Websites:
http://www.sciencepub.net
http://www.sciencepub.net/researcher

Emails:
editor@sciencepub.net
marslandresearcher@gmail.com

Researcher

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Cancer Cell Biology Research Literatures

Mark Herbert, PhD

39 Main Street, Flushing, Queens, New York 11354, USA, ma8080@gmail.com

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 related studies.
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Key words: cancer; life; research; literature; cell

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. This article introduces recent research reports as references in the related studies.

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

Abell, A. N. and G. L. Johnson (2014). "Implications of Mesenchymal Cells in Cancer Stem Cell Populations: Relevance to EMT." Curr Pathobiol Rep **2**(1): 21-26.

The epithelial to mesenchymal transition (EMT) generates tumor cells having stem cell characteristics with phenotypes similar to cancer stem cells (CSCs). Evidence suggests CSCs are in an intermediate state of EMT expressing reduced levels of E-cadherin and exhibiting mesenchymal features including invasiveness associated with metastasis. These findings suggest mechanisms regulating EMT and stemness are closely integrated. Recent reports from multiple laboratories have identified novel mechanisms regulating EMT and stemness involving epigenetics, microenvironment, and dedifferentiation. Circulating tumor cells (CTCs) have also been shown to exhibit features of EMT, but it is unclear what fraction has CSCs properties. EMT characteristics of both CSCs and CTCs are associated with resistance to current clinical treatments, indicating therapies targeting the CSC in addition to the more differentiated tumor cells are required for durable responses. Thus, EMT characteristics of CTCs may prove useful biomarkers for effective therapies for many cancers.

Aktas, B., et al. (2009). "Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients." Breast Cancer Res **11**(4): R46.

INTRODUCTION: The persistence of circulating tumor cells (CTC) in breast cancer patients might be associated with stem cell like tumor cells which have been suggested to be the active source of metastatic spread in primary tumors. Furthermore, these cells also may undergo phenotypic changes, known as epithelial-mesenchymal transition (EMT), which allows them to travel to the site of metastasis formation without getting affected by conventional treatment. Here we evaluated 226 blood samples of 39 metastatic breast cancer patients during a follow-up of palliative chemo-, antibody - or hormonal therapy for the expression of the stem cell marker ALDH1 and markers for EMT and correlated these findings with the presence of CTC and response to therapy. METHODS: 2 x 5 ml blood was analyzed for CTC with the AdnaTest BreastCancer (AdnaGen AG) for the detection of EpCAM, MUC-1 and HER2 transcripts. The recovered c-DNA was additionally multiplex tested for three EMT markers [Twist1, Akt2, PI3Kalpha] and separately for the tumor stem-cell markers ALDH1. The identification of EMT markers was considered positive if at least one marker was detected in the

sample. RESULTS: 97% of 30 healthy donor samples investigated were negative for EMT and 95% for ALDH1 transcripts. CTC were detected in 69/226 (31%) cancer samples. In the CTC (+) group, 62% were positive for at least one of the EMT markers and 69% for ALDH1, respectively. In the CTC (-) group the percentages were 7% and 14%, respectively. In non-responders, EMT and ALDH1 expression was found in 62% and 44% of patients, in responders the rates were 10% and 5%, respectively. CONCLUSIONS: Our data indicate that a major proportion of CTC of metastatic breast cancer patients shows EMT and tumor stem cell characteristics. Further studies are needed to prove whether these markers might serve as an indicator for therapy resistant tumor cell populations and, therefore, an inferior prognosis.

Andriani, F., et al. (2016). "Conversion to stem-cell state in response to microenvironmental cues is regulated by balance between epithelial and mesenchymal features in lung cancer cells." Mol Oncol **10**(2): 253-271.

Cancer cells within a tumor are functionally heterogeneous and specific subpopulations, defined as cancer initiating cells (CICs), are endowed with higher tumor forming potential. The CIC state, however, is not hierarchically stable and conversion of non-CICs to CICs under microenvironment signals might represent a determinant of tumor aggressiveness. How plasticity is regulated at the cellular level is however poorly understood. To identify determinants of plasticity in lung cancer we exposed eight different cell lines to TGFbeta1 to induce EMT and stimulate modulation of CD133(+) CICs. We show that response to TGFbeta1 treatment is heterogeneous with some cells readily switching to stem cell state (1.5-2 fold CICs increase) and others being unresponsive to stimulation. This response is unrelated to original CICs content or extent of EMT engagement but is tightly dependent on balance between epithelial and mesenchymal features as measured by the ratio of expression of CDH1 (E-cadherin) to SNAI2. Epigenetic modulation of this balance can restore sensitivity of unresponsive models to microenvironmental stimuli, including those elicited by cancer-associated fibroblasts both in vitro and in vivo. In particular, tumors with increased prevalence of cells with features of partial EMT (hybrid epithelial/mesenchymal phenotype) are endowed with the highest plasticity and specific patterns of expression of SNAI2 and CDH1 markers identify a subset of tumors with worse prognosis. In conclusion, here we describe a connection between a hybrid epithelial/mesenchymal phenotype and conversion to stem-cell state in response to external stimuli. These findings have implications for current endeavors to identify tumors with increased plasticity.

Ayinde, O., et al. (2017). "Tissue transglutaminase induces Epithelial-Mesenchymal-Transition and the acquisition of stem cell like characteristics in colorectal cancer cells." Oncotarget **8**(12): 20025-20041.

Human colon cancer cell lines (CRCs) RKO, SW480 and SW620 were investigated for TG2 involvement in tumour advancement and aggression. TG2 expression correlated with tumour advancement and expression of markers of epithelial-mesenchymal transition (EMT). The metastatic cell line SW620 showed high TG2 expression compared to the primary tumour cell lines SW480 and RKO and could form tumour spheroids under non- adherent conditions. TG2 manipulation in the CRCs by shRNA or TG2 transduction confirmed the relationship between TG2 and EMT. TGFbeta1 expression in CRC cells, and its level in the cell medium and extracellular matrix was increased in primary tumour CRCs overexpressing TG2 and could regulate TG2 expression and EMT by both canonical (RKO) and non-canonical (RKO and SW480) signalling. TGFbeta1 regulation was not observed in the metastatic SW620 cell line, but TG2 knockdown or inhibition in SW620 reversed EMT. In SW620, TG2 expression and EMT was associated with increased presence of nuclear beta-catenin which could be mediated by association of TG2 with the Wnt signalling co-receptor LRP5. TG2 inhibition/knockdown increased interaction between beta-catenin and ubiquitin shown by co-immunoprecipitation, suggesting that TG2 could be important in beta-catenin regulation. beta-Catenin and TG2 was also upregulated in SW620 spheroid cells enriched with cancer stem cell marker CD44 and TG2 inhibition/knockdown reduced the spheroid forming potential of SW620 cells. Our data suggests that TG2 could hold both prognostic and therapeutic significance in colon cancer.

Bao, B., et al. (2011). "Over-expression of FoxM1 leads to epithelial-mesenchymal transition and cancer stem cell phenotype in pancreatic cancer cells." J Cell Biochem **112**(9): 2296-2306.

FoxM1 is known to play important role in the development and progression of many malignancies including pancreatic cancer. Studies have shown that the acquisition of epithelial-to-mesenchymal transition (EMT) phenotype and induction of cancer stem cell (CSC) or cancer stem-like cell phenotypes are highly inter-related, and contributes to drug resistance, tumor recurrence, and metastasis. The molecular mechanism (s) by which FoxM1 contributes to the acquisition of EMT phenotype and induction of CSC self-renewal

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

Bao, B., et al. (2011). "Notch-1 induces epithelial-mesenchymal transition consistent with cancer stem cell phenotype in pancreatic cancer cells." Cancer Lett **307**(1): 26-36.

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

Bao, B., et al. (2018). "Retraction notice to "Notch-1 induces Epithelial-mesenchymal transition consistent with cancer stem cell phenotype in pancreatic cancer cells"." Cancer Lett **423**: 153.

This article has been retracted: please see Elsevier Policy on Article Withdrawal (http://www.elsevier.com/locate/withdrawalpolicy). This article has been retracted at the request of the Editor in Chief. An investigation by Wayne State University identified a discrepancy between the data reported in Figures 1B, 2B and 3C and the original collected data. The investigation committee concluded that this undermined the scientific basis of the publication, that no credible replacement data were available, and advised that the publication should be retracted.

Bessede, E., et al. (2014). "Helicobacter pylori generates cells with cancer stem cell properties via epithelial-mesenchymal transition-like changes." Oncogene **33**(32): 4123-4131.

Helicobacter pylori infection is the major risk factor for gastric adenocarcinoma. The link with gastric adenocarcinoma is partly due to the H. pylori CagA oncoprotein. CagA is responsible for a particular cell phenotype in vitro, the 'hummingbird' phenotype, that corresponds to an elongation of the cells, mimicking an epithelial-mesenchymal transition (EMT). EMT participates in the carcinogenesis process, and is involved in the generation of cancer stem cells (CSCs). However, its involvement in gastric carcinogenesis has yet not been studied. Therefore, the aim of this study was to determine the role of H. pylori in EMT and in the emergence of gastric CSCs. For this purpose, gastric epithelial cells were cocultured with a cagA-positive H. pylori strain or its isogenic-deleted mutants or were transfected with CagA expression vectors. Study of the expression of epithelial and mesenchymal markers showed that H. pylori, via CagA, is responsible for an EMT phenotype associated with an increase in mesenchymal markers as well as CD44 expression, a known gastric CSC marker. Moreover, infection led to an increased ability to migrate, to invade and to form tumorspheres. Cell

sorting experiments showed that only the CD44(high) cells induced by H. pylori infection displayed the mesenchymal phenotype and CSC properties in vitro, and had higher tumorigenic properties than CD44(low) cells in xenografted mice. Immunohistochemistry analyses on human and mouse gastric mucosa tissue samples confirmed a high expression of CD44 and mesenchymal markers in H. pylori-infected cases, and in gastric dysplasia and carcinoma. All of these data suggest that H. pylori, via CagA, unveils CSC-like properties by induction of EMT-like changes in gastric epithelial cells.

Bhuvanalakshmi, G., et al. (2015). "Secreted frizzled-related protein 4 inhibits glioma stem-like cells by reversing epithelial to mesenchymal transition, inducing apoptosis and decreasing cancer stem cell properties." PLoS One **10**(6): e0127517.

The Wnt pathway is integrally involved in regulating self-renewal, proliferation, and maintenance of cancer stem cells (CSCs). We explored the effect of the Wnt antagonist, secreted frizzled-related protein 4 (sFRP4), in modulating epithelial to mesenchymal transition (EMT) in CSCs from human glioblastoma cells lines, U87 and U373. sFRP4 chemo-sensitized CSC-enriched cells to the most commonly used anti-glioblastoma drug, temozolomide (TMZ), by the reversal of EMT. Cell movement, colony formation, and invasion in vitro were suppressed by sFRP4+TMZ treatment, which correlated with the switch of expression of markers from mesenchymal (Twist, Snail, N-cadherin) to epithelial (E-cadherin). sFRP4 treatment elicited activation of the Wnt-Ca2(+) pathway, which antagonizes the Wnt/ss-catenin pathway. Significantly, the chemo-sensitization effect of sFRP4 was correlated with the reduction in the expression of drug resistance markers ABCG2, ABCC2, and ABCC4. The efficacy of sFRP4+TMZ treatment was demonstrated in vivo using nude mice, which showed minimum tumor engraftment using CSCs pretreated with sFRP4+TMZ. These studies indicate that sFRP4 treatment would help to improve response to commonly used chemotherapeutics in gliomas by modulating EMT via the Wnt/ss-catenin pathway. These findings could be exploited for designing better targeted strategies to improve chemo-response and eventually eliminate glioblastoma CSCs.

Bierie, B., et al. (2017). "Integrin-beta4 identifies cancer stem cell-enriched populations of partially mesenchymal carcinoma cells." Proc Natl Acad Sci U S A **114**(12): E2337-E2346.

Neoplastic cells within individual carcinomas often exhibit considerable phenotypic heterogeneity in their epithelial versus mesenchymal-like cell states. Because carcinoma cells with mesenchymal features are often more resistant to therapy and may serve as a source of relapse, we sought to determine whether such cells could be further stratified into functionally distinct subtypes. Indeed, we find that a basal epithelial marker, integrin-beta4 (ITGB4), can be used to enable stratification of mesenchymal-like triple-negative breast cancer (TNBC) cells that differ from one another in their relative tumorigenic abilities. Notably, we demonstrate that ITGB4(+) cancer stem cell (CSC)-enriched mesenchymal cells reside in an intermediate epithelial/mesenchymal phenotypic state. Among patients with TNBC who received chemotherapy, elevated ITGB4 expression was associated with a worse 5-year probability of relapse-free survival. Mechanistically, we find that the ZEB1 (zinc finger E-box binding homeobox 1) transcription factor activity in highly mesenchymal SUM159 TNBC cells can repress expression of the epithelial transcription factor TAp63alpha (tumor protein 63 isoform 1), a protein that promotes ITGB4 expression. In addition, we demonstrate that ZEB1 and ITGB4 are important in modulating the histopathological phenotypes of tumors derived from mesenchymal TNBC cells. Hence, mesenchymal carcinoma cell populations are internally heterogeneous, and ITGB4 is a mechanistically driven prognostic biomarker that can be used to identify the more aggressive subtypes of mesenchymal carcinoma cells in TNBC. The ability to rapidly isolate and mechanistically interrogate the CSC-enriched, partially mesenchymal carcinoma cells should further enable identification of novel therapeutic opportunities to improve the prognosis for high-risk patients with TNBC.

Boesch, M., et al. (2018). "Concise Review: Aggressive Colorectal Cancer: Role of Epithelial Cell Adhesion Molecule in Cancer Stem Cells and Epithelial-to-Mesenchymal Transition." Stem Cells Transl Med **7**(6): 495-501.

Colorectal cancer (CRC) is one of the most common malignancies worldwide. In spite of various attempts to ameliorate outcome by escalating treatment, significant improvement is lacking particularly in the adjuvant setting. It has been proposed that cancer stem cells (CSCs) and the epithelial-to-mesenchymal transition (EMT) are at least partially responsible for therapy resistance in CRC. The epithelial cell adhesion molecule (EpCAM) was one of the first CSC antigens to be described. Furthermore, an EpCAM-specific antibody (edrecolomab) has the merit of having launched the era of monoclonal antibody treatment in oncology in the 1990s. However, despite great initial enthusiasm, monoclonal antibody treatment has not proven successful in the adjuvant treatment of CRC patients. In the meantime, new insights into the function of

EpCAM in CRC have emerged and new drugs targeting various epitopes have been developed. In this review article, we provide an update on the role of EpCAM in CSCs and EMT, and emphasize the potential predictive selection criteria for novel treatment strategies and refined clinical trial design. Stem Cells Translational Medicine 2018;7:495-501.

Borghese, C., et al. (2013). "Gefitinib inhibits the cross-talk between mesenchymal stem cells and prostate cancer cells leading to tumor cell proliferation and inhibition of docetaxel activity." J Cell Biochem **114**(5): 1135-1144.

Increasing evidence suggests that bone marrow derived mesenchymal stem cells (BM-MSCs) are recruited into the stroma of developing tumors where they contribute to progression by enhancing tumor growth and metastasis, or by inducing anticancer-drug resistance. Prostate cancer cells secrete ligands of epidermal growth factor receptor (EGFR) and EGFR signaling could play an important role in the cross-talk between mesenchymal stem cells and prostate cancer cells. In this study, we showed that treatment of human primary MSCs with conditioned medium (CM) derived from the bone metastatic PC3 carcinoma cells (PC3-CM) resulted in: a significant activation of EGFR; increased proliferation; increased osteoblastic but decreased adipocitic differentiation; inhibition of senescence induced by serum starvation; increased CCL5 secretion. These activities were significantly inhibited in the presence of the EGFR tyrosine kinase inhibitor gefitinib. PC3-CM directly inhibited osteoclastogenesis as well as the ability of osteoblasts to induce osteoclast differentiation. The increased MSCs migration by PC3-CM and PC3 cells was partially mediated by CCL5. MSC-CM increased the formation of colonies by PC3 cells and inhibited the anti-proliferative activity of Docetaxel. Activation of EGFR expressed on MSCs by PC3-CM enhanced their capability to increase PC3 cells proliferation and to inhibit Docetaxel activity. These findings, by showing that the tumor-promoting interactions between PC3 cells and MSCs are mediated, at least in part, by EGFR, suggest a novel application of the EGFR-tyrosine kinase inhibitors in the treatment of prostate cancer.

Borst, P. (2012). "Cancer drug pan-resistance: pumps, cancer stem cells, quiescence, epithelial to mesenchymal transition, blocked cell death pathways, persisters or what?" Open Biol **2**(5): 120066.

Although chemotherapy of tumours has scored successes, drug resistance remains the major cause of death of cancer patients. Initial treatment often leaves residual disease, from which the tumour regrows. Eventually, most tumours become resistant to all available chemotherapy. I call this pan-resistance to distinguish it from multi-drug resistance, usually describing resistance caused by upregulation of drug transporters, such as P-glycoprotein. In this review, I discuss mechanisms proposed to explain both residual disease and pan-resistance. Although plausible explanations are at hand for residual disease, pan-resistance is still a mystery. My conclusion is that it is time for a major effort to solve this mystery using the new genetically modified mouse tumour models that produce real tumours resembling cancer in human patients.

Bose, B., et al. (2018). "Breast Cancer Stem Cell Therapeutics, Multiple Strategies Versus Using Engineered Mesenchymal Stem Cells With Notch Inhibitory Properties: Possibilities and Perspectives." J Cell Biochem **119**(1): 141-149.

Relapse cases of cancers are more vigorous and difficult to control due to the preponderance of cancer stem cells (CSCs). Such CSCs that had been otherwise dormant during the first incidence of cancer gradually appear as radiochemoresistant cancer cells. Hence, cancer therapeutics aimed at CSCs would be an effective strategy for mitigating the cancers during relapse. Alternatively, CSC therapy can also be proposed as an adjuvant therapy, along-with the conventional therapies. As regenerative stem cells (RSCs) are known for their trophic effects, anti-tumorogenicity, and better migration toward an injury site, this review aims to address the use of adult stem cells such as dental pulp derived; cord blood derived pure populations of regenerative stem cells for targeting CSCs. Indeed, pro-tumorogenicity of RSCs is of concern and hence has also been dealt with in relation to breast CSC therapeutics. Furthermore, as notch signaling pathways are upregulated in breast cancers, and anti-notch antibody based and sh-RNA based therapies are already in the market, this review focuses the possibilities of engineering RSCs to express notch inhibitory proteins for breast CSC therapeutics. Also, we have drawn a comparison among various possibilities of breast CSC therapeutics, about, notch1 inhibition. J. Cell. Biochem. 119: 141-149, 2018. (c) 2017 Wiley Periodicals, Inc.

Cronwright, G., et al. (2005). "Cancer/testis antigen expression in human mesenchymal stem cells: down-regulation of SSX impairs cell migration and matrix metalloproteinase 2 expression." Cancer Res **65**(6): 2207-2215.

Several families of genes by and large located on the X chromosome encode proteins of unspecified function. Commonly known as cancer/testis (CT) antigens, they are considered, under normal conditions, only to be expressed in cells of the germ line and

placenta. CT genes are also often expressed in cancer cells, hence their classification. Here we report that their expression in normal cells is wider spread and can be observed in cells with the potential for self-renewal and pleuripotency, namely, stem cells. Several CT genes and their products, CT antigens, including SSX, NY-ESO-1, and N-RAGE, were expressed in undifferentiated mesenchymal stem cells (MSCs) and down-regulated after osteocyte and adipocyte differentiation. To elucidate the possible overlapping function played by these genes in cancer and stem cells, a comparative analysis of the localization of their proteins was made. In addition, localization relative to other MSC markers was examined. This revealed that SSX localizes in the cytoplasm and overlap occurs in regions where matrix metalloproteinase 2 (MMP2) and vimentin accumulate. Nevertheless, it was found that no protein interactions between these molecules occur. Further investigation revealed that the migration of a melanoma cell line (DFW), which expresses SSX, MMP2, and vimentin, decreases when SSX is down-regulated. This decrease in cell migration was paralleled by a reduction in MMP2 levels. Analogous to this, SSX expression is down-regulated in MSCs after differentiation; concomitantly a reduction in MMP2 levels occurs. In addition, E-cadherin expression increases, mimicking a mesenchymal epithelial transition. These results afford SSX a functional role in normal stem cell migration and suggest a potentially similar function in cancer cell metastases.

de Andrade, N. P., et al. (2017). "Cancer stem cell, cytokeratins and epithelial to mesenchymal transition markers expression in oral squamous cell carcinoma derived from ortothopic xenoimplantation of CD44(high) cells." Pathol Res Pract **213**(3): 235-244.

Oral squamous cell carcinoma (OSCC) is the most prevalent neoplasia of oral cavity worldwide and prognosis remains unchanged in decades. Recently, different authors reported that head and neck squamous cell carcinomas have a subpopulation of tumor initiating cells that apparently correspond to cancer stem cells (CSC) and are also responsible for tumor growth and metastasis. The purpose of the present study was to investigate the microscopic and phenotypic characteristics of OSCC tumors induced after orthotopic xenoimplantation of SCC9(WT) cell line and CSC-enriched subpopulation isolated from SCC9 cell line based on high expression of the putative CSC marker CD44. Different numbers of FACS-sorted SCC9 CD44(high) and CD44(low) cells as well as SCC9(WT) (wild type) were transplanted into the tongue of BALB/C nude (NOD/SCID) mice to evaluate their tumorigenic potential. Sixty days post-induction, tumors were morphologically characterized and immunostained for CSC markers (CD44, Nanog and Bmi-1), epithelial-mesenchymal transition (Snail, Slug) and epithelial differentiating cell markers (cytokeratins 4, 13, 15, 17 and 19), as well as E-cadherin and beta-catenin. The data presented here shows that SCC9 CD44(high) cells have higher ability to form tumors than SCC9 CD44(low) cells, even when significantly lower numbers of SCC9 CD44(high) cells were transplanted. Immunoassessment of tumors derived from SCC9 CD44(high) cells revealed high expression of cytokeratin CK19, beta-catenin, E-cadherin and CD44, and negative or low expression of CK17, CK4, CK15, CK13, Nanog, Bmi-1, Snail and Slug. While tumors derived from SCC9(WT) showed high expression of CK17, CK19, CD44, Nanog, Bmi-1, Snail and Slug, and negative or low expression of CK4, CK15, CK13, beta-catenin and E-cadherin. Thus, SCC9 CD44(high) cells were highly tumorigenic, capable of originating heterogeneous tumors and these tumors have a immunohistochemical profile different from those formed by the wild type cell line.

Ding, D. C., et al. (2016). "Interleukin-6 from Ovarian Mesenchymal Stem Cells Promotes Proliferation, Sphere and Colony Formation and Tumorigenesis of an Ovarian Cancer Cell Line SKOV3." J Cancer **7**(13): 1815-1823.

The origin of the majority of epithelial ovarian cancers (EOC) is regarded as extraovarian, with the ovary being the secondary site. The aim of this study was to explore the possible role of ovarian mesenchymal stem cells (OvMSCs) and secreted IL-6 in the development of EOC. OvMSCs were derived from normal ovarian stroma. Cell surface markers and differentiation capability were determined. The effects of IL-6 and conditioned medium of OvMSCs on the malignant phenotype of SKOV3 ovarian cancer cells were tested, and the status of STAT3 and ERK phosphorylation was investigated. OvMSCs had similar surface marker profiles as bone marrow mesenchymal stem cells, i.e., CD44 (+), CD90 (+) and CD45 (-), and was readily inducible to osteogenic, adipogenic and chondrogenic differentiation. OvMSCs secreted an extremely high level (>2500 pg/ml) of IL-6. Treatment of SKOV3 cells with conditioned media from OvMSCs increased cell proliferation, tumor sphere formation and anchorage independent growth, and resulted in activation of STAT3 but not ERK. Coinjection of OvMSCs with SKOV3 cell enhanced tumorigenesis in NOD-SCID mice. All of these behaviors were blocked by IL-6 receptor blocking antibody administered in vitro or in vivo. The OvMSCs alone injected into mice had no tumor growth after 3 months. By secreting high levels of IL-

6, OvMSCs enhance the proliferation, sphere and colony formation and tumorigenesis of SKOV3 cells.

Du, T., et al. (2014). "Microvesicles derived from human Wharton's jelly mesenchymal stem cells promote human renal cancer cell growth and aggressiveness through induction of hepatocyte growth factor." PLoS One **9**(5): e96836.

In our previous study, microvesicles (MVs) released from human Wharton's jelly mesenchymal stem cells (hWJ-MSCs) retard the growth of bladder cancer cells. We would like to know if MVs have a similar effect on human renal cell carcinoma (RCC). By use of cell culture and the BALB/c nu/nu mice xeno-graft model, the influence of MVs upon the growth and aggressiveness of RCC (786-0) was assessed. Cell counting kit-8 (CCK-8) assay, incidence of tumor, tumor size, Ki-67 or TUNEL staining was used to evaluate tumor cell growth in vitro or in vivo. Flow cytometry assay (in vitro) or examination of cyclin D1 expression (in vivo) was carried out to determine the alteration of cell cycle. The aggressiveness was analyzed by Wound Healing Assay (in vitro) or MMP-2 and MMP-9 expression (in vivo). AKT/p-AKT, ERK1/2/p-ERK1/2 or HGF/c-MET expression was detected by real-time PCR or western blot. Our data demonstrated that MVs promote the growth and aggressiveness of RCC both in vitro and in vivo. In addition, MVs facilitated the progression of cell cycle from G0/1 to S. HGF expression in RCC was greatly induced by MVs, associated with activation of AKT and ERK1/2 signaling pathways. RNase pre-treatment abrogated all effects of MVs. In summary, induction of HGF synthesis via RNA transferred by MVs activating AKT and ERK1/2 signaling is one of crucial contributors to the pro-tumor effect.

El-Badawy, A., et al. (2017). "Cancer cell-soluble factors reprogram mesenchymal stromal cells to slow cycling, chemoresistant cells with a more stem-like state." Stem Cell Res Ther **8**(1): 254.

BACKGROUND: Mesenchymal stem cells (MSCs) play different roles in modulating tumor progression, growth, and metastasis. MSCs are recruited to the tumor site in large numbers and subsequently have an important microenvironmental role in modulating tumor progression and drug sensitivity. However, the effect of the tumor microenvironment on MSC plasticity remains poorly understood. Herein, we report a paracrine effect of cancer cells, in which they secrete soluble factors that promote a more stem-like state in bone marrow mesenchymal stem cells (BM-MSCs). METHODS: The effect of soluble factors secreted from MCF7, Hela, and HepG2 cancer cell lines on BM-MSCs was assessed using a Transwell indirect coculture system. After 5 days of coculture, BM-MSCs were characterized by flow cytometry for surface marker expression, by qPCR for gene expression profile, and by confocal immunofluorescence for marker expression. We then measured the sensitivity of cocultured BM-MSCs to chemotherapeutic agents, their cell cycle profile, and their response to DNA damage. The sphere formation, invasive properties, and in-vivo performance of BM-MSCs after coculture with cancer cells were also measured. RESULTS: Indirect coculture of cancer cells and BM-MSCs, without direct cell contact, generated slow cycling, chemoresistant spheroid stem cells that highly expressed markers of pluripotency, cancer cells, and cancer stem cells (CSCs). They also displayed properties of a side population and enhanced sphere formation in culture. Accordingly, these cells were termed cancer-induced stem cells (CiSCs). CiSCs showed a more mesenchymal phenotype that was further augmented upon TGF-beta stimulation and demonstrated a high expression of the beta-catenin pathway and ALDH1A1. CONCLUSIONS: These findings demonstrate that MSCs, recruited to the tumor microenvironment in large numbers, may display cellular plasticity, acquire a more stem-like state, and acquire some properties of CSCs upon exposure to cancer cell-secreted factors. These acquired characteristics may contribute to tumor progression, survival, and metastasis. Our findings provide new insights into the interactions between MSCs and cancer cells, with the potential to identify novel molecular targets for cancer therapy.

Eltoukhy, H. S., et al. (2018). "Immune modulation by a cellular network of mesenchymal stem cells and breast cancer cell subsets: Implication for cancer therapy." Cell Immunol **326**: 33-41.

The immune modulatory properties of mesenchymal stem cells (MSCs) are mostly controlled by the particular microenvironment. Cancer stem cells (CSCs), which can initiate a clinical tumor, have been the subject of intense research. This review article discusses investigative studies of the roles of MSCs on cancer biology including on CSCs, and the potential as drug delivery to tumors. An understanding of how MSCs behave in the tumor microenvironment to facilitate the survival of tumor cells would be crucial to identify drug targets. More importantly, since CSCs survive for decades in dormancy for later resurgence, studies are presented to show how MSCs could be involved in maintaining dormancy. Although the mechanism by which CSCs survive is complex, this article focus on the cellular involvement of MSCs with regard to immune responses. We discuss the immunomodulatory mechanisms of MSC-CSC

interaction in the context of therapeutic outcomes in oncology. We also discuss immunotherapy as a potential to circumventing this immune modulation.

Fan, F., et al. (2012). "Overexpression of snail induces epithelial-mesenchymal transition and a cancer stem cell-like phenotype in human colorectal cancer cells." Cancer Med **1**(1): 5-16.

Epithelial-mesenchymal transition (EMT) is a critical process providing tumor cells with the ability to migrate and escape from the primary tumor and metastasize to distant sites. Recently, EMT was shown to be associated with the cancer stem cell (CSC) phenotype in breast cancer. Snail is a transcription factor that mediates EMT in a number of tumor types, including colorectal cancer (CRC). Our study was done to determine the role of Snail in mediating EMT and CSC function in CRC. Human CRC specimens were stained for Snail expression, and human CRC cell lines were transduced with a retroviral Snail construct or vector control. Cell proliferation and chemosensitivity to oxaliplatin of the infected cells were determined by the MTT (colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Migration and invasion were determined in vitro using modified Boyden chamber assays. EMT and putative CSC markers were analyzed using Western blotting. Intravenous injection of tumor cells was done to evaluate their metastatic potential in mice. Snail was overexpressed in human CRC surgical specimens. This overexpression induced EMT and a CSC-like phenotype in human CRC cells and enhanced cell migration and invasion ($P < 0.002$ vs. control). Snail overexpression also led to an increase in metastasis formation in vivo ($P < 0.002$ vs. control). Furthermore, the Snail-overexpressing CRC cells were more chemoresistant to oxaliplatin than control cells. Increased Snail expression induces EMT and the CSC-like phenotype in CRC cells, which enhance cancer cell invasion and chemoresistance. Thus, Snail is a potential therapeutic target in metastatic CRC.

Faraco, C. C. F., et al. (2018). "Translocation of Epidermal Growth Factor (EGF) to the nucleus has distinct kinetics between adipose tissue-derived mesenchymal stem cells and a mesenchymal cancer cell lineage." J Struct Biol **202**(1): 61-69.

Nuclear Epidermal Growth Factor Receptor (EGFR) has been associated with worse prognosis and treatment resistance for several cancer types. After Epidermal Growth Factor (EGF) binding, the ligand-receptor complex can translocate to the nucleus where it functions in oncological processes. By three-dimensional quantification analysis of super-resolution microscopy images, we verified the translocation kinetics of fluorescent conjugated EGF to the nucleus in two mesenchymal cell types: human adipose tissue-derived stem cells (hASC) and SK-HEP-1 tumor cells. The number of EGF clusters in the nucleus does not change after 10min of stimulation with EGF in both cells. The total volume occupied by EGF clusters in the nucleus of hASC also does not change after 10min of stimulation with EGF. However, the total volume of EGF clusters increases only after 20min in SK-HEP-1 cells nuclei. In these cells the nuclear volume occupied by EGF is 3.2 times higher than in hASC after 20min of stimulation, indicating that translocation kinetics of EGF differs between these two cell types. After stimulation, EGF clusters assemble in larger clusters in the cell nucleus in both cell types, which suggests specific sub-nuclear localizations of the receptor. Super-resolution microscopy images show that EGF clusters are widespread in the nucleoplasm, and can be localized in nuclear envelope invaginations, and in the nucleoli. The quantitative study of EGF-EGFR complex translocation to the nucleus may help to unravel its roles in health and pathological conditions, such as cancer.

Foglietta, F., et al. (2017). "Selective sensitiveness of mesenchymal stem cells to shock waves leads to anticancer effect in human cancer cell co-cultures." Life Sci **173**: 28-35.

AIM: Mesenchymal stem cells (MSC) possess the distinctive feature of homing in on and engrafting into the tumor stroma making their therapeutic applications in cancer treatment very promising. Research into new effectors and external stimuli, which can selectively trigger the release of cytotoxic species from MSC toward the cancer cells, significantly raises their potential. MAIN METHODS: Shock waves (SW) have recently gained recognition for their ability to induce specific biological effects, such as the local generation of cytotoxic reactive oxygen species (ROS) in a non-invasive and tunable manner. We thus investigate whether MSC are able to generate ROS and, in turn, affect cancer cell growth when in co-culture with human glioblastoma (U87) or osteosarcoma (U2OS) cells and exposed to SW. KEY FINDINGS: MSC were found to be the cell line that was most sensitive to SW treatment as shown by SW-induced ROS production and cytotoxicity. Notably, U87 and U2OS cancer cell growth was unaffected by SW exposure. However, significant decreases in cancer cell growth, 1.8 fold for U87 and 2.3 fold for U2OS, were observed 24h after the SW treatment of MSC co-cultures with cancer cells. The ROS production induced in MSC by SW exposure was then responsible for lipid peroxidation and cell death in U87 and U2OS cells co-cultured with MSC. SIGNIFICANCE: This experiment highlights the unique ability of MSC to generate ROS upon SW

treatment and induce the cell death of co-cultured cancer cells. SW might therefore be proposed as an innovative tool for MSC-mediated cancer treatment.

Fomeshi, M. R., et al. (2015). "Evaluation of the expressions pattern of miR-10b, 21, 200c, 373 and 520c to find the correlation between epithelial-to-mesenchymal transition and melanoma stem cell potential in isolated cancer stem cells." Cell Mol Biol Lett **20**(3): 448-465.

Small non-coding RNAs named microRNAs (miRNAs) modulate some functions and signaling pathways in skin epithelial cells and melanocytes. They also function as oncogenes or tumor suppressors in malignancies and tumor metastasis. We investigated the expression patterns of miRNAs, including miR-10b, 21, 200c, 373 and 520c, which regulate epithelial-to-mesenchymal transition (EMT) and metastasis in isolated cancer stem cells (CSCs) and non- CSCs. Six melanoma cell lines were tested for the expressions of stem cell markers. Melanoma stem cells were enriched via fluorescence-activated cell sorting (FACS) using the CD133 cell surface marker or spheroid culture. They were then characterized based on colony and sphere formation, and the expressions of stemness and EMT regulator genes and their invasion potential were assessed using real-time qRT-PCR and invasion assay. Our results indicate that cells enriched via sphere formation expressed all the stemness-related genes and had an enhanced number of colonies, spheres and invaded cells compared to cells enriched using the CD133 cell surface marker. Moreover, miRNAs controlling metastasis increased in the melanospheres. This may be related to the involvement of CSCs in the metastatic process. However, this must be further confirmed through the application of knockdown experiments. The results show that sphere formation is a useful method for enriching melanoma stem cells. Melanospheres were found to upregulate miR-10b, 21, 200c, 373 and 520c, so we suggest that they may control both metastasis and stemness potential.

Ghafarzadeh, M., et al. (2016). "Human amniotic fluid derived mesenchymal stem cells cause an anti-cancer effect on breast cancer cell line in vitro." Cell Mol Biol (Noisy-le-grand) **62**(6): 102-106.

Human amniotic fluid stem cells (hAFSCs) have the ability to self-renew, and multipotent differentiation into three germ layer cells. We obtained 5 ml amniotic fluid from ten 16-20 week pregnant women undergoing amniocentesis. hAFSCs were isolated from all samples, co-cultured with T47D breast cancer cell line and characterized using flow cytometry and RT-PCR. After 3, 4 and 5 days, T47D and HSFCs viability were evaluated with MTT assay. After 5 days of co-culture T47D cells viability were decreased. Our findings showed that hAFSCs can release soluble factors in cell culture, causing an efficient anticancer effect.

Gomez-Casal, R., et al. (2013). "Non-small cell lung cancer cells survived ionizing radiation treatment display cancer stem cell and epithelial-mesenchymal transition phenotypes." Mol Cancer **12**(1): 94.

Ionizing radiation (IR) is used for patients diagnosed with unresectable non small cell lung cancer (NSCLC), however radiotherapy remains largely palliative due to radioresistance. Cancer stem cells (CSCs), as well as epithelial-mesenchymal transition (EMT), may contribute to drug and radiation resistance mechanisms in solid tumors. Here we investigated the molecular phenotype of A549 and H460 NSCLC cells that survived treatment with IR (5Gy) and are growing as floating tumor spheres and cells that are maintained in a monolayer after irradiation.Non-irradiated and irradiated cells were collected after one week, seeded onto ultra low attachment plates and propagated as tumor spheres. Bulk NSCLC cells which survived radiation and grew in spheres express cancer stem cell surface and embryonic stem cell markers and are able to self-renew, and generate differentiated progeny. These cells also have a mesenchymal phenotype. Particularly, the radiation survived sphere cells express significantly higher levels of CSC markers (CD24 and CD44), nuclear beta-catenin and EMT markers (Snail1, Vimentin, and N-cadherin) than non-irradiated lung tumor sphere cells. Upregulated levels of Oct-4, Sox2 and beta-catenin were detected in H460 cells maintained in a monolayer after irradiation, but not in radiation survived adherent A459 cells.PDGFR-beta was upregulated in radiation survived sphere cells and in radiation survived adherent cells in both A549 and H460 cell lines. Combining IR treatment with axitinib or dasatinib, inhibitors with anti-PDFGR activity, potentiates the efficacy of NSCLC radiotherapy in vitro.Our findings suggest that radiation survived cells have a complex phenotype combining the properties of CSCs and EMT. CD44, SNAIL and PDGFR-beta are dramatically upregulated in radiation survived cells and might be considered as markers of radiotherapy response in NSCLC.

Goncalves Ndo, N., et al. (2016). "Effect of Melatonin in Epithelial Mesenchymal Transition Markers and Invasive Properties of Breast Cancer Stem Cells of Canine and Human Cell Lines." PLoS One **11**(3): e0150407.

Cancer stem cells (CSCs) have been associated with metastasis and therapeutic resistance and can be generated via epithelial mesenchymal transition (EMT). Some studies suggest that the hormone

melatonin acts in CSCs and may participate in the inhibition of the EMT. The objectives of this study were to evaluate the formation of mammospheres from the canine and human breast cancer cell lines, CMT-U229 and MCF-7, and the effects of melatonin treatment on the modulation of stem cell and EMT molecular markers: OCT4, E-cadherin, N-cadherin and vimentin, as well as on cell viability and invasiveness of the cells from mammospheres. The CMT-U229 and MCF-7 cell lines were subjected to three-dimensional culture in special medium for stem cells. The phenotype of mammospheres was first evaluated by flow cytometry (CD44(+)/CD24(low/-) marking). Cell viability was measured by MTT colorimetric assay and the expression of the proteins OCT4, E-cadherin, N-cadherin and vimentin was evaluated by immunofluorescence and quantified by optical densitometry. The analysis of cell migration and invasion was performed in Boyden Chamber. Flow cytometry proved the stem cell phenotype with CD44(+)/CD24(low/-) positive marking for both cell lines. Cell viability of CMT-U229 and MCF-7 cells was reduced after treatment with 1mM melatonin for 24 h ($P<0.05$). Immunofluorescence staining showed increased E-cadherin expression ($P<0.05$) and decreased expression of OCT4, N-cadherin and vimentin ($P<0.05$) in both cell lines after treatment with 1 mM melatonin for 24 hours. Moreover, treatment with melatonin was able to reduce cell migration and invasion in both cell lines when compared to control group ($P<0.05$). Our results demonstrate that melatonin shows an inhibitory role in the viability and invasiveness of breast cancer mammospheres as well as in modulating the expression of proteins related to EMT in breast CSCs, suggesting its potential anti-metastatic role in canine and human breast cancer cell lines.

Gottschling, S., et al. (2013). "Mesenchymal stem cells in non-small cell lung cancer--different from others? Insights from comparative molecular and functional analyses." Lung Cancer **80**(1): 19-29.

BACKGROUND: Cancer-associated fibroblasts (CAF) play a vital role in lung cancer initiation and progression. Although mesenchymal stem cells (MSC) are considered progenitor cells of fibroblasts and show cancer modulating abilities themselves, analyses on their presence and properties in lung cancer are lacking so far. METHODS: We performed a comparative molecular and functional analysis of MSC derived from non-small cell lung cancer (NSCLC) and corresponding normal lung tissue (NLT) of a total of 15 patients. MSC were identified and selected according to their mesenchymal multilineage differentiation capability and surface marker profile. RESULTS: Compared to NLT-MSC, NSCLC-MSC showed accelerated growth kinetics and reduced sensitivity to cisplatin. Karyotyping, comparative genomic hybridization and multiplex fluorescence in situ hybridization revealed no chromosomal aberrations. However, gene expression profiling of NSCLC- and NLT-MSC indicated variable expression of 62 genes involved in proliferation, DNA repair, apoptosis, extracellular matrix synthesis, tissue remodeling and angiogenesis. Differential expression of the selected candidate genes butyrylcholinesterase, clusterin and quiescin Q6 sulfhydryl oxidase 1 was validated by quantitative real-time PCR and, on protein level, by immunohistochemical analyses of original tumor tissue. Upon exposure to tumor cell-conditioned medium or transforming growth factor-beta, both, NSCLC-MSC and NLT-MSC acquired expression of alpha-smooth muscle actin (alpha-SMA), a major characteristics of CAF. CONCLUSIONS: This study indicates that NSCLC tissue contains MSC with specific molecular and functional properties. These cells might represent a progenitor reservoir for CAF and thus crucially contribute to lung cancer progression.

Gu, H., et al. (2016). "Exosomes derived from human mesenchymal stem cells promote gastric cancer cell growth and migration via the activation of the Akt pathway." Mol Med Rep **14**(4): 3452-3458.

Mesenchymal stem cells (MSCs) are a component of the tumor microenvironment and can promote the development of gastric cancer through paracrine mechanism. However, the effects of MSCexosomes (MSCex) on gastric cancer are less clear. The present study reported that MSCex promoted the proliferative and metastatic potential of gastric cancer cells ex vivo. It was found that MSCex enhanced the migration and invasion of HGC27 cells via the induction of the epithelialmesenchymal transition. MSCex increased the expression of mesenchymal markers and reduced the expression of epithelial markers in gastric cancer cells. MSCex also enhanced the tumorigenicity of gastric cancer cells ex vivo. MSCex induced the stemness of gastric cancer cells. The expression of octamerbinding transcription factor 4, ex determining region Ybox 2 and Lin28B significantly increased in gastric cancer cells treated with MSCex. The present study further demonstrated that MSCex elicited these biological effects predominantly via the activation of the protein kinase B signaling pathway. Taken together, the present findings provided novel evidence for the role of MSCex in gastric cancer and a new opportunity for improving the efficiency of gastric cancer treatment by targeting MSCex.

Guan, B., et al. (2018). "MicroRNA-218 inhibits the migration, epithelial-mesenchymal transition and cancer stem cell properties of prostate cancer cells." Oncol Lett **16**(2): 1821-1826.

MicroRNA (miRNA) is a class of non-coding single-stranded RNA, able to regulate tumor-associated genes via binding the 3'-UTR of the target gene mRNA. Previous publications have demonstrated that miRNA-218 (miR-218) acts as a tumor-suppressive miRNA in various types of human cancer, including prostate cancer (PCa). However, the role of miR-218 in regulating PCa cell stemness and epithelial-mesenchymal transition remains unknown and requires further research. In the present study, it is demonstrated that miR-218 was downregulated in 2 PCa cell lines and could suppress cell migration, EMT and the exhibition of cancer stem cell-like properties. The expression of GLI family zinc finger 1 (Gli1) was inhibited by miR-218 overexpression, suggesting miR-218-suppression of Gli1 as a potential mechanism for the tumor-suppressive effect of miR-218. Overall, the results indicate that miR-218 served a critical role in the inhibition of PCa development. This may provide new insight for elucidating the mechanisms of PCa oncogenesis and suggests that miR-218 may be a novel therapeutic target for PCa.

Guo, D., et al. (2012). "Cancer stem-like side population cells in the human nasopharyngeal carcinoma cell line cne-2 possess epithelial mesenchymal transition properties in association with metastasis." Oncol Rep **28**(1): 241-247.

It has been recently reported that side population (SP) cells in nasopharyngeal carcinoma (NPC) cell lines display characteristics of cancer stem-like cells. However, the biological behavior and the significance of these cells for NPC progression remain unclear. In this study, we isolated SP cells from the NPC cell line CNE-2 by flow cytometry and investigated their biological characteristics. We discovered that SP cells had stronger colony forming abilities compared to the non-side population (NSP) cells, and observed that some SP cells looked more like the shape of mesenchymal cells when cultured in the common polyHEMA-coated flask. When checked by quantitative real-time PCR, the SP cells expressed higher levels of stemness-related genes Oct4, Sox2 and Nanog, and mesenchymal cell-related genes N-cadherin, vimentin and Snail, while they expressed lower levels of the epithelial cell-related gene, E-cadherin. Western blot and immunofluorescence staining methods further verified that SP cells expressed higher vimentin and expressed lower E-cadherin levels. Finally, Transwell invasion assay results indicated that the SP cells had higher invasive potential compared to NSP cells. Collectively, our data reveal that SP cells in the CNE-2 cell line not only possess the properties of cancer stem cells, but also have more mesenchymal cell characteristics which are associated with epithelial mesenchymal transition (EMT) and cancer cell invasion and metastasis. These findings are helpful for developing novel targets for effective clinical treatment of NPC.

Guo, F., et al. (2015). "EML4-ALK induces epithelial-mesenchymal transition consistent with cancer stem cell properties in H1299 non-small cell lung cancer cells." Biochem Biophys Res Commun **459**(3): 398-404.

The echinoderm microtubule-associated protein-like 4(EML4)--anaplastic lymphoma kinase (ALK) fusion gene has been identified as a driver mutation in non-small-cell lung cancer (NSCLC). However, the role of EML4-ALK in malignant transformation is not entirely clear. Here, for the first time, we showed that H1299 NSCLC cells stably expressing EML4-ALK acquire EMT phenotype, associated with enhanced invasive migration and increased expression of EMT-inducing transcription factors. H1299-EML4-ALK cells also displayed cancer stem cell-like properties with a concomitant up-regulation of CD133 and enhanced ability of mammospheres formation. Moreover, we found that inhibition of ERK1/2 reversed EMT induced by EML4-ALK in H1299 cells. Taken together, these results suggested that EML4-ALK induced ERK activation is mechanistically associated with EMT phenotype. Thus, inhibition of ERK signaling pathway could be a potential strategy in treatment of NSCLC patients with EML4-ALK translocation.

Hall, B., et al. (2007). "Mesenchymal stem cells in cancer: tumor-associated fibroblasts and cell-based delivery vehicles." Int J Hematol **86**(1): 8-16.

Recent evidence suggests that mesenchymal stem cells (MSC) selectively home to tumors, where they contribute to the formation of tumor-associated stroma. This effect can be opposed by genetically modifying MSC to produce high levels of anti-cancer agents that blunt tumor growth kinetics and inhibit the growth of tumors in situ. In this review article, we describe the biological properties of MSC within the tumor microenvironment and discuss the potential use of MSC and other bone marrow-derived cell populations as delivery vehicles for antitumor proteins.

Halpern, J. L., et al. (2011). "Mesenchymal stem cells promote mammary cancer cell migration in vitro via the CXCR2 receptor." Cancer Lett **308**(1): 91-99.

Bone metastasis is a common event during breast cancer progression. Recently, mesenchymal stem cells (MSCs) have been implicated in the metastasis of

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

Han, M., et al. (2012). "Re-expression of miR-21 contributes to migration and invasion by inducing epithelial-mesenchymal transition consistent with cancer stem cell characteristics in MCF-7 cells." Mol Cell Biochem **363**(1-2): 427-436.

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

Han, M., et al. (2012). "MiR-21 regulates epithelial-mesenchymal transition phenotype and hypoxia-inducible factor-1alpha expression in third-sphere forming breast cancer stem cell-like cells." Cancer Sci **103**(6): 1058-1064.

Cancer stem cells (CSCs) are predicted to be critical drivers of tumor progression due to their "stemness", but the molecular mechanism of CSCs in regulating metastasis remains to be elucidated. Epithelial-mesenchymal transition (EMT), hypoxia-inducible factor (HIF)-1alpha, and miR-21, all of which contribute to cell migration for metastasis, are interrelated with CSCs. In the present study, third-sphere forming (3-S) CSC-like cells, which showed elevated CSC surface markers (ALDH1(+) and CD44(+)/CD24(-/low)) and sphereforming capacity as well as migration and invasion capacities, were cultured and isolated from breast cancer MCF-7 parental cells, to evaluate the role of miR-21 in regulating the CSC-like cell biological features, especially EMT. EMT, which was assessed by overexpression of mesenchymal cell markers (N-cadherin, Vimentin, alpha-smooth muscle actin [alpha-SMA]) and suppression of epithelial cell marker (E-cadherin), was induced in 3-S CSC-like cells. Moreover, both of HIF-1alpha and miR-21 were upregulated in the CSC-like cells. Interestingly, antagonism of miR-21 by antagomir led to reversal of EMT, downexpression of HIF-1alpha, as well as suppression of invasion and migration, which indicates a key role of miR-21 involved in regulate CSC-associated features. In conclusion, we demonstrated that the formation of CSC-like cells undergoing process of EMT-like associated with overexpression of HIF-1alpha, both of which are regulated by miR-21.

He, M., et al. (2014). "Casticin inhibits epithelial-mesenchymal transition of liver cancer stem cells of the SMMC-7721 cell line through downregulating Twist." Oncol Lett **7**(5): 1625-1631.

The existence of cancer stem cells (CSCs) is central to the pathogenesis and therapeutic target of human hepatocellular carcinoma. The aim of this study was to investigate the effects of casticin on epithelial-mesenchymal transition (EMT) of liver cancer stem cells (LCSCs) derived from the SMMC-7721 cell line. Our results demonstrated that CD133(+) sphere-forming cells (SFCs) sorted from the SMMC-7721 cell line not only possessed a higher capacity to form tumor spheroids in vitro, but also had a greater potential to form tumors when implanted in Balb/c-nu

mice, indicating that CD133(+) SFCs possessed similar traits to LCSCs. Casticin increased the expression levels of E-cadherin and decreased those of N-cadherin in LCSCs. Treatment of LCSCs with casticin for 48 h also decreased the levels of the EMT-associated transcription factor, Twist. Overexpression of Twist attenuated the casticin-induced regulation of E-cadherin and N-cadherin protein expression, as well as the EMT capacity of LCSCs. In conclusion, CD133(+) SFCs of the SMMC-7721 cell line may represent a subpopulation of LCSCs with the characteristics of EMT. Furthermore, casticin targeted LCSCs through the inhibition of EMT by downregulating Twist.

Hendijani, F., et al. (2015). "Effect of human Wharton's jelly mesenchymal stem cell secretome on proliferation, apoptosis and drug resistance of lung cancer cells." Res Pharm Sci **10**(2): 134-142.

Multipotent mesenchymal stem cells (MSCs) are recently found to alter the tumor condition. However their exact role in tumor development is not yet fully unraveled. MSCs were established to perform many of their actions through paracrine effect. Thus investigation of MSC secretome interaction with tumor cells may provide important information for scientists who are attempting to apply stem cells in the treatment of the disease. In this study we investigated the effect of human Wharton's jelly derived MSC (WJ-MSCs) secretome on proliferation, apoptotic potential of A549 lung cancer cells, and their response to the chemotherapeutic agent doxorubicin. WJ-MSCs were isolated from human umbilical cord and then characterized according to the International Society for Cellular Therapy criteria and WJ-MSC secretome was collected. BrdU cell proliferation assay and Annexin V-PI staining were used for the evaluation of cytotoxic and proapoptotic effects of WJ-MSC secretome on A549 cells. WJ-MSC secretome neither induced proliferation of lung cancer cells nor affected the apoptotic potential of the tumor cells. We also studied the combinatorial effect of WJ-MSC secretome and the anticancer drug doxorubicinwhich showed no induction of drug resistance when A549 cells was treated with combination of WJ-MSC secretome and doxorubicin. Although MSCs did not show antitumor properties, our in vitro results showed that MSC secretome was not tumorigenic and also did not make lung cancer cells resistant to doxorubicin. Thus MSC secretome could be considered safe for other medical purposes such as cardiovascular, neurodegenerative, and autoimmune diseases which may exist or occur in cancer patients.

Hu, W., et al. (2011). "Human umbilical blood mononuclear cell-derived mesenchymal stem cells serve as interleukin-21 gene delivery vehicles for epithelial ovarian cancer therapy in nude mice." Biotechnol Appl Biochem **58**(6): 397-404.

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

Huang, B., et al. (2018). "Suppressed epithelial-mesenchymal transition and cancer stem cell properties mediate the anti-cancer effects of ethyl pyruvate via regulation of the AKT/nuclear factor-kappaB pathway in prostate cancer cells." Oncol Lett **16**(2): 2271-2278.

Castration-resistant prostate cancer (CRPC) is a leading cause of mortality among cases of prostate cancer (PCa). Current treatment options for CRPC are limited. Ethyl pyruvate (EP), a lipophilic derivative of pyruvic acid, has been reported to have antitumor activities. In the present study, the efficacy of EP against PCa was investigated using two human PCa cell lines and a mouse xenograft tumor model. PC3 and CWR22RV1 cells were treated with EP, and cytotoxicity was evaluated via Cell Counting Kit-8 and colony formation assays, while cell cycle distribution was assessed by flow cytometry. Changes in cell migration and invasion caused by EP treatment were

also evaluated with Transwell and wound healing assays, and changes in the expression of intracellular signaling pathway components were detected by western blotting. EP treatment reduced cell viability, induced G1 arrest, and activated the intrinsic apoptosis pathway. Additionally, the in vivo experiments revealed that EP administration markedly inhibited tumor growth. EP also reversed epithelial-mesenchymal transition and suppressed cancer stem cell properties in part through negative regulation of AKT/nuclear factor-kappaB signaling. These results indicate that EP has anticancer activity in vitro and in vivo, and is therefore a promising therapeutic agent for the treatment of PCa.

Izumiya, M., et al. (2012). "Chemoresistance is associated with cancer stem cell-like properties and epithelial-to-mesenchymal transition in pancreatic cancer cells." Anticancer Res **32**(9): 3847-3853.

BACKGROUND: The aim of this study was to evaluate whether apoptosis-resistant cancer cells have cancer stem cell (CSC)-like properties. MATERIALS AND METHODS: Panc-1 pancreatic cancer cells were incubated in the presence of 5-fluorouracil (5-FU) for 24 h, and further incubated without 5-FU for 28 days. To assess the capacity of self-renewal, surviving cells were planted for sphere-forming assay. Epithelial-to-mesenchymal transition (EMT) was induced with TGF-beta, then mRNA expression was evaluated by real-time PCR for E-cadherin, SNAIL, and vimentin. The E-Cadherin protein levels were also examined by immunoblot analysis. The Local invasion ability was analyzed by Matrigel invasion assay. RESULTS: The frequency of cells that were capable of initiating spheres was higher in 5-FU-pre treated cells, which also overexpressed stem cell marker genes, OCT4 and NANOG. Matrigel invasion activity of apoptosis-resistant Panc-1 cells was greater than that of control Panc-1 cells. CONCLUSION: Apoptosis-resistant cancer cells have CSC-like properties, i.e., able to initiate sphere formation, express stem cell genes, and respond to EMT stimulation.

Jeon, E. S., et al. (2010). "Ovarian cancer-derived lysophosphatidic acid stimulates secretion of VEGF and stromal cell-derived factor-1 alpha from human mesenchymal stem cells." Exp Mol Med **42**(4): 280-293.

Lysophosphatidic acid (LPA) stimulates growth and invasion of ovarian cancer cells and tumor angiogenesis. Cancer-derived LPA induces differentiation of human adipose tissue-derived mesenchymal stem cells (hASCs) to alpha-smooth muscle actin (alpha-SMA)-positive cancer-associated fibroblasts. Presently, we explored whether cancer-derived LPA regulates secretion of pro-angiogenic factors from hASCs. Conditioned medium (CM) from the OVCAR-3 and SKOV3 ovarian cancer cell lines stimulated secretion angiogenic factors such as stromal-derived factor-1 alpha (SDF-1 alpha) and VEGF from hASCs. Pretreatment with the LPA receptor inhibitor Ki16425 or short hairpin RNA lentiviral silencing of the LPA ((1)) receptor abrogated the cancer CM-stimulated expression of alpha-SMA, SDF-1, and VEGF from hASCs. LPA induced expression of myocardin and myocardin-related transcription factor-A, transcription factors involved in smooth muscle differentiation, in hASCs. siRNA-mediated depletion of endogenous myocardin and MRTF-A abrogated the expression of alpha-SMA, but not SDF-1 and VEGF. LPA activated RhoA in hASCs and pretreatment with the Rho kinase inhibitor Y27632 completely abrogated the LPA-induced expression of alpha-SMA, SDF-1, and VEGF in hASCs. Moreover, LPA-induced alpha-SMA expression was abrogated by treatment with the ERK inhibitor U0126 or the phosphoinositide-3-kinase inhibitor LY294002, but not the PLC inhibitor U73122. LPA-induced VEGF secretion was inhibited by LY294002, whereas LPA-induced SDF-1 secretion was markedly attenuated by U0126, U73122, and LY294002. These results suggest that cancer-secreted LPA induces differentiation of hASCs to cancer-associated fibroblasts through multiple signaling pathways involving Rho kinase, ERK, PLC, and phosphoinositide-3-kinase.

Jia, Y., et al. (2014). "Transforming growth factor-beta1 regulates epithelial-mesenchymal transition in association with cancer stem-like cells in a breast cancer cell line." Int J Clin Exp Med **7**(4): 865-872.

Epithelial-mesenchymal transition (EMT) is associated with altered connection and junctions between cells and changes in abilities of invasion and migration. In this study, we investigated whether SK-BR-3 breast cancer cells induced to undergo EMT exhibit changes in morphological and invasion abilities after Transforming growth factor beta1 (TGF-beta1) treatment. Serum-deprived SK-BR-3 cells were treated with TGF-beta1 (0, 10 ng/mL) for 24 h. The cells morphological changes were observed and imaged using inverted phase contrast microscope. Scratch experiment and invasion experiment were employed to detect changes of invasion ability, cell-flow experiment was used to assess cell cycle, immunohistochemistry technique was used to detect epithelial and mesenchymal markers after the crawling cells were fixed. Our research reveal that SK-BR-3 cells become larger and more messy, the elongated cells extend pseudopodia, the link of the cells became more loosely and cell gap widened after TGF-beta1

treatment. SK-BR-3 cells showed faster growing and improved invasion abilities after TGF-beta1 treatment, and reduced G1 phase cells proportion in the total number of cells after the conversion, in contrast the S phase cells accounted for the proportion of the total number of cells increased. These findings indicate that TGF-beta1-induced EMT in breast cancer cells may be associated with major alterations in morphological and invasion abilities.

Jiao, J., et al. (2013). "Cyclin D1 affects epithelial-mesenchymal transition in epithelial ovarian cancer stem cell-like cells." Onco Targets Ther **6**: 667-677.

BACKGROUND: The association of cancer stem cells with epithelial-mesenchymal transition (EMT) is receiving attention. We found in our previous study that EMT existed from CD24- phenotype cells to their differentiated cells. It was shown that cyclin D1 functioned in sustaining self-renewal independent of CDK4/CDK6 activation, but its effect on the EMT mechanism in ovarian cancer stem cells is unclear. METHODS: The anchorage-independent spheroids from ovarian adenocarcinoma cell line 3AO were formed in a serum-free medium. CD24- and CD24+ cells were isolated by fluorescence-activated cell sorting. Cell morphology, viability, apoptosis, and migratory ability were observed. Stem-related molecule Bmi-1, Oct-4 and EMT-related marker E-cadherin, and vimentin expressions were analyzed. Cyclin D1 expression in CD24- phenotype enriched spheroids was knocked down with small interfering RNA, and its effects on cell proliferation, apoptosis, migration ability, and EMT-related phenotype after transfection were observed. RESULTS: In our study, CD24- cells presented stronger proliferative, anti-apoptosis capacity, and migratory ability, than CD24+ cells or parental cells. CD24- cells grew with a scattered spindle-shape within 3 days of culture and transformed into a cobblestone-like shape, identical to CD24+ cells or parental cells at 7 days of culture. CD24- cells or spheroids highly expressed cyclin D1, Bmi-1, and vimentin, and seldom expressed E-cadherin, while CD24+ or parental cells showed the opposite expression. Furthermore, cyclin D1-targeted small interfering RNA resulted in decreased vimentin expression in spheroids. Transfected cells also exhibited an obvious decrease in cell viability and migration, but an increase in cell apoptosis. CONCLUSION: Cancer stem cell-like cells possess mesenchymal characteristics and EMT ability, and cyclin D1 involves in EMT mechanism, suggesting that EMT of cancer stem cell-like cells may play a key role in invasion and metastasis of ovarian cancer.

Kanzawa, M., et al. (2013). "WNT5A is a key regulator of the epithelial-mesenchymal transition and cancer stem cell properties in human gastric carcinoma cells." Pathobiology **80**(5): 235-244.

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.

Kasimir-Bauer, S., et al. (2012). "Expression of stem cell and epithelial-mesenchymal transition markers in primary breast cancer patients with circulating tumor cells." Breast Cancer Res **14**(1): R15.

INTRODUCTION: The presence of circulating tumor cells (CTC) in breast cancer might be associated with stem cell-like tumor cells which have been suggested to be the active source of metastatic spread in primary tumors. Furthermore, to be able to disseminate and metastasize, CTC must be able to perform epithelial-mesenchymal transition (EMT). We studied the expression of three EMT markers and the stem cell marker ALDH1 in CTC from 502 primary breast cancer patients. Data were correlated with the presence of disseminated tumor cells (DTC) in the bone marrow (BM) and with clinicopathological data of the patients. METHODS: A total of 2 x 5 ml of blood was analyzed for CTC with the AdnaTest BreastCancer (AdnaGen AG) for the detection of EpCAM, MUC-1, HER2 and beta-Actin transcripts.

The recovered c-DNA was additionally multiplex tested for three EMT markers [TWIST1, Akt2, phosphoinositide kinase-3 (PI3Kalpha)] and separately for the tumor stem cell marker ALDH1. The identification of EMT markers was considered positive if at least one marker was detected in the sample. Two BM aspirates from all patients were analyzed for DTC by immunocytochemistry using the pan-cytokeratin antibody A45-B/B3. RESULTS: Ninety-seven percent of 30 healthy donor samples investigated were negative for EMT and 95% for ALDH1 transcripts, respectively. CTC were detected in 97/502 (19%) patients. At least one of the EMT markers was expressed in 29% and ALDH1 was present in 14% of the samples, respectively. Interestingly, 5% of the ALDH1-positive and 18% of the EMT-positive patients were CTC-negative based on the cut-off level determined for CTC-positivity applying the AdnaTest BreastCancer. DTC in the BM were detected in 107/502 (21%) patients and no correlation was found between BM status and CTC positivity (P = 0.41). The presence of CTC, EMT and ALDH1 expression was not correlated to any of the prognostic clinical markers. CONCLUSIONS: Our data indicate that (1) a subset of primary breast cancer patients shows EMT and stem cell characteristics and (2) the currently used detection methods for CTC are not efficient to identify a subtype of CTC which underwent EMT. (3) The clinical relevance on prognosis and therapy response has to be further evaluated in a prospective trial.

Kim, Y. J., et al. (2017). "Ursodeoxycholic acid suppresses epithelial-mesenchymal transition and cancer stem cell formation by reducing the levels of peroxiredoxin II and reactive oxygen species in pancreatic cancer cells." Oncol Rep **38**(6): 3632-3638.

Reactive oxygen species (ROS) play a key role in cancer development and progression. Ursodeoxycholic acid (UDCA) may possess antioxidant, anti-inflammatory and chemoprophylatic effects. Therefore, we aimed to investigate the effects and mechanisms of UDCA treatment on pancreatic cancer cells. The pancreatic cancer cell lines HPAC and Capan-1 were treated with 0.2 mM UDCA. To examine alterations in the levels of intracellular ROS, the DCF-DA stain was used and both stemness and epithelial-mesenchymal transition (EMT)-related genes were quantified using qRT-PCR and western blot analysis. The pancreatic cancer sphere culture was performed following seven days of treatment with 0.2 mM UDCA, as an indicator of stemness. Following treatment with UDCA, the level of intracellular ROS was decreased in the pancreatic cancer cells. UDCA decreased both the phosphorylation of STAT3 and the expression of peroxiredoxin II (Prx2). Furthermore, the treatment resulted in the upregulation of E-cadherin and in the downregulation of N-cadherin. In addition, UDCA decreased the expression of sex determining region Y-box 2 (Sox2) and it diminished the number of pancreatic cancer spheres formed. In conclusion UDCA suppressed the levels of intracellular ROS and Prx2 and it decreased EMT and stem cell formation in pancreatic cancer cells. Therefore, UDCA may provide favorable therapeutic benefits, through its antioxidant effects, for patients with pancreatic cancer.

Kong, D., et al. (2010). "Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells." PLoS One **5**(8): e12445.

BACKGROUND: Current management of patients diagnosed with prostate cancer (PCa) is very effective; however, tumor recurrence with Castrate Resistant Prostate Cancer (CRPC) and subsequent metastasis lead to poor survival outcome, suggesting that there is a dire need for novel mechanistic understanding of tumor recurrence, which would be critical for designing novel therapies. The recurrence and the metastasis of PCa are tightly linked with the biology of prostate cancer stem cells or cancer-initiating cells that is reminiscent of the acquisition of Epithelial to Mesenchymal Transition (EMT) phenotype. Increasing evidence suggests that EMT-type cells share many biological characteristics with cancer stem-like cells. METHODOLOGY/PRINCIPAL FINDINGS: In this study, we found that PCa cells with EMT phenotype displayed stem-like cell features characterized by increased expression of Sox2, Nanog, Oct4, Lin28B and/or Notch1, consistent with enhanced clonogenic and sphere (prostasphere)-forming ability and tumorigenecity in mice, which was associated with decreased expression of miR-200 and/or let-7 family. Reversal of EMT by re-expression of miR-200 inhibited prostasphere-forming ability of EMT-type cells and reduced the expression of Notch1 and Lin28B. Down-regulation of Lin28B increased let-7 expression, which was consistent with repressed self-renewal capability. CONCLUSIONS/SIGNIFICANCE: These results suggest that miR-200 played a pivotal role in linking the characteristics of cancer stem-like cells with EMT-like cell signatures in PCa. Selective elimination of cancer stem-like cells by reversing the EMT phenotype to Mesenchymal-Epithelial Transition (MET) phenotype using novel agents would be useful for the prevention of tumor recurrence especially by eliminating those cells that are the "Root Cause" of tumor development and recurrence.

Kong, L., et al. (2016). "Overexpression of SDF-1 activates the NF-kappaB pathway to induce epithelial to mesenchymal transition and cancer stem

cell-like phenotypes of breast cancer cells." Int J Oncol **48**(3): 1085-1094.

The formation of EMT and EMT-induced CSC-like phenotype is crucial for the metastasis of tumor cells. The stromal cell-derived factor-1 (SDF-1) is upregulated in various human carcinomas, which is closely associated with proliferation, migration, invasion and prognosis of malignancies. However, limited attention has been directed towards the effect of SDF-1 on epithelial to mesenchymal transition (EMT) or cancer stem cell (CSC)-like phenotype formation in breast cancer cells and the related mechanism. In the present study, we screened MCF-7 cells with low SDF-1 expression level for the purpose of evaluating whether SDF-1 is involved in EMT and CSC-like phenotype formation in MCF-7 cells. The pEGFP-N1-SDF-1 plasmid was transfected into MCF-7 cells, and the stably overexpressed SDF-1 in MCF-7 cells was confirmed by real-time PCR and western blot analysis. Colony formation assay, MTT, wound healing assay and Transwell invasion assay demonstrated that overexpression of SDF-1 significantly boosted the proliferation, migration and invasion of MCF-7 cells compared with parental ($P<0.05$). Flow cytometry analysis revealed a notable increase of CD44+/CD24- subpopulation in SDF-1 overexpressing MCF-7 cells ($P<0.001$), accompanied by the apparently elevated ALDH activity and the upregulation of the stem cell markers OCT-4, Nanog, and SOX2 compared with parental ($P<0.01$). Besides, western blot analysis and immunofluorescence assay observed the significant decreased expression of E-cadherin and enhanced expression of slug, fibronectin and vimentin in SDF-1 overexpressed MCF-7 cells in comparison with parental ($P<0.01$). Further study found that overexpression of SDF-1 induced the activation of NF-kappaB pathway in MCF-7 cells. Conversely, suppressing or silencing p65 expression by antagonist or RNA interference could remarkably increase the expression of E-cadherin in SDF-1 overexpressed MCF-7 cells ($P<0.001$). Overall, the above results indicated that overexpression of SDF-1 enhanced EMT by activating the NF-kappaB pathway of MCF-7 cells and further induced the formation of CSC-like phenotypes, ultimately promoting the proliferation and metastasis of MCF-7 cells. Therefore, SDF-1 may further be assessed as a potential target for gene therapy of breast cancer.

Krawczyk, N., et al. (2014). "Expression of stem cell and epithelial-mesenchymal transition markers in circulating tumor cells of breast cancer patients." Biomed Res Int **2014**: 415721.

Evaluation and characterization of circulating tumor cells (CTCs) have become a major focus of translational cancer research. Presence of CTCs predicts worse clinical outcome in early and metastatic breast cancer. Whether all cells from the primary tumor have potential to disseminate and form subsequent metastasis remains unclear. As part of the metastatic cascade, tumor cells lose their cell-to-cell adhesion and undergo epithelial-mesenchymal transition (EMT) in order to enter blood circulation. During EMT epithelial antigens are downregulated; thus, such tumor cells might elude classical epithelial marker-based detection. Several researchers postulated that some CTCs express stem cell-like phenotype; this might lead to chemoresistance and enhanced metastatic potential of such cells. In the present review, we discuss current data on EMT and stem cell markers in CTCs of breast cancer and their clinical significance.

Kuo, C. H., et al. (2014). "17beta-estradiol inhibits mesenchymal stem cells-induced human AGS gastric cancer cell mobility via suppression of CCL5-Src/Cas/Paxillin signaling pathway." Int J Med Sci **11**(1): 7-16.

Gender differences in terms of mortality among many solid organ malignancies have been proved by epidemiological data. Estrogen has been suspected to cast a protective effect against cancer because of the lower mortality of gastric cancer in females and the benefits of hormone replacement therapy (HRT) in gastric cancer. Hence, it suggests that 17beta-estradiol (E2) may affect the behavior of cancer cells. One of the key features of cancer-related mortality is metastasis. Accumulating evidences suggest that human bone marrow mesenchymal stem cells (HBMMSCs) and its secreted CCL-5 have a role in enhancing the metastatic potential of breast cancer cells. However, it is not clear whether E2 would affect HBMMSCs-induced mobility in gastric cancer cells. In this report, we show that CCL-5 secreted by HBMMSCs enhanced mobility in human AGS gastric cancer cells via activation of Src/Cas/Paxillin signaling pathway. Treatment with specific neutralizing antibody of CCL-5 significantly inhibited HBMMSCs-enhanced mobility in human AGS gastric cancer cells. We further observe that 17beta-estradiol suppressed HBMMSCs-enhanced mobility by down-regulating CCL5-Src/Cas/paxillin signaling pathway in AGS cells. Collectively, these results suggest that 17beta-estradiol treatment significantly inhibits HBMMSCS-induced mobility in human AGS gastric cancer cells.

Lam, S. S., et al. (2014). "Targeting estrogen-related receptor alpha inhibits epithelial-to-mesenchymal transition and stem cell properties of ovarian cancer cells." Mol Ther **22**(4): 743-751.

Epithelial-mesenchymal transition represents a key event in cancer progression and has emerged as a

promising anticancer target. Estrogen-related receptor alpha (ERRalpha) is frequently elevated in advanced-stage ovarian cancer, but its potential role in tumor progression is not known. Here we show that ERRalpha functions in epithelial-mesenchymal transition and in subsequent stem cell traits responsible for the acquisition of high degree of aggressiveness and potential for metastasis that are characteristic of ovarian cancer. Importantly, targeted inhibition of ERRalpha also inhibited the expression of Snail, a repressor of E-cadherin and an inducer of epithelial-mesenchymal transition. Interestingly, induction of Snail resulted from not only changes in mRNA transcription rate but also mRNA stability. We thus identified the miR-200 family as a new player in the ERRalpha-mediated posttranscriptional regulation of Snail, and antagonism of miR-200a/b could revert the decreased expression of Snail and reversal of epithelial-mesenchymal transition and stem cell characteristics due to ERRalpha depletion. Finally, we showed that RNA interference-mediated inhibition of ERRalpha significantly reduced tumor burden, ascites formation, and metastatic peritoneal nodules in vivo in an orthotopic model of ovarian cancer. These results suggest ERRalpha activation as a mechanism of tumor aggressiveness and imply that targeting ERRalpha may be a promising approach in ovarian cancer treatment.

Loebinger, M. R., et al. (2010). "TRAIL-expressing mesenchymal stem cells kill the putative cancer stem cell population." Br J Cancer **103**(11): 1692-1697.

BACKGROUND: Tumours contain stem-like, side population (SP) cells, which have increased tumorigenic potential, resistance to traditional therapies and may be responsible for treatment failures and relapse in patients. METHODS: Mesenchymal stem cells (MSCs) were engineered to express the apoptotic ligand, TNF-related apoptosis-inducing ligand (TRAIL). Squamous (H357) and lung (A549) cancer cell lines were sorted into side and non-side populations (non-SP) by Hoechst flow cytometry. The survival and growth of both SP and non-SP cancer populations, in conjunction with TRAIL-expressing MSCs and mitoxantrone chemotherapy, were assessed by flow cytometry and colony forming ability. RESULTS: Mesenchymal stem cells expressing TRAIL migrate to tumours and reduce the growth of primary cancers and metastases. This report demonstrates that these cells cause apoptosis, death and reduced colony formation of the SP of squamous and adenocarcinoma lung cancer cells and are synergistic when combined with traditional chemotherapy in apoptosis induction. CONCLUSIONS: The sensitivity of putative cancer stem cells to TRAIL-expressing MSCs, suggests their possible role in the prevention of cancer relapse.

Long, H., et al. (2015). "CD133+ ovarian cancer stem-like cells promote non-stem cancer cell metastasis via CCL5 induced epithelial-mesenchymal transition." Oncotarget **6**(8): 5846-5859.

Cancer stem cells (CSCs, also called cancer stem-like cells, CSLCs) can function as "seed cells" for tumor recurrence and metastasis. Here, we report that, in the presence of CD133+ ovarian CSLCs, CD133- non-CSLCs can undergo an epithelial-mesenchymal transition (EMT)-like process and display enhanced metastatic capacity in vitro and in vivo. Highly elevated expression of chemokine (C-C motif) ligand 5 (CCL5) and its receptors chemokine (C-C motif) receptor (CCR) 1/3/5 are observed in clinical and murine metastatic tumor tissues from epithelial ovarian carcinomas. Mechanistically, paracrine CCL5 from ovarian CSLCs activates the NF-kappaB signaling pathway in ovarian non-CSLCs via binding CCR1/3/5, thereby inducing EMT and tumor invasion. Taken together, our results redefine the metastatic potential of non-stem cancer cells and provide evidence that targeting the CCL5:CCR1/3/5-NF-kappaB pathway could be an effective strategy to prevent ovarian cancer metastasis.

Luo, D., et al. (2013). "Differential effects of mesenchymal stem cells on a heterogeneous cell population within lung cancer cell lines." Mol Cell Biochem **378**(1-2): 107-116.

Although mesenchymal stem cells (MSCs) promote lung cancer growth in vivo, in vitro studies indicate that they inhibit the proliferation of lung cancer cells. Because malignant tumors contain a heterogeneous cell population with variable capacity for self-renewal, the aim of this study was to determine whether the inconsistencies between in vitro and in vivo studies are a result of differential effects of MSCs on the heterogeneous cell population within lung cancer cell lines. Human MSCs were isolated from the bone marrow, and their cell surface antigen expression and multi-lineage differentiation capacity was examined at passage 10. CD133+ cells were isolated from A549 and H446 cell lines using immunomagnetic separation. The effects of MSCs on the growth and microsphere formation of heterogeneous cell populations within two lung cancer cell lines (A549 and H446) were compared. MSCs inhibited the in vitro proliferation of both cell lines, but significantly accelerated tumor formation and stimulated tumor growth in vivo ($P < 0.05$). In CD133+ cells isolated from both A549 and H446 cells, co-culture with MSCs for 1-3 days significantly increased their proliferation ($P < 0.05$). MSCs also

significantly increased microsphere formation in both cell lines ($P < 0.05$). Selective stimulation of CD133+ cell growth may account for the discrepant effects of MSCs on lung cancer progression.

Luo, F., et al. (2016). "Bone marrow mesenchymal stem cells participate in prostate carcinogenesis and promote growth of prostate cancer by cell fusion in vivo." Oncotarget **7**(21): 30924-30934.

The tumor microenvironment is comprised of diverse stromal cells that contribute towards tumor progression. As a result, there has been a growing interest in the role of bone marrow derived cells (BMDCs) in cancer progression. However, the role of BMDCs in prostate cancer (PCa) progression still remains unclear. In this study, we established GFP bone marrow transplanted TRAMP and MUN-induced prostate cancer models, in order to investigate the role of BMDCs in prostate cancer progression. By tracing GFP positive cells, we observed that BMDCS were recruited into mouse prostate tissues during tumorigenesis. GFP+/Sca-1+/CD45- BMDCs were significantly increased in the MNU-induced PCa group, as compared to the citrated-treated control group (2.67 +/- 0.25% vs 0.67 +/- 0.31%, $p = 0.006$). However, there were no significant differences found in GFP+/Sca-1+/CD45+ cell populations between the two groups (0.27 +/- 0.15% vs 0.10 +/- 0.10%, $p = 0.334$). Moreover, co-grafting of bone marrow mesenchymal stem cells (BMMSCs) and RM1 cells were found to promote RM1 tumor growth in vivo, and cell fusion was observed in RM-1+BMMSCs xenografts. Therefore, the data suggests that BMDCs can be recruited to the prostate during carcinogenesis, and that BMMSCs may promote the growth of PCa.

Luo, J., et al. (2015). "Infiltrating bone marrow mesenchymal stem cells (BM-MSCs) increase prostate cancer cell invasion via altering the CCL5/HIF2alpha/androgen receptor signals." Oncotarget **6**(29): 27555-27565.

Several infiltrating cells in the tumor microenvironment could influence the cancer progression via secreting various cytokines. Here, we found the CCL5 secreted from BM-MSCs suppressed androgen receptor (AR) signals via enhancing the expression of hypoxia inducible factor 2alpha (HIF2alpha) in prostate cancer (PCa) cells. Mechanism dissection revealed that the increased HIF2alpha might alter the AR-HSP90 interaction to suppress the AR transactivation, and inhibition of HIF2alpha reversed the BM-MSCs-increased PCa stem cell population and PCa cells invasion. Importantly, CCL5 could suppress the prolyl hydroxylases (PHDs) expression, which might then lead to suppress VHL-mediated HIF2alpha ubiquitination. Together, these results demonstrated that the CCL5 signals from infiltrating BM-MSC cells to HIF2alpha signals within PCa cells might play a key role to increase PCa stem cell population and PCa metastasis via altering the AR signals. Targeting this newly identified CCL5/HIF2alpha/AR axis signal axis may allow us to develop a novel way to suppress PCa metastasis.

Luo, J., et al. (2014). "Infiltrating bone marrow mesenchymal stem cells increase prostate cancer stem cell population and metastatic ability via secreting cytokines to suppress androgen receptor signaling." Oncogene **33**(21): 2768-2778.

Although the contribution of the bone marrow mesenchymal stem cells (BM-MSCs) in cancer progression is emerging, their potential roles in prostate cancer (PCa) remain unclear. Here, we showed that PCa cells could recruit BM-MSCs and consequently the metastatic ability of PCa cells was increased. We also found that the increased metastatic ability of PCa cells could be due to the increased PCa stem cell population. Mechanism dissection studies found that the upregulation of Chemokine ligand 5 (CCL5) expression in BM-MSCs and PCa cells, after MSCs infiltrated into the PCa cells, subsequently downregulated androgen receptor (AR) signaling, which was due to inhibition of AR nuclear translocation. Interruption of such signaling led to suppression of the BM-MSCs-induced PCa stem cell population increase and thereby inhibited the metastatic ability of PCa cells. The PCa stem cell increase then led to the upregulation of matrix metalloproteinase 9, ZEB-1, CD133 and CXCR4 molecules, and enhanced the metastatic ability of PCa cells. Therefore, we conclude that the BM-MSCs-mediated increased metastatic ability of PCa cells can be due to the PCa stem cell increase via alteration of the CCL5-AR signaling pathway. Together, these results uncover the important roles of BM-MSCs as key components in the prostate tumor microenvironment to promote PCa metastasis and may provide a new potential target to suppress PCa metastasis by blocking BM-MSCs infiltration into PCa.

Luo, X., et al. (2018). "Inflammatory Human Umbilical Cord-Derived Mesenchymal Stem Cells Promote Stem Cell-Like Characteristics of Cancer Cells in an IL-1beta-Dependent Manner." Biomed Res Int **2018**: 7096707.

To ensure the safety of clinical applications of MSCs, thorough understanding of their impacts on tumor initiation and progression is essential. Here, to further explore the complex dialog between MSCs and tumor cells, umbilical cord-derived mesenchymal stem cells (UC-MSCs) were employed to be cocultured with

either breast or ovarian cancer cells. Though having no obvious influence on proliferation or apoptosis, UC-MSCs exerted intense stem cell-like properties promoting effects on both cancer models. Cocultured cancer cells showed enriched side population, enhanced sphere formation ability, and upregulated pluripotency-associated stem cell markers. Human cytokine array and real-time PCR revealed a panel of MSC-derived prostemness cytokines CCL2, CXCL1, IL-8, and IL-6 which were induced upon coculturing. We further revealed IL-1beta, a well-characterized proinflammatory cytokine, to be the inducer of these prostemness cytokines, which was generated from inflammatory UC-MSCs in an autocrine manner. Additionally, with introduction of IL-1RA (an IL-1 receptor antagonist) into the coculturing system, the stem cell-like characteristics promoting effects of inflammatory UC-MSCs were partially blocked. Taken together, these findings suggest that transduced inflammatory MSCs work as a major source of IL-1beta in tumor microenvironment and initiate the formation of prostemness niche via regulating their secretome in an IL-1beta-dependent manner.

Maffey, A., et al. (2017). "Mesenchymal stem cells from tumor microenvironment favour breast cancer stem cell proliferation, cancerogenic and metastatic potential, via ionotropic purinergic signalling." Sci Rep **7**(1): 13162.

Interaction between tumor cells and the microenvironment is key in initiation, progression, and invasiveness of cancer. In particular, mesenchymal stem cells (MSCs) are recruited to the sites of developing tumors, thus promoting metastasis formation. Although it is well known that MSCs migrate and integrate in the tumor microenvironment (TME), their fate and function inside the tumor is still not clear. In this study, we analyzed the role played by MSCs in breast cancer oncogenesis. Data indicate that interaction of breast cancer cells with MSCs results in an increased proliferation and metabolic activity of breast cancer cells, partially due to MSC-derived microvesicles that are shed in the TME. Moreover, we addressed the question of whether we could modulate such interaction by acting on P2X-mediated intercellular communication. By inhibiting P2X-mediated purinergic signaling, we succeeded in reducing both the cancerogenic as well as the metastatic potential of breast cancer cells co-cultured with MSCs, in 2D as well as in 3D in vitro models. Data obtained demonstrate for the first time that the trophic effect of MSCs on breast cancer cell growth is exerted via ionotropic purinergic signaling, thus suggesting the inhibition of the purinergic signaling system as a potential target for therapeutic intervention.

Maj, M., et al. (2017). "Influence of Mesenchymal Stem Cells Conditioned Media on Proliferation of Urinary Tract Cancer Cell Lines and Their Sensitivity to Ciprofloxacin." J Cell Biochem **118**(6): 1361-1368.

Mesenchymal stem cells (MSCs) are known to interact with cancer cells through direct cell-to-cell contact and secretion of paracrine factors, although their exact influence on tumor progression in vivo remains unclear. To better understand how fetal and adult stem cells affect tumors, we analyzed viability of human renal (786-0) and bladder (T24) carcinoma cell lines cultured in conditioned media harvested from amniotic fluid-derived stem cells (AFSCs) and adipose-derived stem cells (ASCs). Both media reduced metabolic activity of 786-0 cells, however, decreased viability of T24 cells was noted only after incubation with conditioned medium from ASCs. To test the hypothesis that MSCs-secreted factors might be involved in chemoresistance acquisition, we further analyzed influence of mesenchymal stem cell conditioned media (MSC-CM) on cancer cells sensitivity to ciprofloxacin, that is considered as potential candidate agent for urinary tract cancers treatment. Significantly increased resistance to tested drug indicates that MSCs may protect cancer cells from chemotherapy. J. Cell. Biochem. 118: 1361-1368, 2017. (c) 2016 Wiley Periodicals, Inc.

Mandel, K., et al. (2013). "Mesenchymal stem cells directly interact with breast cancer cells and promote tumor cell growth in vitro and in vivo." Stem Cells Dev **22**(23): 3114-3127.

Cellular interactions were investigated between human mesenchymal stem cells (MSC) and human breast cancer cells. Co-culture of the two cell populations was associated with an MSC-mediated growth stimulation of MDA-MB-231 breast cancer cells. A continuous expansion of tumor cell colonies was progressively surrounded by MSC (GFP) displaying elongated cell bodies. Moreover, some MSC (GFP) and MDA-MB-231(cherry) cells spontaneously generated hybrid/chimeric cell populations, demonstrating a dual (green fluorescent protein+cherry) fluorescence. During a co-culture of 5-6 days, MSC also induced expression of the GPI-anchored CD90 molecule in breast cancer cells, which could not be observed in a transwell assay, suggesting the requirement of direct cellular interactions. Indeed, MSC-mediated CD90 induction in the breast cancer cells could be partially blocked by a gap junction inhibitor and by inhibition of the notch signaling pathway, respectively. Similar findings were observed in vivo by which a subcutaneous injection of a co-culture of primary MSC with MDA-MB-231(GFP) cells into NOD/scid mice exhibited an about 10-fold

increased tumor size and enhanced metastatic capacity as compared with the MDA-MB-231(GFP) mono-culture. Flow cytometric evaluation of the co-culture tumors revealed more than 90% of breast cancer cells with about 3% of CD90-positive cells, also suggesting an MSC-mediated in vivo induction of CD90 in MDA-MB-231 cells. Furthermore, immunohistochemical analysis demonstrated an elevated neovascularization and viability in the MSC/MDA-MB-231(GFP)-derived tumors. Together, these data suggested an MSC-mediated growth stimulation of breast cancer cells in vitro and in vivo by which the altered MSC morphology and the appearance of hybrid/chimeric cells and breast cancer-expressing CD90(+) cells indicate mutual cellular alterations.

Marofi, F., et al. (2017). "Mesenchymal Stromal/Stem Cells: A New Era in the Cell-Based Targeted Gene Therapy of Cancer." Front Immunol **8**: 1770.

In recent years, in light of the promising potentials of mesenchymal stromal/stem cells (MSCs) for carrying therapeutic anticancer genes, a complete revisitation on old chemotherapy-based paradigms has been established. This review attempted to bring forward and introduce the novel therapeutic opportunities of using genetically engineered MSCs. The simplicities and advantages of MSCs for medical applications make them a unique and promising option in the case of cancer therapy. Some of the superiorities of using MSCs as therapeutic gene micro-carriers are the easy cell-extraction procedures and their abundant proliferation capacity in vitro without losing their main biological properties. Targeted therapy by using MSCs as the delivery vehicles of therapeutic genes is a new approach in the treatment of various types of cancers. Some of the distinct properties of MSCs, such as tumor-tropism, non-immunogenicity, stimulatory effect on the anti-inflammatory molecules, inhibitory effect on inflammatory responses, non-toxicity against the normal tissues, and easy processes for the clinical use, have formed the basis of attention to MSCs. They can be easily used for the treatment of damaged or injured tissues, regenerative medicine, and immune disorders. This review focused on the drugability of MSCs and their potential for the delivery of candidate anticancer genes. It also briefly reviewed the vectors and methods used for MSC-mediated gene therapy of malignancies. Also, the challenges, limitations, and considerations in using MSCs for gene therapy of cancer and the new methods developed for resolution of these problems are reviewed.

Masui, T., et al. (2014). "Snail-induced epithelial-mesenchymal transition promotes cancer stem cell-like phenotype in head and neck cancer cells." Int J Oncol **44**(3): 693-699.

Head and neck squamous cell carcinoma (HNSCC) is known to have a poor prognosis. The resistance to treatment and distant metastasis are important clinical problems in HNSCC. The epithelial-mesenchymal transition (EMT) is a key process in successful execution of many steps such as the invasion and metastasis for cancer cells. Snail is one of the master regulators that promote EMT in many types of malignancies including HNSCC. Recently, it has been shown that Snail-induced EMT could induce a cancer stem cell (CSC)like phenotype in a number of tumor types. In this study, we investigated the role of Snail in inducing EMT properties and CSC-like phenotype in HNSCC. We established HNSCC cell lines transfected with Snail. E-cadherin was analyzed using western blot analysis and immunofluorescence staining. Cell migration and invasion were assessed using wound-healing assay and modified Boyden chamber assay, respectively. CSC markers of HNSCC, CD44 and aldehyde dehydrogenase 1 (ALDH1), were also evaluated with western blot analysis, and chemosensitivity was assessed with WST-8 assay. Introduction of Snail induced EMT properties in HNSCC cells and enhanced cell migration and invasion. Moreover, Snail-induced EMT gained CSC-like phenotype and was associated with increased chemoresistance. These results suggest that Snail could be one of the attractive targets for the development of therapeutic strategies in HNSCC.

Melzer, C., et al. (2017). "Cancer stem cell niche models and contribution by mesenchymal stroma/stem cells." Mol Cancer **16**(1): 28.

BACKGROUND: The initiation and progression of malignant tumors is driven by distinct subsets of tumor-initiating or cancer stem-like cells (CSCs) which develop therapy/apoptosis resistance and self-renewal capacity. In order to be able to eradicate these CSCs with novel classes of anti-cancer therapeutics, a better understanding of their biology and clinically-relevant traits is mandatory. MAIN BODY: Several requirements and functions of a CSC niche physiology are combined with current concepts for CSC generation such as development in a hierarchical tumor model, by stochastic processes, or via a retrodifferentiation program. Moreover, progressive adaptation of endothelial cells and recruited immune and stromal cells to the tumor site substantially contribute to generate a tumor growth-permissive environment resembling a CSC niche. Particular emphasis is put on the pivotal role of multipotent mesenchymal stroma/stem cells (MSCs) in supporting CSC development by various kinds of interaction and cell fusion to form hybrid tumor cells. CONCLUSION:

A better knowledge of CSC niche physiology may increase the chances that cancer stemness-depleting interventions ultimately result in arrest of tumor growth and metastasis.

Mognetti, B., et al. (2013). "Bone marrow mesenchymal stem cells increase motility of prostate cancer cells via production of stromal cell-derived factor-1alpha." J Cell Mol Med **17**(2): 287-292.

Prostate cancer frequently metastasizes to the bone, and the interaction between cancer cells and bone microenvironment has proven to be crucial in the establishment of new metastases. Bone marrow mesenchymal stem cells (BM-MSCs) secrete various cytokines that can regulate the behaviour of neighbouring cell. However, little is known about the role of BM-MSCs in influencing the migration and the invasion of prostate cancer cells. We hypothesize that the stromal cell-derived factor-1alpha released by BM-MSCs may play a pivotal role in these processes. To study the interaction between factors secreted by BM-MSCs and prostate cancer cells we established an in vitro model of transwell co-culture of BM-MSCs and prostate cancer cells DU145. Using this model, we have shown that BM-MSCs produce soluble factors which increase the motility of prostate cancer cells DU145. Neutralization of stromal cell-derived factor-1alpha (SDF1alpha) via a blocking antibody significantly limits the chemoattractive effect of bone marrow MSCs. Moreover, soluble factors produced by BM-MSCs greatly activate prosurvival kinases, namely AKT and ERK 1/2. We provide further evidence that SDF1alpha is involved in the interaction between prostate cancer cells and BM-MSCs. Such interaction may play an important role in the migration and the invasion of prostate cancer cells within bone.

Mohammadian, F. and B. Negahdari (2017). "Isolation and characterization of mesenchymal stem cells and its antitumor application on ovarian cancer cell line." Artif Cells Nanomed Biotechnol: 1-10.

The molecular interaction network of Oct-4 (POU5F1) and NANOG connected to regulation and growth of mesenchymal stem cells (MSCs) were supplemented with information of miRNA to find an important micro-RNAs and supplemented molecular interaction network. Following co-culturing of Bone marrow mesenchymal stem cells (BMMSCs) with SKOV3 ovarian cancer cell lines and undifferentiated BMMSCs, MTT was analyzed for cell cytotoxicity. The analyses of the expression of miRNA were performed either after oesteogenesis (hsa-miR-34 and hsa-miR-335) or chondrogenic (hsa-miR-145 and hsa-miR-455) differentiation. This molecular interaction network was imaged in using software. The results from these findings gave an understanding of the main molecular mechanisms regulating MSCs therapeutic activity and their undifferentiated state maintenance. We recommend that the downregulation of miR-335 is crucial role for tissue homeostasis.

Mohr, A. and R. Zwacka (2018). "The future of mesenchymal stem cell-based therapeutic approaches for cancer - From cells to ghosts." Cancer Lett **414**: 239-249.

Mesenchymal stem cells (MSCs) are multipotent stromal cells which can differentiate into a variety of cell types including osteoblasts, adipocytes and chondrocytes. They are normally resident in adipose tissue, bone marrow and the umbilical cord, but can also be found in other tissues and are known to be recruited to sites of wound healing as well as growing tumours. The therapeutic potential of MSCs has been explored in a number of phase I/II and III clinical trials, of which several were targeted against graft-versus-host disease and to support engraftment of haematopoietic stem cells (HSCs), but currently only very few in the oncology field. There are now three clinical trials either ongoing or recruiting patients that use MSCs to treat tumour disease. In these, MSCs target gastrointestinal, lung and ovarian cancer, respectively. The first study uses MSCs loaded with a HSV-TK expression construct under the control of the CCL5 promoter, and has recently reported successful completion of Phase I/II. While no adverse side effects were seen during this study, no outcomes with respect to therapeutic benefits have been published. The other clinical trials targeting lung and ovarian cancer will be using MSCs expressing cytokines as therapeutic payload. Despite these encouraging early steps towards their clinical use, many questions are still unanswered regarding the biology of MSCs in normal and pathophysiological settings. In this review, in addition to summarising the current state of MSC-based therapeutic approaches for cancer, we will describe the remaining questions, obstacles and risks, as well as novel developments such as MSC-derived nanoghosts.

Molloy, A. P., et al. (2009). "Mesenchymal stem cell secretion of chemokines during differentiation into osteoblasts, and their potential role in mediating interactions with breast cancer cells." Int J Cancer **124**(2): 326-332.

Over 70% of patients with advanced breast cancer will develop bone metastases for which there is no cure. Mesenchymal Stem Cells (MSCs) and their derivative osteoblasts are subpopulations of cells within the bone marrow environment, postulated as potential interacting targets for disseminating cancer cells because of their ability to secrete a range of chemokines. This study aimed to investigate

chemokine secretion throughout MSC differentiation into osteoblasts and their effect on the breast cancer cells. Primary MSCs and osteoblast progenitors were cultured in appropriate conditions to induce differentiation into mature osteoblasts. Chemokines secreted throughout differentiation were detected using ChemiArray and ELISA. Migration of breast cancer cells in response to the bone-derived cells was quantified using Transwell inserts. Breast cancer cells were cocultured with MSCs, retrieved using magnetic beads, and changes in CCL2 expression were analyzed. MSCs secreted a range of factors including IL-6, TIMP-1 and CCL2, the range and level of which changed throughout differentiation. CCL2 secretion by MSCs increased significantly above control cells as they differentiated into mature osteoblasts ($p<0.05$). The bone-derived cells stimulated migration of breast cancer cells, and this was inhibited (21-50%) in the presence of a CCL2 antibody. CCL2 gene expression in breast cancer cells was upregulated following direct coculture with MSCs. The varying levels of chemokines secreted throughout MSC differentiation may play an important role in supporting tumor cell homing and progression. These results further highlight the distinct effect MSCs have on breast cancer cells and their potential importance in supporting development of metastases.

Morata-Tarifa, C., et al. (2016). "Low adherent cancer cell subpopulations are enriched in tumorigenic and metastatic epithelial-to-mesenchymal transition-induced cancer stem-like cells." Sci Rep **6**: 18772.

Cancer stem cells are responsible for tumor progression, metastasis, therapy resistance and cancer recurrence, doing their identification and isolation of special relevance. Here we show that low adherent breast and colon cancer cells subpopulations have stem-like properties. Our results demonstrate that trypsin-sensitive (TS) breast and colon cancer cells subpopulations show increased ALDH activity, higher ability to exclude Hoechst 33342, enlarged proportion of cells with a cancer stem-like cell phenotype and are enriched in sphere- and colony-forming cells in vitro. Further studies in MDA-MB-231 breast cancer cells reveal that TS subpopulation expresses higher levels of SLUG, SNAIL, VIMENTIN and N-CADHERIN while show a lack of expression of E-CADHERIN and CLAUDIN, being this profile characteristic of the epithelial-to-mesenchymal transition (EMT). The TS subpopulation shows CXCL10, BMI-1 and OCT4 upregulation, differing also in the expression of several miRNAs involved in EMT and/or cell self-renewal such as miR-34a-5p, miR-34c-5p, miR-21-5p, miR-93-5p and miR-100-5p. Furthermore, in vivo studies in immunocompromised mice demonstrate that MDA-MB-231 TS cells form more and bigger xenograft tumors with shorter latency and have higher metastatic potential. In conclusion, this work presents a new, non-aggressive, easy, inexpensive and reproducible methodology to isolate prospectively cancer stem-like cells for subsequent biological and preclinical studies.

Morton, J. J., et al. (2018). "Dual use of hematopoietic and mesenchymal stem cells enhances engraftment and immune cell trafficking in an allogeneic humanized mouse model of head and neck cancer." Mol Carcinog **57**(11): 1651-1663.

In this report, we describe in detail the evolving procedures to optimize humanized mouse cohort generation, including optimal conditioning, choice of lineage for engraftment, threshold for successful engraftment, HNSCC tumor implantation, and immune and stroma cell analyses. We developed a dual infusion protocol of human hematopoietic stem and progenitor cells (HSPCs) and mesenchymal stem cells (MSCs), leading to incremental human bone marrow engraftment, and exponential increase in mature peripheral human immune cells, and intratumor homing that includes a more complete lineage reconstitution. Additionally, we have identified practical rules to predict successful HSPC/MSC expansion, and a peripheral human cell threshold associated with bone marrow engraftment, both of which will optimize cohort generation and management. The tremendous advances in immune therapy in cancer have made the need for appropriate and standardized models more acute than ever, and therefore, we anticipate that this manuscript will have an immediate impact in cancer-related research. The need for more representative tools to investigate the human tumor microenvironment (TME) has led to the development of humanized mouse models. However, the difficulty of immune system engraftment and minimal human immune cell infiltration into implanted xenografts are major challenges. We have developed an improved method for generating mismatched humanized mice (mHM), using a dual infusion of human HSPCs and MSCs, isolated from cord blood and expanded in vitro. Engraftment with both HSPCs and MSCs produces mice with almost twice the percentage of human immune cells in their bone marrow, compared to mice engrafted with HSPCs alone, and yields 9- to 38-fold higher levels of mature peripheral human immune cells. We identified a peripheral mHM blood human B cell threshold that predicts an optimal degree of mouse bone marrow humanization. When head and neck squamous cell carcinoma (HNSCC) tumors are implanted on the flanks of HSPC-MSC engrafted mice, human T cells, B cells, and macrophages infiltrate the stroma of these tumors at 2- to 8-fold higher ratios. In dually HSPC-

MSC engrafted mice we also more frequently observed additional types of immune cells, including regulatory T cells, cytotoxic T cells, and MDSCs. Higher humanization was associated with in vivo response to immune-directed therapy. The complex immune environment arising in tumors from dually HSPC-MSC engrafted mice better resembles that of the originating patient's tumor, suggesting an enhanced capability to accurately recapitulate a human TME.

Narang, H., et al. (2015). "Effect of proton and gamma irradiation on human lung carcinoma cells: Gene expression, cell cycle, cell death, epithelial-mesenchymal transition and cancer-stem cell trait as biological end points." Mutat Res **780**: 35-46.

Proton beam therapy is a cutting edge modality over conventional gamma radiotherapy because of its physical dose deposition advantage. However, not much is known about its biological effects vis-a-vis gamma irradiation. Here we investigated the effect of proton- and gamma- irradiation on cell cycle, death, epithelial-mesenchymal transition (EMT) and "stemness" in human non-small cell lung carcinoma cells (A549). Proton beam (3MeV) was two times more cytotoxic than gamma radiation and induced higher and longer cell cycle arrest. At equivalent doses, numbers of genes responsive to proton irradiation were ten times higher than those responsive to gamma irradiation. At equitoxic doses, the proton-irradiated cells had reduced cell adhesion and migration ability as compared to the gamma-irradiated cells. It was also more effective in reducing population of Cancer Stem Cell (CSC) like cells as revealed by aldehyde dehydrogenase activity and surface phenotyping by CD44(+), a CSC marker. These results can have significant implications for proton therapy in the context of suppression of molecular and cellular processes that are fundamental to tumor expansion.

Ning, X., et al. (2018). "Ectopic expression of miR-147 inhibits stem cell marker and epithelial-mesenchymal transition (EMT)-related protein expression in colon cancer cells." Oncol Res.

Colon cancer is one of the most common cancers in the world. Epithelial-to-mesenchymal transition (EMT) is a crucial step in tumor progression and also involves in the acquisition of stem cell-like properties. Some miRNAs have been shown to function as either tumor suppressors or oncogenes in colon cancer. Here, we investigated the role of miR-147 in the regulation of stem cell-like traits of colon cancer cells. We observed that miR-147 was down-regulated in several colon cancer cell lines and overexpressed miR-147 decreased the expression of cancer stem cell (CSC) markers OCT4, SOX2 and NANOG in colon cancer cells (HCT116, SW480). Besides that, overexpressed miR-147 inhibited EMT by increasing the expression of epithelial markers Ecadherin and alpha-catenin while decreasing the expression of mesenchymal markers fibronectin and vimentin. Moreover, activation of EMT by TGF-beta1 treatment counteracted the inhibitive effect of miR-147 on the expression of CSC markers OCT4, SOX2 and NANOG significantly, supporting that overexpressed miR-147 inhibited stem cell-like traits by suppressing EMT in colon cancer. In addition, we found that overexpressed miR-147 down-regulated the expression of beta-Catenin, c-myc and Survivin which were related to Wnt/beta-Catenin pathway. Moreover, treatment with Wnt/beta-Catenin pathway activator Licl in miR-147 mimic transfected cells attenuated the inhibitive effect of miR-147 mimic on EMT and stem cell-like traits of colon cancer cells, indicating that ectopic expression of miR-147 inhibited stem cell-like traits in colon cancer cells through suppressing EMT via the Wnt/beta-Catenin pathway. In summary, our present study highlighted the crucial role of miR-147 in the inhibition of stem cell-like traits of colon cancer cells and indicated that miR-147 could be a promising therapeutic target for colon cancer treatment.

Pakravan, K., et al. (2017). "MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses in vitro angiogenesis through modulating the mTOR/HIF-1alpha/VEGF signaling axis in breast cancer cells." Cell Oncol (Dordr) **40**(5): 457-470.

BACKGROUND: Human mesenchymal stem cells (MSCs) have been shown to be involved in the formation and modulation of tumor stroma and in interacting with tumor cells, partly through their secretome. Exosomes are nano-sized intraluminal multi-vesicular bodies secreted by most types of cells and have been found to mediate intercellular communication through the transfer of genetic information via coding and non-coding RNAs to recipient cells. Since exosomes are considered as protective and enriched sources of shuttle microRNAs (miRNAs), we hypothesized that exosomal transfer of miRNAs from MSCs may affect tumor cell behavior, particularly angiogenesis. METHODS: Exosomes derived from MSCs were isolated and characterized by scanning electron microscopy analyses, dynamic light scattering measurements, and Western blotting. Fold changes in miR-100 expression levels were calculated in exosomes and their corresponding donor cells by qRT-PCR. The effects of exosomal transfer of miR-100 from MSCs were assessed by qRT-PCR and Western blotting of the mTOR/HIF-1alpha/VEGF signaling axis in breast cancer cells. The quantification of secreted VEGF protein was determined by enzyme-linked immunosorbent assay. The putative paracrine effects of MSC-derived exosomes on tumor

angiogenesis were explored by in vitro angiogenesis assays including endothelial cell proliferation, migration and tube formation assays. RESULTS: We found that MSC-derived exosomes induce a significant and dose-dependent decrease in the expression and secretion of vascular endothelial growth factor (VEGF) through modulating the mTOR/HIF-1alpha signaling axis in breast cancer-derived cells. We also found that miR-100 is enriched in MSC-derived exosomes and that its transfer to breast cancer-derived cells is associated with the down-regulation of VEGF in a time-dependent manner. The putative role of exosomal miR-100 transfer in regulating VEGF expression was substantiated by the ability of anti-miR-100 to rescue the inhibitory effects of MSC-derived exosomes on the expression of VEGF in breast cancer-derived cells. In addition, we found that down-regulation of VEGF mediated by MSC-derived exosomes can affect the vascular behavior of endothelial cells in vitro. CONCLUSIONS: Overall, our findings suggest that exosomal transfer of miR-100 may be a novel mechanism underlying the paracrine effects of MSC-derived exosomes and may provide a means by which these vesicles can modulate vascular responses within the microenvironment of breast cancer cells.

Parascandolo, A., et al. (2017). "Extracellular Superoxide Dismutase Expression in Papillary Thyroid Cancer Mesenchymal Stem/Stromal Cells Modulates Cancer Cell Growth and Migration." Sci Rep **7**: 41416.

Tumor stroma-secreted growth factors, cytokines, and reactive oxygen species (ROS) influence tumor development from early stages to the metastasis phase. Previous studies have demonstrated downregulation of ROS-producing extracellular superoxide dismutase (SOD3) in thyroid cancer cell lines although according to recent data, the expression of SOD3 at physiological levels stimulates normal and cancer cell proliferation. Therefore, to analyze the expression of SOD3 in tumor stroma, we characterized stromal cells from the thyroid. We report mutually exclusive desmoplasia and inflammation in papillary and follicular thyroid cancers and the presence of multipotent mesenchymal stem/stromal cells (MSCs) in non-carcinogenic thyroids and papillary thyroid cancer (PTC). The phenotypic and differentiation characteristics of Thyroid MSCs and PTC MSCs were comparable with bone marrow MSCs. A molecular level analysis showed increased FIBROBLAST ACTIVATING PROTEIN, COLLAGEN 1 TYPE A1, TENASCIN, and SOD3 expression in PTC MSCs compared to Thyroid MSCs, suggesting the presence of MSCs with a fibrotic fingerprint in papillary thyroid cancer tumors and the autocrine-paracrine conversion of SOD3 expression, which was enhanced by cancer cells. Stromal SOD3 had a stimulatory effect on cancer cell growth and an inhibitory effect on cancer cell migration, thus indicating that SOD3 might be a novel player in thyroid tumor stroma.

Patel, S., et al. (2015). "Epigenetic regulators governing cancer stem cells and epithelial-mesenchymal transition in oral squamous cell carcinoma." Curr Stem Cell Res Ther **10**(2): 140-152.

Oral squamous cell carcinoma (OSCC) is amongst the most prevalent form of cancer worldwide with its predominance in the Indian subcontinent due to its etiological behavioral pattern of tobacco consumption. Late diagnosis, low therapeutic response and aggressive metastasis are the foremost confounders accountable for the poor 5 year survival rate of OSCC. These failures are attributed to the existence of "Cancer Stem cell (CSC)" subpopulation within the tumour environment. Quiescence, apoptotic evasion, resistance to DNA damage, abnormal expression of drug transporter pumps and in vivo tumorigenesis are the defining hallmarks of CSC phenotype. These CSCs have been distinguished from the tumor mass by determining the expression patterns of cell surface proteins, specific stemness markers and quantifying the cellular activities such as drug efflux & aldehyde dehydrogenase activity. Hence, it is necessary to understand the underlying mechanisms that regulate the CSC features in tumor development, metastasis and response to chemotherapy. Increasing evidence suggests that majority of malignant cells eventually undergoing Epithelial-Mesenchymal transition (EMT) share many biological characteristics with CSCs. Thus, this review encompasses the functional relevance of CSC and EMT markers in OSCC population with a hope to elucidate the fundamental mechanisms underlying cancer progression and to highlight the most relevant epigenetic mechanisms that contribute to the regulation of CSC features. We further aimed to explore the causal effects of nicotine, a major tobacco carcinogen, on epigenetic mechanisms regulating the OSCC CSCs and EMT markers which unravels the undisputable contribution of tobacco in oral carcinogenesis.

Patel, S. A., et al. (2010). "Mesenchymal stem cells protect breast cancer cells through regulatory T cells: role of mesenchymal stem cell-derived TGF-beta." J Immunol **184**(10): 5885-5894.

Mesenchymal stem cells (MSCs) have been shown to support breast cancer growth. Because MSCs also increase the frequency of regulatory T cells (T (regs)), this study tested the hypothesis that human MSCs, via Tregs, protect breast cancer cells (BCCs) from immune clearance MSCs suppressed the proliferation of PBMCs when the latter were exposed

to gamma-irradiated BCCs. Similarly, MSCs showed significant inhibition of PBMC migration toward BCCs and a corresponding decrease in CXCL12. MSCs also inhibited NK cell and CTL functions, which correlated with reduced numbers of CD8(+) and CD56(+) cells compared with parallel cultures without MSCs. The reduced NK and CTL activities correlated with a decrease in intracellular and secreted granzyme B. To explain these immunosuppressive findings, we compared T (reg) levels after coculture with MSCs and found an approximately 2-fold increase in T (regs), with associated decreases in antitumor Th1 cytokines and increases in Th2 cytokines. MSC-derived TGF-beta1 was largely responsible for the increase in T (regs) based on knockdown studies. In the presence of T (reg) depletion, PBMC proliferation and effector functions were partially restored. Together, these studies show an MSC-mediated increase in T (regs) in cocultures of PBMCs and BCCs. The results could be explained, in part, by the increase in Th2-type cytokines and MSC-generated TGF-beta1. These findings demonstrate immune protection by MSCs to BCCs. The reduction in immune cell proliferation and recruitment mediated by MSCs has implications for treatment of breast cancer with chemotherapy.

Peng, X., et al. (2018). "SOX4 contributes to TGF-beta-induced epithelial-mesenchymal transition and stem cell characteristics of gastric cancer cells." Genes Dis **5**(1): 49-61.

SOX4 is highly expressed in gastric cancer (GC) and is associated with tumor grade, metastasis and prognosis, however the mechanism is not clear. We report herein that SOX4 was upregulated and overexpression of SOX4 was associated with increased expression of the markers of Epithelial-mesenchymal transition (EMT) and stemness in clinic patient samples. In vitro, overexpression of SOX4 promoted the invasion as showed by Transwell assay and stemness of GC cells as assessed by sphere formation assay, which was suppressed by silencing SOX4 with shRNA. Further studies showed that SOX4 up-regulated the expression of EMT transcription factors Twist1, snail1 and zeb1 and stemness transcription factors SOX2 and OCT4, and promoted the nuclear translocation of beta-catenin. Moreover, we revealed that TGF-beta treatment significantly up-regulated the expression of SOX4 and silencing SOX4 reversed TGF-beta induced invasion and sphere formation ability of GC cells. Finally, we showed that SOX4 promoted the lung metastasis and tumor formation ability of gastric cancer cells in nude mice. Our results suggest that SOX4 is a target TGF-beta signaling and mediates TGF-beta-induced EMT and stem cell characteristics of GC cells, revealing a novel role of TGF-beta/SOX4 axis in the regulation of malignant behavior of GC.

Pore, M., et al. (2016). "Cancer Stem Cells, Epithelial to Mesenchymal Markers, and Circulating Tumor Cells in Small Cell Lung Cancer." Clin Lung Cancer **17**(6): 535-542.

BACKGROUND: Small cell lung cancer (SCLC) has a poor prognosis, and even with localized (limited) disease, the 5-year survival has only been around 20%. Elevated levels of circulating tumor cells (CTCs) have been associated with a worse prognosis, and markers of cancer stem cells (CSCs) and epithelial to mesenchymal transition have been associated with increased chemoresistance and metastatic spread in SCLC. PATIENTS AND METHODS: The biopsy specimens of 38 SCLC patients were used for marker evaluation by immunohistochemistry. The markers for CSCs were CD44 and SOX2. The markers for epithelial to mesenchymal transition were E-cadherin, epithelial cell adhesion molecule, cytokeratins 8, 18, and 19, vimentin, and c-MET. Staining was scored as low (weak) or high (strong) intensity for SOX2, epithelial cell adhesion molecule, cytokeratins 8, 18, and 19, and c-MET and using the immunoreactive score for CD44, E-cadherin, and vimentin, expressed as low or high expression. RESULTS: High expression of c-MET (c-MET (H)) and low expression of E-cadherin (E-cad (L)) showed a trend toward a better prognosis (P =.07 and P =.09, respectively). The combination of c-MET (H) and E-cad (L) resulted in significantly better survival (P =.007). The tested markers were not associated with CTCs, although a trend was seen for c-MET (H)E-cad (L) (P =.09) with low CTCs. The CSC markers SOX2 and CD44 were not associated with overall survival in this patient cohort. CONCLUSION: SCLC with a mesenchymal-like phenotype (c-MET (H)E-cad (L)) is associated with longer survival and showed a trend toward lower CTCs.

Qian, H., et al. (2017). "Cancer stemness and metastatic potential of the novel tumor cell line K3: an inner mutated cell of bone marrow-derived mesenchymal stem cells." Oncotarget **8**(24): 39522-39533.

Mesenchymal stem cells (MSCs) transplantation has been used for therapeutic applications in various diseases. Here we report MSCs can malignantly transform in vivo. The novel neoplasm was found on the tail of female rat after injection with male rat bone marrow-derived MSCs (rBM-MSCs) and the new tumor cell line, K3, was isolated from the neoplasm. The K3 cells expressed surface antigens and pluripotent genes similar to those of rBM-MSCs and presented tumor cell features. Moreover, the K3 cells

contained side population cells (SP) like cancer stem cells (CSCs), which might contribute to K3 heterogeneity and tumorigenic capacity. To investigate the metastatic potential of K3 cells, we established the nude mouse models of liver and lung metastases and isolated the corresponding metastatic cell lines K3-F4 and K3-B6. Both K3-F4 and K3-B6 cell lines with higher metastatic potential acquired more mesenchymal and stemness-related features. Epithelial-mesenchymal transition is a potential mechanism of K3-F4 and K3-B6 formation.

Quint, K., et al. (2012). "Pancreatic cancer cells surviving gemcitabine treatment express markers of stem cell differentiation and epithelial-mesenchymal transition." Int J Oncol **41**(6): 2093-2102.

Objective response rates to standard chemotherapeutic regimens remain low in pancreatic cancer. Subpopulations of cells have been identified in various solid tumors which express stem cell-associated markers and are associated with increased resistance against radiochemotherapy. We investigated the expression of stem cell genes and markers of epithelial-mesenchymal transition in pancreatic cancer cells that survived high concentrations of gemcitabine treatment. Capan-1 and Panc-1 cells were continuously incubated with 1 and 10 microM gemcitabine. Surviving cells were collected after 1, 3 and 6 days. Expression of PDX-1, SHH, CD24, CD44, CD133, EpCAM, CBX7, OCT4, SNAIL, SLUG, TWIST, Ki-67, E-cadherin, beta-catenin and vimentin were quantified by qPCR or immunocytochemistry. Migration was assessed by woundhealing assay. SHH was knocked down using RNA interference. Five primary pancreatic cancer cell lines were used to validate the qPCR results. All investigated genes were upregulated after 6 days of gemcitabine incubation. Highest relative expression levels were observed for OCT4 (13.4-fold), CD24 (47.3-fold) and EpCAM (15.9-fold) in Capan-1 and PDX-1 (13.3fold), SHH (24.1-fold), CD44 (17.4-fold), CD133 (20.2-fold) and SLUG (15.2-fold) in Panc-1 cells. Distinct upregulation patterns were observed in the primary cells. Migration was increased in Panc-1 cells and changes in the expression of E-cadherin and beta-catenin were typical of epithelial-mesenchymal transition in both cell lines. SHH knockdown reduced IC (50) from 30.1 to 27.6 nM in Capan-1 while it strongly inhibited proli-feration in Panc-1 cells. Cells surviving high-dose gemcitabine treatment express increased levels of stem cell genes, show characteristics associated with epithelial-mesenchymal transition and retain their proliferative capacity.

Rahn, S., et al. (2018). "Diabetes as risk factor for pancreatic cancer: Hyperglycemia promotes epithelial-mesenchymal-transition and stem cell properties in pancreatic ductal epithelial cells." Cancer Lett **415**: 129-150.

Type 2 diabetes mellitus (T2DM) is associated with hyperglycemia and a risk to develop pancreatic ductal adenocarcinoma (PDAC), one of the most fatal malignancies. Cancer stem cells (CSC) are essential for initiation and maintenance of tumors, and acquisition of CSC-features is linked to epithelial-mesenchymal-transition (EMT). The present study investigated whether hyperglycemia promotes EMT and CSC-features in premalignant and malignant pancreatic ductal epithelial cells (PDEC). Under normoglycemia (5 mM d-glucose), Panc1 PDAC cells but not premalignant H6c7-kras cells exhibited a mesenchymal phenotype along with pronounced colony formation. While hyperglycemia (25 mM d-glucose) did not impact the mesenchymal phenotype of Panc1 cells, CSC-properties were aggravated exemplified by increased Nanog expression and Nanog-dependent formation of holo- and meroclones. In H6c7-kras cells, high glucose increased secretion of Transforming-Growth-Factor-beta1 (TGF-beta1) as well as TGF-beta1 signaling, and in a TGF-beta1-dependent manner reduced E-cadherin expression, increased Nestin expression and number of meroclones. Finally, reduced E-cadherin expression was detected in pancreatic ducts of hyperglycemic but not normoglycemic mice. These data suggest that hyperglycemia promotes the acquisition of mesenchymal and CSC-properties in PDEC by activating TGF-beta signaling and might explain how T2DM facilitates pancreatic tumorigenesis.

Rasanen, K. and M. Herlyn (2012). "Paracrine signaling between carcinoma cells and mesenchymal stem cells generates cancer stem cell niche via epithelial-mesenchymal transition." Cancer Discov **2**(9): 775-777.

Li and colleagues present data that cancer cell-derived interleukin-1 induces prostaglandin E (2) and cytokine secretion in mesenchymal stem cells (MSC) to activate beta-catenin signaling in the cancer cell. This paracrine signaling between carcinoma cells and MSC leads to the creation of a cancer stem cell niche via epithelial-mesenchymal transition.

Reagan, M. R., et al. (2012). "Stem Cell Implants for Cancer Therapy: TRAIL-Expressing Mesenchymal Stem Cells Target Cancer Cells In Situ." J Breast Cancer **15**(3): 273-282.

PURPOSE: Tumor-specific delivery of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), an apoptosis-inducing peptide, at effective doses remains challenging. Herein we demonstrate the utility of a scaffold-based delivery system for

sustained therapeutic cell release that capitalizes on the tumor-homing properties of mesenchymal stem cells (MSCs) and their ability to express genetically-introduced therapeutic genes. METHODS: Implants were formed from porous, biocompatible silk scaffolds seeded with full length TRAIL-expressing MSCs (FLT-MSCs). under a doxycycline inducible promoter. In vitro studies with FLT-MSCs demonstrated TRAIL expression and antitumor effects on breast cancer cells. Next, FLT-MSCs were administered to mice using three administration routes (mammary fat pad co-injections, tail vein injections, and subcutaneous implantation on scaffolds). RESULTS: In vitro cell-specific bioluminescent imaging measured tumor cell specific growth in the presence of stromal cells and demonstrated FLT-MSC inhibition of breast cancer growth. FLT-MSC implants successfully decreased bone and lung metastasis, whereas liver metastasis decreased only with tail vein and co-injection administration routes. Average tumor burden was decreased when doxycycline was used to induce TRAIL expression for co-injection and scaffold groups, as compared to controls with no induced TRAIL expression. CONCLUSION: This implant-based therapeutic delivery system is an effective and completely novel method of anticancer therapy and holds great potential for clinical applications.

Ren, D., et al. (2014). "Double-negative feedback loop between ZEB2 and miR-145 regulates epithelial-mesenchymal transition and stem cell properties in prostate cancer cells." Cell Tissue Res **358**(3): 763-778.

The invasion and metastasis of tumors are triggered by an epithelial to mesenchymal transition (EMT), which is regulated by microRNAs (miRNAs). EMT also promotes malignant tumor progression and the maintenance of the stem cell property, which endows cancer cells with the capabilities of self-renewal and immortalized proliferation. The transcriptional repressor zinc-finger E-box binding homeobox 2 (ZEB2), as an EMT activator, might be an important promoter of metastasis in some tumors. Here, we report that ZEB2 directly represses the transcription of miR-145, which is a strong repressor of EMT. In turn, ZEB2 is also a direct target of miR-145. Further, our findings show that the downregulation of ZEB2 not only represses invasion, migration, EMT, and the stemness of prostate cancer (PCa) cells, but also suppresses the capability of PC-3 cells to invade bone in vivo. Importantly, the expression level of ZEB2 as revealed by immunohistochemical analysis is positively correlated to bone metastasis, the serum free PSA level, the total PSA level, and the Gleason score in PCa patients and is negatively correlated with miR-145 expression in primary PCa specimens. Thus, our findings demonstrate a double-negative feedback loop between ZEB2 and miR-145 and indicate that the ZEB2/miR-145 double-negative feedback loop plays a significant role in the control of EMT and stem cell properties during the bone metastasis of PCa cells. These results suggest that the double-negative feedback loop between ZEB2 and miR-145 contributes to PCa progression and metastasis and might have therapeutic relevance for the bone metastasis of PCa.

Reza, A. M., et al. (2016). "Human adipose mesenchymal stem cell-derived exosomal-miRNAs are critical factors for inducing anti-proliferation signalling to A2780 and SKOV-3 ovarian cancer cells." Sci Rep **6**: 38498.

An enigmatic question exists concerning the pro- or anti-cancer status of mesenchymal stem cells (MSCs). Despite growing interest, this question remains unanswered, and the debate became intensified with new evidences backing each side. Here, we showed that human adipose MSC (hAMSC)-derived conditioned medium (CM) exhibited inhibitory effects on A2780 human ovarian cancer cells by blocking the cell cycle, and activating mitochondria-mediated apoptosis signalling. Explicitly, we demonstrated that exosomes, an important biological component of hAMSC-CM, could restrain proliferation, wound-repair and colony formation ability of A2780 and SKOV-3 cancer cells. Furthermore, hAMSC-CM-derived exosomes induced apoptosis signalling by upregulating different pro-apoptotic signalling molecules, such as BAX, CASP9, and CASP3, as well as downregulating the anti-apoptotic protein BCL2. More specifically, cancer cells exhibited reduced viability following fresh or protease-digested exosome treatment; however, treatment with RNase-digested exosomes could not inhibit the proliferation of cancer cells. Additionally, sequencing of exosomal RNAs revealed a rich population of microRNAs (miRNAs), which exhibit anti-cancer activities by targeting different molecules associated with cancer survival. Our findings indicated that exosomal miRNAs are important players involved in the inhibitory influence of hAMSC-CM towards ovarian cancer cells. Therefore, we believe that these comprehensive results will provide advances concerning ovarian cancer research and treatment.

Ridge, S. M., et al. (2018). "Secreted factors from metastatic prostate cancer cells stimulate mesenchymal stem cell transition to a pro-tumourigenic 'activated' state that enhances prostate cancer cell migration." Int J Cancer **142**(10): 2056-2067.

Mesenchymal stem cells (MSCs) are a heterogeneous population of multipotent cells that are capable of differentiating into osteocytes,

chondrocytes and adipocytes. Recently, MSCs have been found to home to the tumour site and engraft in the tumour stroma. However, it is not yet known whether they have a tumour promoting or suppressive function. We investigated the interaction between prostate cancer cell lines 22Rv1, DU145 and PC3, and bone marrow-derived MSCs. MSCs were 'educated' for extended periods in prostate cancer cell conditioned media and PC3-educated MSCs were found to be the most responsive with a secretory profile rich in pro-inflammatory cytokines. PC3-educated MSCs secreted increased osteopontin (OPN), interleukin-8 (IL-8) and fibroblast growth factor-2 (FGF-2) and decreased soluble fms-like tyrosine kinase-1 (sFlt-1) compared to untreated MSCs. PC3-educated MSCs showed a reduced migration and proliferation capacity that was dependent on exposure to PC3-conditioned medium. Vimentin and alpha-smooth muscle actin (alphaSMA) expression was decreased in PC3-educated MSCs compared to untreated MSCs. PC3 and DU145 education of healthy donor and prostate cancer patient-derived MSCs led to a reduced proportion of FAP+ alphaSMA+ cells contrary to characteristics commonly associated with cancer associated fibroblasts (CAFs). The migration of PC3 cells was increased toward both PC3-educated and DU145-educated MSCs compared to untreated MSCs, while DU145 migration was only enhanced toward patient-derived MSCs. In summary, MSCs developed an altered phenotype in response to prostate cancer conditioned medium which resulted in increased secretion of pro-inflammatory cytokines, modified functional activity and the chemoattraction of prostate cancer cells.

Riggi, N., et al. (2010). "EWS-FLI-1 modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming toward Ewing sarcoma cancer stem cells." Genes Dev **24**(9): 916-932.

Cancer stem cells (CSCs) display plasticity and self-renewal properties reminiscent of normal tissue stem cells, but the events responsible for their emergence remain obscure. We recently identified CSCs in Ewing sarcoma family tumors (ESFTs) and showed that they retain mesenchymal stem cell (MSC) plasticity. In the present study, we addressed the mechanisms that underlie ESFT CSC development. We show that the EWS-FLI-1 fusion gene, associated with 85%-90% of ESFTs and believed to initiate their pathogenesis, induces expression of the embryonic stem cell (ESC) genes OCT4, SOX2, and NANOG in human pediatric MSCs (hpMSCs) but not in their adult counterparts. Moreover, under appropriate culture conditions, hpMSCs expressing EWS-FLI-1 generate a cell subpopulation displaying ESFT CSC features in vitro. We further demonstrate that induction of the ESFT CSC phenotype is the result of the combined effect of EWS-FLI-1 on its target gene expression and repression of microRNA-145 (miRNA145) promoter activity. Finally, we provide evidence that EWS-FLI-1 and miRNA-145 function in a mutually repressive feedback loop and identify their common target gene, SOX2, in addition to miRNA145 itself, as key players in ESFT cell differentiation and tumorigenicity. Our observations provide insight for the first time into the mechanisms whereby a single oncogene can reprogram primary cells to display a CSC phenotype.

Rizvanov, A. A., et al. (2010). "Interaction and self-organization of human mesenchymal stem cells and neuro-blastoma SH-SY5Y cells under co-culture conditions: A novel system for modeling cancer cell micro-environment." Eur J Pharm Biopharm **76**(2): 253-259.

The common drawback of many in vitro cell culture systems is the absence of appropriate micro-environment, which is formed by the combination of factors such as cell-cell contacts, extracellular matrix and paracrine regulation. Micro-environmental factors in a tumor tissue can influence physiological status of the cancer cells and their susceptibility to anticancer therapies. Interaction of cancer cells with their micro-environment and regional stem cells, therefore, is of particular interest. Development of in vitro systems which allow more accurate modeling of complex relations occurring in real tumor environments can increase efficiency of preclinical assays for screening anticancer drugs. The aim of this work was to study interactions between human mesenchymal stem cells (MSCs) and neuro-blastoma cancer SH-SY5Y cells under co-culture conditions on different coated surfaces to determine the effect of co-existence of cancer and stem cells on each cellular population under various stress conditions. We developed an efficient in vitro system for studying individual cancer and stem cell populations during co-culture using differential live fluorescent membrane labeling, and demonstrated self-organization of cancer and stem cells during co-culture on various coated surfaces. Our findings support the evidence that cancer and stem cell interactions play important roles in cellular behavior of cancer cells. These properties can be used in different fields of cancer research, tissue engineering and biotechnology.

Saito, S., et al. (2011). "Use of BAC array CGH for evaluation of chromosomal stability of clinically used human mesenchymal stem cells and of cancer cell lines." Hum Cell **24**(1): 2-8.

Array-based comparative genomic hybridization (aCGH) using bacterial artificial chromosomes (BAC) is a powerful method to analyze DNA copy number

aberrations of the entire human genome. In fact, CGH and aCGH have revealed various DNA copy number aberrations in numerous cancer cells and cancer cell lines examined so far. In this report, BAC aCGH was applied to evaluate the stability or instability of cell lines. Established cell lines have greatly contributed to advancements in not only biology but also medical science. However, cell lines have serious problems, such as alteration of biological properties during long-term cultivation. Firstly, we investigated two cancer cell lines, HeLa and Caco-2. HeLa cells, established from a cervical cancer, showed significantly increased DNA copy number alterations with passage time. Caco-2 cells, established from a colon cancer, showed no remarkable differences under various culture conditions. These results indicate that BAC aCGH can be used for the evaluation and validation of genomic stability of cultured cells. Secondly, BAC aCGH was applied to evaluate and validate the genomic stabilities of three patient's mesenchymal stem cells (MSCs), which were already used for their treatments. These three MSCs showed no significant differences in DNA copy number aberrations over their entire chromosomal regions. Therefore, BAC aCGH is highly recommended for use for a quality check of various cells before using them for any kind of biological investigation or clinical application.

Sato, F., et al. (2015). "EGFR inhibitors prevent induction of cancer stem-like cells in esophageal squamous cell carcinoma by suppressing epithelial-mesenchymal transition." Cancer Biol Ther **16**(6): 933-940.

There exists a highly tumorigenic subset of esophageal squamous cell carcinoma (ESCC) cells defined by high expression of CD44. A novel therapy targeting these cancer stem-like cells (CSCs) is needed to improve prognosis of ESCC. CSCs of ESCC have a mesenchymal phenotype and epithelial-mesenchymal transition (EMT) is critical to enrich and maintain CSCs. EGFR, frequently overexpressed in ESCC, has pivotal roles in EMT induced by TGF-beta in invasive fronts. Thus, EMT in invasive fronts of ESCC might be important for CSCs and EGFR could be a target of a novel therapy eliminating CSCs. However, effects of EGFR inhibitors on CSCs in ESCC have not been fully examined. EGFR inhibitors, erlotinib and cetuximab, significantly suppressed enrichment of CSCs via TGF-beta1-mediated EMT. Importantly, EGFR inhibitors sharply suppressed ZEB1 that is essential for EMT in ESCC. Further, EGFR inhibitors activated Notch1 and Notch3, leading to squamous cell differentiation. EGFR inhibition may suppress expression of ZEB1 and induce differentiation, thereby blocking EMT-mediated enrichment of CSCs. In organotypic 3D culture, a form of human tissue engineering, tumor cells in invasive nests showed high expression of CD44. Erlotinib significantly blocked invasion into the matrix and CD44 high expressing CSCs were markedly suppressed by erlotinib in organotypic 3D culture. In conclusion, EMT is a critical process for generation of CSCs and the invasive front of ESCC, where EMT occurs, might form a CSC niche in ESCC. EGFR inhibitors could suppress EMT in invasive fronts and be one therapeutic option targeting against generation of CSCs in ESCC.

Sawada, R., et al. (2006). "Changes in expression of genes related to cell proliferation in human mesenchymal stem cells during in vitro culture in comparison with cancer cells." J Artif Organs **9**(3): 179-184.

We investigated the expression levels of several genes related to cell proliferation in human mesenchymal stem cells (hMSCs) during in vitro culture for use in clinical applications. In this study, we focused on the relationship between hMSC proliferation and transforming growth factor beta (TGFbeta) signaling during in vitro culture. The proliferation rate of hMSCs gradually decreased and marked changes in hMSC morphology were not observed in 3 months of in vitro culture. The mRNA expressions of TGFbeta1, TGFbeta2, and TGFbeta receptor type I (TGFbetaRI) in hMSCs increased with the length of cell culture. There had been no change in the TGFbeta3, TGFbetaRII, and TGFbetaRIII mRNA expressions by the 12th passage from the primary culture (at about 3 months). The mRNA expression of Smad3 increased, but those of c-myc and nucleostemin decreased with the length of hMSC in vitro culture. In addition, the expression profiles of the genes that regulate cellular proliferation in hMSCs were significantly different from those of cancer cells. In conclusion, hMSCs derived from bone marrow seldom underwent spontaneous transformation during 1-2 months of in vitro culture for use in clinical applications. In hMSCs as well as in epithelial cells, growth might be controlled by the TGFbeta family signaling.

Scherzad, A., et al. (2015). "Human mesenchymal stem cells enhance cancer cell proliferation via IL-6 secretion and activation of ERK1/2." Int J Oncol **47**(1): 391-397.

Human mesenchymal stem cells (hMSC) are frequently used in tissue engineering. Due to their strong tumor tropism, hMSC seem to be a promising vehicle for anticancer drugs. However, interactions between hMSC and cancer are ambiguous. Particularly the cytokines and growth factors seem to play an important role in cancer progression and metastasis.

The present study evaluated the effects of hMSC on head and neck squamous cell carcinoma (HNSCC) cell lines (FaDu and HLaC78) in vitro. hMSC released several cytokines and growth factors. FaDu and HLaC78 showed a significant enhancement of cell proliferation after cultivation with hMSC-conditioned medium as compared to control. This proliferation improvement was inhibited by the addition of anti-IL-6. The western blot showed an activation of Erk1/2 in FaDu and HLaC78 by hMSC-conditioned medium. HNSCC cell lines expressed EGFR. The current study confirms the importance of cytokines secreted by hMSC in cancer biology. Especially IL-6 seems to play a key role in cancer progression. Thus, the use of hMSC as a carrier for cancer therapy must be discussed critically. Future studies should evaluate the possibility of generating genetically engineered hMSC with, for example, the absence of IL-6 secretion.

Seino, S., et al. (2016). "CD44(high) /ALDH1(high) head and neck squamous cell carcinoma cells exhibit mesenchymal characteristics and GSK3beta-dependent cancer stem cell properties." J Oral Pathol Med **45**(3): 180-188.

BACKGROUND: CD44 and aldehyde dehydrogenase 1 (ALDH1) have been shown to be useful markers for identification of cancer stem cells (CSCs). We previously reported that glycogen synthase kinase 3beta (GSK3beta) is involved in regulation of the self-renewal ability of head and neck squamous cell carcinoma (HNSCC) CSCs. The purpose of the present study was to clarify the role of GSK3beta in CD44(high) /ALDH1(high) HNSCC cells. METHODS: Cells with greater expression of CD44 and higher ALDH1 enzymatic activity were FACS sorted from the OM-1 HNSCC cell line. The self-renewal ability of CD44(high) /ALDH1(high) cells was then examined using a tumor sphere formation assay. mRNA expressions of the stem cell markers Sox2, Oct4, and Nanog, as well as GSK3beta were evaluated by real-time RT-PCR. RESULTS: CD44(high) /ALDH1(high) cells exhibited higher tumor sphere forming ability and increased expression of stem cell markers as compared with CD44(high) /ALDH1(low) cells. Interestingly, spindle-shaped cells positive for vimentin were found in the CD44(high) /ALDH1(high) but not the CD44(high) /ALDH1(low) cell population. In addition, the ALDH1 activity and sphere forming ability of CD44(high) /ALDH1(high) cells was significantly inhibited by GSK3beta knockdown. On the other hand, CD44(high) /ALDH1(low) cells exhibited high epidermal growth factor receptor (EGFR) expression and increased cell growth. CONCLUSIONS: Our results show that GSK3beta plays a major role in maintenance of stemness of CD44(high) /ALDH1(high) HNSCC cells. Additionally, they indicate a close relationship between CSC and mesenchymal characteristics in HNSCC.

Shintani, Y., et al. (2013). "Pulmonary fibroblasts induce epithelial mesenchymal transition and some characteristics of stem cells in non-small cell lung cancer." Ann Thorac Surg **96**(2): 425-433.

BACKGROUND: Fibroblasts are key components of the tumor microenvironment. The purpose of this study was to clarify the role of fibroblasts in tumor progression in non-small cell lung cancer (NSCLC). METHODS: Fibroblasts isolated from surgical exploration were co-cultured with human lung adenocarcinoma cell lines. We defined fibroblasts obtained from tumors as cancer associated fibroblasts (CAFs) and those from normal lung tissue as lung normal fibroblasts (LNFs). RESULTS: Expression levels of myofibroblast markers were higher in CAFs than LNFs within 5 passages in the absence of continuing interaction with carcinoma cells. Thus, we used at least 2 pairs of these CAFs and LNFs in the following experiments; conditioned medium (CM) from fibroblast-induced epithelial mesenchymal transition and acquisition of cancer stem cell-like qualities in lung cancer cells (A549 and NCI-H358), indicating that CM from fibroblasts was biologically active. Furthermore, the concentration of the transforming growth factor (TGF)-beta1 was higher in CM from CAFs as compared with that from LNFs, and phenotypic changes of cancer cells by CM from CAFs were greater than those induced by CM from LNFs. These CAF-induced changes were inhibited by addition of the TGF-beta inhibitor SB431542. Subcutaneous co-injection of lung cancer cells and CAFs in mice enhanced tumor growth when compared with cancer cells alone, which was attenuated by administration of SB431542. CONCLUSIONS: Fibroblasts were associated with increased malignant potential and the acquisition of stem cell-like properties in NSCLC tumors. Targeting CAFs as a therapeutic strategy against cancer is an intriguing concept that would benefit from further study.

Sullivan, K. E., et al. (2017). "The stem cell/cancer stem cell marker ALDH1A3 regulates the expression of the survival factor tissue transglutaminase, in mesenchymal glioma stem cells." Oncotarget **8**(14): 22325-22343.

Tissue transglutaminase (tTG), a dual-function enzyme with GTP-binding and acyltransferase activities, has been implicated in the survival and chemotherapy resistance of aggressive cancer cells and cancer stem cells, including glioma stem cells (GSCs). Using a model system comprising two distinct subtypes of GSCs referred to as proneural (PN) and

mesenchymal (MES), we find that the phenotypically aggressive and radiation therapy-resistant MES GSCs exclusively express tTG relative to PN GSCs. As such, the self-renewal, proliferation, and survival of these cells was sensitive to treatment with tTG inhibitors, with a benefit being observed when combined with the standard of care for high grade gliomas (i.e. radiation or temozolomide). Efforts to understand the molecular drivers of tTG expression in MES GSCs revealed an unexpected link between tTG and a common marker for stem cells and cancer stem cells, Aldehyde dehydrogenase 1A3 (ALDH1A3). ALDH1A3, as well as other members of the ALDH1 subfamily, can function in cells as a retinaldehyde dehydrogenase to generate retinoic acid (RA) from retinal. We show that the enzymatic activity of ALDH1A3 and its product, RA, are necessary for the observed expression of tTG in MES GSCs. Additionally, the ectopic expression of ALDH1A3 in PN GSCs is sufficient to induce the expression of tTG in these cells, further demonstrating a causal link between ALDH1A3 and tTG. Together, these findings ascribe a novel function for ALDH1A3 in an aggressive GSC phenotype via the up-regulation of tTG, and suggest the potential for a similar role by ALDH1 family members across cancer types.

Sun, S., et al. (2014). "[Effect of interleukin-18 gene modified human umbilical cord mesenchymal stem cells on proliferation of breast cancer cell]." Zhonghua Yi Xue Za Zhi **94**(26): 2013-2017.

OBJECTIVE: To establish human umbilical cord mesenchymal stem cells (HUMSCs) strain transfected with interleukin-18 (IL-18) gene and examine its effects on the proliferation of breast cancer cell (MCF-7). METHODS: HUMSCs were isolated and cultured. And the lentivirus-IL-18 vector containing human IL-18 gene was constructed and transfected into HUMSCs. The expressions of IL-18 gene mRNA and protein were detected by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and Western blot. After co-culturing for 1, 3, 5 days, Transwell and cell counting kit-8 (CCK-8) assays were performed on MCF-7 to plot the cell growth curve. RESULTS: IL-18-HUMSCs could stably express of IL-18 gene and inhibit the proliferation of breast cancer cells. The IL-18 mRNA relative expression amounts were 1.40 +/- 0.21 for experimental group (IL-18-HUMSCs), 0.59 +/- 0.09 for negative control group (NV-HUMSCs) and 0.71 +/- 0.05 for blank control group (HUMSCs). As compared with control group, the difference was statistically significant ($F = 31.81$, $P = 0.001$). The relative expressions of IL-18 protein were 1.54 +/- 0.27 for experimental group (IL-18-HUMSCs), 0.57 +/- 0.04 for negative control group (NV-HUMSCs) and 0.59 +/- 0.23 for blank control group (HUMSCs). As compared with control group, the difference was statistically significant ($F = 22.32$, $P = 0.002$). After co-culturing for 5 days, the cellular proliferation was significantly inhibited. CONCLUSION: IL-18 gene has been successfully transfected into HUMSCs and has a stable expression. And IL-18-HUMSCs can effectively inhibit the proliferation of breast cancer cells in vitro.

Takigawa, H., et al. (2017). "Mesenchymal Stem Cells Induce Epithelial to Mesenchymal Transition in Colon Cancer Cells through Direct Cell-to-Cell Contact." Neoplasia **19**(5): 429-438.

We previously reported that in an orthotopic nude mouse model of human colon cancer, bone marrow-derived mesenchymal stem cells (MSCs) migrated to the tumor stroma and promoted tumor growth and metastasis. Here, we evaluated the proliferation and migration ability of cancer cells cocultured with MSCs to elucidate the mechanism of interaction between cancer cells and MSCs. Proliferation and migration of cancer cells increased following direct coculture with MSCs but not following indirect coculture. Thus, we hypothesized that direct contact between cancer cells and MSCs was important. We performed a microarray analysis of gene expression in KM12SM colon cancer cells directly cocultured with MSCs. Expression of epithelial-mesenchymal transition (EMT)-related genes such as fibronectin (FN), SPARC, and galectin 1 was increased by direct coculture with MSCs. We also confirmed the upregulation of these genes with real-time polymerase chain reaction. Gene expression was not elevated in cancer cells indirectly cocultured with MSCs. Among the EMT-related genes upregulated by direct coculture with MSCs, we examined the immune localization of FN, a well-known EMT marker. In coculture assay in chamber slides, expression of FN was seen only at the edges of cancer clusters where cancer cells directly contacted MSCs. FN expression in cancer cells increased at the tumor periphery and invasive edge in orthotopic nude mouse tumors and human colon cancer tissues. These results suggest that MSCs induce EMT in colon cancer cells via direct cell-to-cell contact and may play an important role in colon cancer metastasis.

Tang, Y. J., et al. (2011). "[Prostate cancer cell line PC-3 conditioned medium promotes proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells]." Zhonghua Nan Ke Xue **17**(3): 229-236.

OBJECTIVE: To investigate the effects of prostate cancer cell line PC-3 conditioned medium (PC- 3-CM) on the proliferation and osteogenic differentiation of human bone marrow human basalis mesenchymal stem cells (hBMSCs). METHODS:

hBMSCs were isolated and culture-expanded by density gradient centrifugation from normal volunteers. PC-3 cells were cultured till the time of logarithmic growth and then transferred to a fresh medium, which, after 24 hours of incubation, was collected as PC-3-CM. Passage 3 hBMSCs were cultured in the fresh medium alone (the control group) or that with 50% PC-3-CM (the experimental group), and the effect of PC-3-CM on the proliferation activity of the hBMSCs was detected by WST-8 assay. Based on the types of medium used, the hBMSCs were divided into Groups I (control), II (50% PC-3-CM), III (osteoblast inducer) and IV (osteoblast inducer containing 50% PC-3 CM). The effects of PC-3-CM on the osteoblastic differentiation of the hBMSCs were determined by ALP staining, ALP activity detection, Von Kossa staining, and calcium quantitation. RESULTS: At 1, 3, 5 and 7 days of incubation, the absorbance values of the cells in the experimental group were 0.4370 +/- 0.0285, 0.7980 +/- 0.0213, 1.9090 +/- 0.0612 and 2.3023 +/- 0.0610, and those in the control group were 0.4060 +/- 0.0223, 0.6643 +/- 0.0075, 1.3727 +/- 0.0176 and 1.7947 +/- 0.0115, respectively, with significant differences between the two groups ($P < 0.01$) except on day 1 ($P > 0.05$). The positive rate and intensity of ALP staining were gradually increased in the four groups, with the ALP activities of 0.29 +/- 0.03, 1.30 +/- 0.03, 2.13 +/- 0.08, and 3.80 +/- 0.03, respectively ($P < 0.01$), and so was the intensity of Von Kossa staining, with the calcium depositions of 0.04 +/- 0.01, 0.44 +/- 0.05, 0.98 +/- 0.03, and 1.27 +/- 0.04, respectively ($P < 0.01$). CONCLUSION: PC-3-CM can promote the proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells.

Tiran, V., et al. (2017). "Primary patient-derived lung adenocarcinoma cell culture challenges the association of cancer stem cells with epithelial-to-mesenchymal transition." Sci Rep **7**(1): 10040.

The cancer stem cell (CSC) and epithelial-to-mesenchymal transition (EMT) models have been closely associated and used to describe both the formation of metastasis and therapy resistance. We established a primary lung cell culture from a patient in a clinically rare and unique situation of primary resistant disease. This culture consisted of two biologically profoundly distinct adenocarcinoma cell subpopulations, which differed phenotypically and genotypically. One subpopulation initiated and sustained in spheroid cell culture (LT22s) whereas the other subpopulation was only capable of growth and proliferation under adherent conditions (LT22a). In contrast to our expectations, LT22s were strongly associated with the epithelial phenotype, and expressed additionally CSC markers ALDH1 and CD133, whereas the LT22a was characterized as mesenchymal with lack of CSC markers. The LT22s cells also demonstrated an invasive behavior and mimicked gland formation. Finally, LT22s were more resistant to Cisplatin than LT22a cells. We demonstrate a primary lung adenocarcinoma cell culture derived from a patient with resistant disease, with epithelial aggressive subpopulation of cells associated with stem cell features and therapy resistance. Our findings challenge the current model associating CSC and disease resistance mainly to mesenchymal cells and may have important clinical implications.

Touboul, C., et al. (2013). "Mesenchymal stem cells enhance ovarian cancer cell infiltration through IL6 secretion in an amniochorionic membrane based 3D model." J Transl Med **11**: 28.

BACKGROUND: The early peritoneal invasion of epithelial ovarian cancer (EOC) by tumoral aggregates presents in ascites is a major concern. The role of the microenvironment seems to be important in this process but the lack of adequate models to study cellular interactions between cancer cells and stromal cells does not allow to uncover the molecular pathways involved. Our goal was to study the interactions between ovarian cancer cells (OCC) and mesenchymal stem cells (MSC) using a 3D model. METHODS: We used millimetric pieces of amniochorionic membrane - referred to as amniotic membrane scaffold (AMS) - to create 3D peritoneal nodules mimicking EOC early invasion. We were able to measure the distribution and the depth of infiltration using confocal microsopy. We extracted MSC from the amniochorionic membrane using the markers CD34-, CD45-, CD73+, CD90+, CD105+ and CD29+ at the Fluorescence Activated Cell Sorting (FACS) analysis. We used transwell and wound healing tests to test OCC migration and invasion in vitro. RESULTS: Here we show that OCC tumors were located in regions rich in MSC (70%). The tumors infiltrated deeper within AMS in regions rich in MSC ($p<0.001$). In vitro tests revealed that higher IL6 secretion in a context of MSC-OCC co-culture could enhance migration and invasion of OCC. After IL6 receptor antagonism, OCC infiltration was significantly decreased, mostly in regions rich in MSCs, indicating that recruitment and tridimensional invasion of OCC was dependent of IL6 secretion. CONCLUSIONS: The use of tridimensional models using AMS could be a useful tool to decipher early molecular events in ovarian cancer metastasis. Cytokine inhibitors interrupting the cross-talk between OCCs and MSCs such as IL6 should be investigated as a new therapeutic approach in ovarian cancer.

Trivanovic, D., et al. (2014). "Characteristics of human adipose mesenchymal stem cells isolated from healthy and cancer affected people and their interactions with human breast cancer cell line MCF-7 in vitro." Cell Biol Int **38**(2): 254-265.

Adipose tissue is an attractive source of mesenchymal stem/stromal cells (MSCs) with potential applications in reconstructive plastic surgery and regenerative medicine. The aim of this study was to characterise human adipose tissue MSCs (ASCs) derived from healthy individuals and cancer patients and to compare their interactions with tumour cells. ASCs were isolated from adipose tissue of healthy donors, breast cancer-adjacent adipose tissue of breast cancer patients and tumour-adjacent adipose tissue of non-breast cancer patients. Their proliferation, differentiation, immunophenotype and gene expression were assessed and effects on the proliferation of human breast cancer cell line MCF-7 compared. ASCs from all sources exhibited similar morphology, proliferative and differentiation potential, showing the characteristic pattern of mesenchymal surface markers expression (CD90, CD105, CD44H, CD73) and the lack of HLA-DR and hematopoietic markers (CD11a, CD33, CD45, Glycophorin-CD235a), but uneven expression of CD34. ASCs also shared a common positive gene expression of HLA-DR, HLA-A, IL-6, TGF-beta and HIF-1, but were negative for HLA-G, while the expression levels of Cox-2 and IDO-1 varied. All ASCs significantly stimulated the proliferation of MCF-7 tumour cells in direct mixed co-cultures and transwell system, although their conditioned media displayed antiproliferative activity. Data obtained showed that ASCs with similar characteristics are easily isolated from various donors and sites of origin, although ASCs could both suppress and favour tumour cells growth, emphasising the importance of cellular context within the microenvironment and pointing to the significance of safety studies to exclude any potential clinical risk of their application in regenerative medicine.

Visciano, C., et al. (2015). "Mast cells induce epithelial-to-mesenchymal transition and stem cell features in human thyroid cancer cells through an IL-8-Akt-Slug pathway." Oncogene **34**(40): 5175-5186.

There is increasing evidence that mast cells (MCs) and their mediators are involved in the remodeling of the tumor microenvironment and promote tumor growth, angiogenesis and metastasis. We have found that an increased density of MCs in thyroid cancer (TC) correlates with enhanced invasiveness. However, the MC-derived factors responsible for this activity and the mechanisms by which they enhance TC invasiveness remain unidentified. Here, we report that MCs, when activated by TC cells, produce soluble factors that induce epithelial-to-mesenchymal transition (EMT) and stemness features of TC cells. We identified CXCL8/interleukin (IL)-8 as the main mediator contained in activated MC conditioned media (CM) capable of inducing both EMT and stemness of TC cells. Mechanistically, MC CM or exogenous IL-8 stimulated Akt phosphorylation and Slug expression in TC cells. The inhibition of the Akt pathway or depletion of the Slug transcription factor by RNA interference, reverted EMT and stemness responses. TC cells stably transfected with exogenous IL-8 underwent EMT, displayed increased stemness and enhanced tumorigenicity with respect to control cells. The analysis of TC surgical specimens by immunohistochemical analysis demonstrated a positive correlation between MC density (Tryptase (+) cells) and stemness features (OCT4 staining). Taken together, our data identify an MC-dependent IL-8-Akt-Slug pathway that sustains EMT/stemness of TC cells. The blockade of this circuit might be exploited for the therapy of advanced TC.

Wamsley, J. J., et al. (2015). "Activin upregulation by NF-kappaB is required to maintain mesenchymal features of cancer stem-like cells in non-small cell lung cancer." Cancer Res **75**(2): 426-435.

Soluble growth factors and cytokines within the tumor microenvironment aid in the induction of the epithelial-to-mesenchymal transition (EMT). Although EMT promotes the development of cancer-initiating cells (CIC), cellular mechanisms by which cancer cells maintain mesenchymal phenotypes remain poorly understood. Work presented here indicates that induction of EMT stimulates non-small cell lung cancer (NSCLC) to secrete soluble factors that function in an autocrine fashion. Using gene expression profiling of all annotated and predicted secreted gene products, we find that NF-kappaB activity is required to upregulate INHBA/Activin, a morphogen in the TGFbeta superfamily. INHBA is capable of inducing and maintaining mesenchymal phenotypes, including the expression of EMT master-switch regulators and self-renewal factors that sustain CIC phenotypes and promote lung metastasis. Our work demonstrates that INHBA mRNA and protein expression are commonly elevated in primary human NSCLC and provide evidence that INHBA is a critical autocrine factor that maintains mesenchymal properties of CICs to promote metastasis in NSCLC.

Zolochevska, O., et al. (2012). "Pigment epithelial-derived factor and melanoma differentiation associated gene-7 cytokine gene therapies delivered by adipose-derived stromal/mesenchymal stem cells are effective in reducing prostate cancer cell growth." Stem Cells Dev **21**(7): 1112-1123.

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

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