

SOX2 and cancer literaturesMa Hongbao ¹, Margaret Young ²¹ Brookdale Hospital, Brooklyn, NY 11212, USA; ² Cambridge, MA 02138, USA
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Abstract: SRY (sex determining region Y)-box 2 (SOX2), is a transcription factor that is essential for maintaining self-renewal, or pluripotency, of undifferentiated embryonic stem cells. Sox2 is a member of the Sox family of transcription factors. This protein family has conserved DNA binding domains as High-mobility group (HMG) box domains containing approximately 80 amino acids. In squamous cell carcinoma, gene amplifications frequently target the 3q26.3 region. The gene for Sox2 lies within this region, which effectively characterizes Sox2 as an oncogene. Sox2 is a key upregulated factor in some cell carcinoma, directing many genes involved in tumor progression. Sox2 overexpression cooperates with loss of Lkb1 expression to promote cancer in animals. Its overexpression activates cellular migration and anchorage-independent growth, and gives rise to extensive epithelial hyperplasia and eventually carcinoma in both developing and adult animals. Sox2 expression is found in high gleason grade prostate cancer, and promotes castration-resistant prostate cancer growth. The ectopic expression of SOX2 may be related to abnormal differentiation of colorectal cancer cells.

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Key words: DNA; eternal; life; stem cell; universe

1. Introduction

SRY (sex determining region Y)-box 2 (SOX2), is a transcription factor that is essential for maintaining self-renewal, or pluripotency, of undifferentiated embryonic stem cells. Sox2 is a member of the Sox family of transcription factors. This protein family has conserved DNA binding domains as High-mobility group (HMG) box domains containing approximately 80 amino acids.

In squamous cell carcinoma, gene amplifications frequently target the 3q26.3 region. The gene for Sox2 lies within this region, which effectively characterizes Sox2 as an oncogene. Sox2 is a key upregulated factor in some cell carcinoma, directing many genes involved in tumor progression. Sox2 overexpression cooperates with loss of Lkb1 expression to promote cancer in animals. Its overexpression activates cellular migration and anchorage-independent growth, and gives rise to extensive epithelial hyperplasia and eventually carcinoma in both developing and adult animals. Sox2 expression is found in high gleason grade prostate cancer, and promotes castration-resistant prostate cancer growth. The ectopic expression of SOX2 may be related to abnormal differentiation of colorectal cancer cells.

There are many methods to deliver the transcription factors into target cells to generate iPSCs. The first method is retrovirus or lentivirus transduction. The problem of this technique is the genome integration of virus DNA which could possibly alter differentiation potential or other malignant transformation. The second method is adenoviral vectors to induce iPSC. The advantage of

adenovirus vector based expression is that the transgenes will not integrate into the house genome, thus reduces the risk of tumorigenesis. The third one is a plasmid based transfection that can avoid the genome integration also. Recently, the Cre-recombinase excisable systems are used in iPSC induction and subsequent transgene removal making the iPSC technology closer to clinic applications.

Literatures

The following gives some recent reference papers on .

Amini, S., F. Fathi, et al. "The expressions of stem cell markers: Oct4, Nanog, Sox2, nucleostemin, Bmi, Zfx, Tc11, Tbx3, Dppa4, and Esrrb in bladder, colon, and prostate cancer, and certain cancer cell lines." *Anat Cell Biol*. 2014 Mar;47(1):1-11. doi: 10.5115/acb.2014.47.1.1. Epub 2014 Mar 13.

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, Tc11, 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, Tc11, 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.

Askarian-Amiri, M. E., V. Seyfoddin, et al. "Emerging role of long non-coding RNA SOX2OT in SOX2 regulation in breast cancer." *PLoS One*. 2014 Jul 9;9(7):e102140. doi: 10.1371/journal.pone.0102140. eCollection 2014.

The transcription factor SOX2 is essential for maintaining pluripotency in a variety of stem cells. It has important functions during embryonic development, is involved in cancer stem cell maintenance, and is often deregulated in cancer. The mechanism of SOX2 regulation has yet to be clarified, but the SOX2 gene lies in an intron of a long multi-exon non-coding RNA called SOX2 overlapping transcript (SOX2OT). Here, we show that the expression of SOX2 and SOX2OT is concordant in breast cancer, differentially expressed in estrogen receptor positive and negative breast cancer samples and that both are up-regulated in suspension culture conditions that favor growth of stem cell phenotypes. Importantly, ectopic expression of SOX2OT led to an almost 20-fold increase in SOX2 expression, together with a reduced proliferation and increased breast cancer cell anchorage-independent growth. We propose that SOX2OT plays a key role in the induction and/or maintenance of SOX2 expression in breast cancer.

Atakan, S., H. Bayiz, et al. "Autologous anti-SOX2 antibody responses reflect intensity but not frequency of antigen expression in small cell lung cancer." *BMC Clin Pathol*. 2014 Jun 7;14:24. doi: 10.1186/1472-6890-14-24. eCollection 2014.

BACKGROUND: Anti-SOX2 antibody responses are observed in about 10 to 20% of small cell lung cancer (SCLC) patients. The aim of this study was to determine whether such responses reflect a particular pattern of SOX2 protein expression in the tumor and whether this pattern associates with clinical outcome. **METHODS:** Paraffin embedded tumor

tissues, obtained from SCLC patients who had no evidence of paraneoplastic autoimmune degeneration, were evaluated for SOX2 expression by immunohistochemistry for both intensity and extent of staining. Sera from the same patients were tested for autologous antibodies against recombinant SOX2 by enzyme-linked immunosorbent assay (ELISA). Correlates between overall survival and various clinical parameters including SOX2 staining and serology were determined. **RESULTS:** SOX2 protein expression was observed in tumor tissue in 89% of patients. Seventeen patients (29%) were seropositive for SOX2 antibodies and, in contrast to SOX2 staining, the presence of antibody correlated with limited disease stage ($p = 0.05$). SOX2 seropositivity showed a significant association with the intensity of SOX2 staining in the tumor ($p = 0.02$) but not with the frequency of SOX2 expressing cells. **CONCLUSION:** Anti-SOX2 antibodies associate with better prognosis (limited stage disease) while SOX2 protein expression does not; similar to reports from some earlier studies. Our data provides an explanation for this seemingly contrasting data for the first time as SOX2 antibodies can be observed in patients whose tumors contain relatively few but strongly staining cells, thus supporting the possible presence of active immune-surveillance and immune-editing targeting SOX2 protein in this tumor type.

Belotte, J., N. M. Fletcher, et al. "Sox2 Gene Amplification Significantly Impacts Overall Survival in Serous Epithelial Ovarian Cancer." *Reprod Sci*. 2014 Jul 18. pii: 1933719114542021.

Epithelial ovarian cancer (EOC) is the deadliest gynecologic cancer. Recently, the existence of ovarian cancer stem cells has been reported. Sox2, Nanog and Oct4 are key markers of "stemness". The objective of this study was to determine whether Sox2, Nanog, and Oct4 are associated with EOC and poor outcome. The expression of these markers was assessed by immunofluorescence staining and real-time RT-PCR in human EOC cell lines MDAH-2774 and SKOV-3, while the cancer genome atlas (TCGA) dataset was analyzed for associations with survival. Sox2, Nanog and Oct4 (POU5F1) were all detected by immunofluorescence staining and these results were confirmed by real-time RT-PCR. The TCGA dataset revealed a 26%, 9%, and 6% amplification of Sox2, Nanog and POU5F1, respectively. Additionally, K-M survival analyses showed a significant median overall survival difference (41 versus 48.3 months, $P = .01$) for Sox2 amplification, but not for Nanog (44.1 versus 36.2 months, $P > .05$) and POU5F1 (43.5 versus 45.0 months, $P > .05$). Our results suggest that Sox2 gene amplification significantly influences overall survival.

Bornschein, J., K. Toth, et al. "Dysregulation of CDX1, CDX2 and SOX2 in patients with gastric cancer also affects the non-malignant mucosa." *J Clin Pathol.* 2013 Sep;66(9):819-22. doi: 10.1136/jclinpath-2013-201448. Epub 2013 Apr 23.

The interplay between gastric and intestinal transcription factors has an important impact on gastric carcinogenesis. We compared the gene expression of CDX1, CDX2, SOX2 and related downstream genes in tumour and tumour surrounding gastric tissue of 48 gastric cancer patients with 30 healthy controls. There was no difference of gene expression of CDX1 and CDX2 between tumour or tumour-adjacent and tumour-distant mucosa, but both factors were significantly higher expressed in cancer patients compared with controls ($p < 0.01$). SOX2 was downregulated in tumour tissue compared to controls, whereas tumour-adjacent and tumour-distant mucosa showed intermediate SOX2 expression. Lauren type and *Helicobacter pylori* infection had no significant impact on expression of the transcription factors. Expression of CDX1 and CDX2 was higher in the presence of intestinal metaplasia. The differential regulation of the gene expression of CDX1, CDX2 and SOX2 in patients with gastric cancer affects not only the tumour but also the non-neoplastic tumour-distant mucosa.

Boumahdi, S., G. Driessens, et al. "SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma." *Nature.* 2014 Jul 10;511(7508):246-50. doi: 10.1038/nature13305. Epub 2014 Jun 8.

Cancer stem cells (CSCs) have been reported in various cancers, including in skin squamous-cell carcinoma (SCC). The molecular mechanisms regulating tumour initiation and stemness are still poorly characterized. Here we find that Sox2, a transcription factor expressed in various types of embryonic and adult stem cells, was the most upregulated transcription factor in the CSCs of squamous skin tumours in mice. SOX2 is absent in normal epidermis but begins to be expressed in the vast majority of mouse and human pre-neoplastic skin tumours, and continues to be expressed in a heterogeneous manner in invasive mouse and human SCCs. In contrast to other SCCs, in which SOX2 is frequently genetically amplified, the expression of SOX2 in mouse and human skin SCCs is transcriptionally regulated. Conditional deletion of Sox2 in the mouse epidermis markedly decreases skin tumour formation after chemical-induced carcinogenesis. Using green fluorescent protein (GFP) as a reporter of Sox2 transcriptional expression (SOX2-GFP knock-in mice), we showed that SOX2-expressing cells in invasive SCC are greatly enriched

in tumour-propagating cells, which further increase upon serial transplantations. Lineage ablation of SOX2-expressing cells within primary benign and malignant SCCs leads to tumour regression, consistent with the critical role of SOX2-expressing cells in tumour maintenance. Conditional Sox2 deletion in pre-existing skin papilloma and SCC leads to tumour regression and decreases the ability of cancer cells to be propagated upon transplantation into immunodeficient mice, supporting the essential role of SOX2 in regulating CSC functions. Transcriptional profiling of SOX2-GFP-expressing CSCs and of tumour epithelial cells upon Sox2 deletion uncovered a gene network regulated by SOX2 in primary tumour cells in vivo. Chromatin immunoprecipitation identified several direct SOX2 target genes controlling tumour stemness, survival, proliferation, adhesion, invasion and paraneoplastic syndrome. We demonstrate that SOX2, by marking and regulating the functions of skin tumour-initiating cells and CSCs, establishes a continuum between tumour initiation and progression in primary skin tumours.

Bourguignon, L. Y., G. Wong, et al. "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.* 2012 Sep 21;287(39):32800-24. Epub 2012 Jul 30.

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.

Carina, V., G. Zito, et al. "Multiple pluripotent stem cell markers in human anaplastic thyroid cancer: the putative upstream role of SOX2." *Thyroid*. 2013 Jul;23(7):829-37. doi: 10.1089/thy.2012.0372. Epub 2013 Jun 21.

BACKGROUND: Anaplastic thyroid carcinoma (ATC) is a rare and aggressive endocrine tumor with highly undifferentiated morphology. It has been suggested that cancer stem cells (CSCs) might play a central role in ATC. The objectives of this study were (i) to characterize CSCs from ex vivo ATC specimens by investigating the expression of several pluripotent stem cell markers, and (ii) to evaluate in vitro drug resistance modifications after specific CSC transcription factor switch-off. **METHODS:** In ex vivo experiments, eight formalin-fixed, paraffin-embedded ATC specimens were analyzed by reverse-transcription and real-time quantitative PCR and immunohistochemistry. In in vitro experiments using ATC SW1736 cells, the expression levels of OCT-4, NANOG, and ABCG2 and the sensitivity to either cisplatin or doxorubicin were evaluated after silencing. **RESULTS:** OCT-4, KLF4, and SOX2 transcription factors and C-KIT and THY-1 stem surface antigens showed variable up-regulation in all ATC cases. The SW1736 cell line was characterized by a high percentage of stem population (10.4+/-2.1% of cells were aldehyde dehydrogenase positive) and high expression of several CSC markers (SOX2, OCT4, NANOG, C-MYC, and SSEA4). SOX2 silencing down-regulated OCT-4, NANOG, and ABCG2. SOX2 silencing sensitized SW1736 cells, causing a significant cell death increase (1.8-fold) in comparison to control cells with 10 μ M cisplatin (93.9+/-3.4% vs. 52.6+/-9.4%, $p < 0.01$) and 2.7 fold with 0.5 μ M doxorubicin (45.8+/-9.9% vs. 17.1+/-3.4% $p < 0.01$). ABCG2 silencing caused increased cell

death with both cisplatin (74.9+/-1.4%) and doxorubicin treatment (74.1+/-0.1%) vs. no-target-treated cells (respectively, 45.8+/-1.0% and 48.6+/-1.0%, $p < 0.001$). **CONCLUSIONS:** The characterization of CSCs in ATC through the analysis of multiple pluripotent stem cell markers might be useful in identifying cells with a stem-like phenotype capable of resisting conventional chemotherapy. In addition, our data demonstrate that SOX2 switch-off through ABCG2 transporter down-regulation has a major role in overcoming CSC chemotherapy resistance.

Corominas-Faja, B., S. Cufi, et al. "Nuclear reprogramming of luminal-like breast cancer cells generates Sox2-overexpressing cancer stem-like cellular states harboring transcriptional activation of the mTOR pathway." *Cell Cycle*. 2013 Sep 15;12(18):3109-24. doi: 10.4161/cc.26173. Epub 2013 Aug 21.

Energy metabolism plasticity enables stemness programs during the reprogramming of somatic cells to an induced pluripotent stem cell (iPSC) state. This relationship may introduce a new era in the understanding of Warburg's theory on the metabolic origin of cancer at the level of cancer stem cells (CSCs). Here, we used Yamanaka's stem cell technology in an attempt to create stable CSC research lines in which to dissect the transcriptional control of mTOR--the master switch of cellular catabolism and anabolism--in CSC-like states. The rare colonies with iPSC-like morphology, obtained following the viral transduction of the Oct4, Sox2, Klf4, and c-Myc (OSKM) stemness factors into MCF-7 luminal-like breast cancer cells (MCF-7/Rep), demonstrated an intermediate state between cancer cells and bona fide iPSCs. MCF-7/Rep cells notably overexpressed SOX2 and stage-specific embryonic antigen (SSEA)-4 proteins; however, other stemness-related markers (OCT4, NANOG, SSEA-1, TRA-1-60, and TRA-1-81) were found at low to moderate levels. The transcriptional analyses of OSKM factors confirmed the strong but unique reactivation of the endogenous Sox2 stemness gene accompanied by the silencing of the exogenous Sox2 transgene in MCF-7/Rep cells. Some but not all MCF-7/Rep cells acquired strong alkaline phosphatase (AP) activity compared with MCF-7 parental cells. SOX2-overexpressing MCF-7/Rep cells contained drastically higher percentages of CD44(+) and ALDEFLUOR-stained ALDH(bright) cells than MCF-7 parental cells. The overlap between differentially expressed mTOR signaling-related genes in 3 different SOX2-overexpressing CSC-like cell lines revealed a notable downregulation of 3 genes, PRKAA1 (which codes for the catalytic alpha 1 subunit of AMPK), DDIT4/REDD1 (a stress response

gene that operates as a negative regulator of mTOR), and DEPTOR (a naturally occurring endogenous inhibitor of mTOR activity). The insulin-receptor gene (INSR) was differentially upregulated in MCF-7/Rep cells. Consistent with the downregulation of AMPK expression, immunoblotting procedures confirmed upregulation of p70S6K and increased phosphorylation of mTOR in Sox2-overexpressing CSC-like cell populations. Using an in vitro model of the de novo generation of CSC-like states through the nuclear reprogramming of an established breast cancer cell line, we reveal that the transcriptional suppression of mTOR repressors is an intrinsic process occurring during the acquisition of CSC-like properties by differentiated populations of luminal-like breast cancer cells. This approach may provide a new path for obtaining information about preventing the appearance of CSCs through the modulation of the AMPK/mTOR pathway.

Cox, J. L., P. J. Wilder, et al. "The SOX2-interactome in brain cancer cells identifies the requirement of MSI2 and USP9X for the growth of brain tumor cells." *PLoS One*. 2013 May 7;8(5):e62857. doi: [10.1371/journal.pone.0062857](https://doi.org/10.1371/journal.pone.0062857). Print 2013.

Medulloblastomas and glioblastomas, the most common primary brain tumors in children and adults, respectively, are extremely difficult to treat. Efforts to identify novel proteins essential for the growth of these tumors may help to further our understanding of the biology of these tumors, as well as, identify targets for future therapies. The recent identification of multiple transcription factor-centric protein interaction landscapes in embryonic stem cells has identified numerous understudied proteins that are essential for the self-renewal of these stem cells. To identify novel proteins essential for the fate of brain tumor cells, we examined the protein interaction network of the transcription factor, SOX2, in medulloblastoma cells. For this purpose, Multidimensional Protein Identification Technology (MudPIT) identified >280 SOX2-associated proteins in the medulloblastoma cell line DAOY. To begin to understand the roles of SOX2-associated proteins in brain cancer, we focused on two SOX2-associated proteins, Musashi 2 (MSI2) and Ubiquitin Specific Protease 9x (USP9X). Recent studies have implicated MSI2, a putative RNA binding protein, and USP9X, a deubiquitinating enzyme, in several cancers, but not brain tumors. We demonstrate that knockdown of MSI2 significantly reduces the growth of DAOY cells as well as U87 and U118 glioblastoma cells. We also demonstrate that the knockdown of USP9X in DAOY, U87 and U118 brain tumor cells strongly reduces their growth. Together, our studies identify a large set of SOX2-associated proteins in DAOY medulloblastoma

cells and identify two proteins, MSI2 and USP9X, that warrant further investigation to determine whether they are potential therapeutic targets for brain cancer.

Dhodapkar, K. M., S. N. Gettinger, et al. "SOX2-specific adaptive immunity and response to immunotherapy in non-small cell lung cancer." *Oncoimmunology*. 2013 Jul 1;2(7):e25205. Epub 2013 Jun 10.

Immunotherapeutic strategies including the blockade of programmed death 1 (PD-1) receptors hold promise for the treatment of various cancers including non-small cell lung carcinoma (NSCLC). Preclinical data suggest that pre-existing tumor immunity is important for disease regression upon checkpoint blockade-based therapies. However, the nature of antigen-specific T-cell responses that correlate with the clinical response to immunotherapy in NSCLC patients is not known. The embryonic stem cell gene SRY (sex determining region Y)-box 2 (SOX2) has recently emerged as a major oncogenic driver in NSCLC. Here, we show that nearly 50% of a cohort of NSCLC patients mounted both CD4+ and CD8+ T-cell responses against SOX2, which could be readily detected among peripheral blood mononuclear cells. T-cell responses against SOX2 were associated with NSCLC regression upon immunotherapy with anti-PD-1 monoclonal antibodies, whereas none of the patients lacking SOX2-specific T cells experienced disease regression following immune checkpoint blockade. Conversely, cellular and humoral responses against viral antigens or another tumor-associated antigen (NY-ESO-1) failed to correlate with the clinical response of NSCLC patients to immunotherapy. Of note, the administration of PD-1-blocking antibodies was associated with intramolecular epitope spread as well as with the amplification of SOX2-specific immune responses in vivo. These findings identify SOX2 as an important tumor-associated antigen in NSCLC and link the presence of SOX2-specific T cells with the clinical response of lung cancer patients to immunotherapy.

Dogan, I., S. Kawabata, et al. "SOX2 expression is an early event in a murine model of EGFR mutant lung cancer and promotes proliferation of a subset of EGFR mutant lung adenocarcinoma cell lines." *Lung Cancer*. 2014 Jul;85(1):1-6. doi: [10.1016/j.lungcan.2014.03.021](https://doi.org/10.1016/j.lungcan.2014.03.021). Epub 2014 Mar 29.

OBJECTIVES: Primary and acquired resistance to EGFR TKIs in EGFR mutant lung cancer occurs primarily through secondary mutations in EGFR or Met amplification. Drug resistance can also be mediated by expression of pluripotency transcription factors, such as OCT4, SOX2 and NANOG that decrease terminal differentiation. In this

study, we investigated the expression and role of SOX2 in model systems of EGFR mutant tumors. MATERIALS AND METHODS: Immunoblotting or immunohistochemistry was used to assess expression of pluripotency transcription factors in lungs of transgenic mice or in human NSCLC cell lines. Expression of SOX2 was reduced by shRNA knockdown, and response to erlotinib and cellular proliferation were assessed. RESULTS AND CONCLUSION: Induction of mutant EGFR in transgenic CCSP-rtTA/TetO-EGFR(L858R/T790M) mice correlated with increased OCT4 and SOX2 expression in lung tissue prior to tumor development. Established lung tumors retained SOX2 expression. To assess a role for SOX2 in tumorigenesis, a panel of NSCLC cell lines with activating EGFR mutations was assessed for SOX2 expression. Two of six cell lines with mutant EGFR showed detectable SOX2 levels, suggesting SOX2 expression did not correlate with EGFR mutation status. To assess the role of SOX2 in these cell lines, HCC827 and H1975 cells were infected with lentivirus containing SOX2 shRNA. Knockdown of SOX2 decreased proliferation in both cell lines and increased sensitivity to erlotinib in HCC827 cells. Because constitutive activation of the PI3K/Akt pathway is associated with EGFR TKI resistance, cells were treated with PI3K/AKT inhibitors and expression of SOX2 was examined. PI3K/Akt inhibitors decreased SOX2 expression in a time-dependent manner. These data suggest targeting SOX2 may provide therapeutic benefit in the subset of EGFR-mutant tumors with high constitutive levels of SOX2, and that until more direct means of inhibiting SOX2 are developed, PI3K/Akt inhibitors might be useful to inhibit SOX2 in EGFR TKI resistant tumors.

Finicelli, M., G. Benedetti, et al. "Expression of stemness genes in primary breast cancer tissues: the role of SOX2 as a prognostic marker for detection of early recurrence." *Oncotarget*. 2014 May 1.

The events leading to breast cancer (BC) progression or recurrence are not completely understood and new prognostic markers aiming at identifying high risk-patients and to develop suitable therapy are highly demanded. Experimental evidences found in cancer cells a deregulated expression of some genes involved in governance of stem cell properties and demonstrated a relationship between stemness genes overexpression and poorly differentiated BC subtypes. In the present study 140 primary invasive BC specimens were collected. The expression profiles of 13 genes belonging to the OCT3/SOX2/NANOG/KLF4 core circuitry by RT-PCR were analyzed and any correlation between their expression and the BC clinic-pathological features (CPfs) and prognosis was investigated. In our cohort

(117 samples), NANOG, GDF3 and SOX2 significantly correlated with grade 2, Nodes negative status and higher KI67 proliferation index, respectively ($p=0.019$, $p=0.029$, $p=0.035$). According to multivariate analysis, SOX2 expression resulted independently associated with increased risk of recurrence (HR= 2,99; $p=0,004$) as well as Nodes status (HR=2,44; $p=0,009$) and T-size >1 (HR=1,77; $p=0,035$). Our study provides further proof of the suitable use of stemness genes in BC management. Interestingly, a prognostic role of SOX2, which seems to be a suitable marker of early recurrence irrespective of other clinicopathological features.

Herreros-Villanueva, M., J. S. Zhang, et al. "SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells." *Oncogenesis*. 2013 Aug 5;2:e61. doi: 10.1038/oncsis.2013.23.

SOX2 (Sex-determining region Y (SRY)-Box2) has important functions during embryonic development and is involved in cancer stem cell (CSC) maintenance, in which it impairs cell growth and tumorigenicity. However, the function of SOX2 in pancreatic cancer cells is unclear. The objective of this study was to analyze SOX2 expression in human pancreatic tumors and determine the role of SOX2 in pancreatic cancer cells regulating CSC properties. In this report, we show that SOX2 is not expressed in normal pancreatic acinar or ductal cells. However, ectopic expression of SOX2 is observed in 19.3% of human pancreatic tumors. SOX2 knockdown in pancreatic cancer cells results in cell growth inhibition via cell cycle arrest associated with p21(Cip1) and p27(Kip1) induction, whereas SOX2 overexpression promotes S-phase entry and cell proliferation associated with cyclin D3 induction. SOX2 expression is associated with increased levels of the pancreatic CSC markers ALDH1, ESA and CD44. Importantly, we show that SOX2 is enriched in the ESA(+)/CD44(+) CSC population from two different patient samples. Moreover, we show that SOX2 directly binds to the Snail, Slug and Twist promoters, leading to a loss of E-Cadherin and ZO-1 expression. Taken together, our findings show that SOX2 is aberrantly expressed in pancreatic cancer and contributes to cell proliferation and stemness/dedifferentiation through the regulation of a set of genes controlling G1/S transition and epithelial-to-mesenchymal transition (EMT) phenotype, suggesting that targeting SOX2-positive cancer cells could be a promising therapeutic strategy.

Hutz, K., R. Mejias-Luque, et al. "The stem cell factor SOX2 regulates the tumorigenic potential in human gastric cancer cells." *Carcinogenesis*. 2014

Apr;35(4):942-50. doi: 10.1093/carcin/bgt410. Epub 2013 Dec 9.

Gastric cancer (GC) is still one of the most common causes of cancer-related death worldwide, which is mainly attributable to late diagnosis and poor treatment options. Infection with *Helicobacter pylori*, different environmental factors and genetic alterations are known to influence the risk of developing gastric tumors. However, the molecular mechanisms involved in gastric carcinogenesis are still not fully understood, making it difficult to design targeted therapeutic approaches. Aberrant expression of the specific gastric differentiation marker SOX2 has been observed in stomach cancer. However, the role of SOX2 in gastric tumors has not been well established to date. To elucidate the role of SOX2 in gastric tumorigenesis, SOX2 transcriptional activity was blocked in AZ-521 cells. Interestingly, inhibition of SOX2 reduced cell proliferation and migration, increased apoptosis and induced changes in cell cycle. Blocking of SOX2 also reduced the tumorigenic potential of AZ-521 cells in vivo. In addition, correlation of SOX2 expression and proliferation was observed in a subset of human gastric tumors. Finally, target genes of SOX2 were for the first time identified by RNA microarray in GC cells. Taken together, the results presented here indicate that SOX2 controls several aspects related to GC development and progression by regulating the expression of members of important signaling pathways. These findings could provide new therapeutic options for a subset of GCs exhibiting SOX2 deregulation.

Jeon, H. M., Y. W. Sohn, et al. "ID4 imparts chemoresistance and cancer stemness to glioma cells by derepressing miR-9*-mediated suppression of SOX2." Cancer Res. 2011 May 1;71(9):3410-21. doi: 10.1158/0008-5472.CAN-10-3340.

Glioma stem cells (GSC) possess tumor-initiating potential and are relatively resistant to conventional chemotherapy and irradiation. Thus, they are considered to be major drivers for glioma initiation, progression, and recurrence. However, the precise mechanism governing acquisition of their drug resistance remains to be elucidated. Our previous study has shown that inhibitor of differentiation 4 (ID4) dedifferentiates *Ink4a/Arf(-/-)* mouse astrocytes and human glioma cells to glioma stem-like cells (induced GSCs or iGSCs). In this article, we report that ID4-driven iGSCs exhibit chemoresistant behavior to anticancer drugs through activation of ATP-binding cassette (ABC) transporters. We found that ID4 enhanced SOX2 protein expression by suppressing microRNA-9* (miR-9*), which can repress SOX2 by targeting its 3'-untranslated region. Consequently, ID4-mediated SOX2 induction

enhanced ABCC3 and ABCC6 expression through direct transcriptional regulation, indicating that ID4 regulates the chemoresistance of iGSCs by promoting SOX2-mediated induction of ABC transporters. Furthermore, we found that short hairpin RNA-mediated knockdown of SOX2 in ID4-driven iGSCs resulted in loss of cancer stemness. Moreover, ectopic expression of SOX2 could dedifferentiate *Ink4a/Arf(-/-)* astrocytes and glioma cells to iGSCs, indicating a crucial role of SOX2 in genesis and maintenance of GSCs. Finally, we found that the significance of the ID4-miR-9*-SOX2-ABCC3/ABCC6 regulatory pathway is recapitulated in GSCs derived from patients with glioma. Together, our results reveal a novel regulatory mechanism by which ID4-driven suppression of miR-9* induces SOX2, which imparts stemness potential and chemoresistance to glioma cells and GSCs.

Kitamura, H., T. Torigoe, et al. "Prognostic impact of the expression of ALDH1 and SOX2 in urothelial cancer of the upper urinary tract." Mod Pathol. 2013 Jan;26(1):117-24. doi: 10.1038/modpathol.2012.139. Epub 2012 Aug 17.

Aldehyde dehydrogenase 1 (ALDH1) and sex determining region-Y-related high mobility group box 2 (SOX2) have been identified as putative cancer stem-like cell/tumor-initiating cell markers in various cancer tissues. The aim of this study was to elucidate the prognostic impact of these putative cancer stem-like cell/tumor-initiating cell markers in upper urinary tract urothelial cell carcinoma. Immunohistochemical staining for ALDH1 and SOX2 was carried out on archival specimens from 125 patients with upper urinary tract urothelial cell carcinoma who underwent radical nephroureterectomy. The prognostic value of ALDH1 and SOX2 expression and other clinicopathological features was evaluated. On univariate analysis, tumor grade, pathological T stage, pathological N stage, lymphovascular invasion, ALDH1 expression and SOX2 expression were associated with a poor prognosis. On multivariate analysis, the independent factors of prognosis were tumor grade (P=0.014), pathological N stage (P=0.005) and ALDH1 expression (P=0.002). In subgroup analysis, those subgroups with no positive, one positive or two positive results in immunohistochemistry for ALDH1 and SOX2 expression had estimated 5-year cancer-specific survival rates of 80%, 49% and 22%, respectively (P<0.001). Neither ALDH1 nor SOX2 expression correlated with intravesical recurrence after radical nephroureterectomy. These findings suggest that cancer stem-like cells/tumor-initiating cells are linked to more aggressive behavior of upper urinary tract urothelial cell carcinoma, supporting the current

cancer stem cell hypothesis. Thus, therapeutic targeting of cancer stem-like cells/tumor-initiating cells in upper urinary tract urothelial cell carcinoma is a future possibility.

Kregel, S., K. J. Kiriluk, et al. "Sox2 is an androgen receptor-repressed gene that promotes castration-resistant prostate cancer." PLoS One. 2013;8(1):e53701. doi: [10.1371/journal.pone.0053701](https://doi.org/10.1371/journal.pone.0053701). Epub 2013 Jan 11.

Despite advances in detection and therapy, castration-resistant prostate cancer continues to be a major clinical problem. The aberrant activity of stem cell pathways, and their regulation by the Androgen Receptor (AR), has the potential to provide insight into novel mechanisms and pathways to prevent and treat advanced, castrate-resistant prostate cancers. To this end, we investigated the role of the embryonic stem cell regulator Sox2 [SRX (sex determining region Y)-box 2] in normal and malignant prostate epithelial cells. In the normal prostate, Sox2 is expressed in a portion of basal epithelial cells. Prostate tumors were either Sox2-positive or Sox2-negative, with the percentage of Sox2-positive tumors increasing with Gleason Score and metastases. In the castration-resistant prostate cancer cell line CWR-R1, endogenous expression of Sox2 was repressed by AR signaling, and AR chromatin-IP shows that AR binds the enhancer element within the Sox2 promoter. Likewise, in normal prostate epithelial cells and human embryonic stem cells, increased AR signaling also decreases Sox2 expression. Resistance to the anti-androgen MDV3100 results in a marked increase in Sox2 expression within three prostate cancer cell lines, and in the castration-sensitive LAPC-4 prostate cancer cell line ectopic expression of Sox2 was sufficient to promote castration-resistant tumor formation. Loss of Sox2 expression in the castration-resistant CWR-R1 prostate cancer cell line inhibited cell growth. Up-regulation of Sox2 was not associated with increased CD133 expression but was associated with increased FGF5 (Fibroblast Growth Factor 5) expression. These data propose a model of elevated Sox2 expression due to loss of AR-mediated repression during castration, and consequent castration-resistance via mechanisms not involving induction of canonical embryonic stem cell pathways.

Leis, O., A. Eguiara, et al. "Sox2 expression in breast tumours and activation in breast cancer stem cells." Oncogene. 2012 Mar 15;31(11):1354-65. doi: [10.1038/onc.2011.338](https://doi.org/10.1038/onc.2011.338). Epub 2011 Aug 8.

The cancer stem cell (CSC) model does not imply that tumours are generated from transformed tissue stem cells. The target of transformation could be a tissue stem cell, a progenitor cell, or a

differentiated cell that acquires self-renewal ability. The observation that induced pluripotency reprogramming and cancer are related has led to the speculation that CSCs may arise through a reprogramming-like mechanism. Expression of pluripotency genes (Oct4, Nanog and Sox2) was tested in breast tumours by immunohistochemistry and it was found that Sox2 is expressed in early stage breast tumours. However, expression of Oct4 or Nanog was not found. Mammosphere formation in culture was used to reveal stem cell properties, where expression of Sox2, but not Oct4 or Nanog, was induced. Over-expression of Sox2 increased mammosphere formation, effect dependent on continuous Sox2 expression; furthermore, Sox2 knockdown prevented mammosphere formation and delayed tumour formation in xenograft tumour initiation models. Induction of Sox2 expression was achieved through activation of the distal enhancer of Sox2 promoter upon sphere formation, the same element that controls Sox2 transcription in pluripotent stem cells. These findings suggest that reactivation of Sox2 represents an early step in breast tumour initiation, explaining tumour heterogeneity by placing the tumour-initiating event in any cell along the axis of mammary differentiation.

Lundberg, I. V., A. Lofgren Burstrom, et al. "SOX2 expression is regulated by BRAF and contributes to poor patient prognosis in colorectal cancer." PLoS One. 2014 Jul 10;9(7):e101957. doi: [10.1371/journal.pone.0101957](https://doi.org/10.1371/journal.pone.0101957). eCollection 2014.

Sporadic colorectal cancer (CRC) is a common malignancy and also one of the main causes of cancer deaths worldwide. Aberrant expression of the transcription factor SOX2 has recently been observed in several cancer types, but its role in CRC has not been fully elucidated. Here we studied the expression of SOX2 in 441 CRC patients by immunohistochemistry and related the expression to clinicopathological and molecular variables and patient prognosis. SOX2 was expressed in 11% of the tumors and was significantly associated to BRAFV600E mutation, but not to KRAS mutations (codon 12 and 13). SOX2 positivity was correlated to poor patient survival, especially in BRAFV600E mutated cases. In vitro studies showed that cells expressing the constitutively active BRAFV600E had increased SOX2 expression, a finding not found in cells expressing KRASG12V. Furthermore, blocking downstream BRAF signalling using a MEK-inhibitor resulted in a decreased expression of SOX2. Since SOX2 overexpression has been correlated to increased migration and invasion, we investigated the SOX2 expression in human CRC liver metastasis and found that a SOX2 positive primary CRC also had SOX2

expression in corresponding liver metastases. Finally we found that cells overexpressing SOX2 in vitro showed enhanced expression of FGFR1, which has been reported to correlate with liver metastasis in CRC. Our novel findings suggest that SOX2 expression is partly regulated by BRAF signalling, and an increased SOX2 expression may promote CRC metastasis and mediate a poor patient prognosis.

Maddison, P., A. Thorpe, et al. "Autoimmunity to SOX2, clinical phenotype and survival in patients with small-cell lung cancer." Lung Cancer. 2010 Dec;70(3):335-9. doi: 10.1016/j.lungcan.2010.03.002. Epub 2010 Apr 3.

OBJECTIVE: Autoantibodies to SOXB1 antigens are commonly found in patients with small-cell lung cancer (SCLC). It has not been established whether the presence of circulating SOX antibodies is associated with a specific paraneoplastic clinical phenotype, or if a tumour immune response to SOX antigens can affect prognosis in patients with SCLC in relation to other established prognostic factors. **METHODS:** Using recombinant SOX2 in an ELISA, we analysed sera in a prospective study from 212 unselected SCLC patients, which included 35 patients with neurological paraneoplastic disorders, or other well characterised onconeural antibodies. **RESULTS:** Overall, SOX2 antibodies were detected in 70 SCLC patients, with a sensitivity of 33% (95% CI 27-40%) and specificity of 97% (95% CI 94-99%) compared to controls matched for age, gender and smoking history. No single clinical phenotype was seen in relation to the presence of SOX2 antibodies in isolation. Multivariate analysis showed that the presence of SOX2 antibodies in SCLC patients without evidence of neurological paraneoplastic disorders or onconeural antibodies did not have a significant effect on survival when known prognostic factors were accounted for. **CONCLUSIONS:** SOX2 antibodies are very specific markers for SCLC compared to matched non-tumour controls, but their presence does not seem to alter prognosis in this tumour type.

McCaughan, F., J. C. Pole, et al. "Progressive 3q amplification consistently targets SOX2 in preinvasive squamous lung cancer." Am J Respir Crit Care Med. 2010 Jul 1;182(1):83-91. doi: 10.1164/rccm.201001-0005OC. Epub 2010 Mar 18.

RATIONALE: Amplification of distal 3q is the most common genomic aberration in squamous lung cancer (SQC). SQC develops in a multistage progression from normal bronchial epithelium through dysplasia to invasive disease. Identifying the key driver events in the early pathogenesis of SQC will facilitate the search for predictive molecular biomarkers and the identification of novel molecular

targets for chemoprevention and therapeutic strategies. For technical reasons, previous attempts to analyze 3q amplification in preinvasive lesions have focused on small numbers of predetermined candidate loci rather than an unbiased survey of copy-number variation. **OBJECTIVES:** To perform a detailed analysis of the 3q amplicon in bronchial dysplasia of different histological grades. **METHODS:** We use molecular copy-number counting (MCC) to analyze the structure of chromosome 3 in 19 preinvasive bronchial biopsy specimens from 15 patients and sequential biopsy specimens from 3 individuals. **MEASUREMENTS AND MAIN RESULTS:** We demonstrate that no low-grade lesions, but all high-grade lesions, have 3q amplification. None of seven low-grade lesions progressed clinically, whereas 8 of 10 patients with high-grade disease progressed to cancer. We identify a minimum commonly amplified region on chromosome 3 consisting of 17 genes, including 2 known oncogenes, SOX2 and PIK3CA. We confirm that both genes are amplified in all high-grade dysplastic lesions tested. We further demonstrate, in three individuals, that the clinical progression of high-grade preinvasive disease is associated with incremental amplification of SOX2, suggesting this promotes malignant progression. **CONCLUSIONS:** These findings demonstrate progressive 3q amplification in the evolution of preinvasive SQC and implicate SOX2 as a key target of this dynamic process.

Mukhopadhyay, A., K. C. Berrett, et al. "Sox2 cooperates with Lkb1 loss in a mouse model of squamous cell lung cancer." Cell Rep. 2014 Jul 10;8(1):40-9. doi: 10.1016/j.celrep.2014.05.036. Epub 2014 Jun 19.

Squamous cell carcinoma (SCC) of the lung is the second most common subtype of lung cancer. With limited treatment options, the 5-year survival rate of SCC is only 15%. Although genomic alterations in SCC have been characterized, identifying the alterations that drive SCC is critical for improving treatment strategies. Mouse models of SCC are currently limited. Using lentiviral delivery of Sox2 specifically to the mouse lung, we tested the ability of Sox2 to promote tumorigenesis in multiple tumor suppressor backgrounds. Expression of Sox2, frequently amplified in human SCC, specifically cooperates with loss of Lkb1 to promote squamous lung tumors. Mouse tumors exhibit characteristic histopathology and biomarker expression similar to human SCC. They also mimic human SCCs by activation of therapeutically relevant pathways including STAT and mTOR. This model may be utilized to test the contribution of additional driver

alterations in SCC, as well as for preclinical drug discovery.

Neumann, J., F. Bahr, et al. "SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer." *BMC Cancer*. 2011 Dec 14;11:518. doi: 10.1186/1471-2407-11-518.

BACKGROUND: The transcription factor SOX2, which is involved in the induction of pluripotent stem cells and contributes to colorectal carcinogenesis, is associated with a poor prognosis in colon cancer (CC). Furthermore, SOX2 is a repressor of the transcriptional activity of beta-catenin in vitro. Since the majority of CC develop via an activation of the Wnt/beta-catenin signalling pathway, indicated by nuclear expression of beta-catenin, we wanted to investigate the expression patterns of SOX2 and beta-catenin and correlate them with the occurrence of lymph node and distant metastases as indicators of malignant progression. **METHODS:** The expression of SOX2 and beta-catenin was investigated in a case control study utilizing a matched pair collection (N = 114) of right-sided CCs with either corresponding distant metastases (N = 57) or without distant spread (N = 57) by applying immunohistochemistry. **RESULTS:** Elevated protein expression of SOX2 significantly correlated with the presence of lymph node- (p = 0.006) and distant metastases (p = 0.022). Nuclear beta-catenin expression correlated significantly only with distant metastases (p = 0.001). Less than 10% of cases showed a coexpression of high levels of beta-catenin and SOX2. The positivity for both markers was also associated with a very high risk for lymph-node metastases (p = 0.007) and distant spread (p = 0.028). **CONCLUSION:** We demonstrated that increased expression of either SOX2 or nuclear beta-catenin are associated with distant metastases in right-sided CC. Additionally, SOX2 is also associated with lymph-node metastases. These data underline the importance of stemness-associated markers for the identification of CC with high risk for distant spread.

Piva, M., G. Domenici, et al. "Sox2 promotes tamoxifen resistance in breast cancer cells." *EMBO Mol Med*. 2014 Jan;6(1):66-79.

Development of resistance to therapy continues to be a serious clinical problem in breast cancer management. Cancer stem/progenitor cells have been shown to play roles in resistance to chemo- and radiotherapy. Here, we examined their role in the development of resistance to the oestrogen receptor antagonist tamoxifen. Tamoxifen-resistant cells were enriched for stem/progenitors and expressed high levels of the stem cell marker Sox2. Silencing of the SOX2 gene reduced the size of the stem/progenitor cell population and restored sensitivity to tamoxifen.

Conversely, ectopic expression of Sox2 reduced tamoxifen sensitivity in vitro and in vivo. Gene expression profiling revealed activation of the Wnt signalling pathway in Sox2-expressing cells, and inhibition of Wnt signalling sensitized resistant cells to tamoxifen. Examination of patient tumours indicated that Sox2 levels are higher in patients after endocrine therapy failure, and also in the primary tumours of these patients, compared to those of responders. Together, these results suggest that development of tamoxifen resistance is driven by Sox2-dependent activation of Wnt signalling in cancer stem/progenitor cells.

Riggi, N., M. L. Suva, et al. "EWS-FLI-1 modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming toward Ewing sarcoma cancer stem cells." *Genes Dev*. 2010 May;24(9):916-32. doi: 10.1101/gad.1899710. Epub 2010 Apr 9.

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

Rodriguez-Pinilla, S. M., D. Sarrío, et al. "Sox2: a possible driver of the basal-like phenotype in sporadic breast cancer." *Mod Pathol*. 2007 Apr;20(4):474-81. Epub 2007 Mar 2.

Tumours arising in BRCA1 mutation carriers and sporadic basal-like breast carcinomas have similar

phenotypic, immunohistochemical and clinical characteristics. SOX2 is an embryonic transcription factor located at chromosome 3q, a region frequently gained in sporadic basal-like and BRCA1 germline mutated tumours. The aim of the study was to establish whether sox2 expression was related to basal-like sporadic breast tumours. Two hundred and twenty-six sporadic node-negative invasive breast carcinomas were immunohistochemically analysed for oestrogen receptor (ER), progesterone receptor (PR), CK5/6, EGFR, vimentin, HER2, ki67, p53 and sox2 using tissue microarrays. Tumours were considered to have basal-like phenotype if they were ER/HER2-negative and CK5/6 and/or EGFR-positive. Thirty cases of this series (13.7%) displayed a basal-like phenotype. Sox2 expression was observed in 16.7% of cases and was significantly more frequently expressed in basal-like breast carcinomas (43.3% in basal-like, 10.6% in luminal and 13.3% in HER2+ tumours, $P < 0.001$). Moreover, Sox2 showed a statistically significant inverse association with ER and PR ($P = 0.001$ and 0.017 , respectively) and direct association with CK5/6, EGFR and vimentin ($P = 0.022$, 0.005 and < 0.001 , respectively). Sox2 is preferentially expressed in tumours with basal-like phenotype and may play a role in defining their less differentiated/stem cell phenotypic characteristics.

Rybak, A. P. and D. Tang "SOX2 plays a critical role in EGFR-mediated self-renewal of human prostate cancer stem-like cells." *Cell Signal.* 2013 Dec;25(12):2734-42. doi: [10.1016/j.cellsig.2013.08.041](https://doi.org/10.1016/j.cellsig.2013.08.041). Epub 2013 Sep 13.

SOX2 is an essential transcription factor for stem cells and plays a role in tumorigenesis, however its role in prostate cancer stem cells (PCSCs) remains unclear. We report here a significant upregulation of SOX2 at both mRNA and protein levels in DU145 PCSCs propagated as suspension spheres in vitro. The expression of SOX2 in DU145 PCSCs is positively regulated by epidermal growth factor receptor (EGFR) signaling. Activation of EGFR signaling, following the addition of epidermal growth factor (EGF) or ectopic expression of a constitutively-active EGFR mutant (EGFRvIII), increased SOX2 expression and the self-renewal of DU145 PCSCs. Conversely, a small molecule EGFR inhibitor (AG1478) blocked EGFR activation, reduced SOX2 expression and inhibited PCSC self-renewal activity, implicating SOX2 in mediating EGFR-dependent self-renewal of PCSCs. In line with this notion, ectopic SOX2 expression enhanced EGF-induced self-renewal of DU145 PCSCs, while SOX2 knockdown reduced PCSC self-renewal with EGF treatment no longer capable of enhancing their propagation. Furthermore, SOX2 knockdown reduced the capacity of DU145

PCSCs to grow under anchorage-independent conditions. Finally, DU145 PCSCs generated xenograft tumors more aggressively with elevated levels of SOX2 expression compared to xenograft tumors derived from non-PCSCs. Collectively, we provide evidence that SOX2 plays a critical role in EGFR-mediated self-renewal of DU145 PCSCs.

Saigusa, S., K. Tanaka, et al. "Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy." *Ann Surg Oncol.* 2009 Dec;16(12):3488-98. doi: [10.1245/s10434-009-0617-z](https://doi.org/10.1245/s10434-009-0617-z).

BACKGROUND: Cancer stem cells are associated with metastatic potential, treatment resistance, and poor patient prognosis. Distant recurrence remains the major cause of mortality in rectal cancer patients with preoperative chemoradiotherapy (CRT). We investigated the role of three stem cell markers (CD133, OCT4, and SOX2) in rectal cancer and evaluated the association between these gene levels and clinical outcome in rectal cancer patients with preoperative CRT. **METHODS:** Thirty-three patients with rectal cancer underwent preoperative CRT. Total RNAs of rectal cancer cells before and after CRT were isolated. Residual cancer cells after CRT were obtained from formalin-fixed paraffin-embedded (FFPE) specimens using microdissection. The expression levels of three stem cell genes were measured using real-time reverse-transcription polymerase chain reaction (RT-PCR). The association between these gene levels and radiation was evaluated using colon cancer cell lines. Immunohistochemical staining of these markers after CRT was also investigated. **RESULTS:** There were significant positive correlations among the three genes after CRT. Patients who developed distant recurrence had higher levels of the three genes compared with those without recurrence in residual cancer after CRT. These elevated gene levels were significantly associated with poor disease-free survival. The radiation caused upregulation of these gene levels in LoVo and SW480 in vitro. Immunohistochemically, CD133 staining was observed in not only luminal surface but also cytoplasm. **CONCLUSIONS:** Expression of CD133, OCT4, and SOX2 may predict distant recurrence and poor prognosis of rectal cancer patients treated with preoperative CRT. Correlations among these genes may be associated with tumor regrowth and metastatic relapse after CRT.

Schrock, A., F. Goke, et al. "Sex determining region Y-box 2 (SOX2) amplification is an independent indicator of disease recurrence in sinonasal cancer."

PLoS One. 2013;8(3):e59201. doi: 10.1371/journal.pone.0059201. Epub 2013 Mar 27.

OBJECTIVES: The transcription factor SOX2 (3q26.3-q27) is an embryonic stem cell factor contributing to the induction of pluripotency in terminally differentiated somatic cells. Recently, amplification of the SOX2 gene locus has been described in squamous cell carcinoma (SCC) of different organ sites. Aim of this study was to investigate amplification and expression status of SOX2 in sinonasal carcinomas and to correlate the results with clinico-pathological data. **MATERIALS AND METHODS:** A total of 119 primary tumor samples from the sinonasal region were assessed by fluorescence in-situ hybridization and immunohistochemistry for SOX2 gene amplification and protein expression, respectively. Of these, 59 were SSCs, 18 sinonasal undifferentiated carcinomas (SNUC), 10 carcinomas associated with an inverted papilloma (INVC), 19 adenocarcinomas (AD) and 13 adenoid cystic carcinomas (ACC). **RESULTS:** SOX2 amplifications were found in subsets of SCCs (37.5%), SNUCs (35.3%), INVCs (37.5%) and ADs (8.3%) but not in ACCs. SOX2 amplification resulted in increased protein expression. Patients with SOX2-amplified sinonasal carcinomas showed a significantly higher rate of tumor recurrences than SOX2 non-amplified tumors. **CONCLUSION:** This is the first study assessing SOX2 amplification and expression in a large cohort of sinonasal carcinomas. As opposed to AD and ACC, SOX2 amplifications were detected in more than 1/3 of all SCCs, SNUCs and INVCs. We therefore suggest that SNUCs are molecularly closely related to SCCs and INVCs and that these entities represent a subgroup of sinonasal carcinomas relying on SOX2 acquisition during oncogenesis. SOX2 amplification appears to identify sinonasal carcinomas that are more likely to relapse after primary therapy, suggesting that these patients might benefit from a more aggressive therapy regime.

Stolzenburg, S., M. G. Rots, et al. "Targeted silencing of the oncogenic transcription factor SOX2 in breast cancer." *Nucleic Acids Res.* 2012 Aug;40(14):6725-40. Epub 2012 May 4.

The transcription factor (TF) SOX2 is essential for the maintenance of pluripotency and self-renewal in embryonic stem cells. In addition to its normal stem cell function, SOX2 over-expression is associated with cancer development. The ability to selectively target this and other oncogenic TFs in cells, however, remains a significant challenge due to the 'undruggable' characteristics of these molecules. Here, we employ a zinc finger (ZF)-based artificial TF (ATF) approach to selectively suppress SOX2 gene expression in cancer cells. We engineered four

different proteins each composed of 6ZF arrays designed to bind 18 bp sites in the SOX2 promoter and enhancer region, which controls SOX2 methylation. The 6ZF domains were linked to the Kruppel Associated Box (SKD) repressor domain. Three engineered proteins were able to bind their endogenous target sites and effectively suppress SOX2 expression (up to 95% repression efficiencies) in breast cancer cells. Targeted down-regulation of SOX2 expression resulted in decreased tumor cell proliferation and colony formation in these cells. Furthermore, induced expression of an ATF in a mouse model inhibited breast cancer cell growth. Collectively, these findings demonstrate the effectiveness and therapeutic potential of engineered ATFs to mediate potent and long-lasting down-regulation of oncogenic TF expression in cancer cells.

Uozaki, H., R. R. Barua, et al. "Transcriptional factor typing with SOX2, HNF4aP1, and CDX2 closely relates to tumor invasion and Epstein-Barr virus status in gastric cancer." *Int J Clin Exp Pathol.* 2011 Mar;4(3):230-40. Epub 2011 Mar 1.

BACKGROUND: Gastric cancer (GC) is a major cancer, sometimes associated with Epstein-Barr virus (EBV). Some transcriptional factors (TFs) are specific to the digestive tract and related to the character of the tumors. **METHODS:** We studied three TFs, SOX2, CDX2, and hepatocyte nuclear factor 4 alpha-promoter 1 (HNF4aP1) in GC. First, 255 tumors including 31 EBV-associated GC were immunohistochemically examined using tissue arrays and compared TF type and mucin phenotype. We classified them into 4 TF types: N-TF type as SOX2-/HNF4aP1- tumor, G: SOX2+/HNF4aP1-, GI: SOX2+/HNF4aP1+, and I: SOX2-/HNF4aP1+. Next, 915 GCs were intensely investigated and compared with their clinicopathological factors. **RESULTS:** In the first study, 255 GCs were classified into N-TF 44%, G-TF 31%, GI-TF 3%, and I-TF 2%. The TF type did not strictly accord with the mucin phenotype, classified by MUC2/5AC/6/CD10 expression. EBV status was the only factor related to both the TF and mucin phenotype classifications ($P < 0.0001$, < 0.0001). TF classification is related to more factors including tumor stage, than mucin phenotype classification. The second study using 915 GCs revealed that N-TF gradually increased and I-TF decreased as GC invaded deeper. TF classification was not related to nodal involvement in each tumor stage. HNF4aP1 and CDX2 were independent factors for early stage tumor in logistic regression analysis. **CONCLUSIONS:** EBV-associated GC is a discriminating group in both TF and mucin phenotype. TF classification, especially the absence of HNF4aP1 and CDX2, is related to

tumor invasion. TF classification is a useful marker to study the carcinogenesis of GC further.

Vazquez-Martin, A., S. Cufi, et al. "Reprogramming of non-genomic estrogen signaling by the stemness factor SOX2 enhances the tumor-initiating capacity of breast cancer cells." *Cell Cycle*. 2013 Nov 15;12(22):3471-7. doi: 10.4161/cc.26692. Epub 2013 Oct 4.

The restoration of pluripotency circuits by the reactivation of endogenous stemness factors, such as SOX2, may provide a new paradigm in cancer development. The tumoral stem cell reprogramming hypothesis, i.e., the ability of stemness factors to redirect normal and differentiated tumor cells toward a less-differentiated and stem-like state, adds new layers of complexity to cancer biology, because the effects of such reprogramming may remain dormant until engaged later in response to (epi)genetic and/or (micro)environmental events. To test this hypothesis, we utilized an in vitro model of a SOX2-overexpressing cancer stem cell (CSC)-like cellular state that was recently developed in our laboratory by employing Yamanaka's nuclear reprogramming technology in the estrogen receptor alpha (ERalpha)-positive MCF-7 breast cancer cell line. Despite the acquisition of distinct molecular features that were compatible with a breast CSC-like cellular state, such as strong aldehyde dehydrogenase activity, as detected by ALDEFLUOR, and overexpression of the SSEA-4 and CD44 breast CSC markers, the tumor growth-initiating ability of SOX2-overexpressing CSC-like MCF-7 cells solely occurred in female nude mice supplemented with estradiol when compared with MCF-7 parental cells. Ser118 phosphorylation of estrogen receptor alpha (ERalpha), which is a pivotal integrator of the genomic and nongenomic E2/ERalpha signaling pathways, drastically accumulated in nuclear speckles in the interphase nuclei of SOX2-driven CSC-like cell populations. Moreover, SOX2-positive CSC-like cells accumulated significantly higher numbers of actively dividing cells, and the highest levels of phospho-Ser118-ERalpha occurred when chromosomes lined up on a metaphase plate. The previously unrecognized link between E2/ERalpha signaling and SOX2-driven stem cell circuitry may significantly impact our current understanding of breast cancer initiation and progression, i.e., SOX2 can promote non-genomic E2 signaling that leads to nuclear phospho-Ser118-ERalpha, which ultimately exacerbates genomic ER signaling in response to E2. Because E2 stimulation has been recently shown to enhance breast tumor-initiating cell survival by downregulating miR-140, which targets SOX2, the establishment of a bidirectional cross-talk interaction between the stem

cell self-renewal regulator, SOX2, and the local and systemic ability of E2 to increase breast CSC activity may have profound implications for the development of new CSC-directed strategies for breast cancer prevention and therapy.

Wilbertz, T., P. Wagner, et al. "SOX2 gene amplification and protein overexpression are associated with better outcome in squamous cell lung cancer." *Mod Pathol*. 2011 Jul;24(7):944-53. doi: 10.1038/modpathol.2011.49. Epub 2011 Apr 1.

The transcription factor SOX2 (3q26.3-q27) is a key regulator of foregut development and an embryonic stem cell factor cooperating during induction of pluripotency in terminally differentiated somatic cells. Recently, we found SOX2 to be amplified in a subset of squamous cell lung and esophageal cancers. The aim of this study was to explore the prognostic role of SOX2 in a large series of squamous cell carcinomas and adenocarcinomas of the lung. A total of 891 samples from two independent population-based cohorts were assessed by fluorescence in situ hybridization and immunohistochemistry. Furthermore, we assessed for associations between SOX2 amplification/upregulation and clinicopathological features. Similar results were found in the two cohorts. Within squamous cell carcinoma cases, 8% high-level as well as 68 and 65% low-level SOX2 amplifications occurred in the two cohorts, respectively. In adenocarcinomas, no high-level amplification was found and low-level amplification occurred in 6% of the two cohorts. Within squamous cell carcinomas of one cohort, SOX2 amplification was associated with lower tumor grade, while higher levels of SOX2 expression were related to younger age, smaller tumor size, and lower probability of angiolymphatic invasion and metastasis. High SOX2 expression levels proved to be a marker for prolonged overall survival among patients with squamous cell carcinomas. In conclusion, SOX2 amplification and upregulation are frequent events in squamous cell carcinomas of the lung and are associated with indicators of favorable prognosis.

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