are not perfect and are thus unable to identify all cancer stem cells. Our analysis is based on a mathematical model for cancer cell proliferation that takes into account phenotypic switching, imperfect markers and error in the sorting process. Our conclusion is that the observation of reversible expression of surface markers after sorting does not provide sufficient evidence in support of phenotypic switching.

Zhang, C., G. B. Liu, et al. "[Touchdown PCR and overlap extension PCR for generating CD133(+) cancer stem cell-selective adenovirus vector]." Nan Fang Yi Ke Da Xue Xue Bao. 2011 Sep;31(9):1513-7.

OBJECTIVE: To construct a replication-incompetent adenovirus vector targeting cancer stem cells by modified touchdown PCR and overlap extension PCR and investigate its infection efficiency in CD133(+) SW480 cells in vitro. METHODS: The two portions of the fiber gene encoding the Ad5 fiber knob domain with the HI loop deleted were amplified using two pairs of designed primers and then linked by

overlap extension PCR. The product obtained was identified by sequencing and inserted into prokaryotic expression vector pEGFP-N1. The product, pEGFP-N1 KNOBdeltaHI, contained a unique EcoRV restriction site in the deleted portion of the sequence encoding the HI loop. The gene sequences of the adenovirus fiber were amplified using both common PCR and overlap extension PCR, then identified by sequencing and inserted into pNEB193, resulting in pNEB-F5. CD133(+) SW480 cells were infected with the generated adenovirus vectors Ad5-GFP and Ad5FHI-GFP to investigate the infection efficiency using fluorescent microscope. RESULTS: The target fragments of expected sizes were amplified by touchdown PCR and overlap extension PCR, but not by common PCR. Ad5FHI-GFP showed a higher infection efficiency than Ad5-GFP in CD133(+) SW480 cells. CONCLUSION: Compared with common PCR, touchdown PCR and overlap extension PCR can significantly improve the specificity and efficiency of the PCR products for constructing CD133(+) cancer stem cell-selective adenovirus type 5 vector, which provides carriers for tumor-targeted gene therapy.

Zhang, X. Y., M. Varthi, et al. "The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression." Mol Cell. 2008 Jan 18;29(1):102-11. doi: 10.1016/j.molcel.2007.12.015.

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

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