



## Cancer Stem Cell Theory Research Literatures

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

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**Key words:** cancer; stem cell; theory; 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.

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

Aguirre, L. A., et al. (2020). "Tumor stem cells fuse with monocytes to form highly invasive tumor-hybrid cells." *Oncoimmunology* **9**(1): 1773204.

The 'cancer cell fusion' theory is controversial due to the lack of methods available to identify hybrid cells and to follow the phenomenon in patients. However, it seems to be one of the best explanations for both the origin and metastasis of primary tumors. Herein, we co-cultured lung cancer stem cells with human monocytes and analyzed the dynamics and properties of tumor-hybrid cells (THC), as well as the molecular mechanisms beneath this fusion process by several techniques: electron-microscopy, karyotyping, CRISPR-Cas9, RNA-seq, immunostaining, signaling blockage, among others. Moreover, mice models were assessed for in vivo characterization of hybrids colonization and invasiveness. Then, the presence of THCs in bloodstream and samples from primary and metastatic lesions were detected by FACS and immunofluorescence protocols, and their correlations

with TNM stages established. Our data indicate that the generation of THCs depends on the expression of CD36 on tumor stem cells and the oxidative state and polarization of monocytes, the latter being strongly influenced by microenvironmental fluctuations. Highly oxidized M2-like monocytes show the strongest affinity to fuse with tumor stem cells. THCs are able to proliferate, colonize and invade organs. THC-specific cell surface signature CD36(+)CD14(+)PANK(+) allows identifying them in matched primary tumor tissues and metastases as well as in bloodstream from patients with lung cancer, thus functioning as a biomarker. THCs levels in circulation correlate with TNM classification. Our results suggest that THCs are involved in both origin and spread of metastatic cells. Furthermore, they might set the bases for future therapies to avoid or eradicate lung cancer metastasis.

Akiba, J., et al. (2020). "Recent Topics Concerning Combined Hepatocellular Cholangiocarcinoma." *Kurume Med J* **66**(1): 29-36.

Combined hepatocellular-cholangiocarcinoma (CHC) is a relatively rare tumor with an incidence range of 1.0-4.7%. CHC is defined as a tumor containing unequivocal, intimately mixed components of both hepatocellular carcinoma and intrahepatic cholangiocarcinoma. The recent development of biochemical methodologies and cancer stem cell theory have paved the way for a clearer understanding of the histogenesis of CHC. The latest edited WHO classification published in 2010 adopted the concept of stem cell/hepatic progenitor cells in the pathological classification of CHC. Although this classification includes novel and unique concepts of histogenesis and facilitates the recognition of CHC, there are several

problems with it in practice. To reduce confusion, an international group of hepatic pathologists, radiologists, surgeons, and clinicians formulated a nomenclature for CHC and issued a consensus article in 2018. In this review article, we discuss the problems with the latest WHO classification and introduce recent topics concerning CHC from pathologic and genetic points of view.

Alladin, A., et al. (2020). "Tracking cells in epithelial acini by light sheet microscopy reveals proximity effects in breast cancer initiation." *Elife* **9**.

Cancer clone evolution takes place within tissue ecosystem habitats. But, how exactly tumors arise from a few malignant cells within an intact epithelium is a central, yet unanswered question. This is mainly due to the inaccessibility of this process to longitudinal imaging together with a lack of systems that model the progression of a fraction of transformed cells within a tissue. Here, we developed a new methodology based on primary mouse mammary epithelial acini, where oncogenes can be switched on in single cells within an otherwise normal epithelial cell layer. We combine this stochastic breast tumor induction model with inverted light-sheet imaging to study single-cell behavior for up to four days and analyze cell fates utilizing a newly developed image-data analysis workflow. The power of this integrated approach is illustrated by us finding that small local clusters of transformed cells form tumors while isolated transformed cells do not.

There are now drugs to treat many types of cancer, but questions still remain around how these diseases start in the first place. Researchers think that tumor growth begins when a single cell suffers damage to certain sites in its DNA that eventually cause it to divide uncontrollably. That damaged cell, and its descendants, go on to form a lump, or tumor. The trouble with proving this theory is that it is hard to watch it happening in real time. Doctors usually only meet people with cancer when their tumors start to cause health problems. By this point, the tumors contain millions of cells. A way to watch the very beginnings of a cancer could reveal risk factors within a tissue that foster the growth of a tumor. But first, researchers need to test their theory about how the disease begins in the first place. One way to do this is to surround a single cancer cell with healthy cells and watch what happens next. To do this, Alladin, Chaible et al. took healthy cells from the breast tissue of mice and grew them in the laboratory into mini-organs called organoids. These organoids share a lot of features with actual mouse breast tissue; they can even make milk if given the right hormones. Once the organoids were ready, Alladin, Chaible et al then started modifying a small number of single cells inside them by switching on genes called oncogenes, which are known to drive

cancer formation in humans. Using fluorescent proteins and a sheet of laser light it was possible to watch what happened to the cells over time. This revealed that, even though all the oncogene-driven single cells received the same signals, not all of them started to divide uncontrollably. In fact, a single modified cell had a low chance of forming a tumor on its own. The more oncogene-driven cells there were near to each other, the more likely they were to form tumors. Alladin, Chaible et al. think that this is because the healthy tissue interacts with the modified, oncogene-driven cells to suppress tumor formation. It is only when a larger number of modified cells group together and start to communicate with each other that they can override the inhibitory messages of the healthy tissue. How healthy tissue stops single modified cells from forming tumors is not yet clear. But, with this new mini-organ system, researchers now have the tools to investigate. In the future, this could lead to new strategies to stop cancer before it has a chance to get started.

Attia, S., et al. (2019). "Expression of CD133 as a cancer stem cell marker in invasive gastric carcinoma." *Pathologica* **111**(1): 18-23.

Introduction: Gastric cancer is considered to be the fourth most common malignancy worldwide and the second cause of cancer deaths. Regarding the cancer stem cells (CSCs) theory, they are a small group of tumor cells with unrestricted self-renewal and differentiation abilities that help tumor formation. There is an interest in the utility of CD133 as a promising marker to detect the tumor stem cell population for a variety of solid malignancies including gastric cancer. Tumors that express stem cell markers such as CD133 are found to be more aggressive tumors with poor prognosis and high liability for recurrence. This study aimed to evaluate the immunohistochemical expression of CD133 in invasive gastric carcinoma and study the relation between CD133 immunohistochemical expression and different clinicopathological parameters. Material and methods: 77 cases of gastric carcinoma were collected from the surgical pathology unit at the Gastroenterology Center, Mansoura University, Egypt. CD133 expression in tumor tissue was evaluated by immunohistochemistry. Results: CD133 expression positively correlated with tumor metastasis and recurrence. Multivariate analysis revealed CD133 positivity to be an independent prognostic factor for tumor recurrence ( $P = 0.03$ ). Conclusion: CD133 is a good marker that can predict tumor recurrence and metastasis in gastric carcinoma. Even though, studies regarding CSCs are still in their initial stages especially those related to CD133 in gastric cancer.

Azim, R. and S. Wang (2021). "Cell-specific gene association network construction from single-cell RNA sequence." *Cell Cycle*: 1-16.

The recent development of a high throughput single-cell RNA sequence devises the opportunity to study entire transcriptomes in the smallest detail. It also leads to the characterization of molecules and subtypes of a cell. Cancer epigenetics induced not only from individual molecules but also from the dysfunction of the system and the coupling effect of genes. While rapid advances are being made in the development of tools for single-cell RNA-seq data analysis, few slants are noticed in the potential advantages of single-cell network construction. Here, we used network perturbation theory with significant analysis to develop a cell-specific network that provides an insight into gene-gene association based on molecular expressions in a single-cell resolution. Besides, using this method, we can characterize each cell by inspecting how genes are connected and can identify the hub genes using network degree theory. Pathway & Gene enrichment analysis of the identified cell-specific high network degree genes supported the effectiveness of this method. This method could be beneficial for personalized drug design and even therapeutics.

Aziz, A. N., et al. (2018). "Laevifins A-G, clerodane diterpenoids from the Bark of Croton oblongus Burm.f." *Phytochemistry* **156**: 193-200.

A phytochemical investigation of the stem barks of the Malaysian Croton oblongus Burm.f. (Syn. Croton laevifolius Blume) (Euphorbiaceae) yielded seven previously undescribed ent-neo-clerodane diterpenoids, laevifins A - G and the known crovatin (3). Structures were established by a combination of spectroscopic methods including HRESIMS, NMR spectroscopy and X-ray crystallography. The absolute configuration of crovatin and laevifins A-G was established by comparison of experimental ECD and theoretical TDDFT ECD calculated spectra. This is the first report on the occurrence of the sesquiterpenoid cryptomeridiol in a Croton species. In vitro cytotoxicity assays on laevifins A, B and G showed moderate activities against the MCF-7 cancer cell line (IC<sub>50</sub> 102, 115 and 106 μM, respectively) while beta-amyrin and acetyl aleuritic acid showed good anti-inflammatory activity on the LPS-induced NF-κB translocation inhibition in RAW 264.7 cells assay with IC<sub>50</sub> values of 23.5 and 35.4 μg/mL, respectively.

Baker, S. G. (2020). "Rethinking carcinogenesis: The detached pericyte hypothesis." *Med Hypotheses* **144**: 110056.

The limiting step in cancer prevention is a lack of understanding of cancer biology. This limitation is exacerbated by a focus on the dominant somatic mutation theory (that driver mutations cause cancer) with little consideration of alternative theories of

carcinogenesis. The recently proposed detached pericyte hypothesis explains many puzzling phenomena in cancer biology for which the somatic mutation theory offers no obvious explanation. These puzzling phenomena include foreign-body tumorigenesis, the link between denervation and cancer, tumors in transgenic mice that lack the inducing mutation, and non-genotoxic carcinogens. The detached pericyte hypothesis postulates that (1) a carcinogen or chronic inflammation causes pericytes to detach from blood cell walls, (2) some detached pericytes develop into myofibroblasts which alter the extracellular matrix (3) some detached pericytes develop into mesenchymal stem cells, (4) some of the mesenchymal stem cells adhere to the altered extracellular matrix (5) the altered extracellular matrix disrupts regulatory controls, causing the adjacent mesenchymal stem cells to develop into tumors. Results from experimental studies support the detached pericyte hypothesis. If the detached pericyte hypothesis is correct, pericytes should play a key role in metastasis - a testable prediction. Recent experimental results confirm this prediction and motivate a proposed experiment to partially test the detached pericyte hypothesis. If the detached pericyte hypothesis is correct, it could lead to new strategies for cancer prevention.

Barati, M., et al. (2021). "Pluripotent Stem Cells: Cancer Study, Therapy, and Vaccination." *Stem Cell Rev Rep*.

**INTRODUCTION:** Pluripotent stem cells (PSCs) are promising tools for modern regenerative medicine applications because of their stemness properties, which include unlimited self-renewal and the ability to differentiate into all cell types in the body. Evidence suggests that a rare population of cells within a tumor, termed cancer stem cells (CSCs), exhibit stemness and phenotypic plasticity properties that are primarily responsible for resistance to chemotherapy, radiotherapy, metastasis, cancer development, and tumor relapse. Different therapeutic approaches that target CSCs have been developed for tumor eradication. **RESULTS AND DISCUSSION:** In this review, we first provide an overview of different viewpoints about the origin of CSCs. Particular attention has been paid to views believe that CSCs are probably appeared through dysregulation of very small embryonic-like stem cells (VSELs) which reside in various tissues as the main candidate for tissue-specific stem cells. The expression of pluripotency markers in these two types of cells can strengthen the validity of this theory. In this regard, we discuss the common properties of CSCs and PSCs, and highlight the potential of PSCs in cancer studies, therapeutic applications, as well as educating the immune system against CSCs. **CONCLUSION:** In conclusion, the

resemblance of CSCs to PSCs can provide an appropriate source of CSC-specific antigens through cultivation of PSCs which brings to light promising ideas for prophylactic and therapeutic cancer vaccine development.

Belluomini, L., et al. (2021). "A narrative review on tumor microenvironment in oligometastatic and oligoprogressive non-small cell lung cancer: a lot remains to be done." *Transl Lung Cancer Res* **10**(7): 3369-3384.

**Objective:** In this review, we aim to collect and discuss available data about the role and composition of tumor microenvironment (TME) in oligometastatic (OMD) and oligoprogressive (OPD) non-small cell lung cancer (NSCLC). Furthermore, we aim to summarize the ongoing clinical trials evaluating as exploratory objective the TME composition, through tissue and/or blood samples, in order to clarify whether TME and its components could explain, at least partially, the oligometastatic/oligoprogressive process and could unravel the existence of predictive and/or prognostic factors for local ablative therapy (LAT). **Background:** OMD/OPD NSCLC represent a heterogeneous group of diseases. Several data have shown that TME plays an important role in tumor progression and therefore in treatment response. The crucial role of several types of cells and molecules such as immune cells, cytokines, integrins, protease and adhesion molecules, tumor-associated macrophages (TAMs) and mesenchymal stem cells (MSCs) has been widely established. Due to the peculiar activation of specific pathways and expression of adhesion molecules, metastatic cells seem to show a tropism for specific anatomic sites (the so-called "seed and soil" hypothesis). Based on this theory, metastases appear as a biologically driven process rather than a random release of cancer cells. Although the role and the function of TME at the time of progression in patients with NSCLC treated with tyrosine-kinase inhibitors and immune checkpoint inhibitors (ICIs) have been investigated, limited data about the role and the biological meaning of TME are available in the specific OMD/OPD setting. **Methods:** Through a comprehensive PubMed and ClinicalTrials.gov search, we identified available and ongoing studies exploring the role of TME in oligometastatic/oligoprogressive NSCLC. **Conclusions:** Deepening the knowledge on TME composition and function in OMD/OPD may provide innovative implications in terms of both prognosis and prediction of outcome in particular from local treatments, paving the way for future investigations of personalized approaches in both advanced and early disease settings.

Bissoli, I. and C. Muscari (2020). "Doxorubicin and alpha-Mangostin oppositely affect luminal breast cancer cell stemness evaluated by a new retinaldehyde-

dependent ALDH assay in MCF-7 tumor spheroids." *Biomed Pharmacother* **124**: 109927.

According to cancer stem cell theory, only a limited number of self-renewing and cloning cells are responsible for tumor relapse after a period of remittance. The aim of the present study was to investigate the effects of Doxorubicin and alpha-Mangostin, two antiproliferative drugs, on both tumor bulk and stem cells in multicellular tumor spheroids originated from the luminal MCF-7 breast cancer cell line. A new and original fluorimetric assay was used to selectively measure the activity of the retinaldehyde-dependent isoenzymes of aldehyde dehydrogenase (RALDH), which are markers of a subpopulation of breast cancer stem cells. The administration of 5 mug/ml (12.2 muM) alpha-Mangostin for 48 h provoked: i) a marked disaggregation of the spheroids, leading to a doubling of their volume ( $p < 0.01$ ), ii) a 40 % decrease in cell viability ( $p < 0.01$ ), evaluated by the acid phosphatase assay, and iii) a reduction by more than 90 % of RALDH activity. By contrast, Doxorubicin given for 48 h in the range of 0.1-40 muM did not significantly reduce cell viability and caused only a modest modification of the spheroid morphology. Moreover, 40 muM Doxorubicin increased RALDH activity 2.5-fold compared to the untreated sample. When the two drugs were administered together using 5 mug/ml alpha-Mangostin, the IC50 of Doxorubicin referred to cell viability decreased six-fold and the RALDH activity was further reduced. In conclusion, the combined administration of Doxorubicin and alpha-Mangostin provoked a significant cytotoxicity and a remarkable inhibition of RALDH activity in MCF-7 tumor spheroids, suggesting that these drugs could be effective in reducing cell stemness in luminal breast cancer.

Bogen, K. T. (2019). "Inflammation as a Cancer Co-Initiator: New Mechanistic Model Predicts Low/Negligible Risk at Noninflammatory Carcinogen Doses." *Dose Response* **17**(2): 1559325819847834.

Linear-no-threshold (LNT) risk extrapolation has long been applied to estimate risks posed by low-level environmental carcinogen exposures, based on the 60-year-old multistage somatic mutation/clonal expansion (MSM) cancer theory. Recent evidence supports an alternative theory: Malignant tumors arise most efficiently from a stem cell that incurs requisite mutations and also is activated by inflammation to an epigenetically mediated and maintained state of adaptive hyperplasia (AH). This new inflammation-MSM (ISM) theory posits that inflammation-activated stem cells normally restricted to sites of injury-induced inflammation and tissue repair become uniquely susceptible to efficient carcinogenesis if normal post-inflammation AH termination is blocked by mutation.

This theory posits that inflammation generally thus co-initiates cancer and transiently amplifies activated stem cells, implying that MSM theory (eg, the 2-stage stochastic "Moolgavkar, Venzon, Knudson [MVK]" model) is incomplete. Because inflammation dose-response typically is not LNT, the ISM theory predicts this is also true for most (perhaps all) carcinogens. The ISM (but not the MVK) model is shown to be consistent with recent data showing approximately 100% carcinoma incidence (but not DNA adducts) in livers of rats exposed to aflatoxin B1 and was eliminated when that dose was co-administered with a highly potent anti-inflammatory agent. Experimental approaches to test ISM theory more robustly are discussed.

Bort, A., et al. (2020). "Dysregulated lipid metabolism in hepatocellular carcinoma cancer stem cells." *Mol Biol Rep* **47**(4): 2635-2647.

According to the stem cell theory for cancer, hepatocellular carcinomas are sustained by a group of cancer stem cells (CSCs) which are responsible for resistance to chemotherapy. In the present study we aimed to examine lipid metabolism in cancer stem cells induced by long-term treatment with sorafenib and its relationship with acquisition of a CSC-like phenotype. Two cell lines (HepG2SF1 and Huh7SF1) were generated by incubation with a step-wise increase of sorafenib concentrations for 10 months. These cell lines displayed stem-like characteristics like increase in the expression of ABCB1A, Nanog and Oct4 as well as an E-cadherin/N-cadherin switch. HepG2SF1 and Huh7SF1 cells showed intracellular accumulation of neutral lipids, assessed by flow cytometry and confocal microscopy. The exam of lipid metabolism revealed that HepG2SF1 and Huh7SF1 cells increased the expression of the enzymes involved in de novo lipid synthesis ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) and that of the fatty acid transporter CD36. In addition, these CSC-like cells had enhanced expression of the lipogenic transcription factor SREBP1c. Analysis of the key metabolic sensor AMP-activated kinase (AMPK) demonstrated that both AMPK phosphorylation and levels were decreased in the CSC-like cells compared to their parental cells. Interestingly, transfection of HepG2SF1 and Huh7SF1 cells with AMPK, restored the levels of the lipogenic enzymes and SREBP1c and decreased the intracellular lipid accumulation. Furthermore, AMPK transfection decreased the stemness markers and inhibited the E-cadherin/N-cadherin switch. Targeting AMPK and lipid metabolism of hepatocellular cancer stem cells is a promising strategy to face stemness and chemotherapy resistance.

Brauer, E. R., et al. (2019). ""Improving to where?": treatment-related health risks and perceptions of the

future among adolescents and young adults after hematopoietic cell transplantation." *Support Care Cancer* **27**(2): 623-630.

**PURPOSE:** Despite the prevalence of hematological malignancies in early adulthood, very little is known about hematopoietic cell transplantation among adolescents and young adults, and even less is known about their transition from the completion of therapy to early survivorship. In this qualitative study, we investigated the impact of the cancer experience on sense of life potential and perception of the future from the perspectives of adolescents and young adults after hematopoietic cell transplantation. **METHODS:** In-depth interviews were conducted with adolescents and young adults who underwent allogeneic or autologous hematopoietic cell transplantation between the ages of 15-29 years and were 6-60 months post-treatment. Interview transcripts were systematically coded based on constructivist grounded theory. **RESULTS:** Eighteen adolescents and young adults participated and described how they came to understand the lifelong, chronic nature of cancer survivorship. "Improving to where?" was a question raised in the post-treatment period that reflected participants' confusion about the goals of treatment and expectations for survivorship. Participants reported bracing themselves for "something bad" to deal with the uncertainty of medical and psychosocial effects of treatment. They struggled to move forward with their lives given their substantial health risks and found it necessary to "roll with the punches" in order to adjust to this new reality. **CONCLUSIONS:** Adolescents and young adults who undergo hematopoietic cell transplantation are at significant risk for long-term and late effects in survivorship. Age-appropriate interventions are needed to support these survivors as they manage their fears about the future while enhancing health and well-being. Brown, G. (2021). "Oncogenes, Proto-Oncogenes, and Lineage Restriction of Cancer Stem Cells." *Int J Mol Sci* **22**(18).

In principle, an oncogene is a cellular gene (proto-oncogene) that is dysfunctional, due to mutation and fusion with another gene or overexpression. Generally, oncogenes are viewed as deregulating cell proliferation or suppressing apoptosis in driving cancer. The cancer stem cell theory states that most, if not all, cancers are a hierarchy of cells that arises from a transformed tissue-specific stem cell. These normal counterparts generate various cell types of a tissue, which adds a new dimension to how oncogenes might lead to the anarchic behavior of cancer cells. It is that stem cells, such as hematopoietic stem cells, replenish mature cell types to meet the demands of an organism. Some oncogenes appear to deregulate this homeostatic process by restricting leukemia stem cells to a single cell lineage. This review examines whether cancer is a

legacy of stem cells that lose their inherent versatility, the extent that proto-oncogenes play a role in cell lineage determination, and the role that epigenetic events play in regulating cell fate and tumorigenesis.

Brown, G., et al. (2019). "Are Leukaemic Stem Cells Restricted to a Single Cell Lineage?" *Int J Mol Sci* **21**(1).

Cancer-stem-cell theory states that most, if not all, cancers arise from a stem/uncommitted cell. This theory revolutionised our view to reflect that cancer consists of a hierarchy of cells that mimic normal cell development. Elegant studies of twins who both developed acute lymphoblastic leukaemia in childhood revealed that at least two genomic insults are required for cancer to develop. These 'hits' do not appear to confer a growth advantage to cancer cells, nor do cancer cells appear to be better equipped to survive than normal cells. Cancer cells created by investigators by introducing specific genomic insults generally belong to one cell lineage. For example, transgenic mice in which the LIM-only 2 (LMO2, associated with human acute T-lymphoblastic leukaemia) and BCR-ABL(p210) (associated with human chronic myeloid leukaemia) oncogenes were active solely within the haematopoietic stem-cell compartment developed T-lymphocyte and neutrophil lineage-restricted leukaemia, respectively. This recapitulated the human form of these diseases. This 'hardwiring' of lineage affiliation, either throughout leukaemic stem cell development or at a particular stage, is different to the behaviour of normal haematopoietic stem cells. While normal cells directly commit to a developmental pathway, they also remain versatile and can develop into a terminally differentiated cell that is not part of the initial lineage. Many cancer stem cells do not have this versatility, and this is an essential difference between normal and cancer stem cells. In this report, we review findings that support this notion.

Budhwani, M., et al. (2020). "Dysregulation of Stemness Pathways in HPV Mediated Cervical Malignant Transformation Identifies Potential Oncotherapy Targets." *Front Cell Infect Microbiol* **10**: 307.

Human papillomavirus (HPV) infection is associated with a range of malignancies that affect anogenital and oropharyngeal sites. alpha-HPVs dominantly infect basal epithelial cells of mucosal tissues, where they dysregulate cell division and local immunity. The cervix is one of the mucosal sites most susceptible to HPV infections. It consists of anatomically diverse regions, and the majority of cervical intraepithelial neoplasia and cancers arise within the cervical squamo-columnar junction where undifferentiated basal progenitor cells with stem cell properties are found. The cancer stem cell theory particularly associates tumorigenesis, invasion,

dissemination, and metastasis with cancer cells exhibiting stem cell properties. In this perspective, we discuss evidence of a cervical cancer stem cell niche and explore the association of stemness related genes with 5-year survival using a publicly available transcriptomic dataset of a cervical cancer cohort. We report that poor prognosis in this cohort correlates with overexpression of a subset of stemness pathway genes, a majority of which regulate the central Focal Adhesion pathway, and are also found to be enriched in the HPV infection pathway. These observations support therapeutic targeting of stemness genes overexpressed by mucosal cells infected with high-risk HPVs.

Burgio, E., et al. (2018). "Environmental Carcinogenesis and Transgenerational Transmission of Carcinogenic Risk: From Genetics to Epigenetics." *Int J Environ Res Public Health* **15**(8).

The dominant pathogenic model, somatic mutation theory (SMT), considers carcinogenesis as a 'genetic accident' due to the accumulation of 'stochastic' DNA mutations. This model was proposed and accepted by the scientific community when cancer mainly affected the elderly, but it does not explain the epidemiological observation of the continuous increase in cancer incidence among children and young adults. Somatic mutation theory has been proposed for a revision based on the emerging experimental evidence, as it does not fully address some issues that have proven to be crucial for carcinogenesis, namely: the inflammatory context of cancer; the key role played by the stroma, microenvironment, endothelial cells, activated macrophages, and surrounding tissues; and the distorted developmental course followed by the neoplastic tissue. Furthermore, SMT is often not able to consider either the existence of specific mutations resulting in a well-defined cancer type, or a clear relationship between mutations and tumor progression. Moreover, it does not explain the mechanism of action of the non-mutagenic and environmental carcinogens. In the last decade, cancer research has highlighted the prominent role of an altered regulation of gene expression, suggesting that cancer should be considered as a result of a polyclonal epigenetic disruption of stem/progenitor cells, mediated by tumour-inducing genes. The maternal and fetal exposure to a wide range of chemicals and environmental contaminants is raising the attention of the scientific community. Indeed, the most powerful procarcinogenic mechanisms of endocrine disruptors and other pollutants is linked to their potential to interfere epigenetically with the embryo-fetal programming of tissues and organs, altering the regulation of the genes involved in the cell cycle, cell proliferation, apoptosis, and other key signaling pathways. The embryo-fetal exposure to environmental, stressful, and proinflammatory triggers

(first hit), seems to act as a 'disease primer', making fetal cells and tissues more susceptible to the subsequent environmental exposures (second hit), triggering the carcinogenic pathways. Furthermore, even at the molecular level, in carcinogenesis, 'epigenetics precedes genetics' as global DNA hypomethylation, and the hypermethylation of tumor suppressor genes are common both in cancerous and in precancerous cells, and generally precede mutations. These epigenetic models may better explain the increase of cancer and chronic/degenerative diseases in the last decades and could be useful to adopt appropriate primary prevention measures, essentially based on the reduction of maternal-fetal and child exposure to several procarcinogenic agents and factors dispersed in the environment and in the food-chains, as recently suggested by the World Health Organization. Capp, J. P. (2019). "Cancer Stem Cells: From Historical Roots to a New Perspective." *J Oncol* **2019**: 5189232.

The relationships between cancer and stemness have a long history that is traced here. From the mid-19th century when the first theory on the embryonic origin of cancer was formulated to works on embryonal carcinoma cells in the mid-20th century, many steps have been crossed leading to the current cancer stem cell theory postulating that tumor growth is supported by a small fraction of the tumoral cells that have stem-like properties. However, in the last fifteen years, many works regularly encourage us to revise the concept of cancer stem cell. This article mentions key results that lead to a new perspective where cancer stem cells are primarily seen as cells exhibiting increased epigenetic plasticity and increased gene expression variability. This perspective suggests new therapeutical interventions consisting in stabilizing gene expression to control cancer cell proliferation and prevent stochastic gene expression variations that could lead to therapeutic resistance.

Carvalho, J. (2020). "Cell Reversal From a Differentiated to a Stem-Like State at Cancer Initiation." *Front Oncol* **10**: 541.

Even if the Somatic Mutation Theory of carcinogenesis explains many of the relevant experimental results in tumor origin and development, there are frequent events that are not justified, or are even contradictory to this widely accepted theory. A Cell Reversal Theory is presented, putting forward the hypothesis that cancer is originated by reversal of a differentiated cell into a non-differentiated stem-like state, by a change of its intrinsic epigenetic state, following a perturbation on the cell and/or its microenvironment. In the current proposal a cluster of cancer stem cells can be established, without the strict control mechanisms of a normal stem cell niche, and initiate a tumor. It is proposed that a reversal to a

pluripotent state is at tumor origin and not tumor progress that prompts cell dedifferentiation. The uncontrolled proliferation of cancer stem cells causes a microenvironment disorganization, resulting in stressful conditions, like hypoxia and nutrient deprivation, which induces the genetic instability characteristic of a tumor; thus, in most cases, mutations are a consequence and not the direct cause of a tumor. It is also proposed that metastases result from dedifferentiation signaling dispersion instead of cell migration. However, conceivably, once the microenvironment is normalized, the stem cell-like state can differentiate back to a mature cell state and loose its oncogenic capacity. Therefore, this can be a reversible condition, suggesting important therapeutic opportunities.

Carvalho, J. (2021). "A bioelectric model of carcinogenesis, including propagation of cell membrane depolarization and reversal therapies." *Sci Rep* **11**(1): 13607.

As the main theory of carcinogenesis, the Somatic Mutation Theory, increasingly presents difficulties to explain some experimental observations, different theories are being proposed. A major alternative approach is the Tissue Organization Field Theory, which explains cancer origin as a tissue regulation disease instead of having a mainly cellular origin. This work fits in the latter hypothesis, proposing the bioelectric field, in particular the cell membrane polarization state, and ionic exchange through ion channels and gap junctions, as an important mechanism of cell communication and tissue organization and regulation. Taking into account recent experimental results and proposed bioelectric models, a computational model of cancer initiation was developed, including the propagation of a cell depolarization wave in the tissue under consideration. Cell depolarization leads to a change in its state, with the activation and deactivation of several regulation pathways, increasing cell proliferation and motility, changing its epigenetic state to a more stem cell-like behavior without the requirement of genomic mutation. The intercellular communication via gap junctions leads, in certain circumstances, to a bioelectric state propagation to neighbor cells, in a chain-like reaction, till an electric discontinuity is reached. However, this is a reversible process, and it was shown experimentally that, by implementing a therapy targeted on cell ion exchange channels, it is possible to reverse the state and repolarize cells. This mechanism can be an important alternative way in cancer prevention, diagnosis and therapy, and new experiments are proposed to test the presented hypothesis.

Chagay, N. B. and A. M. Mkrtumyan (2019). "[Estrogen metabolism, lifetime methylation disorders,

and breast cancer]." *Probl Endokrinol (Mosk)* **65**(3): 161-173.

Oncogenesis can be caused by an increase in the activity of genes responsible for initiating tumor growth in stem or progenitor cells, as well as a reduction in the functioning of suppressor genes. Endogenous estrogen exposure is associated with an increased risk of breast cancer in both pre- and postmenopausal women. The most important step in the understanding of the pathogenesis of breast cancer was the development of the theory of the switching of estrogen's effect from hormonal to genotoxic, in which the main culprits of carcinogenesis are not chemical metabolites of estrogens, but their derivatives, corresponding to chemical procarcinogens according to their damaging characteristics. The origin of these substances and the formation of estrogen genotoxicity lies in the disruption of the inactivation process of catechol estrogens in methylation reactions. The main epigenetic modification of the human genome is the methylation of cell DNA molecules. DNA methylation does not alter the primary sequence of nucleotides, but is necessary for the functional suppression of certain genes. The phenomenon of hypomethylation-hypermethylation underlies the long-term silencing of various genes, including tumor suppressor genes. Nutrition and a lifestyle associated with smoking and the consumption of excessive quantities of alcohol determine estrogen metabolism and the availability of methyl groups in the body, as well as epigenetic changes in the DNA of the genome. The assessment of individual risk of breast cancer on the basis of an assay for the expression and methylation of the COMT gene responsible for estrogen metabolism seems relevant.

Cobaleda, C. and I. Sanchez-Garcia (2021). "Leukemia Stem Cell Drug Discovery." *Methods Mol Biol* **2185**: 39-48.

The relative survival of cancer patients, when considering the tumoral stage at diagnosis, has not changed significantly in the last three decades, in spite of our increasingly detailed knowledge of the molecular alterations occurring in human tumors. In parallel, despite a growing number of clinical trials being conducted, the absolute number of drugs that are effective in humans is declining, and many new drugs move into the market without having enough evidence of their benefit on survival or quality of life. In part, this failure is due to the discordance between the results from preclinical and clinical trial phases, therefore leading to a high percentage of apparently promising lead compounds being abandoned in the transfer to the clinic. This discordance is caused, to a large degree, by the use of inappropriate animal models in the first stages of drug development. In this chapter, we discuss how the development of cancer therapies needs to be redesigned in order to achieve cancer cure,

and how this redesign must involve the generation of better animal models, based on the tenets of the cancer stem cell theory, and capable of recapitulating all the aspects of human cancer. The use of such improved models should increase the likelihood of success in drug development, reducing the number of agents that go into trial, and the amount of patients undergoing useless trials.

Corallo, C. E., et al. (2020). "Dapsone for *Pneumocystis jirovecii* pneumonia prophylaxis - applying theory to clinical practice with a focus on drug interactions." *Drug Metab Pers Ther* **35**(3).

*Pneumocystis jirovecii* pneumonia (PJP) is a potentially life-threatening infection that occurs in immunocompromised individuals. The incidence can be as high as 80% in some groups but can be reduced to less than 1% with appropriate prophylaxis. HIV-infected patients with a low CD4 count are at the highest risk of PJP. Others at substantial risk include haematopoietic stem cell and solid organ transplant recipients, those with cancer (particularly haematologic malignancies), and those receiving glucocorticoids, chemotherapeutic agents, and other immunosuppressive medications. Trimethoprim-sulfamethoxazole is an established first-line agent for prevention and treatment of PJP. However, in some situations, this medication cannot be used and dapsone is considered a suitable cost-effective second line agent. However, information on potential interactions with drugs commonly used in immunosuppressed patients is lacking or contradictory. In this article we review the metabolic pathway of dapsone with a focus on interactions and clinical significance particularly in patients with haematological malignancies. An understanding of this process should optimise the use of this agent.

Curtarelli, R. B., et al. (2018). "Expression of Cancer Stem Cell Biomarkers in Human Head and Neck Carcinomas: a Systematic Review." *Stem Cell Rev Rep* **14**(6): 769-784.

Malignant neoplasms may be composed of several cell groups, including cancer stem cells (CSC). These cells have been related with the capacity of metastasis, relapse and resistance to multiple drugs during chemotherapy. This study aims to identify CSC biomarkers and their expression pattern in human head and neck carcinomas. This study was conducted following the PRISMA checklist. The search for articles was carried out in five databases (PubMed, Scopus, Web of Science, Lilacs and Scielo). The articles found were selected in two phases: 1) reading the titles and / or abstract and 2) reading the full text. At the end, the selected articles were evaluated by QUADAS-2. Most studies evaluated oral neoplastic tissues and, as a control, samples of normal local mucosa. All studies performed immunohistochemistry



as a method of immunolocalization and some also applied immunofluorescence. The most commonly used biomarker was CD44. However, other such as Sox2, Oct4, Nestin, Nanog, BMI1, ALDH1, CD133 and CD166 were also found. Several biomarkers were (ALDH1, Sox2, Oct4, ABCB5, AGR2 and TAZ) correlated with clinical characteristics of the tumor, such as staging, tumor size and lymph node metastasis. These data reinforce the CSC theory and favor the use of these biomarkers as possible determinants of prognosis.

Daniel, Y., et al. (2021). "Interplay between Metabolism Reprogramming and Epithelial-to-Mesenchymal Transition in Cancer Stem Cells." *Cancers (Basel)* **13**(8).

Tumor cells display important plasticity potential, which contributes to intratumoral heterogeneity. Notably, tumor cells have the ability to retrodifferentiate toward immature states under the influence of their microenvironment. Importantly, this phenotypical conversion is paralleled by a metabolic rewiring, and according to the metabostemness theory, metabolic reprogramming represents the first step of epithelial-to-mesenchymal transition (EMT) and acquisition of stemness features. Most cancer stem cells (CSC) adopt a glycolytic phenotype even though cells retain functional mitochondria. Such adaptation is suggested to reduce the production of reactive oxygen species (ROS), protecting CSC from detrimental effects of ROS. CSC may also rely on glutaminolysis or fatty acid metabolism to sustain their energy needs. Besides pro-inflammatory cytokines that are well-known to initiate the retrodifferentiation process, the release of catecholamines in the microenvironment of the tumor can modulate both EMT and metabolic changes in cancer cells through the activation of EMT transcription factors (ZEB1, Snail, or Slug (SNAI2)). Importantly, the acquisition of stem cell properties favors the resistance to standard care chemotherapies. Hence, a better understanding of this process could pave the way for the development of therapies targeting CSC metabolism, providing new strategies to eradicate the whole tumor mass in cancers with unmet needs.

Dzobo, K., et al. (2020). "Advances in Therapeutic Targeting of Cancer Stem Cells within the Tumor Microenvironment: An Updated Review." *Cells* **9**(8).

Despite great strides being achieved in improving cancer patients' outcomes through better therapies and combinatorial treatment, several hurdles still remain due to therapy resistance, cancer recurrence and metastasis. Drug resistance culminating in relapse continues to be associated with fatal disease. The cancer stem cell theory posits that tumors are driven by specialized cancer cells called cancer stem cells (CSCs). CSCs are a subpopulation of cancer cells known to be resistant to therapy and cause metastasis.

Whilst the debate on whether CSCs are the origins of the primary tumor rages on, CSCs have been further characterized in many cancers with data illustrating that CSCs display great abilities to self-renew, resist therapies due to enhanced epithelial to mesenchymal (EMT) properties, enhanced expression of ATP-binding cassette (ABC) membrane transporters, activation of several survival signaling pathways and increased immune evasion as well as DNA repair mechanisms. CSCs also display great heterogeneity with the consequential lack of specific CSC markers presenting a great challenge to their targeting. In this updated review we revisit CSCs within the tumor microenvironment (TME) and present novel treatment strategies targeting CSCs. These promising strategies include targeting CSCs-specific properties using small molecule inhibitors, immunotherapy, microRNA mediated inhibitors, epigenetic methods as well as targeting CSC niche-microenvironmental factors and differentiation. Lastly, we present recent clinical trials undertaken to try to turn the tide against cancer by targeting CSC-associated drug resistance and metastasis.

Elkashty, O. A., et al. (2019). "Head and neck cancer management and cancer stem cells implication." *Saudi Dent J* **31**(4): 395-416.

Head and neck squamous cell carcinomas (HNSCCs) arise in the mucosal linings of the upper aerodigestive tract and are heterogeneous in nature. Risk factors for HNSCCs are smoking, excessive alcohol consumption, and the human papilloma virus. Conventional treatments are surgery, radiotherapy, chemotherapy, or a combined modality; however, no international standard mode of therapy exists. In contrast to the conventional model of clonal evolution in tumor development, there is a newly proposed theory based on the activity of cancer stem cells (CSCs) as the model for carcinogenesis. This "CSC hypothesis" may explain the high mortality rate, low response to treatments, and tendency to develop multiple tumors for HNSCC patients. We review current knowledge on HNSCC etiology and treatment, with a focus on CSCs, including their origins, identifications, and effects on therapeutic options.

Espinosa-Sanchez, A., et al. (2020). "Therapeutic Targeting of Signaling Pathways Related to Cancer Stemness." *Front Oncol* **10**: 1533.

The theory of cancer stem cells (CSCs) proposes that the different cells within a tumor, as well as metastasis deriving from it, are originated from a single subpopulation of cells with self-renewal and differentiation capacities. These cancer stem cells are supposed to be critical for tumor expansion and metastasis, tumor relapse and resistance to conventional therapies, such as chemo- and radiotherapy. The acquisition of these abilities has been

attributed to the activation of alternative pathways, for instance, WNT, NOTCH, SHH, PI3K, Hippo, or NF-kappaB pathways, that regulate detoxification mechanisms; increase the metabolic rate; induce resistance to apoptotic, autophagic, and senescence pathways; promote the overexpression of drug transporter proteins; and activate specific stem cell transcription factors. The elimination of CSCs is an important goal in cancer therapeutic approaches because it could decrease relapses and metastatic dissemination, which are main causes of mortality in oncology patients. In this work, we discuss the role of these signaling pathways in CSCs along with their therapeutic potential.

Fattore, L., et al. (2020). "Cancer Stem Cells and the Slow Cycling Phenotype: How to Cut the Gordian Knot Driving Resistance to Therapy in Melanoma." *Cancers (Basel)* **12**(11).

Cancer stem cells (CSCs) have historically been defined as slow cycling elements that are able to differentiate into mature cells but without dedifferentiation in the opposite direction. Thanks to advances in genomic and non-genomic technologies, the CSC theory has more recently been reconsidered in a dynamic manner according to a "phenotype switching" plastic model. Transcriptional reprogramming rewires this plasticity and enables heterogeneous tumors to influence cancer progression and to adapt themselves to drug exposure by selecting a subpopulation of slow cycling cells, similar in nature to the originally defined CSCs. This model has been conceptualized for malignant melanoma tailored to explain resistance to target therapies. Here, we conducted a bioinformatics analysis of available data directed to the identification of the molecular pathways sustaining slow cycling melanoma stem cells. Using this approach, we identified a signature of 25 genes that were assigned to four major clusters, namely 1) kinases and metabolic changes, 2) melanoma-associated proteins, 3) Hippo pathway and 4) slow cycling/CSCs factors. Furthermore, we show how a protein-protein interaction network may be the main driver of these melanoma cell subpopulations. Finally, mining The Cancer Genome Atlas (TCGA) data we evaluated the expression levels of this signature in the four melanoma mutational subtypes. The concomitant alteration of these genes correlates with the worst overall survival (OS) for melanoma patients harboring BRAF-mutations. All together these results underscore the potentiality to target this signature to selectively kill CSCs and to achieve disease control in melanoma.

Ferrell, S. D., Jr., et al. (2020). "Why is cancer so common a disease in people yet so rare at a cellular level?" *Med Hypotheses* **144**: 110171.

Cancers are common diseases in people and yet, on a cellular level, are quite rare. The vast majority

of both sporadic, spontaneous cancers and inherited germline cancers arise in single foci from singly transformed cells despite the fact that, in the former, carcinogenic factors bathe fields of millions of potential target cells and, in the latter, the predisposing germline mutations are present in every cell of a given organ and, in fact, every cell of the body. Although the multi-hit theory of carcinogenesis has been invoked to explain such things as cancer latency, which is the period between cancer initiation and emergence and the cancer-aging relationship where an accumulation of "hits" over a period of time are necessary for cancer emergence, the multi-hit theory falls short in explaining the rareness of transformation at a cellular level. This is so because many cancers are not due to multiple hits, and even for those that are, it would be expected that many cells would be exposed to those factors inducing the hits. Although the tumor stem/progenitor cell compartmental theory of tumorigenesis characterizes a tumor compartment that is capable of self-renewal and multipotency, accounting for cancer relapses and recurrences, this compartmental theory alone cannot account for the rareness of initial transformation at a cellular level as the cancer stem/progenitor cell compartment is already transformed and considerable in size. This study advances a different and novel hypothesis that oncogenesis is regulated and ultimately determined by a cell of origin's critical state of differentiation. Before and after this critical state of differentiation has been reached, target cells cannot transform and give rise to cancer even when they receive the necessary carcinogenic insults or have the requisite transforming tumor suppressor genes or oncogenes. As support for this hypothesis, the study cites preliminary evidence using oncogene-containing transgenic mice that develop mammary carcinomas, to derive tail vein fibroblasts converted to iPSCs which, when left undifferentiated, and injected into the cleared fat pads of non-transgenic background mice give rise to mammary gland ontogeny and mammary gland carcinogenesis. However, when first differentiated in vitro into multiply different non-mammary lineages prior to injection, they fail to do so. The hypothesis has widespread implications for chemopreventive strategies.

Fiscion, G., et al. (2018). "SWIM tool application to expression data of glioblastoma stem-like cell lines, corresponding primary tumors and conventional glioma cell lines." *BMC Bioinformatics* **19**(Suppl 15): 436.

**BACKGROUND:** It is well-known that glioblastoma contains self-renewing, stem-like subpopulation with the ability to sustain tumor growth. These cells - called cancer stem-like cells - share certain phenotypic characteristics with untransformed stem cells and are resistant to many conventional cancer therapies, which might explain the limitations in

curing human malignancies. Thus, the identification of genes controlling the differentiation of these stem-like cells is becoming a successful therapeutic strategy, owing to the promise of novel targets for treating malignancies. **METHODS:** Recently, we developed SWIM, a software able to unveil a small pool of genes - called switch genes - critically associated with drastic changes in cell phenotype. Here, we applied SWIM to the expression profiling of glioblastoma stem-like cells and conventional glioma cell lines, in order to identify switch genes related to stem-like phenotype. **RESULTS:** SWIM identifies 171 switch genes that are all down-regulated in glioblastoma stem-like cells. This list encompasses genes like CAV1, COL5A1, COL6A3, FLNB, HMMR, ITGA3, ITGA5, MET, SDC1, THBS1, and VEGFC, involved in "ECM-receptor interaction" and "focal adhesion" pathways. The inhibition of switch genes highly correlates with the activation of genes related to neural development and differentiation, such as the 4-core OLIG2, POU3F2, SALL2, SOX2, whose induction has been shown to be sufficient to reprogram differentiated glioblastoma into stem-like cells. Among switch genes, the transcription factor FOSL1 appears as the brightest star since: it is down-regulated in stem-like cells; it highly negatively correlates with the 4-core genes that are all up-regulated in stem-like cells; the promoter regions of the 4-core genes harbor a consensus binding motif for FOSL1. **CONCLUSIONS:** We suggest that the inhibition of switch genes in stem-like cells could induce the deregulation of cell communication pathways, contributing to neoplastic progression and tumor invasiveness. Conversely, their activation could restore the physiological equilibrium between cell adhesion and migration, hampering the progression of cancer.

Gotz, C., et al. (2018). "ALDH1 as a prognostic marker for lymph node metastasis in OSCC." *Biomed Rep* 9(4): 284-290.

Long-term survival in cases of head and neck squamous cell carcinoma, particularly oral squamous cell carcinoma (OSCC), remains a rare achievement in the field of clinical oncology. In recent years, the theory of cancer stem cells (CSCs) has emerged and been used to offer explanations for tumour recurrence and metastasis. The present aim was to investigate the role of aldehyde dehydrogenase 1 (ALDH1) as a CSC-marker for OSCC and to determine the role of p16(ink4a), which is also a surrogate marker of human papilloma virus (HPV), in the expression of ALDH1. The study cohort comprised of 186 surgically-treated cases of OSCC. The primaries were located in the oral cavity. The expression of the CSC marker (CSCM) ALDH1 was evaluated via immunohistochemistry (IHC) of a tissue microarray. HPV detection was performed by polymerase chain reaction and an HPV

Array kit. Furthermore, the IHC expression of p16(ink4a) was also analysed. Risk regression models as the Kruskal Wallis test was used to assess the association of CSCM and p16(ink4a) expression with tumour size and lymph node metastasis, and cox proportional hazards were analysed. Additionally, coexpression of the markers ALDH1 and p16(ink4a) was analysed with regard to associations with tumour classification. Overall, high expression of ALDH1 in lymph nodes was significantly associated with Union for International Cancer Control (UICC) stage IV (P=0.044) and T4 stage cancer (P=0.03). p16(ink4a) positivity, in cases of HPV negativity, was associated with worse survival rate compared with that of the total cohort (P=0.048). Collectively the data indicate that ALDH1 expression may be suitable for detection of unfavourable prognosis in OSCC patients, based in part on its apparent role as a marker of metastasis. HPV status was not statistically predictive of patient outcome or CSCM expression; however, p16(ink4a) remains a potential marker in HNSCC. Further in vitro studies with ALDH1 and p16(ink4a) should be performed to evaluate the expression of ALDH1 and HPV in cell culture and to clarify the role of ALDH1 as a marker for increased invasiveness of OSCC cells.

Guo, X., et al. (2019). "Enrichment of cancer stem cells by agarose multi-well dishes and 3D spheroid culture." *Cell Tissue Res* 375(2): 397-408.

As the theory of cancer stem cells (CSCs) is maturing, CSC-targeted therapy is emerging as an important therapeutic strategy and seeking the ideal method for rapid enrichment and purification of CSCs has become crucial. So far, based on the known CSC phenotypes and biological characteristics, the methods for enrichment CSCs mainly include low adhesion culture, low oxygen culture, chemotherapy drug stimulation and side population (SP) sorting but these methods cannot realize quick enrichment of the desired CSCs. Herein, we adopt a novel method that efficiently enriches a certain amount of CSCs through agarose multi-well dishes using rubber micro-molds to make cancer cells into cell spheroids (3D). These 3D cancer cell spheroids in the proportions of expression of CSC biomarkers (single stain of CD44, CD44v6 and CD133 or double stain of both CD44 and CD133) were significantly higher than those of the conventional adherent culture (2D) using flow cytometry analysis. In addition, the expression levels of stemness transcription factors such as OCT4, NANOG and SOX2 in 3D were also significantly higher than that in 2D through Western blot (WB) and quantitative polymerase chain reaction (qPCR) assays. In addition, the CSCs in 3D could form colonies with different sizes in soft agar. In conclusion, we developed a new method to enrich some kinds of CSCs, which might be

a benefit for future CSC-targeted therapy studies and anti-CSC drug screening applications.

Harvey, J. and M. Berndt (2021). "Cancer caregiver reports of post-traumatic growth following spousal hematopoietic stem cell transplant." *Anxiety Stress Coping* **34**(4): 397-410.

**BACKGROUND AND OBJECTIVES:** Cancer caregivers are at risk for experiencing health issues due to the stress of caregiving. Despite this, it is possible to prompt adaptive coping during the cancer experience. Adaptive coping is associated with improved health for caregiver populations. Forms of emotional disclosure are associated with caregiver reports of post-traumatic growth (PTG), which is an adaptive coping mechanism that comprises positive change following trauma. This study sought to identify areas of PTG identified by spousal hematopoietic stem-cell transplant (HSCT) cancer caregivers, via emotional disclosure writings. **DESIGN & METHOD:** Twenty-two spousal caregivers of patients who underwent a (HSCT) submitted emotional disclosure writings three times at one-week intervals. Writings centered on positive outcomes arising in light of the cancer experience. A qualitative grounded theory approach was used to evaluate caregiver accounts of PTG that arose while caring for their spouse. **RESULTS & CONCLUSIONS:** Findings suggest seven areas of PTG recognized through the disclosure process: living in the moment, a sense of honor and pride, choosing positivity, uninfluenced self-choice and expression, deprioritizing materialism, personal and/or spiritual connection, and altruistic expansion. The primary theoretical advancement arising from this study includes the notion that PTG largely appears to be a socially dependent process. Trial registration: ClinicalTrials.gov identifier: NCT02339870.

Hassan, G., et al. (2020). "Cancer stem cell generation by silenced MAPK enhancing PI3K/AKT signaling." *Med Hypotheses* **141**: 109742.

Cancer stem cells, which are defined by self-renewal, differentiation potential and tumorigenicity, are proposed to be responsible for cancer initiation and maintain the tumor mass. Cancer stem cells are thought to be resistant to chemotherapy inhibiting different signaling pathways. This might sound stemness is maintained even when the growth is suppressed. Typically, growth factors are known to stimulate MEK/ERK pathways, which is responsible for mitogenic activity, while the PI3K appears more related with the maintenance of stemness. The cross-talk, even in positive or negative ways, between these two pathways could stimulate or accelerate the conversion of normal stem cells into cancer stem cells. Here, we propose a new hypothesis of a mechanism for the conversion of stem cells or progenitors including induced pluripotent stem cells (iPSCs) to tissue-

specific cancer stem cells. This conversion could be prepared by inhibiting MEK/ERK pathway and enhancing PI3K/AKT pathway exploiting conditioned media derived from cancer cell lines, which are good sources of many different cytokines, chemokines, tissues-specific factors, metabolites and so on, together with some inhibitors. The feasibility of this combination will be explained in this hypothesis through the reports published somewhere. Generation of cancer stem cells using embryonic stem cells/iPSCs will bring new theory in the mechanisms of tumorigenesis and assist drug screening that applies for the precision medicines for individuals.

Hatfield, K. J., et al. (2021). "Future perspective: metabolism as a therapeutic target in acute myeloid leukemia - from Warburg to precision medicine." *Curr Med Res Opin*: 1-5.

Acute myeloid leukemia (AML) is a highly malignant blood cancer disease, with dismal prognosis. The theory that cancer cells utilize metabolism to their growth advantage was postulated almost hundred years ago. However, only recently have been able to take advantage of this Achilles heel of malignant cell growth. Current observations suggest a crucial role for various metabolic pathways in AML, and special in leukemia stem cells, believed to be responsible for re-initiation of the leukemic clone, and hence relapse of this devastating disease. In the present article we discuss the features for metabolism in AML based on recent research, and special emphasizing the potential of pharmacological inhibiting metabolism as new treatment approaches.

Heng, W. S., et al. (2019). "Lung cancer stem cells: origin, features, maintenance mechanisms and therapeutic targeting." *Biochem Pharmacol* **160**: 121-133.

Lung cancer remains the leading cause of cancer-related deaths despite recent breakthroughs in immunotherapy. The widely embraced cancer stem cell (CSC) theory has also been applied for lung cancer, postulating that an often small proportion of tumor cells with stem cell properties are responsible for tumor growth, therapeutic resistance and metastasis. The identification of these CSCs and underlying molecular maintenance mechanisms is considered to be absolutely necessary for developing therapies for their riddance, hence achieving remission. In this review, we will critically address the CSC concept in lung cancer and its advancement thus far. We will describe both normal lung stem cells and their malignant counterparts in order to identify common aspects with respect to their emergence and regulation. Subsequently, the importance of CSCs and their molecular features in lung cancers will be discussed in a preclinical and clinical context. We will highlight some examples on how lung CSCs attain stemness through different

molecular modifications and cellular assistance from the tumor microenvironment. The exploitation of these mechanistic features for the development of pharmacological therapy will also be discussed. In summary, the validity of the CSC concept has been evidenced by various studies. Ongoing research to identify molecular mechanisms driving lung CSC have revealed potential new cell intrinsic as well as tumor microenvironment-derived therapeutic targets. Although successfully demonstrated in preclinical models, the clinical benefit of lung CSC targeted therapies has thus far not been demonstrated. Therefore, further research to validate the therapeutic value of CSC concept is required.

Horwitz, K. B. and C. A. Sartorius (2020). "90 YEARS OF PROGESTERONE: Progesterone and progesterone receptors in breast cancer: past, present, future." *J Mol Endocrinol* **65**(1): T49-T63.

Progesterone and progesterone receptors (PR) have a storied albeit controversial history in breast cancers. As endocrine therapies for breast cancer progressed through the twentieth century from oophorectomy to antiestrogens, it was recognized in the 1970s that the presence of estrogen receptors (ER) alone could not efficiently predict treatment responses. PR, an estrogen regulated protein, became the first prognostic and predictive marker of response to endocrine therapies. It remains today as the gold standard for predicting the existence of functional, targetable ER in breast malignancies. PRs were subsequently identified as highly structured transcription factors that regulate diverse physiological processes in breast cancer cells. In the early 2000s, the somewhat surprising finding that prolonged use of synthetic progestin-containing menopausal hormone therapies was associated with increased breast cancer incidence raised new questions about the role of PR in 'tumorigenesis'. Most recently, PR have been linked to expansion of cancer stem cells that are postulated to be the principal cells reactivated in occult or dormant disease. Other studies establish PR as dominant modulators of ER activity. Together, these findings mark PR as bona fide targets for progestin or anti-progestin therapies, yet their diverse actions have confounded that use. Here we summarize the early history of PR in breast cancer; debunk the theory that progesterone causes cancer; discuss recent discoveries that PR regulate cell heterogeneity; attempt to unify theories describing PR as either good or bad actors in tumors; and discuss emerging areas of research that may help explain this enigmatic hormone and receptor.

Houshmand, M., et al. (2019). "Bone marrow microenvironment: The guardian of leukemia stem cells." *World J Stem Cells* **11**(8): 476-490.

Bone marrow microenvironment (BMM) is the main sanctuary of leukemic stem cells (LSCs) and

protects these cells against conventional therapies. However, it may open up an opportunity to target LSCs by breaking the close connection between LSCs and the BMM. The elimination of LSCs is of high importance, since they follow cancer stem cell theory as a part of this population. Based on cancer stem cell theory, a cell with stem cell-like features stands at the apex of the hierarchy and produces a heterogeneous population and governs the disease. Secretion of cytokines, chemokines, and extracellular vesicles, whether through autocrine or paracrine mechanisms by activation of downstream signaling pathways in LSCs, favors their persistence and makes the BMM less hospitable for normal stem cells. While all details about the interactions of the BMM and LSCs remain to be elucidated, some clinical trials have been designed to limit these reciprocal interactions to cure leukemia more effectively. In this review, we focus on chronic myeloid leukemia and acute myeloid leukemia LSCs and their milieu in the bone marrow, how to segregate them from the normal compartment, and finally the possible ways to eliminate these cells.

Javadi, A., et al. (2021). "Finite-Set Model Predictive Control of Melanoma Cancer Treatment Using Signaling Pathway Inhibitor of Cancer Stem Cell." *IEEE/ACM Trans Comput Biol Bioinform* **18**(4): 1504-1511.

Drug delivery is one of the most important issues in the treatment of cancer and surviving the patient. Recently, with a combination of mathematical models of the tumor growth and control theory, optimal drug delivery can be planned, individually. The goal is reducing the tumor volume with minimum side effects on the patient. One of the most important challenges of the modeling is considering the drug resistance, which may lead to failure of the treatment. In this paper, a mathematical model is proposed for describing the growth dynamics of the melanoma tumor cells. It is assumed that the melanoma cancer is treated with Notch signaling pathway inhibitors of the cancer stem cells. The model parameters are identified based on experimental data obtained from 13 male nude mice with an induced melanoma cancer involved in a dual antiplatelet therapy (DAPT) program. The mathematical model is used to determine if DAPT can reduce the growth rate of the tumor. Then an optimal drug delivery plan for the treatment of every animal model is presented, individually using finite-set model predictive control method. The results show that the proposed model can estimate the drug's effect on the treatment of melanoma cancer.

Jones, S. M. W., et al. (2020). "Age and gender differences in financial distress among hematopoietic cell transplant survivors." *Support Care Cancer* **28**(9): 4361-4371.

**PURPOSE:** Cancer has long-term financial consequences. Adolescent and young adult (AYA) and middle-aged cancer survivors may experience more financial toxicity than older adults. This study examined age differences in financial distress in hematopoietic cell transplant survivors and whether these differences result from measurement bias, more financial barriers to care, or an overall higher level of distress. **METHODS:** Hematologic malignancy survivors ( $n = 1135$ , 2-10 years post-transplant) completed the Cancer and Treatment Distress Scale (CTXD) and demographics as part of the baseline assessment for a randomized clinical trial. The CTXD has seven subscales, but for this study, we examined the financial distress subscale and the overall score. Item response theory analyses tested for bias by age and gender. Multivariate linear regression tested the association of age and gender with the CTXD scores while controlling for financial barriers to care. **RESULTS:** No bias was found on the CTXD. AYA ( $p < 0.01$ ) and middle-aged adults ( $p < 0.001$ ) reported more financial and overall distress than older (age 65+) adults. The same association of age and financial distress was observed in women ( $p < 0.01$ ). However, only middle-aged men ( $p < 0.01$ ) reported more financial and overall distress than older men; AYA men did not ( $p > 0.18$ ). Financial barriers to care were not associated with financial or overall distress.

Kars, M. D. and G. Yildirim (2019). "Determination of the target proteins in chemotherapy resistant breast cancer stem cell-like cells by protein array." *Eur J Pharmacol* **848**: 23-29.

Breast cancer comes second among the causes of cancer deaths of women. Although new generation hormone therapy is a promising strategy, re-occurrence or emergence of drug resistance limits the success. According to the theory of cancer stem cells (CSCs); CSCs are immortal, tumor inducing and self renewing pluripotent cells and multiply as chemotherapy proceeds, making the chemotherapy inefficient. Emerging scientific reports indicate that the mechanisms of drug resistance are the main features that CSCs gain actually. Due to this fact, cancer stem cell markers should be clarified to target CSCs and this will play important role to reverse drug resistance. In this study, MCF-7/Pac, a cell line resistant to microtubule inhibitor paclitaxel and multiple drugs permanently, was used as a reference cell line for drug resistant mammary cancer. It has some properties that breast cancer stem cells possess so it is considerable to isolate breast cancer stem cell-like cells from MCF-7/Pac population. The chemotherapy resistant breast cancer stem-like (BCSC-like) cells were sorted from MCF-7/Pac population by using markers CD44, CD24 and ALDH. At the next step the proteins that are up-regulated in BCSC-like cells were determined by

protein array analysis. Additionally the effect of paclitaxel on BCSC-like cell proliferation was determined. The MCF-7/Pac population contains 12.4% BCSC-like cells. The cells bearing BCSC-like cell phenotype exhibited resistance to paclitaxel. The over-expressed growth factors, MMP proteins, Frizzled proteins and IL-23 were found to be related to the BCSC-like cell proliferation. These results will guide both basic science and medical science.

Kaushik, V., et al. (2021). "Alternative models of cancer stem cells: The stemness phenotype model, 10 years later." *World J Stem Cells* **13**(7): 934-943.

The classical cancer stem cell (CSCs) theory proposed the existence of a rare but constant subpopulation of CSCs. In this model cancer cells are organized hierarchically and are responsible for tumor resistance and tumor relapse. Thus, eliminating CSCs will eventually lead to cure of cancer. This simplistic model has been challenged by experimental data. In 2010 we proposed a novel and controversial alternative model of CSC biology (the Stemness Phenotype Model, SPM). The SPM proposed a non-hierarchical model of cancer biology in which there is no specific subpopulation of CSCs in tumors. Instead, cancer cells are highly plastic in term of stemness and CSCs and non-CSCs can interconvert into each other depending on the microenvironment. This model predicts the existence of cancer cells ranging from a pure CSC phenotype to pure non-CSC phenotype and that survival of a single cell can originate a new tumor. During the past 10 years, a plethora of experimental evidence in a variety of cancer types has shown that cancer cells are indeed extremely plastic and able to interconvert into cells with different stemness phenotype. In this review we will (1) briefly describe the cumulative evidence from our laboratory and others supporting the SPM; (2) the implications of the SPM in translational oncology; and (3) discuss potential strategies to develop more effective therapeutic regimens for cancer treatment.

Kenda Suster, N. and I. Virant-Klun (2019). "Presence and role of stem cells in ovarian cancer." *World J Stem Cells* **11**(7): 383-397.

Ovarian cancer is the deadliest gynecological malignancy. It is typically diagnosed at advanced stages of the disease, with metastatic sites disseminated widely within the abdominal cavity. Ovarian cancer treatment is challenging due to high disease recurrence and further complicated pursuant to acquired chemoresistance. Cancer stem cell (CSC) theory proposes that both tumor development and progression are driven by undifferentiated stem cells capable of self-renewal and tumor-initiation. The most recent evidence revealed that CSCs in terms of ovarian cancer are not only responsible for primary tumor growth, metastasis and relapse of disease, but also for the

development of chemoresistance. As the elimination of this cell population is critical for increasing treatment success, a deeper understanding of ovarian CSCs pathobiology, including epithelial-mesenchymal transition, signaling pathways and tumor microenvironment, is needed. Finally, before introducing new therapeutic agents for ovarian cancer, targeting CSCs, accurate identification of different ovarian stem cell subpopulations, including the very small embryonic-like stem cells suggested as progenitors, is necessary. To these ends, reliable markers of ovarian CSCs should be identified. In this review, we present the current knowledge and a critical discussion concerning ovarian CSCs and their clinical role.

Koll, T. T., et al. (2020). "Returning to life activities after hematopoietic cell transplantation in older adults." *J Geriatr Oncol* **11**(2): 304-310.

**OBJECTIVES:** The prevalence of hematopoietic cell transplant (HCT) among older adults with hematological malignancies has more than doubled over the last decade and continues to grow. HCT is an intense process that can impact functional status and health-related quality of life. The objective of this paper is to describe the experience of returning to life activities after HCT in patients 60 years of age and older and the resources required to adapt and cope to limitations in physical, psychological, and cognitive function. **MATERIALS AND METHODS:** Twenty English speaking adults 60 years and older with hematological malignancy 3 to 12 months post-HCT completed semi-structured interviews. Open-ended questions and probes were guided by the Transactional Model of Stress and Coping to explore adaptive functioning, coping resources, and coping strategies. An integrated grounded theory approach was used to code the textual data to identify themes. The study took place at a tertiary comprehensive cancer center in the Midwest United States. **RESULTS:** Eight allogeneic and twelve autologous HCT recipients participated in the interviews. Nineteen participants were within 6-12 months and 1 participant was at 3 months post-HCT. Our findings identify the significant role of engaging in life activities and social support in the recovery of physical, psychological and cognitive function. **CONCLUSION:** Older HCT recipients are an understudied population. They are at high risk for functional decline. Our findings may provide community oncologists and primary care physicians with a context for providing care to older HCT survivors during their recovery.

Kroemeke, A., et al. (2019). "Dyadic support and affect in patient-caregiver dyads following hematopoietic stem-cell transplantation: A diary study." *J Consult Clin Psychol* **87**(6): 541-550.

**OBJECTIVE:** Cancer and its treatment are highly stressful events that may significantly affect the daily emotional well-being of patients and their informal caregivers. Patient- and caregiver-reported received and provided support may contribute to both dyad members' fluctuation in daily affect, but few studies have examined these associations from a dyadic perspective so far. The current study examined predictions derived from 3 theories on patterns of relations between subjectively assessed dyadic provided and received support and daily affect within dyad members: (a) invisible support theory, (b) the suggestion that providing support may be better than receiving it, and (c) beneficial supportive equity. **METHOD:** Actor-partner interdependence models were tested using 28-day diary data from 200 patient-caregiver dyads. Diary assessments started on the first day following patients' discharge from the hospital, that is, about 3 weeks following patients' hematopoietic stem cell transplantation (HSCT). **RESULTS:** Daily invisible support was not related to more positive indicators of patients' or caregivers' daily affect. For patients' affect, findings generally supported the hypothesis of psychological benefits of support provision over receipt, in both concurrent and lagged analyses. For caregivers, visible received support from patients and supportive equity (i.e., both provided and received support relatively high), both concurrently and lagged, were related with better emotional state. **CONCLUSIONS:** The findings highlight the costs, benefits, and complexities of daily support transactions in dyads following HSCT, thus indicating the practical implications of the study: the importance of screening for support needs and abilities in both patients and caregivers. (PsycINFO Database Record (c) 2019 APA, all rights reserved).

Lacina, L., et al. (2019). "Evolution of Cancer Progression in the Context of Darwinism." *Anticancer Res* **39**(1): 1-16.

Our review compares evolution of cancer in the human body to the origin of new species from a common ancestor organism with respect to the theory of Charles Darwin. Moreover, the functional role of the tumor microenvironment as a selective pressure actively participating in cancer progression is also demonstrated. Evolutionary aspects of tumor growth and invasion from the point of view of modern therapeutic challenges and opportunities in precision personalized medicine are also discussed.

Lai, S. (2018). "Carcinogenesis is consequence of failure of tissue development." *Med Hypotheses* **119**: 84-87.

Cancer has become a public health problem. The exploration of pathogenesis and therapy of cancer is mainly under the guidance of gene mutation theory. But the therapeutic effect of cancer is not satisfactory,

and many predictions of gene mutation theory do not conform actual phenomena of cancer. The research results of mechanism of genetic molecular mutation trap us in an intricate molecular maze hopelessly. The dilemma compels us to doubt about the correctness of gene mutation theory and re-understand the nature of tumor. This study explores the nature of cancer by the method of theoretical analysis by the view of tissue regeneration, and draws a conclusion that the carcinogenesis is consequence of failure of tissue development. Tumors originate from tissue regeneration, tumor cells originate from normal tissue stem cells. The tumor cells are only normal immature cells. Tumor promoters stimulate stem cells to proliferate. Carcinogens obstruct the inducers from inducing tissue stem cells differentiation outside of cells. With tumor promoters and carcinogens, the tissue stem cells proliferate, but cannot differentiate into mature cells, and stop in different phases of differentiation forming atypical hyperplasia of different degrees and tumors of various differentiation grades in tissue.

Li, L., et al. (2019). "Metabolic and epigenetic reprogramming in the arsenic-induced cancer stem cells." *Semin Cancer Biol* **57**: 10-18.

At present, the belief that genetic mutations control every aspect of tumorigenesis is still very popular. Even for the highly debated "bad luck" theory of cancers, it ascertained that random mutation of genes during the self-renewal of somatic stem cells is responsible for cancer initiation. Logically, most of the new therapeutic strategies so far, from molecular targeting to precision medicine or personalized medicine, are genome-obsessed and focused on identifying and targeting these mutated genes. Accordingly, a rather simplified therapeutic regimen was formulated: cancers with the same mutations, e.g., lung cancer, pancreatic cancer, breast cancer, ovarian cancer, etc, were managed with the same chemo or targeting medicine, whereas for a particular cancer, such as breast cancer or lung cancer, with different mutational spectrums was treated with different, so-called personalized medicine. The outcomes of this strategy, however, are mixed with encouraging and disappointing findings. In this review article, we will address the importance of non-genetic factors, the metabolic and epigenetic reprogramming, during the induction of cancer stem cells in response to arsenic, a major environmental human carcinogen. The information provided may not only advance our understanding of carcinogenic mechanism to a new level but also help in designing new strategies through targeting the metabolic and epigenetic signaling pathways for cancer therapy.

Li, Y., et al. (2021). "Apigenin Enhanced Antitumor Effect of Cisplatin in Lung Cancer via Inhibition of Cancer Stem Cells." *Nutr Cancer* **73**(8): 1489-1497.

Cancer stem cell theory has been proposed to explain tumor heterogeneity and the carcinogenesis process. Highly tumorigenic lung cancer stem cells develop resistance to cisplatin (CDDP), a common chemotherapy drug. Herein, we attempted to clarify whether apigenin (API) can improve the antitumor efficiency of CDDP in lung cancer using cancer stem cells. Lung cancer stem cells were identified as CD 133 positive cancer cells in non-small cell lung cancer (NSCLC) A549, H1299 cells and CDDP-resistant NSCLC A549R cells. The cytotoxic effect of API was measured in CDDP-treated A549, H1299, and A549R cells. API repressed CD 133 positive cells and enhanced the antitumor effect of CDDP in A549, H1299, and A549R cells. The synergistic antitumor effect of API and CDDP was blocked by addition of the p53 inhibitor Pifithrin-alpha, and siRNA targeting the p53 gene in A549R cells. Furthermore, API eliminates CDDP-induced CSC via p53, since A549R cells lacking p53 and Pifithrin-alpha addition derepressed the decrease in CD 133 positive cells after API treatment in CDDP-treated A549 and A549R cells. The findings indicate that API might eliminate cancer stem cells and enhance the antitumor effects of CDDP in NSCLC via p53.

Liao, Y., et al. (2020). "Weighted Gene Coexpression Network Analysis of Features That Control Cancer Stem Cells Reveals Prognostic Biomarkers in Lung Adenocarcinoma." *Front Genet* **11**: 311.

Purpose We aimed to identify new prognostic biomarkers of lung adenocarcinoma (LUAD) based on cancer stem cell theory. Materials and Methods: RNA-seq and microarray data were obtained with clinical information downloaded from The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) databases. Weighted gene coexpression network analysis (WGCNA) was applied to identify significant module and hub genes. The hub genes were validated via microarray data from GEO, and a prognostic signature with prognostic hub genes was constructed. Results LUAD patients enrolled from TCGA had a higher mRNA expression-based stemness index (mRNAsi) in tumor tissue than in adjacent normal tissue. Some clinical features and prognoses were found to be highly correlated with mRNAsi. WGCNA found that the green module and blue module were the most significant modules related to mRNAsi; 50 key genes were identified in the green module and were enriched mostly in the cell cycle, chromosome segregation, chromosomal region and microtubule binding. Six hub genes were revealed through the protein-protein interaction (PPI) network and Molecular Complex Detection (MCODE) plugin of



Cytoscape software. Based on external verification with the GEO database, these six genes are not only expressed at different levels in LUAD and normal tissues but also associated with different clinical features.

Liao, Y., et al. (2020). "Bioinformatics Analysis Reveals Biomarkers With Cancer Stem Cell Characteristics in Lung Squamous Cell Carcinoma." *Front Genet* **11**: 427.

**Background:** Tumor stem cells play important roles in the survival, proliferation, metastasis and recurrence of tumors. We aimed to identify new prognostic biomarkers for lung squamous cell carcinoma (LUSC) based on the cancer stem cell theory. **Methods:** RNA-seq data and relevant clinical information were downloaded from The Cancer Genome Atlas (TCGA) database. Weighted gene coexpression network analysis (WGCNA) was applied to identify significant modules and hub genes, and prognostic signatures were constructed with the prognostic hub genes. **Results:** LUSC patients in the TCGA database have higher mRNA expression-based stemness index (mRNAsi) in tumor tissue than in adjacent normal tissue. In addition, some clinical features and outcomes were highly correlated with the mRNAsi. WGCNA revealed that the pink and yellow modules were the most significant modules related to the mRNAsi; the top 10 hub genes in the pink module were enriched mostly in epidermal development, the secretory granule membrane, receptor regulator activity and the cytokine-cytokine receptor interaction. The protein-protein interaction (PPI) network revealed that the top 10 hub genes were significantly correlated with each other at the transcriptional level. In addition, the top 10 hub genes were all highly expressed in LUSC, and some were differentially expressed in different TNM stages. Regarding the survival analysis, the nomogram of a prognostic signature with three hub genes showed high predictive value. **Conclusion:** mRNAsi-related hub genes could be a potential biomarker of LUSC.

Liu, J. (2020). "The "life code": A theory that unifies the human life cycle and the origin of human tumors." *Semin Cancer Biol* **60**: 380-397.

Tumors arise from the transformation of normal stem cells or mature somatic cells. Intriguingly, two types of tumors have been observed by pathologists for centuries: well-differentiated tumors and undifferentiated tumors. Well-differentiated tumors are architecturally similar to the tissues from which they originate, whereas undifferentiated tumors exhibit high nuclear atypia and do not resemble their tissue of origin. The relationship between these two tumor types and the human life cycle has not been clear. Here I propose a unifying theory that explains the processes of transformation of both tumor types with our life cycle.

Human life starts with fertilization of an egg by a sperm to form a zygote. The zygote undergoes successive rounds of cleavage division to form blastomeres within the zona pellucida, with progressive decreases in cell size, and the cleaved blastomeres then compact to form a 32-cell or a "64n" morula [ $n=1$  full set of chromosomes]. Thus early embryogenesis can be interpreted as a progressive increase in ploidy, and if the zona pellucida is considered a cell membrane and cleavage is interpreted as endomitosis, then the 32-cell morula can be considered a multinucleated giant cell (or 64n syncytium). The decrease in cell size is accompanied by an increase in the nuclear-to-cytoplasmic (N/C) ratio, which then selectively activates a combined set of embryonic transcription factors that dedifferentiate the parental genome to a zygotic genome. This process is associated with a morphologic transition from a morula to a blastocyst and formation of an inner cell mass that gives rise to a new embryonic life. If the subsequent differentiation proceeds to complete maturation, then a normal life results. However, if differentiation is blocked at any point along the continuum of primordial germ cell to embryonic maturation to fetal organ maturation, a well-differentiated tumor will develop. Depending on the level of developmental hierarchy at which the stem cell differentiation is blocked, the resulting tumor can range from highly malignant to benign. Undifferentiated tumors are derived from mature somatic cells through dedifferentiation via a recently described reprogramming mechanism named the giant cell life cycle or the giant cell cycle. This mechanism can initiate "somatic embryogenesis" via an increase in ploidy ranging from 4n to 64n or more, similar to that in normal embryogenesis. This dedifferentiation mechanism is initiated through an endocycle and is followed by endomitosis, which leads to the formation of mononucleated or multinucleated polyploid giant cancer cells (PGCCs), that is, cancer stem-like cells that mimic the blastomere-stage embryo. The giant cell life cycle leads to progressive increases in the N/C ratio and awakens the suppressed embryonic reprogram, resulting in mature somatic transformation into undifferentiated tumors. Thus, the increase in ploidy explains not only normal embryogenesis for well-differentiated tumors but also "somatic embryogenesis" for undifferentiated tumors. I refer to this ploidy increase as the "life code". The concept of the "life code" may provide a simple theoretical framework to guide our immense efforts to understand cancer and fight this disease.

Liu, X., et al. (2020). "ATM Paradoxically Promotes Oncogenic Transformation via Transcriptional Reprogramming." *Cancer Res* **80**(8): 1669-1680.

The role of the ataxia-telangiectasia-mutated (ATM) gene in human malignancies, especially in solid

tumors, remains poorly understood. In the present study, we explored the involvement of ATM in transforming primary human cells into cancer stem cells. We show that ATM plays an unexpected role in facilitating oncogene-induced malignant transformation through transcriptional reprogramming. Exogenous expression of an oncogene cocktail induced a significant amount of DNA double-strand breaks in human fibroblasts that caused persistent activation of ATM, which in turn enabled global transcriptional reprogramming through chromatin relaxation, allowing oncogenic transcription factors to access chromatin. Consistently, deficiencies in ATM significantly attenuated oncogene-induced transformation of human cells. In addition, ATM inhibition significantly reduced tumorigenesis in a mouse model of mammary cancer. ATM and cellular DNA damage response therefore play a previously unknown role in facilitating rather than suppressing oncogene-induced malignant transformation of mammalian cells. **SIGNIFICANCE:** These findings uncover a novel pro-oncogenic role for ATM and show that contrary to established theory, ATM does not always function as a tumor suppressor; its function is however dependent on cell type.

Ma, Y. S., et al. (2020). "Targeting Colorectal Cancer Stem Cells as an Effective Treatment for Colorectal Cancer." Technol Cancer Res Treat **19**: 1533033819892261.

As one of the common cancers that threaten human life, the recurrence and metastasis of colorectal cancer seriously affect the prognosis of patients. Although new drugs and comprehensive treatments have been adopted, the current treatment effect on this tumor, especially in advanced colorectal cancer, is still not satisfactory. More and more evidence shows that tumors are likely to be a stem cell disease. In recent years, the rise of cancer stem cell theory has provided a new way for cancer treatment. Studies have found that a small number of special cells in colorectal cancer tissues that induce tumorigenesis, proliferation, and promote tumor migration and metastasis, namely, colorectal cancer stem cells. Colorectal cancer stem cells are defined with a group of cell-surface markers, such as CD44, CD133, CD24, epithelial cell adhesion factor molecule, LGR5, and acetaldehyde dehydrogenase. They are highly tumorigenic, aggressive, and chemoresistant and thus are critical in the metastasis and recurrence of colorectal cancer. Therefore, targeting colorectal cancer stem cells may become an important research direction for the future cure of colorectal cancer.

Mansour, H., et al. (2020). "Metastasis Model of Cancer Stem Cell-Derived Tumors." Methods Protoc **3**(3).

Metastasis includes the dissemination of cancer cells from a malignant tumor and seed in distant

sites inside the body forming secondary tumors. Metastatic cells from the primary tumor can move even before the cancer is detected. Therefore, metastases are responsible for more than 90% of cancer-related deaths. Over recent decades there has been adequate evidence suggesting the existence of CSCs with self-renewing and drug-resistant potency within heterogeneous tumors. Cancer stem cells (CSCs) act as a tumor initiating cells and have roles in tumor retrieve and metastasis. Our group recently developed a unique CSC model from mouse induced pluripotent stem cells cultured in the presence of cancer cell-conditioned medium that mimics tumors microenvironment. Using this model, we demonstrated a new method for studying metastasis by intraperitoneal transplantation of tumors and investigate the metastasis ability of cells from these segments. First of all, CSCs were injected subcutaneously in nude mice. The developed malignant tumors were minimized then transplanted into the peritoneal cavity. Following this, the developed tumor in addition to lung, pancreas and liver were then excised and analyzed. Our method showed the metastatic potential of CSCs with the ability of disseminated and moving to blood circulation and seeding in distant organs such as lung and pancreas. This method could provide a good model to study the mechanisms of metastasis according to CSC theory.

Motohara, T. and H. Katabuchi (2019). "Ovarian Cancer Stemness: Biological and Clinical Implications for Metastasis and Chemotherapy Resistance." Cancers (Basel) **11**(7).

Epithelial ovarian cancer is a highly lethal gynecological malignancy that is characterized by the early development of disseminated metastasis. Though ovarian cancer has been generally considered to preferentially metastasize via direct transcoelomic dissemination instead of the hematogenous route, emerging evidence has indicated that the hematogenous spread of cancer cells plays a larger role in ovarian cancer metastasis than previously thought. Considering the distinctive biology of ovarian cancer, an in-depth understanding of the biological and molecular mechanisms that drive metastasis is critical for developing effective therapeutic strategies against this fatal disease. The recent "cancer stem cell theory" postulates that cancer stem cells are principally responsible for tumor initiation, metastasis, and chemotherapy resistance. Even though the hallmarks of ovarian cancer stem cells have not yet been completely elucidated, metastasized ovarian cancer cells, which have a high degree of chemoresistance, seem to manifest cancer stem cell properties and play a key role during relapse at metastatic sites. Herein, we review our current understanding of the cell-biological mechanisms that regulate ovarian cancer metastasis and chemotherapy resistance, with a pivotal focus on

ovarian cancer stem cells, and discuss the potential clinical implications of evolving cancer stem cell research and resultant novel therapeutic approaches.

Mukherjee, S. (2020). "Quiescent stem cell marker genes in glioma gene networks are sufficient to distinguish between normal and glioblastoma (GBM) samples." *Sci Rep* **10**(1): 10937.

Grade 4 glioma or GBM has poor prognosis and is the most aggressive grade of glioma. Accurate diagnosis and classification of tumor grade is a critical determinant for development of treatment pathway. Extensive genomic sequencing of gliomas, different cell types, brain tissue regions and advances in bioinformatics algorithms, have presented an opportunity to identify molecular markers that can complement existing histology and imaging methods used to diagnose and classify gliomas. 'Cancer stem cell theory' purports that a minor population of stem cells among the heterogeneous population of different cell types in the tumor, drive tumor growth and resistance to therapies. However, characterization of stem cell states in GBM and ability of stem cell state signature genes to serve as diagnostic or prognostic molecular markers are unknown. In this work, two different network construction algorithms, Weighted correlation network analysis (WGCNA) and Multiscale Clustering of Geometric Network (MEGENA), were applied on publicly available glioma, control brain and stem cell gene expression RNA-seq datasets, to identify gene network regulatory modules associated with GBM. Both gene network algorithms identified consensus or equivalent modules, HuAgeGBsplit\_18 (WGCNA) and c1\_HuAgeGBsplit\_32/193 (MEGENA), significantly associated with GBM. Characterization of HuAgeGBsplit\_18 (WGCNA) and c1\_HuAgeGBsplit\_32/193 (MEGENA) modules showed significant enrichment of rodent quiescent stem cell marker genes (GSE70696\_QNPbyTAP). A logistic regression model built with eight of these quiescent stem cell marker genes (GSE70696\_QNPbyTAP) was sufficient to distinguish between control and GBM samples. This study demonstrates that GBM associated gene regulatory modules are characterized by diagnostic quiescent stem cell marker genes, which may potentially be used clinically as diagnostic markers and therapeutic targets in GBM.

Nazio, F., et al. (2019). "Autophagy and cancer stem cells: molecular mechanisms and therapeutic applications." *Cell Death Differ* **26**(4): 690-702.

Autophagy and mitophagy act in cancer as bimodal processes, whose differential functions strictly depend on cancer ontogenesis, progression, and type. For instance, they can act to promote cancer progression by helping cancer cells survive stress or, instead, when mutated or abnormal, to induce carcinogenesis by influencing cell signaling or

promoting intracellular toxicity. For this reason, the study of autophagy in cancer is the main focus of many researchers and several clinical trials are already ongoing to manipulate autophagy and by this way determine the outcome of disease therapy. Since the establishment of the cancer stem cell (CSC) theory and the discovery of CSCs in individual cancer types, autophagy and mitophagy have been proposed as key mechanisms in their homeostasis, dismissal or spread, even though we still miss a comprehensive view of how and by which regulatory molecules these two processes drive cell fate. In this review, we will dive into the deep water of autophagy, mitophagy, and CSCs and offer novel viewpoints on possible therapeutic strategies, based on the modulation of these degradative systems.

Norskov, K. H., et al. (2021). "Social support as a moderator of healthcare adherence and distress in long-term hematopoietic cell transplantation survivors." *J Cancer Surviv*.

**BACKGROUND:** Treatment with hematopoietic cell transplantation (HCT) has potentially severe effects on physical and psychosocial functioning. Poor social support has been linked with physical morbidity and mortality as well as psychological distress in HCT survivors. This study tested a theory-driven hypothesis that social support buffers adverse effects of health stressors of comorbidities and graft-versus-host disease (cGVHD) on distress and adherence to recommended healthcare among long-term HCT survivors. **METHODS:** This cross-sectional study analyzed baseline data from a randomized controlled trial in adult survivors 3-18 years post-HCT. Data included medical records and patient-reported outcomes including cancer and treatment distress (CTXD), healthcare adherence (HCA), comorbidity index, cGVHD, ENRICH Social Support Instrument (ESSI), Social Activity Log, and Health Self-Efficacy. We tested hypothesized models for HCA and CTXD using blocked hierarchical linear regressions. **RESULTS:** Among the 781 HCT survivors completing baseline assessment, 38% had > 3 comorbidities, 8% had moderate-severe cGVHD, 30% reported low social support, 30% reported elevated distress, and 49% reported low healthcare adherence. Social support and self-efficacy were directly related to both adherence and distress. Regression models supported the hypothesized moderated relationships for distress but not for healthcare adherence. **CONCLUSIONS:** The two tested models confirm that the health stressors of comorbidities and cGVHD are moderated by better social support and self-efficacy in their associations with lower distress but without moderating effects for healthcare adherence. **IMPLICATIONS FOR CANCER SURVIVORS:** Social support and self-efficacy confer protective

benefits on healthcare adherence and psychological distress. Interventions are needed that focus on maintaining social networks or finding new networks if necessary. CLINICAL TRIAL REGISTRATION NUMBER: NCT00799461.

Ottesen, J. T., et al. (2019). "Bridging blood cancers and inflammation: The reduced Cancitis model." *J Theor Biol* **465**: 90-108.

A novel mechanism-based model - the Cancitis model - describing the interaction of blood cancer and the inflammatory system is proposed, analyzed and validated. The immune response is divided into two components, one where the elimination rate of malignant stem cells is independent of the level of the blood cancer and one where the elimination rate depends on the level of the blood cancer. A dimensional analysis shows that the full 6-dimensional system of nonlinear ordinary differential equations may be reduced to a 2-dimensional system - the reduced Cancitis model - using Fenichel theory. The original 18 parameters appear in the reduced model in 8 groups of parameters. The reduced model is analyzed. Especially the steady states and their dependence on the exogenous inflammatory stimuli are analyzed. A semi-analytic investigation reveals the stability properties of the steady states. Finally, positivity of the system and the existence of an attracting trapping region in the positive octahedron guaranteeing global existence and uniqueness of solutions are proved. The possible topologies of the dynamical system are completely determined as having a Janus structure, where two qualitatively different topologies appear for different sets of parameters. To classify this Janus structure we propose a novel concept in blood cancer - a reproduction ratio  $R$ . It determines the topological structure depending on whether it is larger or smaller than a threshold value. Furthermore, it follows that inflammation, affected by the exogenous inflammatory stimulation, may determine the onset and development of blood cancers. The body may manage initial blood cancer as long as the self-renewal rate is not too high, but fails to manage it if an inflammation appears.

Padua, D., et al. (2020). "The Relevance of Transcription Factors in Gastric and Colorectal Cancer Stem Cells Identification and Eradication." *Front Cell Dev Biol* **8**: 442.

Gastric and colorectal cancers have a high incidence and mortality worldwide. The presence of cancer stem cells (CSCs) within the tumor mass has been indicated as the main reason for tumor relapse, metastasis and therapy resistance, leading to poor overall survival. Thus, the elimination of CSCs became a crucial goal for cancer treatment. The identification of these cells has been performed by using cell-surface markers, a reliable approach, however it lacks

specificity and usually differs among tumor type and in some cases even within the same type. In theory, the ideal CSC markers are those that are required to maintain their stemness features. The knowledge that CSCs exhibit characteristics comparable to normal stem cells that could be associated with the expression of similar transcription factors (TFs) including SOX2, OCT4, NANOG, KLF4 and c-Myc, and signaling pathways such as the Wnt/beta-catenin, Hedgehog (Hh), Notch and PI3K/AKT/mTOR directed the attention to the use of these similarities to identify and target CSCs in different tumor types. Several studies have demonstrated that the abnormal expression of some TFs and the dysregulation of signaling pathways are associated with tumorigenesis and CSC phenotype. The disclosure of common and appropriate biomarkers for CSCs will provide an incredible tool for cancer prognosis and treatment. Therefore, this review aims to gather the new insights in gastric and colorectal CSC identification specially by using TFs as biomarkers and divulge promising drugs that have been found and tested for targeting these cells.

Petrova, S. C., et al. (2020). "Regulation of breast cancer oncogenesis by the cell of origin's differentiation state." *Oncotarget* **11**(43): 3832-3848.

Human breast cancer which affects 1/8 women is rare at a cellular level. Even in the setting of germline BRCA1/BRCA2, which is present in all breast cells, solitary cancers or cancers arising at only several foci occur. The overwhelming majority of breast cells (10<sup>9</sup>-10<sup>12</sup> cells) resist transformation. Our hypothesis to explain this rareness of transformation is that mammary oncogenesis is regulated by the cell of origin's critical window of differentiation so that target cells outside of this window cannot transform. Our novel hypothesis differs from both the multi-hit theory of carcinogenesis and the stem/progenitor cell compartmental theory of tumorigenesis and utilizes two well established murine transgenic models of breast oncogenesis, the FVB/N-Tg (MMTV-PyVT)634Mul/J and the FVB-Tg (MMTV-ErbB2) NK1Mul/J. Tail vein fibroblasts from each of these transgenics were used to generate iPSCs. When select clones were injected into cleared mammary fat pads, but not into non-orthotopic sites of background mice, they exhibited mammary ontogenesis and oncogenesis with the expression of their respective transgenes. iPSC clones, when differentiated along different non-mammary lineages in vitro, were also not able to exhibit either mammary ontogenesis or oncogenesis in vivo. Therefore, in vitro and in vivo regulation of differentiation is an important determinant of breast cancer oncogenesis.

Pieterse, Z., et al. (2019). "Ovarian cancer stem cells and their role in drug resistance." *Int J Biochem Cell Biol* **106**: 117-126.

Ovarian cancer is typically diagnosed at advanced stages (III or IV), with metastasis ensuing at stage III. Complete remission is infrequent and is not achieved in almost half of the women diagnosed with ovarian cancer. Consequently, management and treatment of this disease is challenging as many patients are faced with tumour recurrence disseminating to surrounding organs further complicated with acquired chemo-resistance. The cancer stem cell theory proposes the idea that a drug resistant subset of tumour cells drive tumour progression, metastasis and ultimately, recurrent disease. In the ovarian cancer field, cancer stem cells remain elusive with significant gaps in our knowledge. The characteristics and specific role of ovarian cancer stem cells in recurrence still requires further research since different studies often arrive at contradictory conclusions. Here we present a review and critical analysis of current research conducted in the field of ovarian cancer stem cells and their potential role in drug resistance including several signalling pathways within these cells that affect the viability of targeted therapies.

Pozzi, V., et al. (2020). "Cancer stem cell enrichment is associated with enhancement of nicotinamide N-methyltransferase expression." *IUBMB Life* **72**(7): 1415-1425.

The cancer stem cell theory states that a subset of tumor cells, termed cancer stem cells (CSCs), has the ability to self-renew and differentiate within the tumors. According to this theory, CSCs would be mainly responsible for tumor initiation, progression, resistance to therapy, recurrence, and metastasis. In this study, a culture system was setup to enrich CSCs from bladder cancer (T24), lung cancer (A549), colorectal cancer (CaCo-2), and osteosarcoma (MG63) cell lines, through sphere formation. Magnetic-activated cell sorting was also used to further increase CSC enrichment. Subsequently, molecular characterization of CSC-enriched cell populations and parental cells was carried out, by exploring the expression levels of stem markers and the enzyme nicotinamide N-methyltransferase (NNMT). Results obtained showed a significant upregulation of stem cell markers in CSC-enriched populations, obtained upon sphere formation, compared with parental counterparts. Moreover, NNMT expression levels were markedly increased in samples enriched with CSCs with respect to control cells. Considering the fundamental role played by CSCs in carcinogenesis, reported data strengthen the hypothesis that sustains a pivotal role of NNMT in cancer growth and metastasis. In addition, these findings could represent an important achievement for the development of new and effective anticancer therapies, based on CSC-associated targets.

Rabinovich, I., et al. (2018). "Cancer stem cell markers ALDH1 and CD44+/CD24- phenotype and their prognosis impact in invasive ductal carcinoma." *Eur J Histochem* **62**(3).

Breast cancer is a very heterogeneous disease. The intrinsic molecular subtypes can explain the intertumoral heterogeneity and the cancer stem cell (CSC) hypothesis can explain the intratumoral heterogeneity of this kind of tumor. CD44+/CD24- phenotype and ALDH1 expression are the major CSC markers described in invasive breast cancer. In the present study, 144 samples of invasive breast carcinoma, no special type were distributed in 15 tissue microarrays (TMA) and then evaluated for expression of the CD44+/CD24- phenotype and ALDH1 to understand the importance of these CSC markers and the clinical aspects of breast cancer. The samples were classified into four molecular subtypes according to clinicopathological criteria: Luminal A, Luminal B, HER2, and Basal-like. A statistical association was found between the molecular subtypes and the CSC markers, with HER2 the most frequent subtype for both markers. ALDH1 was also associated with other poor prognostic variables, such as a high histological grade and larger tumors, but it was not associated with the patients' prognosis in this sample and nor was the CD44+/CD24- phenotype in a multivariate analysis. There are still many controversies about the role of these markers in breast cancer molecular subtypes. The identification of these populations of cells, through immunohistochemical markers, can help to better understand the CSC theory in clinical practice and, in the near future, contribute to developing new target therapies.

Recio, A. (2019). "Tumour growth activation by the central nervous system-An integrative theory of cancer." *Stress Health* **35**(4): 569-581.

The currently recognized mechanisms of the biology of cancer are not yet enough to explain the high incidence of the disease in industrialized countries. Survival and proliferation of cancer cells demand a well-orchestrated combination of functional capabilities, or hallmarks, which requires complex signalling networks that often exceed the tumour boundaries. Based on latest research on environmental health and aiming to provide cancer with a coherent set of organizing principles, we propose an integrative model of carcinogenesis founded on tumour growth activation by the central nervous system as an adaptive, allostatic response to both environmental and emotional challenges. In this way, chronicity of physical as well as psychological stressors may be directly involved in cancer genesis and progression, after an early inflammatory stage. The model also contemplates accidental activation of the tumour growth programme following direct DNA damage, but

as a rare event that does not account for most cancers in humans. Bodily and cellular mechanisms designed to facilitate tumorigenesis may include exacerbation of the sympathetic activity, overexpression of membrane ion channels, promotion of selected mutations and methylations, degradation of the mitochondria and reprogramming of adult stem cells.

Reshadmanesh, A., et al. (2018). "Evaluation of cellular and transcriptional targeting of breast cancer stem cells via anti-HER2 nanobody conjugated PAMAM dendrimers." *Artif Cells Nanomed Biotechnol* **46**(sup3): S105-S115.

According to the cancer stem cell (CSC) theory, a small subset of cells with stem cell-like characteristics is responsible for tumor initiation, progression, and recurrence. CD44(+)/CD24(-) phenotype is assumed to be one of the main characteristics of the breast CSCs. We developed an MDA-MB-231 cell line overexpressing cell surface HER2 antigen for the evaluation of targeting efficiency of anti-HER2 nanobody (Nb)-conjugated polyamidoamine (PAMAM) polyplexes. Apoptosis-inducing tBid gene under control of CXCR1 promoter was delivered by this nanoparticle. Cellular uptake study showed higher uptake of Nb-targeted PAMAM carriers compared to non-targeted nanoparticles after 6 h of incubation. Gene expression analysis showed a significant rise in the expression of tBid in both MDA-MB-231/HER2(+) and MDA-MB-231 compared to the two other cell lines. The same effect was observed after transfection with Nb-conjugated polyplexes within MDA-MB-231/HER2(+) cell line compared to non-conjugated PAMAM polyplexes. We confirmed the killing efficiency of the gene construct in both MDA-MB-231/HER2(+) and MDA-MB-231 cell lines by caspase 3 activity assay. These findings suggest that imposing pre-entry and post-entry restrictions on tBid killer gene might be a promising approach to specifically target the breast CSCs.

Rhodes, A. and T. Hillen (2019). "A mathematical model for the immune-mediated theory of metastasis." *J Theor Biol* **482**: 109999.

Accumulating experimental and clinical evidence suggest that the immune response to cancer is not exclusively anti-tumor. Indeed, the pro-tumor roles of the immune system - as suppliers of growth and pro-angiogenic factors or defenses against cytotoxic immune attacks, for example - have been long appreciated, but relatively few theoretical works have considered their effects. Inspired by the recently proposed "immune-mediated" theory of metastasis, we develop a mathematical model for tumor-immune interactions at two anatomically distant sites, which includes both anti- and pro-tumor immune effects, and the experimentally observed tumor-induced phenotypic plasticity of immune cells (tumor "education" of the

immune cells). Upon confrontation of our model to experimental data, we use it to evaluate the implications of the immune-mediated theory of metastasis. We find that tumor education of immune cells may explain the relatively poor performance of immunotherapies, and that many metastatic phenomena, including metastatic blow-up, dormancy, and metastasis to sites of injury, can be explained by the immune-mediated theory of metastasis. Our results suggest that further work is warranted to fully elucidate the pro-tumor effects of the immune system in metastatic cancer.

Riley, P. A. (2018). "Epimutation and Cancer: Carcinogenesis Viewed as Error-Prone Inheritance of Epigenetic Information." *J Oncol* **2018**: 2645095.

The epimutation concept, that is, malignancy is a result of deranged patterns of gene expression due to defective epigenetic control, proposes that in the majority of adult cancers the primary (initiating) lesion adversely affects the mechanism of vertical transmission of the epigenetic pattern existing in the stem cells of differentiated tissue. Such an error-prone mechanism will result in deviant gene expression capable of accumulation at each mitosis of the affected stem cell clone. It is argued that a proportion of these proliferation products will express combinations of genes which endow them with malignant properties, such as the ability to transgress tissue boundaries and migrate to distant locations. Since the likelihood of this occurrence is dependent on the proliferation of cells manifesting the defective epigenetic transmission, the theory predicts that cancer incidence will be strongly influenced by factors regulating the turnover rate of the stem cells of the tissue in question. Evidence relating to this stipulation is examined. In addition, it would be anticipated on the basis of the selection of genes involved that the susceptibility to malignant transformation will vary according to the tissue of origin and this is also discussed.

Rozhok, A. and J. DeGregori (2019). "A generalized theory of age-dependent carcinogenesis." *Elife* **8**.

The Multi-Stage Model of Carcinogenesis (MMC), developed in the 1950 s-70s, postulated carcinogenesis as a Darwinian somatic selection process. The cellular organization of tissues was then poorly understood, with almost nothing known about cancer drivers and stem cells. The MMC paradigm was later confirmed, and cancer incidence was explained as a function of mutation occurrence. However, the MMC has never been tested for its ability to account for the discrepancies in the number of driver mutations and the organization of the stem cell compartments underlying different cancers that still demonstrate nearly universal age-dependent incidence patterns. Here we demonstrate by Monte Carlo modeling the impact of key somatic evolutionary parameters on the MMC performance,

revealing that two additional major mechanisms, aging-dependent somatic selection and life history-dependent evolution of species-specific tumor suppressor mechanisms, need to be incorporated into the MMC to make it capable of generalizing cancer incidence across tissues and species. Editorial note: This article has been through an editorial process in which the authors decide how to respond to the issues raised during peer review. The Reviewing Editor's assessment is that all the issues have been addressed (see decision letter).

Sato, M., et al. (2020). "Immortalized normal human lung epithelial cell models for studying lung cancer biology." *Respir Investig* **58**(5): 344-354.

Primary cultures of human lung epithelial cells are ideal representatives of normal lung epithelial cells, and while there are certain novel approaches for the long-term culture of lung epithelial cells, the cells eventually undergo irreversible growth arrest, limiting their experimental utility, particularly the ability to widely distribute these cultures and their clonal derivatives to the broader research community. Therefore, the establishment of immortalized normal human lung epithelial cell strains has garnered considerable attention. The number and type of oncogenic changes necessary for the tumorigenic transformation of normal cells could be determined using "normal" cell lines immortalized with the simian virus 40 (SV40) large T antigen (LT). A primary report suggested that LT, human telomerase reverse transcriptase (hTERT), and oncogenic RAS transformed normal lung epithelial cells into tumorigenic cells. Since LT inactivates the tumor suppressors p53 and RB, at least four alterations would be necessary. However, the SV40 small T antigen (ST), a different oncoprotein, was also introduced simultaneously with LT in the above-mentioned study. Furthermore, the possible uncharacterized functions of LT remained largely obscure. Therefore, no definitive conclusion could be arrived in these studies. Subsequent studies used methods that did not involve the use of oncoproteins and revealed that at least five genetic changes were necessary for full tumorigenic transformation. hTERT-immortalized normal human lung epithelial cell lines established without using viral oncoproteins were also used for investigating several aspects of lung cancer, such as epithelial to mesenchymal transition and the cancer stem cell theory.

Sharp, J. A., et al. (2019). "Optimal control of acute myeloid leukaemia." *J Theor Biol* **470**: 30-42.

Acute myeloid leukaemia (AML) is a blood cancer affecting haematopoietic stem cells. AML is routinely treated with chemotherapy, and so it is of great interest to develop optimal chemotherapy treatment strategies. In this work, we incorporate an immune response into a stem cell model of AML, since

we find that previous models lacking an immune response are inappropriate for deriving optimal control strategies. Using optimal control theory, we produce continuous controls and bang-bang controls, corresponding to a range of objectives and parameter choices. Through example calculations, we provide a practical approach to applying optimal control using Pontryagin's Maximum Principle. In particular, we describe and explore factors that have a profound influence on numerical convergence. We find that the convergence behaviour is sensitive to the method of control updating, the nature of the control, and to the relative weighting of terms in the objective function. All codes we use to implement optimal control are made available.

Silvestris, E., et al. (2019). "Ddx4(+) Oogonial Stem Cells in Postmenopausal Women's Ovaries: A Controversial, Undefined Role." *Cells* **8**(7).

Recent studies support the existence of oogonial stem cells (OSCs) in the ovarian cortex of different mammals, including women. These cells are characterized by small size, membrane expression of DEAD(Asp-Glu-Ala-Asp)-box polypeptide-4 (Ddx4), and stemness properties (such as self-renewal and clonal expansion) as well as the ability to differentiate in vitro into oocyte-like cells. However, the discovery of OSCs contrasts with the popular theory that there is a numerically defined oocyte pool for female fertility which undergoes exhaustion with menopause. Indeed, in the ovarian cortex of postmenopausal women OSCs have been detected that possess both viability and capability to differentiate into oocytes, which is similar to those observed in younger patients. The pathophysiological role of this cell population in aged women is still debated since OSCs, under appropriate stimuli, differentiate into somatic cells, and the occurrence of Ddx4(+) cells in ovarian tumor samples also suggests their potential involvement in carcinogenesis. Although further investigation into these observations is needed to clarify OSC function in ovary physiology, clinical investigators and researchers studying female infertility are presently focusing on OSCs as a novel opportunity to restore ovarian reserve in both young women undergoing early ovarian failure and cancer survivors experiencing iatrogenic menopause.

Soderholm, S. and C. Cantu (2020). "The WNT/beta-catenin dependent transcription: A tissue-specific business." *Wiley Interdiscip Rev Syst Biol Med*: e1511.

beta-catenin-mediated Wnt signaling is an ancient cell-communication pathway in which beta-catenin drives the expression of certain genes as a consequence of the trigger given by extracellular WNT molecules. The events occurring from signal to transcription are evolutionarily conserved, and their

final output orchestrates countless processes during embryonic development and tissue homeostasis. Importantly, a dysfunctional Wnt/beta-catenin pathway causes developmental malformations, and its aberrant activation is the root of several types of cancer. A rich literature describes the multitude of nuclear players that cooperate with beta-catenin to generate a transcriptional program. However, a unified theory of how beta-catenin drives target gene expression is still missing. We will discuss two types of beta-catenin interactors: transcription factors that allow beta-catenin to localize at target regions on the DNA, and transcriptional co-factors that ultimately activate gene expression. In contrast to the presumed universality of beta-catenin's action, the ensemble of available evidence suggests a view in which beta-catenin drives a complex system of responses in different cells and tissues. A malleable armamentarium of players might interact with beta-catenin in order to activate the right "canonical" targets in each tissue, developmental stage, or disease context. Discovering the mechanism by which each tissue-specific beta-catenin response is executed will be crucial to comprehend how a seemingly universal pathway fosters a wide spectrum of processes during development and homeostasis. Perhaps more importantly, this could ultimately inform us about which are the tumor-specific components that need to be targeted to dampen the activity of oncogenic beta-catenin. This article is categorized under: Cancer > Molecular and Cellular Physiology Cancer > Genetics/Genomics/Epigenetics Cancer > Stem Cells and Development.

Somarelli, J. A., et al. (2020). "Molecular Biology and Evolution of Cancer: From Discovery to Action." *Mol Biol Evol* **37**(2): 320-326.

Cancer progression is an evolutionary process. During this process, evolving cancer cell populations encounter restrictive ecological niches within the body, such as the primary tumor, circulatory system, and diverse metastatic sites. Efforts to prevent or delay cancer evolution and progression require a deep understanding of the underlying molecular evolutionary processes. Herein we discuss a suite of concepts and tools from evolutionary and ecological theory that can inform cancer biology in new and meaningful ways. We also highlight current challenges to applying these concepts, and propose ways in which incorporating these concepts could identify new therapeutic modes and vulnerabilities in cancer.

Sun, H., et al. (2021). "Analysis of Age-Related Circular RNA Expression Profiles in Mesenchymal Stem Cells of Rat Bone Marrow." *Front Genet* **12**: 600632.

As multicellular organisms age, they undergo a reduction in tissue and organ function. Researchers have put forward a theory that stem cell aging is the

main factor responsible for decreased tissue and organ function. The adult stem cells guarantee the maintenance and repair of adult tissues and organs. Among adult stem cells, mesenchymal stem cells (MSCs) are emerging as hopeful candidates for cell-based therapy of numerous diseases. In recent years, high-throughput sequencing technologies have evolved to identify circular RNAs (circRNAs) associated with an increasing number of diseases, such as cancer and age-related diseases. It has been reported that circRNAs can compete with microRNAs (miRNAs) to affect the stability or translation of target RNAs and further regulate gene expression at the transcriptional level. However, the role of circRNAs expressed in MSCs in aging mechanisms has not yet been deciphered. The aim of this study was to explore and analyze the expression profiles of age-related circRNAs in MSCs. In this study, bone marrow MSCs were extracted from aged and young rats and analyzed using high-throughput sequencing and bioinformatics. The reliability of high-throughput RNA sequencing was verified by quantitative real-time polymerase chain reaction. The most important circRNA functions and pathways were further selected by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomics (KEGG) analysis. Age-related circRNAs were found in the circRNA-miRNA-mRNA interaction network. The results of high-throughput sequencing showed that 4,229 circRNAs were involved in age-related senescence of MSCs. Compared with the young group, there were 29 differentially expressed circRNAs in the aged group, of which four were upregulated and 25 were downregulated. GO analysis covered three domains: biological process (BP), cellular component (CC), and molecular function (MF). The terms assigned to the BP domain were cellular metabolic processes and cellular macromolecule metabolic processes. The identified CC terms were intracellular and intracellular part, and the identified MF terms were binding and protein binding. The top five KEGG pathways were mitophagy-animal-Rattus norvegicus, prostate cancer-Rattus norvegicus, pathways in cancer-Rattus norvegicus, lysosome-Rattus norvegicus, and autophagy-animal-Rattus norvegicus. Altogether, circRNAs may play a major role in age-related MSC senescence. This study provides new mechanistic insights into MSC senescence, possibly leading to novel therapeutic strategies for age-related diseases.

Tamazashvili, T. (2019). "Systemic Stem Cells Equilibrium Theory - the Law of Life." *Georgian Med News*(296): 144-149.

Despite progress in physiology, there unfortunately still remains a gap that does not explain what the governing nature of periodical morpho-functional changes of the body during normal development and if it can be associated with the



different pathological processes. The aim of the current study was to determine the role of systemic stem cells during different periods of life via introducing a new theory. Systematic stem cells equilibrium theory is a new concept in physiology which reflects the lifetime balance of systematic stem cells via their synchronized production, utilization and distribution/redistribution, determined by the need of a certain period of life with predominant support of reproductive function of the body. According to this theory, all mammalian organisms have innate total systemic stem cell pools, which consist of three major categories of stem cells: 1) central - embryonic stem cells. 2) distributary stem cells circulating in the blood stream and lymphatic system. 3) stationary/regional somatic stem cells, situated in the "niche" of tissues and organs. Redistribution of systematic stem cells occurs throughout the life and is an integral property of a living organism. Periodical change in systematic stem cell distribution is usually a normal process, that serves as a base for morpho-functional remodeling of the body necessary for a certain period of life. Changes in distribution of systemic stem cells, unfortunately, may occur situationally as well, causing pathological morpho-functional changes in the body and trigger development of different pathological processes including oncogenesis. Equilibrium of systemic stem cells has vital importance for the living organism because disbalance in this system can cause accumulation of excessive number of stem cells in the circulatory system and their unnecessary delivery to the regions, creating a favorable condition for cancer development. Tandon, I., et al. (2019). "Cancer Stem Cells Equipped with Powerful Hedgehog Signaling and Better Epigenetic Memory: Avenues to Look for Cancer Therapeutics." *Curr Cancer Drug Targets* **19**(11): 877-884.

Complex nature of the tumor is depicted at the cellular landscape by showing heterogeneity in the presence of cancer cells, cancer-associated stromal cells, mesenchymal stem cells and cancer stem cells (CSCs). One of the plausible views in cancer formation is suggested as the theory of cancer CSCs that is known as a source of initiation of tumorigenesis. In essence, these powerful CSCs are equipped with high Sonic Hedgehog (SHH) signaling and epigenetic memory power that support various tumor hallmarks. Truly, nature justifies its intent by limiting these stem cells with a potential to turn into CSCs and in turn suppressing the high risk of humans and other organisms. In short, this mini-review addresses the contribution of SHH signaling to allow reprogramming of epigenetic memory within CSCs that support tumor hallmarks. Besides, this paper explores therapeutic approaches to mitigate SHH signaling that may lead to a blockade of the pro-tumor potential of CSCs.

Tanimura, Y., et al. (2021). "Long-term model of colitis-associated colorectal cancer suggests tumor spread mechanism and nature of cancer stem cells." *Oncol Lett* **21**(1): 7.

Although chemical-induced animal models of colorectal cancer (CRC) suggest a lot about the disease, more efforts are required to establish metastasis models. Azoxymethane (AOM) and dextran sodium sulfate (DSS)-treated (AOM/DSS) Crl:CD-1 mice were sacrificed after 10 or 20 weeks in our previous study, and most colon tumors exhibited intramucosal adenocarcinomas. Our observations were extended until 30 weeks to study a colitis-associated advanced CRC mouse model, and explore whether linker threonine-phosphorylated Smad2/3 (pSmad2/3L-Thr) immunostaining-positive cells were involved in the progressive course of colitis-associated CRC as cancer stem cells. AOM/DSS mice were sacrificed at 10, 20 and 30 weeks after AOM administration. Following the histopathological analysis, immunohistochemical staining was performed for the following markers: CD34, podoplanin, beta-catenin, E-cadherin, Ki67, Bmi1 and pSmad2/3L-Thr. Compared with AOM/DSS mice at 10 and 20 weeks, submucosal tumor infiltration and tumor invasion into vessels were markedly increased at 30 weeks. In the parts of colon tumors from AOM/DSS mice, particularly in mice at 30 weeks, the positive signal of E-cadherin was clearly reduced in the cell membranes. The percentage of Ki67-positive tumor cells in mucosal areas of AOM/DSS mice was higher than that in the sites of submucosal infiltration. In mucosal areas of colon tumors, pSmad2/3L-Thr-positive cells were scattered among tumor cells. At sites of submucosal infiltration and vessel invasion of these tumors, pSmad2/3L-Thr-positive cells were also observed among tumor cells. In colon tumors from AOM/DSS mice at 30 weeks, the percentage of pSmad2/3L-Thr-positive cells among the nuclear beta-catenin-positive tumor cells was higher than that among the cytoplasmic beta-catenin-positive tumor cells. For both non-neoplastic and neoplastic epithelial cells, pSmad2/3L-Thr-positive cells exhibited immunohistochemical co-localization with Bmi1. The present study developed an advanced CRC mouse model that exhibited tumor infiltration into the submucosa and invasion into vessels. The present study re-confirmed the theory that pSmad2/3L-Thr-positive cells may be cancer stem cells.

Tarbeeve, D. V., et al. (2019). "Cytotoxic polyphenolic compounds from *Lespedeza bicolor* stem bark." *Fitoterapia* **135**: 64-72.

Four new pterocarpanes (6aR,11aR)-6a,11a-dihydrolespedezol A2 (2), (6aR,11aR)-2-isoprenyl-6a,11a-dihydrolespedezol A2 (3), (6aR,11aR,3'R)-6a,11a-dihydrolespedezol A3 (4), (6aR,11aR,3'S)-6a,11a-dihydrolespedezol A3 (5) and one new

stilbenoid with 1,2-diketone fragment named bicoloketone (6) along with one previously known pterocarpene lepedezol A2 (1) have been isolated from *Lespedeza bicolor* stem bark using multistage column chromatography on polyamide and silica gel. The structures of the isolated polyphenolic compounds were determined by spectroscopic methods. The absolute configurations of 4 and 5 were determined by comparison of their electronic circular dichroism (ECD) spectra obtained experimentally and the spectra calculated using time-dependent density functional theory (TDDFT). The isolated compounds exhibited a moderate DPPH scavenging effect and ferric reducing power compared to the reference antioxidant quercetin. The cytotoxicity of compounds against three human cancer cell lines, HTB-19, Kyse-30, and HEPG-2, and two normal cell lines, RPE-1 and HEK-293, was tested using the MTT assay. Compound 3 showed the strongest cytotoxic activity against all cell lines (IC<sub>50</sub> 6.0-19.1 μM) compared with the positive control cisplatin. The other tested compounds possessed moderate cytotoxic activity against cancer cells.

Trucco, M. M., et al. (2018). "A phase II study of temsirolimus and liposomal doxorubicin for patients with recurrent and refractory bone and soft tissue sarcomas." *Clin Sarcoma Res* **8**: 21.

**Background:** Relapsed and refractory sarcomas continue to have poor survival rates. The cancer stem cell (CSC) theory provides a tractable explanation for the observation that recurrences occur despite dramatic responses to upfront chemotherapy. Preclinical studies demonstrated that inhibition of the mechanistic target of rapamycin (mTOR) sensitizes the CSC population to chemotherapy. **Methods:** Here we present the results of the Phase II portion of a Phase I/II clinical trial that aimed to overcome the chemoresistance of sarcoma CSC by combining the mTOR inhibitor temsirolimus (20 mg/m<sup>2</sup> weekly) with the chemotherapeutic agent liposomal doxorubicin (30 mg/m<sup>2</sup> monthly). **Results:** Fifteen patients with relapsed/refractory sarcoma were evaluable at this recommended Phase 2 dose level. The median progression free survival was 315 days (range 27-799). Response rate, defined as stable disease or better for 60 days, was 53%. Nine of the patients had been previously treated with doxorubicin. Therapy was well tolerated. In a small number of patients, pre- and post-treatment tumor biopsies were available for assessment of ALDH expression as a marker of CSCs and showed a correlation between response and decreased ALDH expression. We also found a correlation between biopsy-proven inhibition of mTOR and response. **Conclusions:** Our study adds to the literature supporting the addition of mTOR inhibition to chemotherapy agents for the treatment of sarcomas, and proposes that a mechanism by which mTOR

inhibition enhances the efficacy of chemotherapy may be through sensitizing the chemoresistant CSC population. Further study, ideally with pre- and post-therapy assessment of ALDH expression in tumor cells, is warranted. Trial registration The trial was registered on clinicaltrials.gov (NCT00949325) on 30 July 2009. <http://www.editorialmanager.com/csrj/default.aspx>.

Tu, S. M. and L. L. Pisters (2021). "Curing Cancer: Lessons from a Prototype." *Cancers (Basel)* **13**(4).

Germ cell tumor of the testis (TGCT) is a remarkably curable solid tumor even when it is widely metastatic and patently heterogeneous. It provides invaluable clues about the origin and nature of metastasis and heterogeneity, cancer dormancy and late recurrence, drug sensitivity and resistance, tumor immunity, and spontaneous remission that would enable us to enhance the cure and improve the care of patients with other currently intractable solid tumors. After all, germ cells are primeval stem cells and TGCT are a perfect stem cell tumor for us to investigate a stem cell versus genetic origin of cancer. In many respects, TGCT is a prototype stem cell tumor that will enable us to elucidate the role of differentiation versus dedifferentiation in the evolution of a complex mixed tumor. It will help us decipher relevance of the genome versus the epi-genome in a progenitor cancer stem cell versus a progeny differentiated cancer cell. Importantly, clarification of a cellular context versus the genetic makeup in cancer has immense clinical implications. We postulate a unified theory of cancer derived from seminal TGCT research to improve personalized cancer care. Contrary to current norms and conventional wisdom, we propose that when it concerns a complex rather than simple cancer and a mixed rather than pure tumor (which is practically all solid tumors) multimodal therapy trumps targeted therapy and integrated medicine overrides precision medicine.

van der Heijden, M. and L. Vermeulen (2019). "Stem cells in homeostasis and cancer of the gut." *Mol Cancer* **18**(1): 66.

The intestinal epithelial lining is one of the most rapidly renewing cell populations in the body. As a result, the gut has been an attractive model to resolve key mechanisms in epithelial homeostasis. In particular the role of intestinal stem cells (ISCs) in the renewal process has been intensely studied. Interestingly, as opposed to the traditional stem cell theory, the ISC is not a static population but displays significant plasticity and in situations of tissue regeneration more differentiated cells can revert back to a stem cell state upon exposure to extracellular signals. Importantly, normal intestinal homeostasis provides important insight into mechanisms that drive colorectal cancer (CRC) development and growth. Specifically, the dynamics of cancer stem cells bear important

resemblance to ISC functionality. In this review we present an overview of the current knowledge on ISCs in homeostasis and their role in malignant transformation. Also, we discuss the existence of stem cells in intestinal adenomas and CRC and how these cells contribute to (pre-)malignant growth. Furthermore, we will focus on new paradigms in the field of dynamical cellular hierarchies in CRC and the intimate relationship between tumor cells and their niche.

Vellingiri, B., et al. (2020). "Understanding the Role of the Transcription Factor Sp1 in Ovarian Cancer: from Theory to Practice." *Int J Mol Sci* **21**(3).

Ovarian cancer (OC) is one of the deadliest cancers among women contributing to high risk of mortality, mainly owing to delayed detection. There is no specific biomarker for its detection in early stages. However, recent findings show that over-expression of specificity protein 1 (Sp1) is involved in many OC cases. The ubiquitous transcription of Sp1 apparently mediates the maintenance of normal and cancerous biological processes such as cell growth, differentiation, angiogenesis, apoptosis, cellular reprogramming and tumorigenesis. Sp1 exerts its effects on cellular genes containing putative GC-rich Sp1-binding site in their promoters. A better understanding of the mechanisms underlying Sp1 transcription factor (TF) regulation and functions in OC tumorigenesis could help identify novel prognostic markers, to target cancer stem cells (CSCs) by following cellular reprogramming and enable the development of novel therapies for future generations. In this review, we address the structure, function, and biology of Sp1 in normal and cancer cells, underpinning the involvement of Sp1 in OC tumorigenesis. In addition, we have highlighted the influence of Sp1 TF in cellular reprogramming of iPSCs and how it plays a role in controlling CSCs. This review highlights the drugs targeting Sp1 and their action on cancer cells. In conclusion, we predict that research in this direction will be highly beneficial for OC treatment, and chemotherapeutic drugs targeting Sp1 will emerge as a promising therapy for OC.

Wang, J., et al. (2020). "LY6D as a Chemoresistance Marker Gene and Therapeutic Target for Laryngeal Squamous Cell Carcinoma." *Stem Cells Dev* **29**(12): 774-785.

Laryngeal squamous cell carcinoma (LSCC) is a common head and neck cancer that is unresponsive to chemotherapy; therefore, understanding the causes of chemotherapy resistance is important. The cancer stem cell (CSC) theory postulates that CSCs are the source of tumor chemoresistance. We enrich laryngeal CSCs to overcome chemoresistance of LSCC. A laryngeal cancer xenograft model was established, and a low dose of cisplatin was administered until

chemoresistance arose. A next-generation xenograft model was established using surviving tumor cells, and the test was repeated four times to screen for CSCs. Cell function experiments were performed on each tumor cell generation (m1, m2, m3, and m4). The m3 line, with the highest stemness, was selected for transcriptome sequencing. LY6D was selected for clinical sample validation and functional verification. LY6D expression was detected in 107 laryngeal cancer samples, with high expression in 91 of these samples. LY6D expression was correlated with pathological T and clinical stages, and with cervical lymph node metastasis. The siLY6D group exhibited reduced adhesion and chemoresistance to cisplatin, 5-fluorouracil, and paclitaxel. LY6D is upregulated in laryngeal cancer and may serve as a biomarker for chemoresistance in CSCs. Moreover, LY6D could serve as an alternative antigenic peptide in the targeted treatment of laryngeal cancer.

Wang, X., et al. (2021). "It takes a village: microbiota, parainflammation, paligenosis and bystander effects in colorectal cancer initiation." *Dis Model Mech* **14**(5).

Sporadic colorectal cancer (CRC) is a leading cause of worldwide cancer mortality. It arises from a complex milieu of host and environmental factors, including genetic and epigenetic changes in colon epithelial cells that undergo mutation, selection, clonal expansion, and transformation. The gut microbiota has recently gained increasing recognition as an additional important factor contributing to CRC. Several gut bacteria are known to initiate CRC in animal models and have been associated with human CRC. In this Review, we discuss the factors that contribute to CRC and the role of the gut microbiota, focusing on a recently described mechanism for cancer initiation, the so-called microbiota-induced bystander effect (MIBE). In this cancer mechanism, microbiota-driven parainflammation is believed to act as a source of endogenous mutation, epigenetic change and induced pluripotency, leading to the cancerous transformation of colon epithelial cells. This theory links the gut microbiota to key risk factors and common histologic features of sporadic CRC. MIBE is analogous to the well-characterized radiation-induced bystander effect. Both phenomena drive DNA damage, chromosomal instability, stress response signaling, altered gene expression, epigenetic modification and cellular proliferation in bystander cells. Myeloid-derived cells are important effectors in both phenomena. A better understanding of the interactions between the gut microbiota and mucosal immune effector cells that generate bystander effects can potentially identify triggers for parainflammation, and gain new insights into CRC prevention.

Wang, Z. and C. Yang (2019). "Metal carcinogen exposure induces cancer stem cell-like property

through epigenetic reprogramming: A novel mechanism of metal carcinogenesis." *Semin Cancer Biol* **57**: 95-104.

Arsenic, cadmium, nickel and hexavalent chromium are among the most common environmental pollutants and potent carcinogens. Chronic exposure to these metals causes various types of cancer in humans, representing a significant environmental health issue. Although under active investigation, the mechanisms of metal carcinogenesis have not been clearly defined. One common feature of these metal carcinogens is that they are all able to cause various epigenetic dysregulations, which are believed to play important roles in their carcinogenicity. However, how metal carcinogen-caused epigenetic dysregulation contributes to metal carcinogenesis remains largely unknown. The evolution of cancer stem cell (CSC) theory has opened exciting new avenues for studying the mechanism of metal carcinogenesis. Increasing evidence indicates that chronic metal carcinogen exposure produces CSC-like cells through dysregulated epigenetic mechanisms. This review will first provide some brief introductions about CSC, epigenetics and epigenetic regulation of CSCs; then summarize progresses in recent studies on metal carcinogen-induced CSC-like property through epigenetic reprogramming as a novel mechanism of metal carcinogenesis. Some perspectives for future studies in this field are also presented.

Wojdyla, T., et al. (2019). "Mutation, drift and selection in single-driver hematologic malignancy: Example of secondary myelodysplastic syndrome following treatment of inherited neutropenia." *PLoS Comput Biol* **15**(1): e1006664.

Cancer development is driven by series of events involving mutations, which may become fixed in a tumor via genetic drift and selection. This process usually includes a limited number of driver (advantageous) mutations and a greater number of passenger (neutral or mildly deleterious) mutations. We focus on a real-world leukemia model evolving on the background of a germline mutation. Severe congenital neutropenia (SCN) evolves to secondary myelodysplastic syndrome (sMDS) and/or secondary acute myeloid leukemia (sAML) in 30-40%. The majority of SCN cases are due to a germline ELANE mutation. Acquired mutations in CSF3R occur in >70% sMDS/sAML associated with SCN. Hypotheses underlying our model are: an ELANE mutation causes SCN; CSF3R mutations occur spontaneously at a low rate; in fetal life, hematopoietic stem and progenitor cells expands quickly, resulting in a high probability of several tens to several hundreds of cells with CSF3R truncation mutations; therapeutic granulocyte colony-stimulating factor (G-CSF) administration early in life exerts a strong selective pressure, providing mutants with a growth advantage. Applying population genetics

theory, we propose a novel two-phase model of disease development from SCN to sMDS. In Phase 1, hematopoietic tissues expand and produce tens to hundreds of stem cells with the CSF3R truncation mutation. Phase 2 occurs postnatally through adult stages with bone marrow production of granulocyte precursors and positive selection of mutants due to chronic G-CSF therapy to reverse the severe neutropenia. We predict the existence of the pool of cells with the mutated truncated receptor before G-CSF treatment begins. The model does not require increase in mutation rate under G-CSF treatment and agrees with age distribution of sMDS onset and clinical sequencing data.

Yamulla, R. J., et al. (2020). "Most Commonly Mutated Genes in High-Grade Serous Ovarian Carcinoma Are Nonessential for Ovarian Surface Epithelial Stem Cell Transformation." *Cell Rep* **32**(9): 108086.

High-grade serous ovarian carcinoma (HGSOC) is the fifth leading cause of cancer-related deaths of women in the United States. Disease-associated mutations have been identified by the Cancer Genome Atlas Research Network. However, aside from mutations in TP53 or the RB1 pathway that are common in HGSOC, the contributions of mutation combinations are unclear. Here, we report CRISPR mutagenesis of 20 putative HGSOC driver genes to identify combinatorial disruptions of genes that transform either ovarian surface epithelium stem cells (OSE-SCs) or non-stem cells (OSE-NSs). Our results support the OSE-SC theory of HGSOC initiation and suggest that most commonly mutated genes in HGSOC have no effect on OSE-SC transformation initiation. Our results indicate that disruption of TP53 and PTEN, combined with RB1 disruption, constitutes a core set of mutations driving efficient transformation in vitro. The combined data may contribute to more accurate modeling of HGSOC development.

Zefferino, R., et al. (2019). "Gap Junction Intercellular Communication in the Carcinogenesis Hallmarks: Is This a Phenomenon or Epiphenomenon?" *Cells* **8**(8).

If occupational tumors are excluded, cancer causes are largely unknown. Therefore, it appeared useful to work out a theory explaining the complexity of this disease. More than fifty years ago the first demonstration that cells communicate with each other by exchanging ions or small molecules through the participation of connexins (Cx) forming Gap Junctions (GJs) occurred. Then the involvement of GJ Intercellular Communication (GJIC) in numerous physiological cellular functions, especially in proliferation control, was proven and accounts for the growing attention elicited in the field of carcinogenesis. The aim of the present paper is to verify and discuss the role of Cxs, GJs, and GJIC in cancer hallmarks,

pointing on the different involved mechanisms in the context of the multi-step theory of carcinogenesis. Functional GJIC acts both as a tumor suppressor and as a tumor enhancer in the metastatic stage. On the contrary, lost or non-functional GJs allow the uncontrolled proliferation of stem/progenitor initiated cells. Thus, GJIC plays a key role in many biological phenomena or epiphenomena related to cancer. Depending on this complexity, GJIC can be considered a tumor suppressor in controlling cell proliferation or a cancer ally, with possible preventive or therapeutic implications in both cases.

Zehra, S., et al. (2020). "Water soluble ionic Co(II), Cu(II) and Zn(II) diimine-glycinate complexes targeted to tRNA: structural description, in vitro comparative binding, cleavage and cytotoxic studies towards chemoresistant prostate cancer cells." *Dalton Trans* **49**(46): 16830-16848.

Four new water soluble Co(ii), Cu(ii) and Zn(ii) ionic metal complexes (1-4) [Cu(diimine)(H<sub>2</sub>O)<sub>2</sub>(glycinate)]+[glycinate]<sup>-</sup>, [Co(diimine)(H<sub>2</sub>O)<sub>4</sub>]+[glycinate]<sup>-</sup> and [Zn(diimine)(H<sub>2</sub>O)<sub>4</sub>]+[glycinate]<sup>-</sup>, where diimine = 2,2'-bipyridine (1-3) and 1,10-phenanthroline (4) were synthesized and thoroughly characterized by spectroscopic and single X-ray crystallographic studies. Complex 1 possesses a triclinic crystal system with a penta-coordinated geometry whereas complexes 2-4 crystallized in an isostructural monoclinic system having distorted octahedral geometry. Density functional theory (DFT) studies for complexes 1-4 were performed to correlate their geometrical parameters and to calculate the energy of frontier molecular orbitals. The corroborative results of spectroscopic and voltammetric studies with ct-DNA and tRNA revealed that the complexes bind noncovalently via an electrostatic mode of binding with specificity for tRNA as compared to ct-DNA. Gel electrophoresis experiments revealed that all the complexes unwind the plasmid pBR322 DNA at low micromolar concentrations (2-9 μM) following an oxidative mechanism for Cu(ii) and Co(ii) complexes (1, 2 and 4) whereas the Zn(ii) complex (3) mediates DNA cleavage by the hydrolytic pathway. The tRNA cleavage showed concentration and time dependent activity of the complexes to promote RNA hydrolysis. Furthermore, the BSA binding ability of complexes 1-4 was monitored, which revealed that the complexes could quench the intrinsic fluorescence in a static manner. Complexes 1-4 were found to be non-toxic towards normal prostate epithelial cells, PNT2, but were potent against chemoresistant metastatic prostate cancer cells, Du145, with GI50 values ranging from 12.75-37 μM. Complexes 1 and 2 also showed cytotoxic activity against cancer stem cells having GI50 values of 14.70 and 14.90 μM, respectively. Molecular docking studies were performed with DNA

and tRNA which further validated the spectroscopic analysis demonstrating the higher binding affinity of the complexes towards tRNA.

Zhai, Y., et al. (2019). "Effect of NELL1 on lung cancer stemlike cell differentiation." *Oncol Rep* **41**(3): 1817-1826.

The cancer stem cell theory recently has received enormous attention in cancer biology. Lung cancer stemlike cells are a subpopulation of undifferentiated lung tumor cells critical for lung cancer tumorigenesis, metastasis and resistance to therapy and disease relapse. The neural EGFL like 1 (NELL1) is a potent growth factor believed to preferentially target cells committed to the osteochondral lineage; yet, its expression and function in lung cancer are largely unknown. In the present study, we used specific medium to accumulate lung cancer stemlike cells of 95D cells in spheres and obtained these highly expressed CD133 cells through flow cytometric cell sorting of CD133stained cells which were termed 95D lung cancer stemlike cells (95D LCSCs). These 95D LCSCs highly expressed stemness genes CD133, Oct4 and Sox2 determined by western blot analysis and quantitative realtime polymerase chain reaction (qPCR) analysis. Notably, we found that overexpression of NELL1 significantly reduced colony formation and invasion of 95D LCSCs tested by soft agar colony formation and cell invasion assay. In addition, as determined by cell proliferation assay, overexpression of NELL1 increased the chemotherapeutic sensitivity of 95D LCSCs to carboplatin and cisplatin. NELL1 also reduced the expression of phosphoMET (pMET), Notch3 and HES1, which suggests that NELL1 may induce 95D LCSC differentiation by inhibiting the expression of cMETNotch signaling. Our results suggest that NELL1 induces lung cancer stemlike cell differentiation, which provides a new potential therapeutic target for cancer stem cells.

Zhang, X., et al. (2020). "Integrative analysis of the common genetic characteristics in ovarian cancer stem cells sorted by multiple approaches." *J Ovarian Res* **13**(1): 116.

**BACKGROUND:** Ovarian cancer is the second fatal malignancy of the female reproductive system. Based on the cancer stem cell (CSC) theory, its poor prognosis of ovarian cancer attributed to tumor recurrence caused by CSCs. A variety of cell surface-specific markers have been employed to identify ovarian cancer stem cells (OCSCs). In this study, we attempted to explore the common feature in ovarian cancer stem cells sorted by multiple approaches. **METHODS:** We collected the gene expression profiles of OCSCs were from 5 public cohorts and employed R software and Bioconductor packages to establish differently expressed genes (DEGs) between OCSCs

and parental cells. We extracted the integrated DEGs by protein-protein interaction (PPI) network construction and explored potential treatment by the Cellminer database. RESULTS: We identified and integrated the DEGs of OCSCs sorted by multiple isolation approaches. Besides, we identified OCSCs share characteristics in the lipid metabolism and extracellular matrix changes. Moreover, we obtained 16 co-expressed core genes, such as FOXQ1, MMP7, AQP5, RBM47, ETV4, NPW, SUSD2, SFRP2, IDO1, ANPEP, CXCR4, SCNN1A, SPP1 and IFI27 (upregulated) and SERPINE1, DUSP1, CD40, and IL6 (downregulated). Through correlation analysis, we screened out ten potential drugs to target the core genes. CONCLUSION: Based on the comprehensive analysis of the genomic datasets with different sorting methods of OCSCs, we figured out the common driving genes to regulating OCSC and obtained ten new potential therapies for eliminating ovarian cancer stem cells. Hence, the findings of our study might have potential clinical significance.

Zhang, Z., et al. (2021). "Bioinformatics analysis reveals biomarkers with cancer stem cell characteristics in kidney renal clear cell carcinoma." *Transl Androl Urol* 10(8): 3501-3514.

Background: Kidney renal clear cell carcinoma (KIRC) is a renal cortical tumor. KIRC is the most common subtype of kidney cancer, accounting for 70%-80% of kidney cancer. Early identification of the risk of KIRC patients can facilitate more accurate clinical treatment, but there is a lack of effective prognostic markers. We aimed to identify new prognostic biomarkers for KIRC on the basis of the cancer stem cell (CSC) theory. Methods: RNA-sequencing (RNA-seq) data and related clinical information were downloaded from The Cancer Genome Atlas (TCGA) database. Weighted gene co-expression network analysis (WGCNA) was used to identify significant modules and hub genes, and predictive hub genes were used to construct prognostic characteristics. Results: The messenger RNA expression-based stemness index (mRNAsi) in tumor tissues of patients in the TCGA database is higher than that of the corresponding normal tissues. In addition, some clinical features and results are highly correlated with mRNAsi. WGCNA found that the green module is

the most prominent module associated with mRNAsi; the genes in the green module are mainly concentration in Notch binding, endothelial cell development, Notch signaling pathway, and Rap 1 signaling pathway. A protein-protein interaction (PPI) network showed that the top 10 central genes were significantly associated with the transcriptional level. Moreover, the 10 hub genes were up-regulated in KIRC. Regarding survival analysis, the nomogram of the prognostic markers of the seven pivotal genes showed a higher predictive value. The classical receiver operating characteristic (ROC) curve analysis showed that risk score biomarkers had the highest accuracy and specificity with an area under the curve (AUC) value of 0.701. Conclusions: mRNAsi-related genes may be good prognostic biomarkers for KIRC.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

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