Nanog and Cancer Biology 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.
[Mark Herbert. **Nanog and Cancer Biology Research Literatures.** *Researcher* 2019;11(1):96-122]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). http://www.sciencepub.net/researcher. 8. doi:10.7537/marsrsj110119.08.

Key words: cancer; nanog; life; research; literature; cell; biology

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.

Almozyan, S., et al. (2017). "PD-L1 promotes OCT4 and Nanog expression in breast cancer stem cells by sustaining PI3K/AKT pathway activation." Int J Cancer **141**(7): 1402-1412.

The expression of PD-L1 in breast cancer is associated with estrogen receptor negativity, chemoresistance and epithelial-to-mesenchymal transition (EMT), all of which are common features of a highly tumorigenic subpopulation of cancer cells termed cancer stem cells (CSCs). Hitherto, the expression and intrinsic role of PD-L1 in the dynamics of breast CSCs has not been investigated. To address this issue, we used transcriptomic datasets, proteomics and several in vitro and in vivo assays. Expression profiling of a large breast cancer dataset (530 patients) showed statistically significant correlation ($p < 0.0001$, $r = 0.36$) between PD-L1 expression and stemness score of breast cancer. Specific knockdown of PD-L1 using ShRNA revealed its critical role in the expression of the embryonic stem cell transcriptional factors: OCT-4A, Nanog and the stemness factor, BMI1. Conversely, these factors could be induced upon PD-L1 ectopic expression in cells that are normally PD-L1 negative. Global proteomic analysis hinted for the central role of AKT in the biology of PD-L1 expressing cells. Indeed, PD-L1 positive effect on OCT-4A and Nanog was dependent on AKT activation. Most importantly, downregulation of PD-L1 compromised the self-renewal capability of breast CSCs in vitro and in vivo as shown by tumorsphere formation assay and extreme limiting dilution assay, respectively. This study demonstrates a novel role for PD-L1 in sustaining stemness of breast cancer cells and identifies the subpopulation and its associated molecular pathways that would be targeted upon anti-PD-L1 therapy.

Amini, S., et al. (2014). "The expressions of stem cell markers: Oct4, Nanog, Sox2, nucleostemin, Bmi, Zfx, Tcl1, Tbx3, Dppa4, and Esrrb in bladder, colon, and prostate cancer, and certain cancer cell lines." Anat Cell Biol **47**(1): 1-11.

Uncontrolled self-renewal plays a direct function in the progression of different types of carcinomas. The same molecular pathway that manages self-renewal in normal stem cells also seems to manage cancer stem cells. Here, we examine the expressions of self-renewal regulatory factors Oct4, Nanog, Sox2, nucleostemin, Zfx, Esrrb, Tcl1, Tbx3, and Dppa4 in tissue samples of colon, prostate, and bladder carcinomas as well as cancer cell lines HT-29, Caco-2, HT-1376, LNCaP, and HepG2. We used reverse transcriptase polymerase chain reaction to examine expressions of the above mentioned regulatory factors in cancer cell lines HT-29, Caco-2, HT-1376, LNCaP, and HepG2 and in 20 tumor tissue samples. Total RNA was isolated by the ISOGEN method. RNA integrity was checked by agarose gel electrophoresis and spectrophotometry. Expressions of Oct4 and nucleostemin at the protein level were determined by immunocytochemistry. A significant relationship was found between tumor grade and self-renewal gene

expression. Expressions of stem cell specific marker genes were detected in all examined cancer cell lines, in 40% to 100% of bladder cancer samples, and in 60% to 100% of colon and prostate cancer samples. Oct4 expressed in 100% of tumor tissue samples. Our data show that stem cell markers Oct4, Nanog, Sox2, nucleostemin, Bmi, Zfx, Esrrb, Tcl1, Tbx3, and Dppa4 significantly express in cancer cell lines and cancer tissues. Hence, these markers might be useful as potential tumor markers in the diagnosis and/or prognosis of tumors.

Amsterdam, A., et al. (2013). "Differential localization of LGR5 and Nanog in clusters of colon cancer stem cells." Acta Histochem **115**(4): 320-329.

One paradigm of cancer development claims that cancer emerges at the niche of tissue stem cells and these cells continue to proliferate in the tumor as cancer stem cells. LGR5, a membrane receptor, was recently found to be a marker of normal colon stem cells in colon polyps and is also expressed in colon cancer stem cells. Nanog, an embryonic stem cell nuclear factor, is expressed in several embryonic tissues, but Nanog expression is not well documented in cancerous stem cells. Our aim was to examine whether both LGR5 and Nanog are expressed in the same clusters of colon stem cells or cancer stem cells, using immunocytochemistry with specific antibodies to each antigen. We analyzed this aspect using paraffin embedded tumor tissue sections obtained from 18 polyps and 36 colon cancer specimens at stages I-IV. Antibodies to LGR5 revealed membrane and cytoplasm immunostaining of scattered labeled cells in normal crypts, with no labeling of Nanog. However, in close proximity to the tumors, staining to LGR5 was much more intensive in the crypts, including that of the epithelial cells. In cancer tissue, positive LGR5 clusters of stem cells were observed mainly in poorly differentiated tumors and in only a few scattered cells in the highly differentiated tumors. In contrast, antibodies to Nanog mainly stained the growing edges of carcinoma cells, leaving the poorly differentiated tumor cells unlabeled, including the clustered stem cells that could be detected even by direct morphological examination. In polyp tissues, scattered labeled cells were immunostained with antibodies to Nanog and to a much lesser extent with antibodies to LGR5. We conclude that expression of LGR5 is probably specific to stem cells of poorly differentiated tumors, whereas Nanog is mainly expressed at the edges of highly differentiated tumors. However, some of the cell layers adjacent to the carcinoma cell layers that still remained undifferentiated, expressed mainly Nanog with only a few cells labeled with antibodies to LGR5. Considering the different sites and pattern of expression in the tumor, our data imply that targeting the clustered stem cells expressing LGR5 in poorly differentiated colon cancer may require different strategies than targeting the stem cells expressing Nanog in the highly differentiated tumors. Alternatively, combined application of specific inhibitory miRNAs to Nanog and to LGR5 expression may assist therapeutically.

Amsterdam, A., et al. (2013). "LGR5 and Nanog identify stem cell signature of pancreas beta cells which initiate pancreatic cancer." Biochem Biophys Res Commun **433**(2): 157-162.

Pancreas cancer, is the fourth leading cause of cancer death but its cell of origin is controversial. We compared the localization of stem cells in normal and cancerous pancreas using antibodies to the stem cell markers Nanog and LGR5. Here we show, for the first time, that LGR5 is expressed in normal pancreas, exclusively in the islets of Langerhans and it is co-localized, surprisingly, with Nanog and insulin in clusters of beta cells. In cancerous pancreas Nanog and LGR5 are expressed in the remaining islets and in all ductal cancer cells. We observed insulin staining among the ductal cancer cells, but not in metastases. This indicates that the islet's beta cells, expressing LGR5 and Nanog markers are the initiating cells of pancreas cancer, which migrated from the islets to form the ductal cancerous tissue, probably after mutation and de-differentiation. This discovery may facilitate treatment of this devastating cancer.

Arif, K., et al. (2015). "The role of Nanog expression in tamoxifen-resistant breast cancer cells." Onco Targets Ther **8**: 1327-1334.

There is an accumulation of evidence that shows a significant role of cancer stem cells in tumor initiation, proliferation, relapse, and metastasis. Nanog is the most important core transcription marker of stem cells, known by its role in maintaining pluripotency, proliferation, and differentiation. Therefore, this study aimed to examine the role of Nanog in breast cancer cell tamoxifen resistance and its implications in breast cancer treatment. In this study, the expression of the three core transcription markers Nanog, Oct3/4, and Sox2 were quantitatively evaluated using flow cytometry. Then, small interfering RNA (siRNA) against human Nanog was transfected into tamoxifen-resistant breast cancer cells via Lipofectamine 2000. Nanog gene expression in the cells was detected using reverse transcription polymerase chain reaction (RT-PCR). The change in cell proliferation was evaluated using the tetrazolium bromide method. An enzyme-linked immunosorbent assay was used to detect apoptosis of the transfected cells alone and in combination with 4-hydroxytamoxifen. The results showed a high level expression of Nanog, Oct3/4, and

Sox2 in MDA-MB-231 and MCF7/tamoxifen resistant cells compared with MCF7/wild-type. siRNA-mediated Nanog gene silencing can efficiently inhibit cell proliferation and induce apoptosis of tamoxifen-resistant breast cancer cells. This study provides a basis for further study of the role of Nanog in developing resistance to tamoxifen, its implication in breast cancer management, and as a new strategy to enhance response to endocrine therapy.

Bahl, K., et al. (2012). "Increased Levels of Circulating and Tissue mRNAs of Oct-4, Sox-2, Bmi-1 and Nanog is ESCC Patients: Potential Tool for Minimally Invasive Cancer Diagnosis." Biomark Insights **7**: 27-37.

BACKGROUND: Early stages of esophageal cancer lack a specific symptom, a reliable biomarker and accurate non-invasive diagnostic modalities prompting the pressing need for identification of a marker for early diagnosis of this disease. METHODS: In the present study we investigated the levels of circulating and tissue mRNAs of Oct-3/4, Sox-2, Nanog and Bmi-1 in esophageal cancer patients using Reverse-Transcription Polymerase Chain Reaction (RT-PCR) with the aim of evaluating their potential as minimally invasive diagnostic markers. RESULT: Increased transcript levels of Oct-4, Sox-2, Bmi-1 and Nanog were detected in (92%), (95%), (75%) and (67%) of the esophageal cancer tissues, respectively as compared with the matched distant normals. CONCLUSION: Interestingly, most of the preneoplastic tissues exhibited increased transcript levels of these stemness markers suggesting their role in early stages of esophageal tumorigenesis. Furthermore, the detection of elevated levels of circulating mRNAs of Oct-4 and Nanog in sera of esophageal cancer patients emphasizes their potential as minimally invasive diagnostic markers for esophageal cancer.

Bourguignon, L. Y., et al. (2012). "Hyaluronan-CD44v3 interaction with Oct4-Sox2-Nanog promotes miR-302 expression leading to self-renewal, clonal formation, and cisplatin resistance in cancer stem cells from head and neck squamous cell carcinoma." J Biol Chem **287**(39): 32800-32824.

Human head and neck squamous cell carcinoma (HNSCC) is a highly malignant cancer associated with major morbidity and mortality. In this study, we determined that human HNSCC-derived HSC-3 cells contain a subpopulation of cancer stem cells (CSCs) characterized by high levels of CD44v3 and aldehyde dehydrogenase-1 (ALDH1) expression. These tumor cells also express several stem cell markers (the transcription factors Oct4, Sox2, and Nanog) and display the hallmark CSC properties of self-renewal/clonal formation and the ability to generate heterogeneous cell populations. Importantly, hyaluronan (HA) stimulates the CD44v3 (an HA receptor) interaction with Oct4-Sox2-Nanog leading to both a complex formation and the nuclear translocation of three CSC transcription factors. Further analysis reveals that microRNA-302 (miR-302) is controlled by an upstream promoter containing Oct4-Sox2-Nanog-binding sites, whereas chromatin immunoprecipitation (ChIP) assays demonstrate that stimulation of miR-302 expression by HA-CD44 is Oct4-Sox2-Nanog-dependent in HNSCC-specific CSCs. This process results in suppression of several epigenetic regulators (AOF1/AOF2 and DNMT1) and the up-regulation of several survival proteins (cIAP-1, cIAP-2, and XIAP) leading to self-renewal, clonal formation, and cisplatin resistance. These CSCs were transfected with a specific anti-miR-302 inhibitor to silence miR-302 expression and block its target functions. Our results demonstrate that the anti-miR-302 inhibitor not only enhances the expression of AOF1/AOF2 and DNMT1 but also abrogates the production of cIAP-1, cIAP-2, and XIAP and HA-CD44v3-mediated cancer stem cell functions. Taken together, these findings strongly support the contention that the HA-induced CD44v3 interaction with Oct4-Sox2-Nanog signaling plays a pivotal role in miR-302 production leading to AOF1/AOF2/DNMT1 down-regulation and survival of protein activation. All of these events are critically important for the acquisition of cancer stem cell properties, including self-renewal, clonal formation, and chemotherapy resistance in HA-CD44v3-activated head and neck cancer.

Es-Haghi, M., et al. (2016). "Perspective: Cooperation of Nanog, NF-kappaBeta, and CXCR4 in a regulatory network for directed migration of cancer stem cells." Tumour Biol **37**(2): 1559-1565.

Directed cell migration is a crucial mobility phase of cancer stem cells having stemness and tumorigenic characteristics. It is known that CXCR4 plays key roles in the perception of chemotactic gradients throughout the directed migration of CSCs. There are a number of complex signaling pathways and transcription factors that coordinate with CXCR4/CXCL12 axis during directed migration. In this review, we focus on some transcription factors such as Nanog, NF-kappaB, and Bmi-1 that cooperate with CXCR4/CXCL12 for the maintenance of stemness and induction of metastasis behavior in cancer stem cells.

Gao, S., et al. (2016). "Nanog Predicts Poor Prognosis in Human Pancreatic Cancer and Is Downregulated by QingyihuaJi Formula in Pancreatic

Cancer Stem Cells." Evid Based Complement Alternat Med **2016**: 7028289.

Qingyihuaji formula (QYHJ), confirmed efficacious in a series of clinical trials, has been applied to human pancreatic carcinoma treatment in Shanghai Cancer Center for years. Recent evidence highlighted that pluripotent stem cells transcription factor Nanog plays a pivotal role in carcinogenesis. However, there is little published information regarding the underlying clinical significance and mechanisms of transcription factor Nanog in pancreatic cancer. In this study, our results indicated that Nanog is overexpressed in human pancreatic cancer stem cells and downregulated by QYHJ, which may contribute to explain the clinical effectiveness of QYHJ and provide advanced pancreatic cancer patients with a new therapeutic option, supporting our hypothesis that the degradation pathway is another mechanism by which QYHJ affects Nanog expression.

Gao, S., et al. (2016). "ROR functions as a ceRNA to regulate Nanog expression by sponging miR-145 and predicts poor prognosis in pancreatic cancer." Oncotarget **7**(2): 1608-1618.

lncRNAs have emerged as key regulators of tumor development and progression. ROR is a typical lncRNA that plays important regulatory roles in the pathogenesis and progression of tumors. Nevertheless, current understanding of the involvement of ROR in pancreatic adenocarcinoma tumorigenesis remains limited. In this study, we measured ROR in 61 paired cancerous and noncancerous tissue samples by qRT-PCR and investigated the biological role of ROR on the phenotypes of pancreatic cancer stem cells (PCSCs) in vitro and in vivo. The effects of ROR on PCSCs were studied by RNA interference approaches in vitro and in vivo. Insights of the mechanism of competitive endogenous RNAs (ceRNAs) were gained from bioinformatic analysis, luciferase assays and RNA binding protein immunoprecipitation. The positive ROR/Nanog interaction was identified and verified by immunohistochemistry assay. Compared with adjacent non-tumor tissues, ROR was up-regulated in most tumor tissues. Knockdown of ROR by RNA interference in PCSCs inhibited proliferation, induced apoptosis and decreased migration. Moreover, ROR silencing resulted in significantly decreased tumourigenicity of PCSCs in nude mice than controls. In particular, ROR may act as a ceRNA, effectively becoming a sink for miR-145, thereby activating the derepression of core transcription factors Nanog. In conclusions, we demonstrated that decreased ROR expression could inhibit cell proliferation, invasion, and tumourigenicity by modulating Nanog. Therefore, ROR is a potential novel prognostic marker to predict the clinical outcome of pancreatic cancer patients after surgery and may be a rational target for therapy.

Gawlik-Rzemieniewska, N. and I. Bednarek (2016). "The role of NANOG transcriptional factor in the development of malignant phenotype of cancer cells." Cancer Biol Ther **17**(1): 1-10.

NANOG is a transcription factor that is involved in the self-renewal of embryonic stem cells (ES) and is a critical factor for the maintenance of the undifferentiated state of pluripotent cells. Extensive data in the literature show that the NANOG gene is aberrantly expressed during the development of malignancy in cancer cells. ES and cancer stem cells (CSCs), a subpopulation of cancer cells within the tumor, are thought to share common phenotypic properties. This review describes the role of NANOG in cancer cell proliferation, epithelial-mesenchymal transition (EMT), apoptosis and metastasis. In addition, this paper illustrates a correlation between NANOG and signal transducer and activator of transcription 3 (STAT3) in the maintenance of cancer stem cell properties and multidrug resistance. Together, the available data demonstrate that NANOG is strictly involved in the process of carcinogenesis and is a potential prognostic marker of malignant tumors.

Gawlik-Rzemieniewska, N., et al. (2016). "Silencing expression of the NANOG gene and changes in migration and metastasis of urinary bladder cancer cells." Arch Med Sci **12**(4): 889-897.

INTRODUCTION: It has been proved that expression of the NANOG gene is observed not only in embryonic-derived malignancies, but also in breast cancer, ovarian cancer, cervix cancer and bladder cancer. NANOG overexpression is correlated with high activity of MMP-2 and MMP-9. The aim of the study was to evaluate the changes in the malignant phenotype of T24 bladder cancer cells with modulated expression of the NANOG gene. MATERIAL AND METHODS: Human urinary bladder cancer cells T24 (HTB-4) were cultivated under standard conditions. Transfection of the cells with silencing constructions was performed with the application of Lipofectamine 2000 (Invitrogen) reagent. Evaluation of changes in the expression level of individual genes was performed using qRTPCR. Changes in the protein level were evaluated using the Human ELISA Kit (Abcam). The invasion capability of transfected cells was tested using Matrigel Invasion Chambers (BD Biosciences). The changes in cell migration were assessed with a wound-healing assay. RESULTS: The qRTPCR evaluation showed that silencing the NANOG gene in T24 cells led to the decrease of mRNA for the MMP-2 gene to the level of 62.4% and the MMP-9 gene to the

level of 76%. The cells with modulated expression of the NANOG gene migrated slower in the Matrigel invasion assay and in the wound-healing assay. The immunoenzymatic test showed a decrease in the protein level of MMP-9. CONCLUSIONS: The transcriptional activity of the NANOG gene might be connected with some aspects of bladder cancer cell metastasis in vitro and has an influence on MMP-2 and MMP-9 expression levels.

Gialmanidis, I. P., et al. (2013). "Expression of Bmi1, FoxF1, Nanog, and gamma-catenin in relation to hedgehog signaling pathway in human non-small-cell lung cancer." Lung **191**(5): 511-521.

BACKGROUND: Hedgehog signaling is known to be involved in both lung organogenesis and lung carcinogenesis. The aim of this study was to examine potential downstream targets of the hedgehog signaling pathway in non-small-cell lung cancer. METHODS: Protein expression of Bmi1, FoxF1, Nanog, and gamma-catenin was examined by immunohistochemistry in 80 non-small-cell lung cancer samples. Correlations with the previously immunohistochemically recovered results for sonic hedgehog, Ptch1, Smo, Gli1, and Gli2 in the same cohort of tumors as well as the clinicopathological characteristics of the tumors were also evaluated. RESULTS: Bmi1 was expressed in 78/80 (97.5 %) cases of non-small-cell lung cancer and correlated with male gender and expression of Gli1. Positive expression of FoxF1 was found in 62/80 (77.5 %) cases. Expression of FoxF1 correlated with lymph node metastases, Bmi1, and hedgehog pathway activation. Overexpression of Nanog was also noted in 74/80 (92.5 %) tumors and correlated with Bmi1. Cytoplasmic accumulation of gamma-catenin was observed in 85 % (68/80) of the tumors and correlated with the expression of Bmi1, FoxF1, and Nanog. CONCLUSION: Several developmental pathways seem to be implicated in non-small-cell lung cancer. It is also suggested that Bmi1 and FoxF1 may cooperate with hedgehog signaling in non-small-cell lung carcinogenesis.

Golubovskaya, V. M. (2013). "FAK and Nanog cross talk with p53 in cancer stem cells." Anticancer Agents Med Chem **13**(4): 576-580.

This review is focused on the role of Focal Adhesion Kinase (FAK) signaling in cancer stem cells. The recent data demonstrate the important role of FAK in cancer stem cell proliferation, differentiation, motility, and invasion. We showed recently that the transcription factor Nanog binds the FAK promoter and up-regulates FAK expression, and that FAK binds Nanog and phosphorylates it. This review discusses the interaction of FAK, Nanog, Oct-3/4, and Sox-2 signaling pathways that are critical for the regulation of cancer stem cells. The cross-linked signaling of FAK with p53 and Nanog signaling in cancer stem cell and function and targeted therapeutics approaches are discussed.

Gong, C., et al. (2012). "Implication of expression of Nanog in prostate cancer cells and their stem cells." J Huazhong Univ Sci Technolog Med Sci **32**(2): 242-246.

Recent studies suggested that the prostate cancer may arise from prostate cancer stem cells that share some same characteristics with normal stem cells. The purpose of this study was to detect the differences of Nanog expression between PC3 prostate cancer cell line and its tumor stem cells, and the relationship was preliminarily examined between Nanog and prostate cancer and its tumor stem cells. By using magnetic active cell sorting (MACS), we isolated a population of CD44(+)/CD133(+) prostate cancer cells that display stem cell characteristics from PC3 cell line. Immunohistochemistry revealed positive expressions of CD44, CD133 and alpha (2)beta (1)-integin in the isolated cells. CCK-8 analysis showed that isolated cells had a strong proliferative ability. The formation of the cell spheres in serum-free medium and holoclones in serum-supplied medium showed that the cells were capable of self-renewing, indicating that the isolated cells were a population of cancer stem-like cells derived from PC3 cell line. Western blotting exhibited that the isolated cells had higher experession of Nanog, an embryonic stem marker, as compared with PC3 cells. Our study showed that Nanog might be helpful in sustaining the self-renewal and the undifferentiation of prostate cancer stem cells, and may serve as a marker for prostate cancer stem cells for isolation and identification.

Gong, S., et al. (2015). "Regulation of NANOG in cancer cells." Mol Carcinog **54**(9): 679-687.

As one of the key pluripotency transcription factors, NANOG plays a critical role in maintaining the self-renewal and pluripotency in normal embryonic stem cells. Recent data indicate that NANOG is expressed in a variety of cancers and its expression correlates with poor survival in cancer patients. Of interest, many studies suggest that NANOG enhances the defined characteristics of cancer stem cells and may thus function as an oncogene to promote carcinogenesis. Therefore, NANOG expression determines the cell fate not only in pluripotent cells but also in cancer cells. Although the regulation of NANOG in normal embryonic stem cells is reasonably well understood, the regulation of NANOG in cancer cells has only emerged recently. The current review provides a most updated summary on how NANOG

expression is regulated during tumor development and progression.

Gu, T. T., et al. (2012). "Cytoplasmic NANOG-positive stromal cells promote human cervical cancer progression." Am J Pathol **181**(2): 652-661.

Tumor development has long been known to resemble abnormal embryogenesis. The embryonic stem cell gene NANOG, a divergent homeodomain transcription factor that is independent of leukemia inhibitory factor, has been reported to be expressed in germ cells and in several tumor types. However, the short-term expression and role of NANOG in cervical cancer remain unclear. In the present study, we demonstrate that NANOG exhibits cellular shuttling behavior and increasing stromal distribution during the progression of cervical cancer. Our molecular data using RT-PCR and restriction enzyme digestion show that NANOG is mainly transcribed from the NANOG gene in cervical cancer. In addition, IHC using confocal microscopy suggests that mesenchymal stem cells (MSCs) are one type of cytoplasmic NANOG-positive cells in cervical cancer stroma. Co-culture of cervical cancer-derived MSCs with SiHa cells showed increased proliferation characteristics in vitro and enhanced tumor growth in vivo. Our results show, for the first time to our knowledge, that MSCs are a source of cytoplasmic NANOG expression in the cervical cancer stroma and that they participate in the progression of cervical cancer both in vitro and in vivo. Our study provides evidence that NANOG is a cervical cancer progression marker and also serves as a starting point for a more extensive exploration of the cellular translocation of NANOG and the multifunctionality of the stromal microenvironment.

Guo, T., et al. (2017). "Transcriptional activation of NANOG by YBX1 promotes lung cancer stem-like properties and metastasis." Biochem Biophys Res Commun **487**(1): 153-159.

Aberrant overexpression of the transcription/translation factor Y-box-binding protein-1 (YBX1) is associated with non-small cell lung cancer (NSCLC) aggressiveness. Cancer stem cells (CSCs) contribute to the tumorigenesis and metastasis of NSCLC. Hitherto, the mechanism by which YBX1 regulates CSCs and metastasis in NSCLC remains unclear. Here, we demonstrated that YBX1 levels were elevated in NSCLC tissues and cell lines. Enforced expression of YBX1 promoted NSCLC cells invasion, sphere forming ability and ALDH1(+) population. Conversely, reduced YBX1 impaired CSC properties of NSCLC cells in vitro and tumor-initiating frequencies, as well as metastasis in vivo. Importantly, we described a mechanism whereby YBX1 directly promoted NANOG, a transcription factor, transcriptional activation. Depletion of NANOG abolished the enhanced ability of invasion and sphere formation in YBX1 elevated-A549 cells. Collectively, these findings demonstrate a novel role of YBX1 in maintaining the stemness of CSCs and metastasis, unveiling YBX1 as promising therapeutic target for NSCLC treatments.

Han, J., et al. (2012). "RNA interference-mediated silencing of NANOG reduces cell proliferation and induces G0/G1 cell cycle arrest in breast cancer cells." Cancer Lett **321**(1): 80-88.

Since the processes of normal embryogenesis and neoplasia share many of similar pathways, tumor development has been interpreted as an abnormal form of organogenesis. NANOG is a homeodomain-containing transcription factor that functions to maintain self-renewal and proliferation of embryonic stem cells (ESCs). Aberrant expression of NANOG has been observed in many types of human malignancies. However, its potential implication in tumorigenesis has not been fully clarified. In this study, we have employed small interference RNA (RNAi) technology to silence endogenous NANOG expression in breast cancer cells and successfully selected three independent clones with stably inhibited NANOG expression of MCF-7 cells. Functional analysis revealed that down-regulation of NANOG reduced cell proliferation, colony formation and migration ability of MCF-7 cells. Consistently, proliferation of breast cancer MDA-MB-231 cells was also significantly inhibited after the knockdown of NANOG expression. Interestingly, we found that the expression levels of cyclinD1 and c-myc were markedly down-regulated and the cell cycle were blocked at the G0/G1 phases after the knockdown of NANOG, while the expression of cyclinE and signal transducers and activators of transcription3 (STAT3) remained unaffected. In addition, the expression of NANOG and cyclinD1 can be rescued after the transfection of pcDNA3.1 (-)-NANOG expression vector into the three clones. Finally, our chromatin immunoprecipitation (ChIP) experiment showed that NANOG protein can bind to the promoter region of cyclinD1 and regulate cells cycle. Taken together, our findings may not only establish a molecular basis for the role of NANOG in modulating cell cycle progression of breast cancer cells but also suggest a potential target for the treatment of at least some subtypes of breast cancer.

Han, M. L., et al. (2016). "MicroR-760 suppresses cancer stem cell subpopulation and breast cancer cell proliferation and metastasis: By down-regulating NANOG." Biomed Pharmacother **80**: 304-310.

BACKGROUND AND OBJECTIVE: Emerging evidences suggest that cancer stem cells are responsible for tumor aggressive, metastasis and therapeutic resistance. To data, the mechanism underlying breast cancer stem cell (BCSC) population within tumor metastasis remains to be fully elucidated. The current study was to investigate the potential role of microRNA-760 (miR-760) and its associated target gene in population and metastasis of BCSC. METHODS: Characteristic BCSCs surface markers (CD44(+)/CD24(-/low)) were determined by flow cytometry in breast cancer MCF-7 and BT-549 cells. Quantitative RT-PCR was used to evaluate miR-760 and NANOG mRNA expression. Expression of NANOG protein was determined using western blot. Cell proliferation was determined by MTT assay. The model of breast cancer cell xenograft was used to evaluate the effect of miR-760 on tumor growth. RESULTS: BT-549 cell has substantially more CD44(+)/CD24(-/low) subpopulation than MCF-7 cell. Moreover, BT-549 cell expressed lower level of miR-760 and higher level of NANOG than MCF-7cell. By result from cellular miR-760 modulation, we found that miR-760 overexpression suppressed CD44(+)/CD24(-/low) population as well as inhibited cell proliferation and migration of BT-549. On the contrary, knockdown of miR-760 promoted CD44(+)/CD24(-/low) population and migration of MCF-7 cells. By luciferase reporter assay, miR-760 was proved to be functional associated with NANOG via regulating its expression. This functional interaction was showed to be involved in controlling proliferation and migration of MCF-7 and BT-549 cell. CONCLUSION: These data suggest that the target of miR-760/NANOG axis may represent a new therapeutic approach to suppress breast cancer stem cell subpopulation thereby prevent cancer metastasis.

Hu, C., et al. (2016). "Lentivirus-mediated shRNA targeting Nanog inhibits cell proliferation and attenuates cancer stem cell activities in breast cancer." J Drug Target **24**(5): 422-432.

Emerging evidences suggest that cancer stem cells (CSCs) are responsible for tumor growth, metastasis and treatment resistance. Nanog is one of the transcription factors that are essential for stem cellular physiology process. Previous studies reported that Nanog was detected in breast cancer and other solid tumors and indicated that it has oncogenic characteristics. However, expression feature of Nanog in breast cancer stem cells (BCSCs) enriched population and its biological function in BCSCs is poorly understood. In this study, CD44 + CD24- fraction sorting with Fluorescence Activated Cell Sorter and mammosphere culture were used for enriching BCSCs. We report here that Nanog was highly expressed in CSCs-enriched population from the breast cancer cells, as well as stemness-associated genes. In addition, we employed the lentivirus-mediated shRNA targeting Nanog to investigate function of Nanog in BCSCs. We found that targeted inhibition of Nanog could suppress proliferation and colony formation in breast cancer cells. Further studies showed that targeted inhibition of Nanog resulted in a decrease of BCSCs activities, including mammosphere formation, CD44 + CD24- proportion and expressions of stemness-associated genes. These data therefore suggest that Nanog possesses important function in BCSCs and targeted inhibition of Nanog may provide a novel means of targeting and eliminating BCSCs.

Hu, J., et al. (2016). "Ino80 promotes cervical cancer tumorigenesis by activating Nanog expression." Oncotarget **7**(44): 72250-72262.

Ino80 ATPase is an integral component of the INO80 ATP-dependent chromatin-remodeling complex, which regulates transcription, DNA repair and replication. We found that Ino80 was highly expressed in cervical cancer cell lines and tumor samples. Ino80 knockdown inhibited cervical cancer cell proliferation, induced G0/G1 phase cell cycle arrest in vitro and suppressed tumor growth in vivo. However, Ino80 knockdown did not affect cell apoptosis, migration or invasion in vitro. Ino80 overexpression promoted proliferation in the H8 immortalized cervical epithelial cell line, which has low endogenous Ino80 expression as compared to cervical cancer cell lines. Ino80 bound to the Nanog transcription start site (TSS) and enhanced its expression in cervical cancer cells. Nanog overexpression in Ino80 knockdown cell lines promoted cell proliferation. This study demonstrated for the first time that Ino80 was upregulated in cervical cancer and promoted cell proliferation and tumorigenesis. Our findings suggest that Ino80 may be a potential therapeutic target for the treatment of cervical cancer.

Hu, Q., et al. (2015). "OY-TES-1 may regulate the malignant behavior of liver cancer via NANOG, CD9, CCND2 and CDCA3: a bioinformatic analysis combine with RNAi and oligonucleotide microarray." Oncol Rep **33**(4): 1965-1975.

Given its tumor-specific expression, including liver cancer, OY-TES-1 is a potential molecular marker for the diagnosis and immunotherapy of liver cancers. However, investigations of the mechanisms and the role of OY-TES-1 in liver cancer are rare. In the present study, based on a comprehensive bioinformatic analysis combined with RNA interference (RNAi) and oligonucleotide microarray, we report for the first time that downregulation of OY-

TES-1 resulted in significant changes in expression of NANOG, CD9, CCND2 and CDCA3 in the liver cancer cell line BEL-7404. NANOG, CD9, CCND2 and CDCA3 may be involved in cell proliferation, migration, invasion and apoptosis, yet also may be functionally related to each other and OY-TES-1. Among these molecules, we identified that NANOG, containing a Kazal-2 binding motif and homeobox, may be the most likely candidate protein interacting with OY-TES-1 in liver cancer. Thus, the present study may provide important information for further investigation of the roles of OY-TES-1 in liver cancer.

Ibrahim, E. E., et al. (2012). "Embryonic NANOG activity defines colorectal cancer stem cells and modulates through AP1- and TCF-dependent mechanisms." Stem Cells **30**(10): 2076-2087.

Embryonic NANOG (NANOG1) is considered as an important regulator of pluripotency while NANOGP8 (NANOG-pseudogene) plays a role in tumorigenesis. Herein, we show NANOG is expressed from both NANOG1 and NANOGP8 in human colorectal cancers (CRC). Enforced NANOG1-expression increases clonogenic potential and tumor formation in xenograft models, although it is expressed only in a small subpopulation of tumor cells and is colocalized with endogenous nuclear beta-catenin (High). Moreover, single NANOG1-CRCs form spherical aggregates, similar to the embryoid body of embryonic stem cells (ESCs), and express higher levels of stem-like Wnt-associated target genes. Furthermore, we show that NANOG1-expression is positively regulated by c-JUN and beta-catenin/TCF4. Ectopic expression of c-Jun in murine Apc (Min/+) -ESCs results in the development of larger xenograft tumors with higher cell density compared to controls. Chromatin immunoprecipitation assays demonstrate that c-JUN binds to the NANOG1-promoter via the octamer M1 DNA element. Collectively, our data suggest that beta-Catenin/TCF4 and c-JUN together drive a subpopulation of CRC tumor cells that adopt a stem-like phenotype via the NANOG1-promoter.

Iv Santaliz-Ruiz, L. E., et al. (2014). "Emerging role of nanog in tumorigenesis and cancer stem cells." Int J Cancer **135**(12): 2741-2748.

Nanog is a transcription factor that is well-established as a key regulator of embryonic stem cell (ESC) maintenance. Recent evidence demonstrates that Nanog is dysregulated and intimately involved in promoting tumorigenesis in part through regulation of the cancer stem cell (CSC) population. Elevated Nanog is associated with poorer outcome in numerous epithelial malignancies. Nanog is enriched in CSCs and ablation of Nanog is sufficient to reduce the CSC pool. Nanog has also been implicated to promote chemoresistance and epithelial-mesenchymal transition (EMT). Insight into the Nanog signaling cascade, upstream regulators and downstream effectors, is beginning to emerge but remains to be fully elucidated. This review highlights the current literature on the emerging role of Nanog in tumorigenesis and CSCs.

Jeter, C. R., et al. (2011). "NANOG promotes cancer stem cell characteristics and prostate cancer resistance to androgen deprivation." Oncogene **30**(36): 3833-3845.

Cancer cell molecular mimicry of stem cells (SC) imbues neoplastic cells with enhanced proliferative and renewal capacities. In support, numerous mediators of SC self-renewal have been evinced to show oncogenic potential. We have recently reported that short-hairpin RNA-mediated knockdown of the embryonic stem cell (ESC) self-renewal gene NANOG significantly reduced the clonogenic and tumorigenic capabilities of various cancer cells. In this study, we sought to test the potential pro-tumorigenic functions of NANOG, particularly, in prostate cancer (PCa). Using qRT-PCR, we first confirmed that PCa cells expressed NANOG mRNA primarily from the NANOGP8 locus on chromosome 15q14. We then constructed a lentiviral promoter reporter in which the -3.8-kb NANOGP8 genomic fragment was used to drive the expression of green fluorescence protein (GFP). We observed that NANOGP8-GFP (+) PCa cells showed cancer stem cell (CSC) characteristics such as enhanced clonal growth and tumor regenerative capacity. To further investigate the functions and mechanisms of NANOG in tumorigenesis, we established tetracycline-inducible NANOG-overexpressing cancer cell lines, including both PCa (Du145 and LNCaP) and breast (MCF-7) cancer cells. NANOG induction promoted drug resistance in MCF-7 cells, tumor regeneration in Du145 cells and, most importantly, castration-resistant tumor development in LNCaP cells. These pro-tumorigenic effects of NANOG were associated with key molecular changes, including an upregulation of molecules such as CXCR4, IGFBP5, CD133 and ALDH1. The present gain-of-function studies, coupled with our recent loss-of-function work, establish the integral role for NANOG in neoplastic processes and shed light on its mechanisms of action.

Jeter, C. R., et al. (2016). "NANOG reprograms prostate cancer cells to castration resistance via dynamically repressing and engaging the AR/FOXA1 signaling axis." Cell Discov **2**: 16041.

The pluripotency transcription factor NANOG has been implicated in tumor development, and NANOG-expressing cancer cells manifest stem cell

properties that sustain tumor homeostasis, mediate therapy resistance and fuel tumor progression. However, how NANOG converges on somatic circuitry to trigger oncogenic reprogramming remains obscure. We previously reported that inducible NANOG expression propels the emergence of aggressive castration-resistant prostate cancer phenotypes. Here we first show that endogenous NANOG is required for the growth of castration-resistant prostate cancer xenografts. Genome-wide chromatin immunoprecipitation sequencing coupled with biochemical assays unexpectedly reveals that NANOG co-occupies a distinctive proportion of androgen receptor/Forkhead box A1 genomic loci and physically interacts with androgen receptor and Forkhead box A1. Integrative analysis of chromatin immunoprecipitation sequencing and time-resolved RNA sequencing demonstrates that NANOG dynamically alters androgen receptor/Forkhead box A1 signaling leading to both repression of androgen receptor-regulated pro-differentiation genes and induction of genes associated with cell cycle, stem cells, cell motility and castration resistance. Our studies reveal global molecular mechanisms whereby NANOG reprograms prostate cancer cells to a clinically relevant castration-resistant stem cell-like state driven by distinct NANOG-regulated gene clusters that correlate with patient survival. Thus, reprogramming factors such as NANOG may converge on and alter lineage-specific master transcription factors broadly in somatic cancers, thereby facilitating malignant disease progression and providing a novel route for therapeutic resistance.

Jeter, C. R., et al. (2015). "Concise Review: NANOG in Cancer Stem Cells and Tumor Development: An Update and Outstanding Questions." Stem Cells **33**(8): 2381-2390.

The homeobox domain transcription factor NANOG, a key regulator of embryonic development and cellular reprogramming, has been reported to be broadly expressed in human cancers. Functional studies have provided strong evidence that NANOG possesses protumorigenic attributes. In addition to promoting self-renewal and long-term proliferative potential of stem-like cancer cells, NANOG-mediated oncogenic reprogramming may underlie clinical manifestations of malignant disease. In this review, we examine the molecular origin, expression, biological activities, and mechanisms of action of NANOG in various malignancies. We also consider clinical implications such as correlations between NANOG expression and cancer prognosis and/or response to therapy. We surmise that NANOG potentiates the molecular circuitry of tumorigenesis, and thus may represent a novel therapeutic target or biomarker for the diagnosis, prognosis, and treatment outcome of cancer. Finally, we present critical pending questions relating NANOG to cancer stem cells and tumor development.

Ji, W. and Z. Jiang (2013). "Effect of shRNA-mediated inhibition of Nanog gene expression on the behavior of human gastric cancer cells." Oncol Lett **6**(2): 367-374.

The aim of the present study was to employ RNA interference (RNAi) technology to construct and select shRNA-Nanog recombinant plasmids for the inhibition of Nanog gene expression and transfer these plasmids into the human gastric cancer cell line, SGC-7901, as well as to detect the expression of Nanog and the effects on the proliferation, migration, invasion, cell cycle and apoptosis of SGC-7901 cells. The pshRNA-Nanog interference plasmids were constructed and used to transfect SGC-7901 cells using lipofectamine. The expression of the Nanog gene was detected by fluorescence microscopy, RT-PCR and western blotting, and the most markedly inhibited group was identified. The SGC-7901 cells were transfected with recombinant shRNA-Nanog plasmids from the most markedly inhibited group using lipofectamine and the effect on proliferation was determined by CCK-8 assay. The migration and invasion of the SGC-7901 cells was determined by Transwell assays, while the cell cycle and apoptosis were analyzed by flow cytometry. The group with the highest inhibition rate was successfully constructed and identified. It was observed that the proliferation, invasion and migration capacity of the cells was reduced, that the cell cycle was arrested at the S phase and that apoptosis was significantly increased. The Nanog gene in gastric cancer cells is closely associated with cell proliferation, the cell cycle, apoptosis and migration and invasion abilities. The present study establishes the foundations for a novel approach for the genetic treatment of gastric cancer.

Jiang, L., et al. (2016). "Androgen/androgen receptor axis maintains and promotes cancer cell stemness through direct activation of Nanog transcription in hepatocellular carcinoma." Oncotarget **7**(24): 36814-36828.

Hepatocellular carcinoma (HCC) is one of the most common and malignant cancers. The HCC incidence gets a strong sexual dimorphism as men are the major sufferers in this disaster. Although several studies have uncovered the presentative correlation between the axis of androgen/androgen receptor (AR) and HCC incidence, the mechanism is still largely unknown. Cancer stem cells (CSCs) are a small subgroup of cancer cells contributing to multiple tumors malignant behaviors, which play an important

role in oncogenesis of various cancers including HCC. However, whether androgen/AR axis involves in regulation of HCC cells stemness remains unclear. Our previous study had identified that the pluripotency factor Nanog is not only a stemness biomarker, but also a potent regulator of CSCs in HCC. In this study, we revealed androgen/AR axis can promote HCC cells stemness by transcriptional activation of Nanog expression through directly binding to its promoter. In HCC tissues, we found that AR expression was abnormal high and got correlation with Nanog. Then, by labeling cellular endogenous Nanog with green fluorescent protein (GFP) through CRISPR/Cas9 system, it verified the co-localization of AR and Nanog in HCC cells. With in vitro experiments, we demonstrated the axis can promote HCC cells stemness, which effect is in a Nanog-dependent manner and through activating its transcription. And the xenografted tumor experiments confirmed the axis effect on tumorigenesis facilitation in vivo. Above all, we revealed a new sight of androgen/AR axis roles in HCC and provided a potential way for suppressing the axis in HCC therapy.

Kawamura, N., et al. (2015). "CRISPR/Cas9-mediated gene knockout of NANOG and NANOGP8 decreases the malignant potential of prostate cancer cells." Oncotarget **6**(26): 22361-22374.

NANOG expression in prostate cancer is highly correlated with cancer stem cell characteristics and resistance to androgen deprivation. However, it is not clear whether NANOG or its pseudogenes contribute to the malignant potential of cancer. We established NANOG- and NANOGP8-knockout DU145 prostate cancer cell lines using the CRISPR/Cas9 system. Knockouts of NANOG and NANOGP8 significantly attenuated malignant potential, including sphere formation, anchorage-independent growth, migration capability, and drug resistance, compared to parental DU145 cells. NANOG and NANOGP8 knockout did not inhibit in vitro cell proliferation, but in vivo tumorigenic potential decreased significantly. These phenotypes were recovered in NANOG- and NANOGP8-rescued cell lines. These results indicate that NANOG and NANOGP8 proteins are expressed in prostate cancer cell lines, and NANOG and NANOGP8 equally contribute to the high malignant potential of prostate cancer.

Kenda Suster, N., et al. (2017). "Cancer Stem Cell-Related Marker NANOG Expression in Ovarian Serous Tumors: A Clinicopathological Study of 159 Cases." Int J Gynecol Cancer **27**(9): 2006-2013.

OBJECTIVE: The objectives of this study were to assess cancer stem cell-related marker NANOG expression in ovarian serous tumors and to evaluate its prognostic significance in relation to ovarian serous carcinoma. METHODS: NANOG protein expression was immunohistochemically evaluated in the ovarian tissue microarrays of 20 patients with benign ovarian serous tumors, 30 patients with borderline ovarian serous tumors, and 109 patients with ovarian serous carcinomas, from which 106 were of high-grade and 3 of low-grade morphology Immunohistochemical reaction was scored according to signal intensity and the percentage of positive cells in tumor samples. Pursuant to our summation of signal intensity and positive cell occurrence, we divided our samples into 4 groups: NANOG-negative, NANOG-slightly positive, NANOG-moderately positive, and NANOG-strongly positive group. Complete clinical data were obtained for the ovarian serous carcinoma group, and correlation between clinical data and NANOG expression was analyzed. RESULTS: A specific brown nuclear, or cytoplasmic reaction, was considered a positive NANOG staining. In terms of the ovarian serous carcinoma group, 69.7% were NANOG positive, 22.9% slightly positive, 22.9% moderately positive, and 23.9% strongly positive. All NANOG-positive cases were of high-grade morphology. Benign and borderline tumors and low-grade serous carcinomas were NANOG negative. There was no significant correlation between NANOG expression and clinical parameters in terms of the ovarian serous carcinoma group. CONCLUSIONS: Positive NANOG expression is significantly associated with high-grade ovarian serous carcinoma and is absent in benign, borderline, and low-grade serous lesions. In our study, there was no correlation between NANOG expression and clinical parameters, including its use in the prognosis of ovarian serous carcinoma.

Kenda Suster, N., et al. (2016). "The significance of the pluripotency and cancer stem cell-related marker NANOG in diagnosis and treatment of ovarian carcinoma." Eur J Gynaecol Oncol **37**(5): 604-612.

Ovarian cancer is among the most common gynecologic cancers and unfortunately the most common cause of death from gynecologic malignancies. Due to few early symptoms and insufficient screening programs, an early diagnosis of ovarian cancer is very difficult and new biomarkers related to early ovarian carcinogenesis are needed. In the last years a growing scientific knowledge about cancer stem cells and their markers opened a new perspective on screening and early diagnosis of ovarian cancer. The transcription factor NANOG is not only a pluripotency and cancer stem cell-related marker, but also promotes cancer stem cell-like characteristics of tumor, tumor growth, dissemination, immune evasion, and resistance to conventional therapy. The recent data showed that small stem cells

resembling very small embryonic-like stem cells are present in the ovarian surface epithelium of adult human ovaries. These cells expressed several genes related to primordial germ cells, germinal lineage, and pluripotency, including NANOG, therefore their involvement in the manifestation of ovarian cancer are not excluded. As majority of cancer cells within a tumor are non tumorigenic, the therapies targeting these cells cause tumor regression, but the survived cancer stem cells regenerate the tumor, so tumor relapse or reoccur. The eradication of cancer actually requires the elimination of cancer stem cells, therefore new strategies in treatment that specifically target cancer stem cells are urgently needed. Although the therapeutic efficacy of targeting NANOG as a cancer treatment method is still in experimental phase, the gene therapy with small interfering RNA or short hairpin RNA have already shown some promising therapeutic potential. The authors can conclude that NANOG represents a promising diagnostic marker and agent for target therapy of ovarian cancer.

Kregel, S., et al. (2014). "The pluripotency factor Nanog is directly upregulated by the androgen receptor in prostate cancer cells." Prostate **74**(15): 1530-1543.

BACKGROUND: The Androgen Receptor (AR) is a nuclear hormone receptor that functions as a critical oncogene in all stages of prostate cancer progression, including progression to castration-resistance following androgen-deprivation therapy. Thus, identifying and targeting critical AR-regulated genes is one potential method to block castration-resistant cancer proliferation. Of particular importance are transcription factors that regulate stem cell pluripotency; many of these genes are emerging as critical oncogenes in numerous tumor cell types. Of these, Nanog has been previously shown to increase the self-renewal and stem-like properties of prostate cancer cells. Thus, we hypothesized that Nanog is a candidate AR target gene that may impart castration-resistance. METHODS: We modulated AR signaling in LNCaP prostate cancer cells and assayed for Nanog expression. Direct AR binding to the NANOG promoter was tested using AR Chromatin Immunoprecipation (ChIP) and analyses of publically available AR ChIP-sequencing data-sets. Nanog over-expressing cells were analyzed for cell growth and cytotoxicity in response to the AR antagonist enzalutamide and the microtubule stabilizing agent docetaxel. RESULTS: AR signaling upregulates Nanog mRNA and protein. AR binds directly to the NANOG promoter, and was not identified within 75 kb of the NANOGP8 pseudogene, suggesting the NANOG gene locus was preferentially activated. Nanog overexpression in LNCaP cells increases overall growth, but does not increase resistance to enzalutamide or docetaxel. CONCLUSIONS: Nanog is a novel oncogenic AR target gene in prostate cancer cells, and stable expression of Nanog increases proliferation and growth of prostate cancer cells, but not resistance to enzalutamide or docetaxel.

Kumar, B., et al. (2015). "Suberoylanilide hydroxamic acid (SAHA) reverses chemoresistance in head and neck cancer cells by targeting cancer stem cells via the downregulation of nanog." Genes Cancer **6**(3-4): 169-181.

Acquisition of chemoresistance and metastatic phenotype are the major causes of treatment failure and mortality in head and neck squamous cell carcinoma (HNSCC) patients. Histone deacetylases (HDACs) have been shown to be overexpressed in many tumor types and directly linked to poor prognosis. In this study, we demonstrate that HDACs are markedly elevated in HNSCC. HDACs expression was further increase in cisplatin resistant cell lines (CisR). In addition, cisplatin-resistant cells showed enhanced stem cell properties and tumor metastasis. Depletion of HDAC1 and 2 in CisR cell lines significantly reversed cisplatin resistance and tumorsphere formation. Next, we tested the efficacy of Suberoylanilide hydroxamic acid (SAHA), an HDAC inhibitor, by using both in vitro and in vivo models. SAHA significantly inhibited cell proliferation and synergistically enhanced the anti-proliferative effects of cisplatin. In addition, SAHA significantly decreased tumorsphere formation by markedly reducing nanog expression. In a SCID mouse xenograft model, SAHA significantly enhanced the anti-tumor effects of cisplatin treatment with no added systemic toxicity. Furthermore, SAHA and cisplatin combination treatment significantly decreased tumor metastasis and nanog expression, in vivo. Taken together, our results suggest that targeting HDACs with SAHA could be an effective treatment strategy for the treatment of HNSCC patients.

Lee, M., et al. (2012). "Prognostic impact of the cancer stem cell-related marker NANOG in ovarian serous carcinoma." Int J Gynecol Cancer **22**(9): 1489-1496.

OBJECTIVE: The objective of this study was to evaluate the prognostic significance of NANOG expression in ovarian serous carcinoma. METHODS: The expression of NANOG was evaluated in 6 ovarian carcinoma cell lines, paclitaxel-resistant SKOV3 cells, and SKOV3 spheroid cells with semiquantitative reverse transcription-polymerase chain reaction and Western blotting. NANOG expression was also measured immunohistochemically in a tissue microarray containing ovarian tissues from 74 patients with ovarian serous carcinoma and 24 with ovarian

serous cystadenoma. Each sample was scored based on signal intensity and proportion, and a score greater than 4 was considered "positive." RESULTS: NANOG mRNA expression was variable in different ovarian cancer cell lines. The mRNA level of NANOG was increased in the paclitaxel-resistant SKOV3 cells and SKOV3 spheroid cells compared with that in the SKOV3 cells. NANOG expression was positive in 21.6% of 74 ovarian serous carcinoma tissues, but none of the ovarian serous cystadenoma tissues were positive. Positive NANOG expression was associated with residual tumor size after surgery (P = 0.032). The overall survival of the patients with positive NANOG expression was poorer than that of the patients with negative NANOG expression (P = 0.020). In patients with stage I and II disease, positive NANOG expression was independently associated with shorter overall survival compared with negative NANOG expression (40 vs 120 months, respectively; P = 0.031). CONCLUSIONS: Positive NANOG expression is associated with poor prognosis of ovarian serous carcinoma. NANOG has potential as a predictor of survival for patients with ovarian carcinomas and may be involved in the mechanism of chemoresistance.

Lee, S., et al. (2017). "Crosstalks between Raf-kinase inhibitor protein and cancer stem cell transcription factors (Oct4, KLF4, Sox2, Nanog)." Tumour Biol **39**(4): 1010428317692253.

Raf-kinase inhibitor protein has been reported to inhibit both the Raf/mitogen extracellular signal-regulated kinase/extracellular signal-regulated kinase and nuclear factor kappa-light-chain of activated B cells pathways. It has also been reported in cancers that Raf-kinase inhibitor protein behaves as a metastatic suppressor as well as a chemo-immunosensitizing factor to drug/immune-mediated apoptosis. The majority of cancers exhibit low or no levels of Raf-kinase inhibitor protein. Hence, the activities of Raf-kinase inhibitor protein contrast, in part, to those mediated by several cancer stem cell transcription factors for their roles in resistance and metastasis. In this review, the existence of crosstalks in the signaling pathways between Raf-kinase inhibitor protein and several cancer stem cell transcription factors (Oct4, KLF4, Sox2 and Nanog) was assembled. Oct4 is induced by Lin28, and Raf-kinase inhibitor protein inhibits the microRNA binding protein Lin28. The expression of Raf-kinase inhibitor protein inversely correlates with the expression of Oct4. KLF4 does not interact directly with Raf-kinase inhibitor protein, but rather interacts indirectly via Raf-kinase inhibitor protein's regulation of the Oct4/Sox2/KLF4 complex through the mitogen-activated protein kinase pathway. The mechanism by which Raf-kinase inhibitor protein inhibits Sox2 is via the inhibition of the mitogen-activated protein kinase pathway by Raf-kinase inhibitor protein. Thus, Raf-kinase inhibitor protein's relationship with Sox2 is via its regulation of Oct4. Inhibition of extracellular signal-regulated kinase by Raf-kinase inhibitor protein results in the upregulation of Nanog. The inhibition of Oct4 by Raf-kinase inhibitor protein results in the failure of the heterodimer formation of Oct4 and Sox2 that is necessary to bind to the Nanog promoter for the transcription of Nanog. The findings revealed that there exists a direct correlation between the expression of Raf-kinase inhibitor protein and the expression of each of the above transcription factors. Based on these analyses, we suggest that the expression level of Raf-kinase inhibitor protein may be involved in the regulation of the cancer stem cell phenotype.

Lemos, C., et al. (2016). "MACC1 Induces Tumor Progression in Transgenic Mice and Colorectal Cancer Patients via Increased Pluripotency Markers Nanog and Oct4." Clin Cancer Res **22**(11): 2812-2824.

PURPOSE: We have previously identified the gene MACC1 as a strong prognostic biomarker for colorectal cancer metastasis and patient survival. Here, we report for the first time the generation of transgenic mouse models for MACC1. EXPERIMENTAL DESIGN: We generated mice with transgenic overexpression of MACC1 in the intestine driven by the villin promoter (vil-MACC1) and crossed them with Apc (Min) mice (vil-MACC1/Apc (Min)). RESULTS: vil-MACC1/Apc (Min) mice significantly increased the total number of tumors (P = 0.0056). This was particularly apparent in large tumors (>/=3-mm diameter; P = 0.0024). A detailed histopathologic analysis of these lesions demonstrated that the tumors from the vil-MACC1/Apc (Min) mice had a more invasive phenotype and, consequently, showed a significantly reduced survival time than Apc (Min) mice (P = 0.03). Molecular analysis revealed an increased Wnt and pluripotency signaling in the tumors of vil-MACC1/Apc (Min) mice. Specifically, we observed a prominent upregulation of the pluripotency markers Oct4 and Nanog in these tumors compared with Apc (Min) controls. Finally, we could also validate that Oct4 and Nanog are regulated by MACC1 in vitro and strongly correlate with MACC1 levels in a cohort of 60 tumors of colorectal cancer patients (r = 0.7005 and r = 0.6808, respectively; P > 0.0001 and P > 0.0002, respectively). CONCLUSIONS: We provide proof of principle that MACC1-induced tumor progression in colorectal cancer acts, at least in part, via the newly discovered MACC1/Nanog/Oct4 axis. These findings might have important implications for the design of novel therapeutic intervention strategies to restrict tumor

progression. Clin Cancer Res; 22(11); 2812-24. (c)2016 AACR.

Ma, Y., et al. (2017). "Sanguinarine inhibits pancreatic cancer stem cell characteristics by inducing oxidative stress and suppressing sonic hedgehog-Gli-Nanog pathway." Carcinogenesis **38**(10): 1047-1056.

Sonic hedgehog pathway is highly activated in pancreatic cancer stem cells (CSC) which play crucial roles in cancer initiation, progression and metastasis. However, the molecular mechanisms by which sanguinarine regulates pancreatic CSC characteristics is not well understood. The objectives of this study were to examine the molecular mechanisms by which sanguinarine regulates pancreatic CSC characteristics. Sanguinarine inhibited cell proliferation and colony formation and induced apoptosis through oxidative damage. Sanguinarine inhibited self-renewal capacity of pancreatic CSCs isolated from human and KrasG12D mice. Furthermore, sanguinarine suppressed epithelial-mesenchymal transition (EMT) by up-regulating E-cadherin and inhibiting N-cadherin. Significant decrease in expression level of Snail, Slug and Zeb1 corroborated the suppression of EMT in sanguinarine treated pancreatic CSCS. The ability of sanguinarine to inhibit pluripotency maintaining factors and CSC markers suggest that sanguinarine can be an effective agent for inhibiting pancreatic cancer growth and development by targeting CSCs. Furthermore, sanguinarine inhibited Shh-Gli pathway leading to modulation of Gli target genes in pancreatic CSCs. Chromatin immunoprecipitation assay demonstrated that Nanog directly binds to promoters of Cdk2, Cdk6, FGF4, c-Myc and Oct4, and sanguinarine inhibits the binding of Nanog with these genes, suggesting the direct involvement of Nanog in cell cycle, pluripotency and self-renewal. To further investigate the role of Shh-Gli-Nanog pathway, we regulated Shh signaling either by Shh protein or Nanog overexpression. Enforced activation of Shh or overexpression of Nanog counteracted the inhibitory effects of sanguinarine on pancreatic CSC proliferation, suggesting the actions of sanguinarine are mediated, at least in part, through Shh-Gli-Nanog pathway. Our studies suggest that sanguinarine can be used for the treatment and/or prevention of pancreatic cancer by targeting CSCs.

Mao, C. P., et al. (2014). "Immune-mediated tumor evolution: Nanog links the emergence of a stem like cancer cell state and immune evasion." Oncoimmunology **3**(7): e947871.

Tumor cells undergo molecular evolution under immune pressure. Using a murine metastatic lung cancer model, we recently reported that evolutionary pressure enforced through vaccination incites gain of Nanog, a master transcription factor that mediates both emergence of a stem-like cancer cell state and immune evasion. Thus, therapeutic strategies aiming to blunt NANOG's expression in patient tumors may improve the clinical management of cancer.

Mattoo, A. R., et al. (2014). "Inhibition of NANOG/NANOGP8 downregulates MCL-1 in colorectal cancer cells and enhances the therapeutic efficacy of BH3 mimetics." Clin Cancer Res **20**(21): 5446-5455.

PURPOSE: High levels of BCL-2 family members in colorectal carcinoma cause resistance to treatment. Inhibition of NANOG or its paralog NANOGP8 reduces the proliferation, stemness, and tumorigenicity of colorectal carcinoma cells. Our hypothesis was that inhibition of NANOG/NANOGP8 enhances the cytotoxic effect of BH3 mimetics targeting BCL-2 family members in colorectal carcinoma cells through reducing expression of MCL-1, a prosurvival BCL-2 protein. EXPERIMENTAL DESIGN: Lentiviral vector (LV) shRNA to NANOG (shNG-1) or NANOGP8 (shNp8-1) transduced colorectal carcinoma cells that were also exposed to the BH3 mimetics ABT-737 or ABT-199 in vivo in colorectal carcinoma xenografts and in vitro where proliferation, protein and gene expression, and apoptosis were measured. RESULTS: Clone A and CX-1 were sensitive to ABT-737 and ABT-199 at IC50s of 2 to 9 mumol/L but LS174T was resistant with IC50s of 18 to 30 mumol/L. Resistance was associated with high MCL-1 expression in LS174T. LVshNG-1 or LVshNp8-1 decreased MCL-1 expression, increased apoptosis, and decreased replating efficiency in colorectal carcinoma cells treated with either ABT-737 or ABT-199 compared with the effects of either BH3 mimetic alone. Inhibition or overexpression of MCL-1 alone replicated the effects of LVshNG-1 or LVshNp8-1 in increasing or decreasing the apoptosis caused with the BH3 mimetic. The combination therapy inhibited the growth of LS174T xenografts in vivo compared with untreated controls or treatment with only LV shRNA or ABT-737. CONCLUSIONS: Inhibition of NANOGP8 or NANOG enhances the cytotoxicity of BH3 mimetics that target BCL-2 family members. Gene therapy targeting the NANOGs may increase the efficacy of BH3 mimetics in colorectal carcinoma.

Meng, H. M., et al. (2010). "Over-expression of Nanog predicts tumor progression and poor prognosis in colorectal cancer." Cancer Biol Ther **9**(4): 295-302.

We studied the expression and regulatory effects of ESC self-renewal molecule Nanog in colorectal cancer (CRC). Immunohistochemical analysis of 175 colorectal tumor samples showed that overexpression

of Nanog was strongly correlated with poor prognosis, lymph node metastasis and Dukes classification for CRC. Univariate and multivariate survival analyses further indicated that Nanog expression was a potential prognostic factor for CRC. Gain-of-function analysis revealed that lentivirus-mediated Nanog overexpression promoted proliferation, motility and migration of human CRC cells. Interestingly, we found that Nanog played as both an inducer and a receipt of epithelial-mesenchymal transition (EMT) related signals. Nanog induced expression of Slug and Snail, two major regulator of EMT. Meanwhile, Nanog could also be regulated by Snail and initiated by TGF-beta1. Our data demonstrate self-renewal gene Nanog has a prognostic role in CRC, which functions in progression of CRC by promoting proliferation, invasion, and motility of human CRC cells, and participates EMT process during CRC progression.

Migita, T., et al. (2017). "Epithelial-mesenchymal transition promotes SOX2 and NANOG expression in bladder cancer." Lab Invest.

Bladder cancer is the most common malignant tumor of the urothelium and is classified into non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). Stemness markers such as SOX2 and NANOG are frequently overexpressed in various aggressive cancers, including MIBC; epithelial-mesenchymal transition (EMT) has been proposed as a potential trigger of stemness in cancers. To determine whether cancer stemness is acquired via EMT in bladder cancer, we studied the effect of EMT on the expression of SOX2 and NANOG in bladder cancer cell lines. We also analyzed their expression in clinical tissue samples. Our results revealed that a potent EMT inducer (transforming growth factor beta1) reduced the expression of the epithelial marker E-cadherin and increased expression of both SOX2 and NANOG in epithelial-type bladder cancer cells. As for clinical bladder cancer samples, in NMIBC, E-cadherin expression was slightly diminished, and the expression of both SOX2 and NANOG was negligible. In contrast, in MIBC, E-cadherin expression was highly and heterogeneously diminished, while the expression of both SOX2 and NANOG was increased. We also noticed that either E-cadherin or SOX2 (or NANOG) was expressed (ie, in a manner exclusive of each other). In addition, the concentration of E-cadherin showed a significant negative correlation with tumor grade and stage, while expression of SOX2 and NANOG positively correlated with those clinicopathological parameters. These findings suggest that EMT promotes stemness of bladder cancer cells, contributing to tumor aggressiveness. This EMT-cancer stemness axis may also play an important role in the pathogenesis of NMIBC and MIBC.Laboratory Investigation advance online publication, 27 February 2017; doi:10.1038/labinvest.2017.17.

Miyazawa, K., et al. (2014). "Immunohistochemical expression of four different stem cell markers in prostate cancer: High expression of NANOG in conjunction with hypoxia-inducible factor-1alpha expression is involved in prostate epithelial malignancy." Oncol Lett **8**(3): 985-992.

Cancer stem cells (CSCs) have been identified in a variety of cancer types, including prostate cancer. The aim of the present study was to evaluate the immunohistochemical expression of NANOG, octamer 4 (OCT4), cluster of differentiation 133 (CD133) and NESTIN, which are all CSC markers, and assess their function in prostate carcinogenesis. A total of 114 patients were referred to the Kanazawa Medical University Hospital (Uchinada, Japan) having presented with elevated serum prostate-specific antigen levels and/or abnormal digital rectal examinations, and underwent transrectal ultrasound sonography guided eight core biopsies. The prostate pathological specimens were re-evaluated for selection in this study. When specimens were diagnosed as prostate cancer, immunohistochemical analysis of the four different stem cell markers (NANOG, OCT4, CD133 and NESTIN) and hypoxia-inducible factor (HIF)-1alpha was performed. Prostate cancer was found in 38 cases (33.3%), while the other patients had benign prostate hyperplasia with prostatitis. All prostate cancers were histopathologically identified as adenocarcinomas of various grades, and cancer cells and intraepithelial neoplasia (high grade) were immunohistochemically shown to express NANOG and OCT4, but not CD133 and NESTIN. The intensity of NANOG expression was much greater than that of OCT4, and the positivity and intensity of the four stem cell markers, including NANOG, were elevated with high Gleason scores. A significant correlation was observed between the NANOG- and HIF-1alpha-positive regions. The CSC markers, in particular OCT4 and NANOG, were immunohistochemically expressed in prostate cancers. Furthermore, HIF-1alpha expression may affect NANOG and/or OCT4 expression. The findings of the current study suggested that NANOG expression may be a biomarker for the diagnosis of prostate cancer, and the coexpression of NANOG and HIF-1alpha may be involved in prostate carcinogenesis.

Moon, J. H., et al. (2011). "Nanog-induced dedifferentiation of p53-deficient mouse astrocytes into brain cancer stem-like cells." Biochem Biophys Res Commun **412**(1): 175-181.

Self-renewal, differentiation, and tumorigenicity characterize cancer stem cells (CSCs), which are rare and maintained by specific cell fate regulators. CSCs are isolated from glioblastoma multiforme (GBM) and may be responsible for the lethality of incurable brain tumors. Brain CSCs may arise from the transformation of undifferentiated, nestin-positive neural stem or progenitor cells and GFAP-expressing astrocytes. Here, we report a role of Nanog in the genesis of cancer stem-like cells. Using primary murine p53-knockout astrocytes (p53(-/-) astrocytes), we provide evidence that enforced Nanog expression can increase the cellular growth rate and transform phenotypes in vitro and in vivo. In addition, Nanog drives p53(-/-) astrocytes toward a dedifferentiated, CSC-like phenotype with characteristic neural stem cell/progenitor marker expression, neurosphere formation, self-renewal activity, and tumor development. These findings suggest that Nanog promotes dedifferentiation of p53-deficient mouse astrocytes into cancer stem-like cells by changing the cell fate and transforming cell properties.

Nagata, T., et al. (2014). "Prognostic significance of NANOG and KLF4 for breast cancer." Breast Cancer **21**(1): 96-101.

BACKGROUND: Some of the induced pluripotent stem cell (iPS cell)-inducing factors have been reported to be expressed in breast cancer. The aim of the present study was to examine the relationship between the expression of iPS cell-inducing factors and the prognosis of breast cancer patients. METHODS: In 100 breast cancer patients, the expression of c-MYC, KLF4, NANOG, OCT4, and SOX2 was determined by immunohistochemistry using a tissue microarray analysis. RESULTS: Patients with strong expression of NANOG had significantly lower disease-free survival (DFS) and overall survival rates than those with weak expression of NANOG (P = 0.004 and 0.033, respectively). In contrast, patients with strong expression of KLF4 had better DFS (P = 0.014). CONCLUSIONS: Strong expression of NANOG is an indicator of a poor prognosis for breast cancer patients, whereas KLF4 is a favorable prognostic indicator. Our results suggest that NANOG stimulates the growth and metastasis of breast cancer cells, whereas KLF4 inhibits these processes.

Nagata, T., et al. (2017). "KLF4 and NANOG are prognostic biomarkers for triple-negative breast cancer." Breast Cancer **24**(2): 326-335.

BACKGROUND: Prognosis of breast cancer patients has been reported to depend on the expression of induced pluripotent stem (iPS) cell-inducing factors: KLF4 and NANOG. However, the relationship between KLF4 or NANOG expression in each breast cancer subtype and the life prognosis has not been elucidated. METHOD: KLF4 and NANOG expression levels were evaluated in 208 patients using a newly developed tissue microarray (TMA). In vitro, siRNA against klf4 (siKLF4) was transfected in TNBC cell line MDA-MB-231, and the expression of KLF4 was inhibited. RESULTS: Triple-negative breast cancer (TNBC) patients in KLF4 high-expression (upper) group had more favorable overall survival (OS) and disease-free survival (DFS) rates than KLF4 lower group ($p = 0.0453$ and $p = 0.0427$). In contrast, patients in the NANOG upper group had significantly poorer prognosis than lower group in TNBC breast cancer subtypes ($p < 0.0001$). Multivariate analysis showed that KLF4 ($p = 0.0313$), NANOG ($p = 0.0002$), and TNM stage ($p = 0.0001$) are mutually independent prognostic factors. It was also shown that the proliferation and invasion ability of siKLF4-induced TNBC cells were up-regulated significantly. CONCLUSION: Our findings suggested that KLF4 and NANOG expression levels were favorable prognostic factors for TNBC patients. KLF4 also had an ability to inhibit the proliferation and invasion of TNBC.

Noh, K. H., et al. (2012). "Nanog signaling in cancer promotes stem-like phenotype and immune evasion." J Clin Invest **122**(11): 4077-4093.

Adaptation of tumor cells to the host is a major cause of cancer progression, failure of therapy, and ultimately death. Immune selection drives this adaptation in human cancer by enriching tumor cells with a cancer stem cell-like (CSC-like) phenotype that makes them resistant to CTL-mediated apoptosis; however, the mechanisms that mediate CSC maintenance and proliferation are largely unknown. Here, we report that CTL-mediated immune selection drives the evolution of tumor cells toward a CSC-like phenotype and that the CSC-like phenotype arises through the Akt signaling pathway via transcriptional induction of Tcl1a by Nanog. Furthermore, we found that hyperactivation of the Nanog/Tcl1a/Akt signaling axis was conserved across multiple types of human cancer. Inhibition of Nanog in a murine model of colon cancer rendered tumor cells susceptible to immune-mediated clearance and led to successful, long-term control of the disease. Our findings establish a firm link among immune selection, disease progression, and the development of a stem-like tumor phenotype in human cancer and implicate the Nanog/Tcl1a/Akt pathway as a central molecular target in this process.

Noh, K. H., et al. (2012). "Cancer vaccination drives Nanog-dependent evolution of tumor cells

toward an immune-resistant and stem-like phenotype." Cancer Res **72**(7): 1717-1727.

Due to the exquisite specificity and potency of the immune system, vaccination is in theory the most precise and powerful approach for controlling cancer. However, current data from clinical trials indicate that vaccination rarely yields significant benefits for cancer patients in terms of tumor progression and long-term survival. The poor clinical outcomes of vaccination are primarily caused by mechanisms of immune tolerance, especially within the tumor microenvironment. Here, we report that vaccination drives the evolution of tumor cells toward an immune-resistant and stem-like phenotype that promotes tumor growth and nullifies the CTL response. The emergence of this phenotype required the transcription factor Nanog, which is induced as a consequence of immune selection. Nanog expression enhanced the stem-like features of tumor cells and protected them from killing by tumor-reactive CTLs. Delivery of siNanog into tumor-bearing mice rendered the tumor vulnerable to immune surveillance and strongly suppressed its growth. Together, our findings show tumor adaptation to vaccination through gain of an immune-resistant, stem-like phenotype and identify Nanog as a central molecular target in this process. Future vaccination technology should consider Nanog an important target to enhance the immunotherapeutic response.

Oh, S. J., et al. (2018). "Targeting Cyclin D-CDK4/6 Sensitizes Immune-Refractory Cancer by Blocking the SCP3-NANOG Axis." Cancer Res **78**(10): 2638-2653.

Immunoediting caused by antitumor immunity drives tumor cells to acquire refractory phenotypes. We demonstrated previously that tumor antigen-specific T cells edit these cells such that they become resistant to CTL killing and enrich NANOG (high) cancer stem cell-like cells. In this study, we show that synaptonemal complex protein 3 (SCP3), a member of the Cor1 family, is overexpressed in immunoedited cells and upregulates NANOG by hyperactivating the cyclin D1-CDK4/6 axis. The SCP3-cyclin D1-CDK4/6 axis was preserved across various types of human cancer and correlated negatively with progression-free survival of cervical cancer patients. Targeting CDK4/6 with the inhibitor palbociclib reversed multiaggressive phenotypes of SCP3(high) immunoedited tumor cells and led to long-term control of the disease. Collectively, our findings establish a firm molecular link of multiaggressiveness among SCP3, NANOG, cyclin D1, and CDK4/6 and identify CDK4/6 inhibitors as actionable drugs for controlling SCP3(high) immune-refractory cancer.Significance: These findings reveal cyclin D1-CDK4/6 inhibition as an effective strategy for controlling SCP3(high) immune-refractroy cancer. Cancer Res; 78(10); 2638-53. (c)2018 AACR.

Palla, A. R., et al. (2014). "Reprogramming activity of NANOGP8, a NANOG family member widely expressed in cancer." Oncogene **33**(19): 2513-2519.

NANOG is a key transcription factor for pluripotency in embryonic stem cells. The analysis of NANOG in human cells is confounded by the presence of multiple and highly similar paralogs. In particular, there are three paralogs encoding full-length proteins, namely, NANOG1, NANOG2 and NANOGP8, and at least eight additional paralogs that do not encode full-length NANOG proteins. Here, we have examined NANOG family expression in human embryonic stem cells (hESCs) and in human cancer cell lines using a multi-NANOG PCR that amplifies the three functional paralogs and most of the non-functional ones. As anticipated, we found that hESCs express large amounts of NANOG1 and, interestingly, they also express NANOG2. In contrast, most human cancer cells tested express NANOGP8 and the non-coding paralogs NANOGP4 and NANOGP5. Notably, in some cancer cell lines, the NANOG protein levels produced by NANOGP8 are comparable to those produced by NANOG1 in pluripotent cells. Finally, we show that NANOGP8 is as active as NANOG1 in the reprogramming of human and murine fibroblasts into induced pluripotent stem cells. These results show that cancer-associated NANOGP8 can contribute to promote de-differentiation and/or cellular plasticity.

Pan, Q., et al. (2017). "Transcriptional repression of miR-200 family members by Nanog in colon cancer cells induces epithelial-mesenchymal transition (EMT)." Cancer Lett **392**: 26-38.

Nanog is an important embryonic stem cell (ESC) gene that does not function as a classical oncogene, but needs to cooperate with other molecules to potentiate tumorigenic activity. The question addressed by the present study was whether a miRNA link exists between Nanog and epithelial-mesenchymal transition (EMT)-mesenchymal-epithelial transition (MET) plasticity. Here, we found that Nanog mRNA expression level was inversely correlated with miR-200c and miR-200b expression levels in colon cancer cell lines and human colorectal cancer tissues. Forced Nanog expression in low-Nanog colon cancer cells inhibited miR-200c and miR-200b expression, and interfered Nanog expression in high-Nanog colon cancer cells promoted miR-200c and miR-200b expression. Furthermore, we confirmed that Nanog directly repressed transcription of the miR-200c and miR-200b genes, and miR-200c and miR-200b mediated Nanog-induced EMT occurrence. Luciferase

and ChIP assays determined that Nanog bound directly to the potential Nanog binding sites in the miR-200c and miR-200b promoters and repressed their transcription. In conclusion, our findings suggest that Nanog modulates EMT-MET plasticity by regulating miR-200 clusters via a direct transcriptional mechanism, and the Nanog-miR-200 axis may be a good therapeutic target for CRC control.

Paranjape, A. N., et al. (2014). "Bmi1 regulates self-renewal and epithelial to mesenchymal transition in breast cancer cells through Nanog." BMC Cancer **14**: 785.

BACKGROUND: The Bmi1 polycomb ring finger oncogene, a transcriptional repressor belonging to the Polycomb group of proteins plays an important role in the regulation of stem cell self-renewal and is elevated in several cancers. In the current study, we have explored the role of Bmi1 in regulating the stemness and drug resistance of breast cancer cells. METHODS: Using real time PCR and immunohistochemistry primary breast tissues were analyzed. Retro- and lentiviruses were utilized to overexpress and knockdown Bmi1, RT-PCR and Western blot was performed to evaluate mRNA and protein expression. Stemness properties were analyzed by flow cytometry and sphere-formation and tumor formation was determined by mouse xenograft experiments. Dual luciferase assay was employed to assess promoter activity and MTT assay was used to analyze drug response. RESULTS: We found Bmi1 overexpression in 64% of grade III invasive ductal breast adenocarcinomas compared to normal breast tissues. Bmi1 overexpression in immortalized and transformed breast epithelial cells increased their sphere-forming efficiency, induced epithelial to mesenchymal transition (EMT) with an increase in the expression of stemness-related genes. Knockdown of Bmi1 in tumorigenic breast cells induced epithelial morphology, reduced expression of stemness-related genes, decreased the IC50 values of doxorubicin and abrogated tumor-formation. Bmi1-high tumors showed elevated Nanog expression whereas the tumors with lower Bmi1 showed reduced Nanog levels. Overexpression of Bmi1 increased Nanog levels whereas knockdown of Bmi1 reduced its expression. Dual luciferase promoter-reporter assay revealed Bmi1 positively regulated the Nanog and NFkappaB promoter activity. RT-PCR analysis showed that Bmi1 overexpression activated the NFkappaB pathway whereas Bmi1 knockdown reduced the expression of NFkappaB target genes, suggesting that Bmi1 might regulate Nanog expression through the NFkappaB pathway. CONCLUSIONS: Our study showed that Bmi1 is overexpressed in several high-grade, invasive ductal breast adenocarcinomas, thus supporting its role as a prognostic marker. While Bmi1 overexpression increased self-renewal and promoted EMT, its knockdown reversed EMT, reduced stemness, and rendered cells drug sensitive, thus highlighting a crucial role for Bmi1 in regulating the stemness and drug response of breast cancer cells. Bmi1 may control self-renewal through the regulation of Nanog expression via the NFkappaB pathway.

Patel, S. and R. Rawal (2016). "Role of miRNA dynamics and cytokine profile in governing CD44v6/Nanog/PTEN axis in oral cancer: modulating the master regulators." Tumour Biol **37**(11): 14565-14575.

Late diagnosis, low therapeutic response, and metastasis are accountable for poor 5-year survival rate of OSCC. These failures are attributed to the existence of "cancer stem cell (CSC)" subpopulation. Hence, it is necessary to identify and understand the mechanism of CSCs in tumor development, metastasis, and chemotherapeutic response. Propelling evidences suggest that microRNA (miRNA)-mediated regulation and cytokines of tumor microenvironment have the ability to modulate CSC signalling pathway; however, their exact mechanism needs to be elucidated. Thus, in this study, we characterized CSC markers and highlighted the miRNA dynamics and cytokine profile regulating these CSCs in a pathway-dependent manner. Our results demonstrated CD44+ subpopulation as tumor-initiating cells with self-renewal capability, tumorigenic growth potential and intrinsic chemoresistance. These tumors exhibited increased expression of CSC markers (CD44v3, CD44v6, Nanog, and Bmi1) and significantly reduced expression of PTEN and ATM in OSCC patients. Pathway analysis of these CSC markers demonstrated a prospective pathway regulated by miRNA and cytokine network. On analyzing these modulators, we observed decreased expression of miRNA542-3p, miRNA34a and miRNA9, and significant upregulation of miRNA21, thus forming an unexplored axis. Cytokine profiling revealed significantly increased levels of IL-6 and IL-8 compared to normals and demonstrated their strong association with CD44v6. Collectively, this study indicates that miR5423p and miR34a targets the CD44v6-Nanog-PTEN axis, thus playing a vital role in regulating the CSC properties. Furthermore, we speculate an impinging role of cytokines IL-6 and IL-8 in regulating this CSC-mediated pathway which can have prognostic and therapeutic implications.

Qin, S., et al. (2017). "NANOG regulates epithelial-mesenchymal transition and chemoresistance in ovarian cancer." Biosci Rep **37**(1).

A key transcription factor associated with poor prognosis and resistance to chemotherapy in ovarian cancer is NANOG. However, the mechanism by which NANOG functions remains undefined. It has been suggested that epithelial-to-mesenchymal transition (EMT) also contributes to development of drug resistance in different cancers. We thus determined whether NANOG expression was associated with EMT and chemoresistance in epithelial ovarian cancer cells. NANOG expression was increased in epithelial ovarian cancer cell lines compared with its expression in normal epithelial ovarian cell lines. NANOG expression in SKOV-3 or OV2008 cells directly correlated with high expression of mesenchymal cell markers and inversely with low expression of epithelial cell marker. RNAi-mediated silencing of NANOG in SKOV-3 reversed the expression of mesenchymal cell markers and restored expression of E-cadherin. Reversibly, stable overexpression of NANOG in Moody cells increased expression of N-cadherin whereas down-regulating expression of E-cadherin, cumulatively indicating that NANOG plays an important role in maintaining the mesenchymal cell markers. Modulating NANOG expression did not have any effect on proliferation or colony formation. Susceptibility to cisplatin increased in SKOV-3 cells on down-regulating NANOG and reversible results were obtained in Moody cells post-overexpression of NANOG. NANOG silencing in SKOV-3 and OV2008 robustly attenuated in vitro migration and invasion. NANOG expression exhibited a biphasic pattern in patients with ovarian cancer and expression was directly correlated to chemoresistance retrospectively. Cumulatively, our data demonstrate that NANOG expression modulates chemosensitivity and EMT resistance in ovarian cancer.

Radwan, A. A., et al. (2016). "Target beta-catenin/CD44/Nanog axis in colon cancer cells by certain N'-(2-oxoindolin-3-ylidene)-2-(benzyloxy)benzohydrazides." Bioorg Med Chem Lett **26**(7): 1664-1670.

Cell surface molecule CD44 plays a major role in regulation of cancer stem cells CSCs on both phenotypic and functional level, however chemical inhibition approach of CD44 to targets CSCs is poorly studied. Herein, we report the discovery of certain N'-(2-oxoindolin-3-ylidene)-2-(benzyloxy)benzohydrazides as a novel inhibitor of CD44. Molecular docking study showed interference of the scaffold of these compounds with beta-catenin/TCF-4 complex, building a direct relationship between CD44 inhibition and observed well-fitted binding domain. Compound 11a, most potent member elicits inhibition effect on TCF/LEF reporter activity conformed the involvement of Wnt pathway inhibition as a mechanism of action. Furthermore, the treatment by the mentioned compound leads to inhibition of embryonic transcriptional factor Nanog but not Sox2 or Oct-4 suggested specific targeted effect. Moreover, the cytotoxicity and cell cycle effect of this series seems to be dependent on CD44 expression.

Rasti, A., et al. (2018). "Co-expression of Cancer Stem Cell Markers OCT4 and NANOG Predicts Poor Prognosis in Renal Cell Carcinomas." Sci Rep **8**(1): 11739.

Many renal cancer patients experience disease recurrence after combined treatments or immunotherapy due to permanence of cancer stem cells (CSCs). This study was conducted to evaluate the expression patterns and clinical significance of octamer-binding transcription factor 4 (OCT4) and NANOG as the key stem cell factors in renal cell carcinoma (RCC). A total of 186 RCC tissues were immunostained on a tissue microarray (TMA) for the putative CSC markers OCT4 and NANOG. Subsequently, the correlation among the expression of these markers, the clinicopathological variables and survival outcomes were determined. OCT4 and NANOG were expressed in both the nucleus and the cytoplasm of RCC cells. Coexpression of OCT4 and NANOG in renal cancer was significantly associated with RCC subtypes. A significant association was found among nuclear coexpression of OCT4 and NANOG, worse PFS in RCC, and the clear cell renal cell carcinomas (ccRCC) subtype. The OCT4-nuclear (high)/NANOG-nuclear (high) phenotype in RCC and ccRCC subtype indicated aggressive tumor behavior and predicted a worse clinical outcome, which may be a useful biomarker to identify patients at high risk of postoperative recurrence and metastasis. Cytoplasmic expression of NANOG could be considered as a novel independent prognostic predictor in patients with renal cancer.

Rodrigo, J. P., et al. (2017). "A Novel Role For Nanog As An Early Cancer Risk Marker In Patients With Laryngeal Precancerous Lesions." Sci Rep **7**(1): 11110.

NANOG is a master regulator of embryonic stem cell pluripotency, found to be frequently aberrantly expressed in a variety of cancers, including laryngeal carcinomas. This study investigates for the first time the role of NANOG expression in early stages of laryngeal tumourigenesis and its potential utility as cancer risk marker. NANOG protein expression was evaluated by immunohistochemistry using two large independent cohorts of patients with laryngeal precancerous lesions, and correlated with clinicopathological parameters and laryngeal cancer risk. NANOG expression was detected by

immunohistochemistry in 49 (60%) of 82 laryngeal dysplasias, whereas expression was negligible in patient-matched normal epithelia. Strong NANOG expression was found in 22 (27%) lesions and was established as cut-off point, showing the most robust association with laryngeal cancer risk (P = 0.003) superior to the histological classification (P = 0.320) the current gold standard in the clinical practice. Similar trends were obtained using a multicenter validation cohort of 86 patients with laryngeal dysplasia. Our findings uncover a novel role for NANOG expression in laryngeal tumourigenesis, and its unprecedented application as biomarker for cancer risk assessment.

Schreiber, L., et al. (2014). "CD24 and Nanog identify stem cells signature of ovarian epithelium and cysts that may develop to ovarian cancer." Acta Histochem **116**(2): 399-406.

Ovarian cancer is the most lethal gynecological cancer. There is a general debate whether ovarian cancer is an intrinsic or an imported disease. We investigated whether in normal morphological appearance and in early stages of ovarian tumorgenesis typical cancer cell markers such as CD24 and Nanog are expressed. In 25% of normal appearing ovaries of post-menopausal women there was co-localization of CD24 and Nanog in the walls of the ovarian cysts, leaving the epithelial cells on the surface of these ovaries free of Nanog or CD24 expression. In benign ovarian tumors 37% of specimens were positive to CD24 and Nanog labeling while 26% of them were localized in the cyst walls. In contrast, in serous borderline tumors 79% specimens were labeled with CD24, 42% of them were localized in cysts and in 32% of them showed co-localization with CD24 and Nanog was evident: the rest were labeled in the ovarian epithelial cells. In serous ovarian carcinomas 81% specimens were labeled with CD24 antibodies. In 45% of them co-localization with Nanog was evident in the bulk of the cancerous tissue. In mucinous carcinomas no labeling with CD24 or Nanog was evident. In view of the synergistic effect of CD24 and Nanog expressed in malignant cancer development in other systems, it is suggested that such an analysis can be valuable for early detection of ovarian cancer. Moreover, the abundance of these markers in cysts in the development of ovarian cancer may suggest that they present an intrinsic source of the development of the highly malignant disease. Finally, since CD24 is exposed on the surface of the cancer cells, it may be highly beneficial to target these cells with antibodies to CD24 conjugated to cytotoxic drugs for more efficient treatment of this malignant disease.

Shan, J., et al. (2012). "Nanog regulates self-renewal of cancer stem cells through the insulin-like growth factor pathway in human hepatocellular carcinoma." Hepatology **56**(3): 1004-1014.

UNLABELLED: Hepatocellular carcinoma (HCC) exhibits cellular heterogeneity and embryonic stem-cell-related genes are preferentially overexpressed in a fraction of cancer cells of poorly differentiated tumors. However, it is not known whether or how these cancer cells contribute to tumor initiation and progression. Here, our data showed that increased expression of pluripotency transcription factor Nanog in cancer cells correlates with a worse clinical outcome in HCC. Using the Nanog promoter as a reporter system, we could successfully isolate a small subpopulation of Nanog-positive cells. We demonstrate that Nanog-positive cells exhibited enhanced ability of self-renewal, clonogenicity, and initiation of tumors, which are consistent with crucial hallmarks in the definition of cancer stem cells (CSCs). Nanog (Pos) CSCs could differentiate into mature cancer cells in in vitro and in vivo conditions. In addition, we found that Nanog (Pos) CSCs exhibited resistance to therapeutic agents (e.g., sorafenib and cisplatin) and have a high capacity for tumor invasion and metastasis. Knock-down expression of Nanog in Nanog (Pos) CSCs could decrease self-renewal accompanied with decreased expression of stem-cell-related genes and increased expression of mature hepatocyte-related genes. Overexpression of Nanog in Nanog (Neg) cells could restore self-renewal. Furthermore, we found that insulin-like growth factor (IGF)2 and IGF receptor (IGF1R) were up-regulated in Nanog (Pos) CSCs. Knock-down expression of Nanog in Nanog (Pos) CSCs inhibited the expression of IGF1R, and overexpression of Nanog in Nanog (Neg) cells increased the expression of IGF1R. A specific inhibitor of IGF1R signaling could significantly inhibit self-renewal and Nanog expression, indicating that IGF1R signaling participated in Nanog-mediated self-renewal. CONCLUSION: These data indicate that Nanog could be a novel biomarker for CSCs in HCC, and that Nanog could play a crucial role in maintaining the self-renewal of CSCs through the IGF1R-signaling pathway.

Shao, W. F., et al. (2016). "[Nanog promotes the invasion of breast cancer cells by increasing PKCepsilon expression]." Nan Fang Yi Ke Da Xue Xue Bao **36**(5): 639-644.

OBJECTIVE: To study the relationship between Nanog-promoted metastasis of breast cancer and ezrin (T567) phosphorylation, and explore the possible mechanism by which Nanog regulates ezrin (T567) phosphorylation. METHODS: A siRNA construct

targeting Nanog was transfected in breast cancer cells to knock down Nanog expression, and the changes in the cell invasion was detected using Transwell assay. The expression levels of Nanog and PKC and the phosphorylation level of ezrin (T567) were detected using Western blotting and immunofluorescent staining; the protein interaction between PKCepsilon and ezrin was assayed by co-immunoprecipitation and Western blotting. RESULTS: Nanog knockdown significantly decreased the expression of PKCepsilon protein, phosphorylation level of ezrin (T567) and the invasion ability of breast cancer cells. PKCepsilon knockdown obviously decreased the phosphorylation level of ezrin (T567) in the cells, and PKCepsilon and ezrin were co-immunoprecipitated. CONCLUDIONS: Nanogcan can upregulate the expression of PKCepsilon to promote the phosphorylation of ezrin (T567), which can be a new mechanism by which Nanog promotes tumor metastasis.

Siddique, H. R., et al. (2015). "NUMB phosphorylation destabilizes p53 and promotes self-renewal of tumor-initiating cells by a NANOG-dependent mechanism in liver cancer." Hepatology **62**(5): 1466-1479.

UNLABELLED: Stem cell populations are maintained through self-renewing divisions in which one daughter cell commits to a particular fate whereas the other retains the multipotent characteristics of its parent. The NUMB, a tumor suppressor, in conjunction with another tumor-suppressor protein, p53, preserves this property and acts as a barrier against deregulated expansion of tumor-associated stem cells. In this context, NUMB-p53 interaction plays a crucial role to maintain the proper homeostasis of both stem cells, as well as differentiated cells. Because the molecular mechanism governing the assembly and stability of the NUMB-p53 interaction/complex are poorly understood, we tried to identify the molecule (s) that govern this process. Using cancer cell lines, tumor-initiating cells (TICs) of liver, the mouse model, and clinical samples, we identified that phosphorylations of NUMB destabilize p53 and promote self-renewal of TICs in a pluripotency-associated transcription factor NANOG-dependent manner. NANOG phosphorylates NUMB by atypical protein kinase C zeta (aPKCzeta), through the direct induction of Aurora A kinase (AURKA) and the repression of an aPKCzeta inhibitor, lethal (2) giant larvae. By radioactivity-based kinase activity assays, we showed that NANOG enhances kinase activities of both AURKA and aPKCzeta, an important upstream process for NUMB phosphorylation. Phosphorylation of NUMB by aPKCzeta destabilizes the NUMB-p53 interaction and p53 proteolysis and deregulates self-renewal in TICs. CONCLUSION: Post-translational modification of NUMB by the NANOG-AURKA-aPKCzeta pathway is an important event in TIC self-renewal and tumorigenesis. Hence, the NANOG-NUMB-p53 signaling axis is an important regulatory pathway for TIC events in TIC self-renewal and liver tumorigenesis, suggesting a therapeutic strategy by targeting NUMB phosphorylation. Further in-depth in vivo and clinical studies are warranted to verify this suggestion.

Sodja, E., et al. (2016). "The prognostic value of whole blood SOX2, NANOG and OCT4 mRNA expression in advanced small-cell lung cancer." Radiol Oncol **50**(2): 188-196.

BACKGROUND: The data on expression and clinical impact of cancer stem cell markers SOX2, NANOG and OCT4 in lung cancer is still lacking. The aim of our study was to compare SOX2, NANOG and OCT4 mRNA expression levels in whole blood between advanced small-cell lung cancer (SCLC) patients and healthy controls, and to correlate mRNA expression with progression-free survival (PFS) after first-line chemotherapy and overall survival (OS) in advanced SCLC patients. PATIENTS AND METHODS: 50 advanced SCLC patients treated with standard chemotherapy and followed at University Clinic Golnik, Slovenia, between 2009 and 2013 were prospectively included. SOX2, NANOG and OCT4 mRNA expression levels were determined using TaqMan qPCR in whole blood collected prior to chemotherapy. Whole blood of 34 matched healthy individuals with no cancerous disease was also tested. RESULTS: SOX2 mRNA expression was significantly higher in whole blood of SCLC patients compared to healthy controls ($p = 0.006$). Significant correlation between SOX2 mRNA expression levels and the number of distant metastatic sites was established ($p = 0.027$). In survival analysis, patients with high SOX2 expression had shorter OS ($p = 0.017$) and PFS ($p = 0.046$). In multivariate Cox analysis, an independent value of high SOX2 expression for shorter OS ($p = 0.002$), but not PFS was confirmed. No significant differences were observed for NANOG or OCT4 expression levels when comparing SCLC patients and healthy controls neither when analysing survival outcomes in SCLC patients. CONCLUSIONS: SOX2 mRNA expression in whole blood might be a promising non-invasive marker for molecular screening of SCLC and important prognostic marker in advanced chemotherapy-treated SCLC patients, altogether indicating important role of cancer stem-like cell (CSC) regulators in cancer spread. Further evaluation of SOX2 as a possible screening/prognostic marker and a therapeutic target of SCLC is warranted.

Tamura, S., et al. (2018). "Ecadherin regulates proliferation of colorectal cancer stem cells through NANOG." Oncol Rep **40**(2): 693-703.

Cancer stem cells (CSCs) possess a selfrenewal ability and display tumorigenic potential in immunodeficient mice. Colorectal CSCs are thought to be a uniform population and no functionally distinct subpopulations have been identified. Because Ecadherin is an essential molecule for selfrenewal of embryonic stem cells, we examined Ecadherin expression, which may play a role in maintaining the properties of CSCs, in EpCAMhigh/CD44+ colorectal CSCs from human primary colorectal cancers. We obtained 18 surgical specimens of human primary colorectal cancer. CD44, EpCAM, and Ecadherin expression were analyzed by fluorescenceactivated cell sorting. Sorted EpCAMhigh/CD44+ colorectal CSCs were injected into immunodeficient mice to estimate the tumorigenic potential. Genetic profiles were analyzed by cDNA microarray. Notably, colorectal CSCs could be divided into two populations based on the Ecadherin expression status, and they exhibited different pathological characteristics. Compared to Ecadherinnegative colorectal CSCs, Ecadherinpositive (EC+) colorectal CSCs demonstrated higher tumor growth potential in vivo. EC+ colorectal CSCs revealed a higher expression of the pluripotency factor NANOG, which contributed to the higher tumor growth potential of EC+ colorectal CSCs through control of cyclin D1 expression. These findings are the first demonstration of functionally distinct subpopulations of colorectal CSCs in human clinical samples.

Thiagarajan, P. S., et al. (2018). "Cx26 drives self-renewal in triple-negative breast cancer via interaction with NANOG and focal adhesion kinase." Nat Commun **9**(1): 578.

Tumors adapt their phenotypes during growth and in response to therapies through dynamic changes in cellular processes. Connexin proteins enable such dynamic changes during development, and their dysregulation leads to disease states. The gap junction communication channels formed by connexins have been reported to exhibit tumor-suppressive functions, including in triple-negative breast cancer (TNBC). However, we find that connexin 26 (Cx26) is elevated in self-renewing cancer stem cells (CSCs) and is necessary and sufficient for their maintenance. Cx26 promotes CSC self-renewal by forming a signaling complex with the pluripotency transcription factor NANOG and focal adhesion kinase (FAK), resulting in NANOG stabilization and FAK activation. This FAK/NANOG-containing complex is not formed in mammary epithelial or luminal breast cancer cells. These findings challenge the paradigm that connexins are tumor suppressors in TNBC and reveal a unique function for Cx26 in regulating the core self-renewal signaling that controls CSC maintenance.

Tomiyama, N., et al. (2018). "S100A16 up-regulates Oct4 and Nanog expression in cancer stem-like cells of Yumoto human cervical carcinoma cells." Oncol Lett **15**(6): 9929-9933.

Cancer stem-like cells (CSCs), which possess the ability to self-renewal and are multipotent, are regarded as the cause of tumor formation, recurrence, metastasis and drug resistance. It is necessary to understand the properties of CSCs in order to treat them effectively. It has been previously reported that S100 family proteins, which carry calcium-binding EF-hand motifs and are associated with tumorigenic processes, serve crucial roles in maintaining cancer stem-like properties. S100A16 is upregulated in various types of cancer, including bladder, lung and pancreatic. However, the roles of S100A16 in cancer cells, particularly CSCs, are not clear. The present study investigated the roles of S100A16 in CSCs using the sphere formation assay of Yumoto cells, which are a human cervical carcinoma cell line. The mRNA expression levels were evaluated by reverse transcription-polymerase chain reaction and the protein expression levels were detected by western blot analysis. Following the sphere formation of Yumoto cells, the mRNA and protein expression level of Oct4, Nanog and S100A16 were increased compared with the control cells. Following transfection with S100A16 small interfering RNA (siRNA), the mRNA and protein expression of Oct4 and Nanog were decreased and the spheroid size was significantly decreased in the sphere formation of Yumoto cells compared with control siRNA treated cells. There was no change in the p53 mRNA expression level, whereas the p53 protein expression level, which was decreased by the sphere formation, was recovered by S100A16 knockdown. In addition, the protein expression levels of Oct4 and Nanog, which were increased in the sphere formation, were decreased by the proteasome inhibitor lactacystin. No differences were observed in the S100A16 protein expression between the presence or absence of lactacystin. These results suggest that S100A16 serves an important role in the CSCs of human cervical carcinoma and is a positive regulator of Oct4 and Nanog.

Tulsyan, S., et al. (2014). "Significant association of combination of OCT4, NANOG, and SOX2 gene polymorphisms in susceptibility and response to treatment in North Indian breast cancer patients." Cancer Chemother Pharmacol **74**(5): 1065-1078.

PURPOSE: Dysregulations of regulatory genes in embryonic stem cells (ESCs) gene polymorphisms may lead to breast cancer cell growth, differentiation, and tumor metastasis. METHODS: Polymorphisms in OCT4 (rs3130932), NANOG (rs11055786), LIN28 (rs4274112), and SOX2 (rs11915160) genes were evaluated for susceptibility in 297 breast cancer females and 273 healthy controls from north Indian population. Response to neo-adjuvant chemotherapy was followed in 128 locally advanced breast cancer patients along with clinicopathological features. Genotyping was done using TaqMan allelic discrimination assays. Statistical analysis was performed using SPSS and multifactor dimensionality reduction (MDR). RESULTS: For OCT4 gene polymorphism, protective effect of genotypes AC [P corr = 0.031, OR = 0.63 (0.44-0.91)] and AC+CC [P corr = 0.031, OR = 0.68 (0.48-0.95)] was seen in patients. However, no association of NANOG, LIN28, and SOX2 gene polymorphisms was found with overall breast cancer susceptibility. Further, significant association of AG+GG genotype [P corr = 0.021, OR = 6.08 (1.83-20.15)] and G allele [P corr = 0.021, OR = 3.07 (1.21-7.77)] of rs4274112 polymorphism was seen with positive lymph node. For OCT4, significant association of allele C was seen with patients having negative hormone receptor [P corr = 0.021, OR = 0.51 (0.29-0.90)], but no association of any of the studied polymorphisms individually was found with response to NACT. On MDR analysis, we found combination of SNPs SOX2 rs11915160, OCT4 rs3130932, and NANOG rs11055786 to be the best interaction model for predicting breast cancer risk [p for permutation test <10(-3), OR = 2.04 (1.43-2.910] and response to NACT [p for permutation test = 0.005, OR = 2.09 (1.24-3.52)]. CONCLUSION: Combination of genetic variants of ESCs gene may have a profound effect in breast cancer risk and response to NACT.

Uchino, K., et al. (2012). "Human Nanog pseudogene8 promotes the proliferation of gastrointestinal cancer cells." Exp Cell Res **318**(15): 1799-1807.

There is emerging evidence that human solid tumor cells originate from cancer stem cells (CSCs). In cancer cell lines, tumor-initiating CSCs are mainly found in the side population (SP) that has the capacity to extrude dyes such as Hoechst 33342. We found that Nanog is expressed specifically in SP cells of human gastrointestinal (GI) cancer cells. Nucleotide sequencing revealed that NanogP8 but not Nanog was expressed in GI cancer cells. Transfection of NanogP8 into GI cancer cell lines promoted cell proliferation, while its inhibition by anti-Nanog siRNA suppressed the proliferation. Immunohistochemical staining of primary GI cancer tissues revealed NanogP8 protein to be strongly expressed in 3 out of 60 cases. In these cases, NanogP8 was found especially in an infiltrative part of the tumor, in proliferating cells with Ki67 expression. These data suggest that NanogP8 is involved in GI cancer development in a fraction of patients, in whom it presumably acts by supporting CSC proliferation.

Vaidya, M., et al. (2018). "Differential sequences of exosomal NANOG DNA as a potential diagnostic cancer marker." PLoS One **13**(5): e0197782.

NANOG has been demonstrated to play an essential role in the maintenance of embryonic stem cells, and its pseudogene, NANOGP8, is suggested to promote the cancer stem cell phenotype. As the roles of these genes are intimately involved with glioblastoma multiforme progression and exosomes are critical in intercellular communication, we conducted a detailed analysis of the association of the NANOG gene family with exosomes to identify diagnostic markers for cancer. Exosomes were precipitated from conditioned culture media from various cell lines, and NANOG gene fragments were directly amplified without DNA isolation using multiple primer sets. The use of the enzymes AlwNI and SmaI with restriction fragment length polymorphism analysis functioned to distinguish NANOGP8 from other NANOG family members. Collectively, results suggest that the NANOG DNA associated with exosomes is not full length and that mixed populations of the NANOG gene family exist. Furthermore, sequence analysis of exosomal DNA amplified with a NANOGP8 specific primer set frequently showed an insertion of a 22 bp sequence into the 3' UTR. The occurrence rate of this insertion was significantly higher in exosomal DNA clones from cancer cells as compared to normal cells. We have detected mixed populations of NANOG DNA associated with exosomes and have identified preferential modulations in the sequences from cancer samples. Our findings, coupled with the properties of exosomes, may allow for the detection of traditionally inaccessible cancers (i.e. GBM) through minimally invasive techniques. Further analysis of exosomal DNA sequences of NANOG and other embryonic stemness genes (OCT3/4, SOX2, etc.) may establish a robust collection of exosome based diagnostic markers, and further elucidate the mechanisms of cancer formation, progression, and metastasis.

van Schaijik, B., et al. (2018). "Subcellular localisation of the stem cell markers OCT4, SOX2, NANOG, KLF4 and c-MYC in cancer: a review." J Clin Pathol **71**(1): 88-91.

The stem cell markers octamer-binding transcription factor 4, sex-determining region Y-box 2,

NANOG, Kruppel-like factor 4 and c-MYC are key factors in inducing pluripotency in somatic cells, and they have been used to detect cancer stem cell subpopulations in a range of cancer types. Recent literature has described the subcellular localisation of these markers and their potential implications on cellular function. This is a relatively complex and unexplored area of research, and the extent of the effect that subcellular localisation has on cancer development and growth is largely unknown. This review analyses this area of research in the context of the biology of stem cells and cancer and explores the potential modulating effect of subcellular localisation of these proteins as supported by the literature.

Virant-Klun, I., et al. (2016). "Small putative NANOG, SOX2, and SSEA-4-positive stem cells resembling very small embryonic-like stem cells in sections of ovarian tissue in patients with ovarian cancer." J Ovarian Res **9**: 12.

BACKGROUND: In previous studies it has been found that in cell cultures of human adult ovaries there is a population of small stem cells with diameters of 2-4 mum, which are present mainly in the ovarian surface epithelium and are comparable to very small embryonic-like stem cells (VSELs) from bone marrow. These cells are not observed by histopathologists in the ovarian tissue due to their small size and unknown clinical significance. Because these cells express a degree of pluripotency, they might be involved in the manifestation of ovarian cancer. Therefore we studied the ovarian tissue sections in women with borderline ovarian cancer and serous ovarian carcinoma to perhaps identify the small putative stem cells in situ. METHODS: In 27 women with borderline ovarian cancer and 20 women with high-grade serous ovarian carcinoma the ovarian tissue sections were stained, per standard practice, with eosin and hematoxylin staining and on NANOG, SSEA-4 and SOX2 markers, related to pluripotency, using immunohistochemistry. We focused on the presence and localization of small putative stem cells with diameters of up to 5 mum and with the nuclei spread over nearly the full cell volume. RESULTS: In ovarian sections of both borderline ovarian cancer and serous ovarian carcinoma patients we were able to identify the presence of small round cells complying with the above criteria. Some of these small cells were NANOG-positive, were located among epithelial cells in the ovarian surface epithelium and as a single cell or groups of cells/clusters in typical "chambers", were found only in the presence of ovarian cancer and not in healthy ovaries and are comparable to those in fetal ovaries. We envision that these small cells could be related to NANOG-positive tumor-like structures and oocyte-like cells in similar "chambers" found in sections of cancerous ovaries, which could support their stemness and pluripotency. Further immunohistochemistry revealed a similar population of SSEA-4 and SOX2-positive cells. CONCLUSIONS: We may conclude that putative small stem cells expressing markers, related to pluripotency, are present in the ovarian tissue sections of women with borderline ovarian cancer and high-grade serous ovarian carcinoma thus indicating their potential involvement in ovarian cancer.

Wang, B., et al. (2016). "LGR5 Is a Gastric Cancer Stem Cell Marker Associated with Stemness and the EMT Signature Genes NANOG, NANOGP8, PRRX1, TWIST1, and BMI1." PLoS One **11**(12): e0168904.

BACKGROUND: Accumulating evidence supports the hypothesis that cancer stem cells (CSCs) are essential for cancer initiation, metastasis and drug resistance. However, the functional association of gastric CSC markers with stemness and epithelial-mesenchymal transition (EMT) signature genes is unclear. METHODS: qPCR was performed to measure the expression profiles of stemness and EMT signature genes and their association with putative CSC markers in gastric cancer tissues, cancer cell lines and sphere cells. Western blot analysis was used to confirm the results of the transcript analysis. Cell proliferation, cell migration, drug resistance and sphere cell growth assays were conducted to measure the expansion and invasion abilities of the cells. Tumor xenograft experiments were performed in NOD/SCID mice to test cell stemness in vivo. Flow cytometry and immunofluorescence staining were used to analyze cell subpopulations. RESULTS: The expression of LGR5 was strikingly up-regulated in sphere cells but not in cancer tissues or parental adherent cells. The up-regulation of LGR5 was also positively associated with stemness regulators (NANOG, OCT4, SOX2, and AICDA) and EMT inducers (PRRX1, TWIST1, and BMI1). In addition, sphere cells exhibited up-regulated vimentin and down-regulated E-cadherin expression. Using gene-specific primers, we found that the NANOG expression primarily originates from the retrogene NANOGP8. Western blot analysis showed that the expression of both LGR5 and NANOG is significantly higher in sphere cells. LGR5 over-expression significantly enhanced sphere cell growth, cell proliferation, cell migration and drug resistance in MGC803 cells. Tumor xenografts in nude mice showed that sphere cells are at least 10 times more efficient at tumor initiation than adherent cells. Flow cytometry analysis showed that ~20% of sphere cells are LGR5+/CD54+, but only ~3% of adherent cells are Lgr5+/CD54+. Immunofluorescence staining supports the above results. CONCLUSION: The LGR5-

expressing fraction of CD54+ cells represents gastric cancer CSCs, in which LGR5 is closely associated with stemness and EMT core genes, and NANOG expression is mainly contributed by the retrogene NANOGP8. Sphere cells are the best starting materials for the characterization of CSCs.

Wang, D., et al. (2014). "Oct-4 and Nanog promote the epithelial-mesenchymal transition of breast cancer stem cells and are associated with poor prognosis in breast cancer patients." Oncotarget **5**(21): 10803-10815.

Oct-4 and Nanog in regulating the epithelial-mesenchymal transition (EMT) and metastasis of breast cancer has not been clarified. We found that both Oct-4 and Nanog expression were significantly associated with tumor pathology and poor prognosis in 126 breast cancer patients. Characterization of CD44+CD24-Cancer stem cell (CSC) derived from breast cancer cells indicated that CSC rapidly formed mammospheres and had potent tumorigenicity in vivo. Furthermore, TGF-beta up-regulated the expression of Oct-4, Nanog, N-cadherin, vimentin, Slug, and Snail, but down-regulated E-cadherin and cytokeratin 18 expression, demonstrating that CSC underwent EMT. Knockdown of both Oct-4 and Nanog expression inhibited spontaneous changes in the expression of EMT-related genes, while induction of both Oct-4 and Nanog over-expression enhanced spontaneous changes in the expression of EMT-related genes in CSC. However, perturbing alternation of Oct-4 and Nanog expression also modulated TGF-beta-induced EMT-related gene expression in CSC. Induction of Oct-4 and Nanog over-expression enhanced the invasiveness of CSC, but knockdown of both Oct-4 and Nanog inhibited the migration of CSC in vitro. Our data suggest that both Oct-4 and Nanog may serve as biomarkers for evaluating breast cancer prognosis. Our findings indicate that Oct-4 and Nanog positively regulate the EMT process, contributing to breast cancer metastasis.

Wang, H., et al. (2017). "Reduction of NANOG Mediates the Inhibitory Effect of Aspirin on Tumor Growth and Stemness in Colorectal Cancer." Cell Physiol Biochem **44**(3): 1051-1063.

BACKGROUND/AIMS: Cancer stem cells (CSCs) are considered to be responsible for tumor relapse and metastasis, which serve as a potential therapeutic target for cancer. Aspirin has been shown to reduce cancer risk and mortality, particularly in colorectal cancer. However, the CSCs-suppressing effect of aspirin and its relevant mechanisms in colorectal cancer remain unclear. METHODS: CCK8 assay was employed to detect the cell viability. Sphere formation assay, colony formation assay, and ALDH1 assay were performed to identify the effects of aspirin on CSC properties. Western blotting was performed to detect the expression of the stemness factors. Xenograft model was employed to identify the anti-cancer effects of aspirin in vivo. Unpaired Student t test, ANOVA test and Kruskal-Wallis test were used for the statistical comparisons. RESULTS: Aspirin attenuated colonosphere formation and decreased the ALDH1 positive cell population of colorectal cancer cells. Aspirin inhibited xenograft tumor growth and reduced tumor cells stemness in nude mice. Consistently, aspirin decreased the protein expression of stemness-related transcription factors, including c-Myc, OCT4 and NANOG. Suppression of NANOG blocked the effect of aspirin on sphere formation. Conversely, ectopic expression of NANOG rescued the aspirin-repressed sphere formation, suggesting that NANOG is a key downstream target. Moreover, we found that aspirin repressed NANOG expression in protein level by decreasing its stability. CONCLUSION: We have provided new evidence that aspirin attenuates CSC properties through down-regulation of NANOG, suggesting aspirin as a promising therapeutic agent for colorectal cancer treatment.

Wang, M. L., et al. (2013). "Targeting cancer stem cells: emerging role of Nanog transcription factor." Onco Targets Ther **6**: 1207-1220.

The involvement of stemness factors in cancer initiation and progression has drawn much attention recently, especially after the finding that introducing four stemness factors in somatic cells is able to reprogram the cells back to an embryonic stem cell-like state. Following accumulating data revealing abnormal elevated expression levels of key stemness factors, like Nanog, Oct4, and Sox2, in several types of cancer stem cells; the importance and therapeutic potential of targeting these stemness regulators in cancers has turned to research focus. Nanog determines cell fate in both embryonic and cancer stem cells; activating Nanog at an inappropriate time would result in cancer stem cells rather than normal pluripotent stem cells or differentiated somatic cells. Upregulated Nanog is correlated with poor survival outcome of patients with various types of cancer. The discoveries of downstream regulatory pathways directly or indirectly mediated by Nanog indicate that Nanog regulates several aspects of cancer development such as tumor cell proliferation, self-renewal, motility, epithelial-mesenchymal transition, immune evasion, and drug-resistance, which are all defined features for cancer stem cells. The current review paper illustrates the central role of Nanog in the regulatory networks of cancer malignant development and stemness acquirement, as well as in the

communication between cancer cells and the surrounding stroma. Though a more defined model is needed to test the therapeutic efficacy of targeting Nanog as a cancer treatment method, current animal experiments using siNanog or shNanog have shown the promising therapeutic potential of Nanog targeting in several types of cancer.

Wefers, C., et al. (2018). "Immune Curbing of Cancer Stem Cells by CTLs Directed to NANOG." Front Immunol **9**: 1412.

Cancer stem cells (CSCs) have been identified as the source of tumor growth and disease recurrence. Eradication of CSCs is thus essential to achieve durable responses, but CSCs are resistant to current anti-tumor therapies. Novel therapeutic approaches that specifically target CSCs will, therefore, be crucial to improve patient outcome. Immunotherapies, which boost the body's own immune system to eliminate cancerous cells, could be an alternative approach to target CSCs. Vaccines of dendritic cells (DCs) loaded with tumor antigens can evoke highly specific anti-tumor T cell responses. Importantly, DC vaccination also promotes immunological memory formation, paving the way for long-term cancer control. Here, we propose a DC vaccination that specifically targets CSCs. DCs loaded with NANOG peptides, a protein required for maintaining stem cell properties, could evoke a potent anti-tumor immune response against CSCs. We hypothesize that the resulting immunological memory will also control newly formed CSCs, thereby preventing disease recurrence.

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