The SOX2 and stem cell literatures

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Abstract: SOX2, also known as SRY (sex determining region Y)-box 2, 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 that plays key roles in many stages of mammalian development, and it plays important roles in induced pluripotency. Leukemia inhibitory factor (LIF) signaling activates Sox2 downstream of the JAK-STAT signaling pathway and subsequent activation of Klf4. Oct-4, Sox2 and Nanog positively regulate transcription of all pluripotency circuitry proteins in the LIF pathway. Sox2 in conjunction with Oct4, c-Myc and Klf4 are sufficient for producing induced pluripotent stem cells. Loss of pluripotency is regulated by hypermethylation of some Sox2 and Oct4 binding sites in male germ cells and post-transcriptional suppression of Sox2 by miR134.
[Ma H, Young M. **The SOX2 and stem cell literatures.** *Stem Cell* 2014;5(3):42-57] (ISSN 1545-4570). http://www.sciencepub.net/stem. 6

Key words: life; stem cell; SOX2, SRY (sex determining region Y)-box 2

1. Introduction

SOX2, also known as SRY (sex determining region Y)-box 2, 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 that plays key roles in many stages of mammalian development, and it plays important roles in induced pluripotency. Leukemia inhibitory factor (LIF) signaling activates Sox2 downstream of the JAK-STAT signaling pathway and subsequent activation of Klf4. Oct-4, Sox2 and Nanog positively regulate transcription of all pluripotency circuitry proteins in the LIF pathway.

Sox2 in conjunction with Oct4, c-Myc and Klf4 are sufficient for producing induced pluripotent stem cells. Loss of pluripotency is regulated by hypermethylation of some Sox2 and Oct4 binding sites in male germ cells and post-transcriptional suppression of Sox2 by miR134.

Varying levels of Sox2 affect embryonic stem cells' fate of differentiation. Sox2 inhibits differentiation into the mesendoderm germ layer and promotes differentiation into neural ectoderm germ layer. Npm1/Sox2 complexes are sustained when differentiation is induced along the ectodermal lineage, emphasizing an important functional role for Sox2 in ectodermal differentiation. Induced pluripotency is possible using adult neural stem cells, which express higher levels of Sox2 and c-Myc than embryonic stem cells.

There are three thyroid hormone response elements (TREs) in the region upstream of the Sox2 promoter. This region is known as the enhancer region. Hypothyroidism can arise from a multitude of causes, and is commonly remedied with hormone treatments such as the commonly used Levothyroxine. Sox2 has a critical role in maintenance of embryonic and neural stem 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 tumorogenesis. The third one is a plasmid based transfection that can avoid the genome integration also. Recently, the Cre-recombinase excisable systems are used in iPSC induction and subsequent transgene removal making the iPSC technology closer to clinic applications.

Literatures

The following gives some recent reference papers on .

Adachi, K., I. Nikaido, et al. "Context-dependent wiring of Sox2 regulatory networks for self-renewal of embryonic and trophoblast stem cells." Mol Cell **52**(3): 380-92.

Sox2 is a transcription factor required for the maintenance of pluripotency. It also plays an essential role in different types of multipotent stem cells, raising the possibility that Sox2 governs the common stemness phenotype. Here we show that Sox2 is a critical downstream target of fibroblast growth factor (FGF) signaling, which mediates self-renewal of trophoblast stem cells (TSCs). Sustained expression of Sox2 together with Esrrb or Tfap2c can replace FGF dependency. By comparing genome-wide binding sites of Sox2 in embryonic stem cells (ESCs) and TSCs

combined with inducible knockout systems, we found that, despite the common role in safeguarding the stem cell state, Sox2 regulates distinct sets of genes with unique functions in these two different yet developmentally related types of stem cells. Our findings provide insights into the functional versatility of transcription factors during embryogenesis, during which they can be recursively utilized in a variable manner within discrete network structures.

Ahlfeld, J., R. Favaro, et al. "Sox2 requirement in sonic hedgehog-associated medulloblastoma." Cancer Res **73**(12): 3796-807.

The transcription factor Sox2 has been shown to play essential roles during embryonic development as well as in cancer. To more precisely understand tumor biology and to identify potential therapeutical targets, we thoroughly investigated the expression and function of Sox2 in medulloblastoma, a malignant embryonic brain tumor that initiates in the posterior fossa and eventually spreads throughout the entire cerebrospinal axis. We examined a large series of tumor samples (n = 188) to show that SOX2 is specifically expressed in Sonic hedgehog (SHH)-associated medulloblastoma with an interesting preponderance in adolescent and adult cases. We further show that cerebellar granule neuron precursors (CGNP), which are believed to serve as the cell of origin for this medulloblastoma subgroup, express Sox2 in early stages. Also, Shh-associated medulloblastoma can be initiated from such Sox2-positive CGNPs in mice. Independent of their endogenous Sox2 expression, constitutive activation of Shh signaling in CGNPs resulted in significantly enhanced proliferation and ectopic expression of Sox2 in vitro and Sox2-positive medulloblastoma in vivo. Genetic ablation of Sox2 from murine medulloblastoma did not affect survival, most likely due to a compensatory overexpression of Sox3. However, acute deletion of Sox2 from primary cultures of CGNPs with constitutive Shh signaling significantly decreased proliferation, whereas overexpression of Sox2 enhanced proliferation of murine medulloblastoma cells. We conclude that Sox2 is a marker for Shh-dependent medulloblastomas where it is required and sufficient to drive tumor cell proliferation.

Aksoy, I., R. Jauch, et al. "Oct4 switches partnering from Sox2 to Sox17 to reinterpret the enhancer code and specify endoderm." Embo J **32**(7): 938-53.

How regulatory information is encoded in the genome is poorly understood and poses a challenge when studying biological processes. We demonstrate here that genomic redistribution of Oct4 by alternative partnering with Sox2 and Sox17 is a fundamental regulatory event of endodermal specification. We show that Sox17 partners with Oct4 and binds to a unique 'compressed' Sox/Oct motif that earmarks endodermal genes. This is in contrast to the pluripotent state where Oct4 selectively partners with Sox2 at 'canonical' binding sites. The distinct selection of binding sites by alternative Sox/Oct partnering is underscored by our demonstration that rationally point-mutated Sox17 partners with Oct4 on pluripotency genes earmarked by the canonical Sox/Oct motif. In an endodermal differentiation assay, we demonstrate that the compressed motif is required for proper expression of endodermal genes. Evidently, Oct4 drives alternative developmental programs by switching Sox partners that affects enhancer selection, leading to either an endodermal or pluripotent cell fate. This work provides insights in understanding cell fate transcriptional regulation by highlighting the direct link between the DNA sequence of an enhancer and a developmental outcome.

Aktug, H., A. Uysal, et al. "The detrimental effects of diabetes on pluripotency determined by KLF4, SOX2, C-MYC and OCT4 immunoreactivity in rat testes." Adv Clin Exp Med **22**(3): 327-35.

BACKGROUND: Diabetes mellitus (DM) is a multisystem disorder. Type 1 DM can be experimentally induced in rats with streptozotocin (STZ). Diabetic conditions result in testicular oxidative stress and suppressed male reproductive activity as well as decreases in both testicular organ weights and subject weights. OBJECTIVES: The purpose of this study was to investigate immunohistochemical differences in testicular tissue due to STZ induced diabetes regarding pluripotency via transcription factors like Klf4, Sox2, c-Myc and Oct4, and to determine weight changes in both the subjects and the testes during the experiment. MATERIAL AND METHODS: Diabetes was induced in male adult rats for this study. A healthy control group and a diabetic group were observed for one month. Blood glucose levels over 250 mg/dL were considered diabetic. RESULTS: On days 0, 3, 15 and 30, the subjects' weights and testicular organ weights were determined and analyzed. The results revealed statistically significant decreases ($p < 0.05$ and $p < 0.001$, respectively). Semiquantitative immunohistochemical analyses of Klf4, Sox2, c-Myc and Oct4 were studied in testes paraffin sections via light microscopy. Decreased immunoreactivity of Klf4 was observed in the diabetic group in comparison to the controls. Spermatogonial cells and Sertoli cells showed increased immunostaining for Sox2 and c-Myc, while decreased immunoreactivity of Oct4 was noted for both spermatogenic and Sertoli cells compared to the control group. CONCLUSIONS: This study clearly demonstrated that Klf4, Sox2 and Oct4 immunopositive cells in adult male rat testes manifested sustainable pluripotency and that diabetes has dramatically detrimental effects on this trait.

Albayrak, C., W. C. Yang, et al. "Pluripotency transcription factor Sox2 is strongly adsorbed by heparin but requires a protein transduction domain for cell internalization." Biochem Biophys Res Commun **431**(3): 641-5.

The binding of protein transduction domain (PTD)-conjugated proteins to heparan sulfate is an important step in cellular internalization of macromolecules. Here, we studied the pluripotency transcription factor Sox2, with or without the nonaarginine (R9) PTD. Unexpectedly, we observed that Sox2 is strongly adsorbed by heparin and by the fibroblasts without the R9 PTD. However, only the R9Sox2 fusion protein is internalized by the cells. These results collectively show that binding to heparan sulfate is not sufficient for cellular uptake, thereby supporting a recent hypothesis that other proteins play a role in cell internalization of PTD-conjugated proteins.

Amini, S., F. Fathi, et al. "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.

Andoniadou, C. L., D. Matsushima, et al. "Sox2(+) stem/progenitor cells in the adult mouse pituitary support organ homeostasis and have tumor-inducing potential." Cell Stem Cell **13**(4): 433-45.

Sox2(+) adult mouse pituitary cells can self-renew and terminally differentiate in vitro, but their physiological role in vivo and possible contribution to oncogenesis remain largely unknown. Using genetic lineage tracing, we show here that the Sox2(+) cell compartment of both the embryonic and adult pituitary contains stem/progenitor cells that are able to differentiate into all hormone-producing lineages and contribute to organ homeostasis during postnatal life. In addition, we show that targeted expression of oncogenic beta-catenin in Sox2(+) cells gives rise to pituitary tumors, but, unexpectedly, the tumor mass is not derived from the Sox2(+) mutation-sustaining cells, suggesting a paracrine role of Sox2(+) cells in pituitary oncogenesis. Our data therefore provide in vivo evidence of a role for Sox2(+) stem/progenitor cells in long-term physiological maintenance of the adult pituitary, and highlight an unexpected non-cell-autonomous role for these cells in the induction of pituitary tumors.

Asadi, M. H., A. Derakhshani, et al. "Concomitant upregulation of nucleostemin and downregulation of Sox2 and Klf4 in gastric adenocarcinoma." Tumour Biol **35**(7): 7177-85.

Nucleostemin (NS) is a nucleolar protein involved in stem cell (SC) self-renewal by controlling cell cycle progression. In addition to SCs, NS is also expressed in some highly proliferating cells including several adult stem cells and cancer cell lines. NS knock-down in different cell lines demonstrated its cell type-dependent function in arresting cell cycle in either G1 or G2/M phases. Here, we have evaluated the expression of NS and iPS genes in 36 gastric cancer and their matched marginal nontumor tissues by means of real-time polymerase chain reaction (RT-PCR). We have also examined a potential causative role of NS in gastric tumorigenesis by suppressing its expression in a gastric cancer cell line, AGS. Our data revealed that NS expression level is much higher in tumor tissues ($p = 0.046$), especially in high-grade ones ($p < 0.001$), whereas the expression of Klf4 and Sox2 is downregulated in tumor tissues compared to marginal nontumor samples ($p < 0.001$). Furthermore, NS suppression in the AGS cell line caused some morphological alterations, a cell cycle arrest at G1 phase, and an upregulation of iPS genes: Nanog, Sox2, and Klf4. Based on our results, NS overexpression seems to have a causative role in gastric tumorigenesis and/or progression, and it could be considered as a potential tumor marker for diagnosis, molecular classification, and molecular therapy of gastric adenocarcinoma.

Askarian-Amiri, M. E., V. Seyfoddin, et al. "Emerging role of long non-coding RNA SOX2OT in SOX2 regulation in breast cancer." PLoS One **9**(7): e102140.

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.

Berezovsky, A. D., L. M. Poisson, et al. "Sox2 promotes malignancy in glioblastoma by regulating plasticity and astrocytic differentiation." Neoplasia **16**(3): 193-206, 206 e19-25.

The high-mobility group-box transcription factor sex-determining region Y-box 2 (Sox2) is essential for the maintenance of stem cells from early development to adult tissues. Sox2 can reprogram differentiated cells into pluripotent cells in concert with other factors and is overexpressed in various cancers. In glioblastoma (GBM), Sox2 is a marker of cancer stemlike cells (CSCs) in neurosphere cultures and is associated with the proneural molecular subtype. Here, we report that Sox2 expression pattern in GBM tumors and patient-derived mouse xenografts is not restricted to a small percentage of cells and is coexpressed with various lineage markers, suggesting that its expression extends beyond CSCs to encompass more differentiated neoplastic cells across molecular subtypes. Employing a CSC derived from a patient with GBM and isogenic differentiated cell model, we show that Sox2 knockdown in the differentiated state abolished dedifferentiation and acquisition of CSC phenotype. Furthermore, Sox2 deficiency specifically impaired the astrocytic component of a biphasic gliosarcoma xenograft model while allowing the formation of tumors with sarcomatous phenotype. The expression of genes associated with stem cells and malignancy were commonly downregulated in both CSCs and serum-differentiated cells on Sox2 knockdown. Genes previously shown to be associated with pluripontency and CSCs were only affected in the CSC state, whereas embryonic stem cell self-renewal genes and cytokine signaling were downregulated, and the Wnt pathway activated in differentiated Sox2-deficient cells. Our results indicate that Sox2 regulates the expression of key genes and pathways involved in GBM malignancy, in both cancer stemlike and differentiated cells, and maintains plasticity for bidirectional conversion between the two states, with significant clinical implications.

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 **66**(9): 819-22.

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.

Brafman, D. A., N. Moya, et al. "Analysis of SOX2-Expressing Cell Populations Derived from Human Pluripotent Stem Cells." Stem Cell Reports **1**(5): 464-78.

SOX2 is involved in several cell and developmental processes, including maintenance of embryonic stem cells, differentiation of neural progenitor cells, and patterning of gut endoderm. To study its role in a human system, we generated a human embryonic stem cell (hESC) line harboring a reporter gene encoding GFP in the SOX2 locus. This SOX2 reporter line faithfully recapitulates expression of the SOX2 gene in undifferentiated human pluripotent stem cells (hPSCs), neural progenitor cells (NPCs), and anterior foregut endoderm (AFE). In undifferentiated hESCs, GFP expression corresponds to those cells with highest levels of expression of genes associated with the pluripotent state. In NPCs, expression of GFP can be employed to isolate cells expressing markers associated with NPC multipotency. In AFE, we used transcriptome-wide expression analysis to identify cell surface markers with elevated expression in this population,

thereby facilitating isolation and purification of this hPSC-derived cell population.

Brustmann, H. and A. Brunner "Immunohistochemical expression of SOX2 in vulvar intraepithelial neoplasia and squamous cell carcinoma." Int J Gynecol Pathol **32**(3): 323-8.

SOX2 is a transcription factor controlling pluripotency in both embryonic stem cells and adult tissue-specific stem cells. SOX2 has been reported as an important factor in squamous cell carcinomas (SCC) of different locations and is involved in tumorigenesis. We evaluated the expression of SOX2 in vulvar non-neoplastic and neoplastic epithelia to test whether it is related to neoplastic progression. SOX2 immunoexpression was evaluated in 101 formalin-fixed, paraffin-embedded archival vulvar epithelia consisting of normal squamous vulvar epithelia (n=25), lichen sclerosus (n=9), high-grade classic vulvar intraepithelial neoplasia (HG-VIN, n=16), differentiated vulvar intraepithelial neoplasia (d-VIN, n=18), and vulvar invasive keratinizing SCC (n=33). Immunoexpression of SOX2 was nuclear and increased stepwise from normal vulvar epithelia/lichen sclerosus to HG-VIN and d-VIN (P<0.0001), from HG-VIN and d-VIN to invasive SCC (P=0.0029), and followed the morphologic distribution of atypical squamous epithelial cells. Scores for normal vulvar epithelia versus lichen sclerosus and HG-VIN versus d-VIN, respectively, did not differ significantly. SOX2 expression increased from tumor Grade 1 to 3 (P=0.0124); there was no relation to recurrence and survival. This is the first study presenting SOX2 as overexpressed in vulvar intraepithelial and invasive squamous lesions. This overexpression apparently reflects an early event in the neoplastic transformation of vulvar squamous epithelia. However, SOX2 seems to play a role in histologic dedifferentiation to Grade 3 invasive SCC too, and may be relevant to vulvar carcinogenesis.

Campolo, F., M. Gori, et al. "Essential role of Sox2 for the establishment and maintenance of the germ cell line." Stem Cells **31**(7): 1408-21.

Sox2 is a pluripotency-conferring gene expressed in primordial germ cells (PGCs) and postnatal oocytes, but the role it plays during germ cell development and early embryogenesis is unknown. Since Sox2 ablation causes early embryonic lethality shortly after blastocyst implantation, we generated mice bearing Sox2-conditional deletion in germ cells at different stages of their development through the Cre/loxP recombination system. Embryos lacking Sox2 in PGCs show a dramatic decrease of germ cell numbers at the time of their specification. At later stages, we found that Sox2 is strictly required for PGC proliferation. On the contrary, Sox2 deletion in meiotic oocytes does not impair postnatal oogenesis and early embryogenesis, indicating that it is not essential for oocyte maturation or for zygotic development. We also show that Sox2 regulates Kit expression in PGCs and binds to discrete transcriptional regulatory sequences of this gene, which is known to be important for PGCs survival and proliferation. Sox2 also stimulates the expression of Zfp148, which is required for normal development of fetal germ cells, and Rif1, a potential regulator of PGC pluripotency.

Carina, V., G. Zito, et al. "Multiple pluripotent stem cell markers in human anaplastic thyroid cancer: the putative upstream role of SOX2." Thyroid **23**(7): 829-37.

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 muM cisplatin (93.9+/-3.4% vs. 52.6+/-9.4%, p<0.01) and 2.7 fold with 0.5 muM 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 **12**(18): 3109-24.

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.

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

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 **85**(1): 1-6.

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.

Eini, R., H. Stoop, et al. "Role of SOX2 in the etiology of embryonal carcinoma, based on analysis of the NCCIT and NT2 cell lines." PLoS One **9**(1): e83585.

The transcription factor SOX2, associated with amongst others OCT3/4, is essential for maintenance of pluripotency and self-renewal of embryonic stem cells. SOX2 is highly expressed in embryonal carcinoma (EC), the stem cell component of malignant nonseminomatous germ cell tumors, referred to as germ cell cancer (GCC). In fact, OCT3/4 together with SOX2 is an informative diagnostic tool for EC in a clinical setting. Several studies support the hypothesis that SOX2 is a relevant oncogenic factor in various cancers and recently, SOX2 has been suggested as a putative therapeutic target for early stage EC. We demonstrate the presence of genomic amplification of SOX2 in an EC cell line, NCCIT, using array comparative genome hybridization and fluorescence in situ hybridization. Down-regulation of SOX2 by targeted siRNA provokes NCCIT cells towards apoptosis, while inhibition of OCT3/4 expression induced differentiation, with retained SOX2 levels. Mice pluripotent xenografts from NCCIT (N-NCCIT and N2-NCCIT) show a consistent SOX2 expression, in spite of loss of the expression of OCT3/4, and differentiation, with retained presence of genomic amplification. No SOX2 amplification has been identified in primary pure and mixed EC in vivo patient samples so far. The data presented in this study are based on a single EC cell line with a SOX2 amplification, with NT2 as control EC cell line, showing no profound induction of apoptosis upon SOX2 downregulation. The findings are of relevance to identify mechanisms involved in the pathogenesis of EC tumors, and support the model of SOX2-oncogene dependency of EC, which however, does not exclude induction of differentiation. This finding is likely related to the presence of wild type p53 in GCC, resulting in expression of downstream target genes, amongst others miR-34a, miR-145 and SOX2, associated to the unique sensitivity of GCC to DNA damaging agents.

Evsen, L., S. Sugahara, et al. "Progression of neurogenesis in the inner ear requires inhibition of Sox2 transcription by neurogenin1 and neurod1." J Neurosci **33**(9): 3879-90.

Sox2 is required for proper neuronal formation in the CNS, but the molecular mechanisms involved are not well characterized. Here, we addressed the role of Sox2 in neurogenesis of the developing chicken inner ear. Overexpressing Sox2 from a constitutive (beta-actin) promoter induces the expression of the proneural gene, Neurogenin1 (Ngn1); however, the expression of a downstream target of Ngn1, Neurod1, is unchanged. As a result, there is a reduction of neural precursors to delaminate and populate the developing cochleo-vestibular ganglion. In contrast, overexpression of either Ngn1 or Neurod1 is sufficient to promote the neural fate in this system. These results suggest that high levels of Sox2 inhibit progression of neurogenesis in the developing inner ear. Furthermore, we provide evidence that Ngn1 and Neurod1 inhibit Sox2 transcription through a phylogenetically conserved Sox2 enhancer to mediate neurogenesis. We propose that Sox2 confers neural competency by promoting Ngn1 expression, and that negative feedback inhibition of Sox2 by Ngn1 is an essential step in the progression from neural precursor to nascent neuron.

Forghanifard, M. M., S. Ardalan Khales, et al. "Stemness state regulators SALL4 and SOX2 are involved in progression and invasiveness of esophageal squamous cell carcinoma." Med Oncol **31**(4): 922.

Cancer stem cells, as a subgroup of tumor cells, resemble critical properties of embryonic stem cells (ESCs) such as self-renewal and maintenance of stemness state. SALL4 and SOX2 are two main transcription factors involving in maintenance of pluripotency, self-renewal and cell fate decision in ESCs. In this study, we aimed to elucidate the expression levels of these

important transcription factors in esophageal squamous cell carcinoma (ESCC) and to reveal their probable roles in maintenance and progression of the disease. The expression level of SALL4 and SOX2 was analyzed in fresh tumoral tissues in comparison with distant tumor-free tissues of 50 ESCC patients by relative comparative real-time PCR. SALL4 and SOX2 were overexpressed in 64 and 32% of tumor samples, respectively, in significant correlation with each other ($p = 0.028$). There was a significantly inverse correlation between low level of SALL4 expression and metastasis of tumor cells into the lymph nodes ($p = 0.035$). Furthermore, co-overexpression of the genes was significantly correlated with the depth of tumor invasion ($p = 0.045$) and metastasis to the lymph nodes ($p = 0.049$). SALL4 and SOX2 are co-overexpressed in ESCC and have a significant correlation with invasion and metastasis of the disease. To the best of our knowledge, this is the first report of SALL4 clinical relevance in ESCC to date. The clinical consequences of SALL4-SOX2 association suggest a possible functional interaction between these factors in regulation of ESCC maintenance and aggressiveness and introduce these regulators of stemness state as potentially interesting therapeutic targets to bring new opportunities for onco-therapeutic modalities.

Gagliardi, A., N. P. Mullin, et al. "A direct physical interaction between Nanog and Sox2 regulates embryonic stem cell self-renewal." Embo J **32**(16): 2231-47.

Embryonic stem (ES) cell self-renewal efficiency is determined by the Nanog protein level. However, the protein partners of Nanog that function to direct self-renewal are unclear. Here, we identify a Nanog interactome of over 130 proteins including transcription factors, chromatin modifying complexes, phosphorylation and ubiquitination enzymes, basal transcriptional machinery members, and RNA processing factors. Sox2 was identified as a robust interacting partner of Nanog. The purified Nanog-Sox2 complex identified a DNA recognition sequence present in multiple overlapping Nanog/Sox2 ChIP-Seq data sets. The Nanog tryptophan repeat region is necessary and sufficient for interaction with Sox2, with tryptophan residues required. In Sox2, tyrosine to alanine mutations within a triple-repeat motif (S X T/S Y) abrogates the Nanog-Sox2 interaction, alters expression of genes associated with the Nanog-Sox2 cognate sequence, and reduces the ability of Sox2 to rescue ES cell differentiation induced by endogenous Sox2 deletion. Substitution of the tyrosines with phenylalanine rescues both the Sox2-Nanog interaction and efficient self-renewal. These results suggest that aromatic stacking of Nanog tryptophans and Sox2 tyrosines mediates an interaction central to ES cell self-renewal.

Galatro, T. F., M. Uno, et al. "Differential expression of ID4 and its association with TP53 mutation, SOX2, SOX4 and OCT-4 expression levels." PLoS One **8**(4): e61605.

Inhibitor of DNA Binding 4 (ID4) is a member of the helix-loop-helix ID family of transcription factors, mostly present in the central nervous system during embryonic development, that has been associated with TP53 mutation and activation of SOX2. Along with other transcription factors, ID4 has been implicated in the tumorigenic process of astrocytomas, contributing to cell dedifferentiation, proliferation and chemoresistance. In this study, we aimed to characterize the ID4 expression pattern in human diffusely infiltrative astrocytomas of World Health Organization (WHO) grades II to IV of malignancy (AGII-AGIV); to correlate its expression level to that of SOX2, SOX4, OCT-4 and NANOG, along with TP53 mutational status; and to correlate the results with the clinical end-point of overall survival among glioblastoma patients. Quantitative real time PCR (qRT-PCR) was performed in 130 samples of astrocytomas for relative expression, showing up-regulation of all transcription factors in tumor cases. Positive correlation was found when comparing ID4 relative expression of infiltrative astrocytomas with SOX2 ($r = 0.50$; $p<0.005$), SOX4 ($r = 0.43$; $p<0.005$) and OCT-4 ($r = 0.39$; $p<0.05$). The results from TP53 coding exon analysis allowed comparisons between wild-type and mutated status only in AGII cases, demonstrating significantly higher levels of ID4, SOX2 and SOX4 in mutated cases ($p<0.05$). This pattern was maintained in secondary GBM and further confirmed by immunohistochemistry, suggesting a role for ID4, SOX2 and SOX4 in early astrocytoma tumorigenesis. Combined hyperexpression of ID4, SOX4 and OCT-4 conferred a much lower (6 months) median survival than did hypoexpression (18 months). Because both ID4 alone and a complex of SOX4 and OCT-4 activate SOX2 transcription, it is possible that multiple activation of SOX2 impair the prognosis of GBM patients. These observational results of associated expression of ID4 with SOX4 and OCT-4 may be used as a predictive factor of prognosis upon further confirmation in a larger GBM series.

Gonzalez-Marquez, R., J. L. Llorente, et al. "SOX2 expression in hypopharyngeal, laryngeal, and sinonasal squamous cell carcinoma." Hum Pathol **45**(4): 851-7.

Squamous cell carcinoma (SCC) of the head and neck display high frequencies of DNA copy number gains at chromosomal region 3q26-27. Recently SOX2 has been postulated as a driver oncogene for these amplifications; however, its role as a prognostic marker is still a matter of debate. The aim of this study was to evaluate the involvement of SOX2 protein expression in three different sublocalizations of head and neck SCC and its possible role as prognostic marker. SOX2 expression was analyzed by immunohistochemistry in 102 pharyngeal, 67 laryngeal, and 51 sinonasal SCCs, and the relation to clinicopathological and follow-up data was studied by chi(2) and Kaplan-Meier analysis. SOX2 expression was significantly ($P = .002$) more frequent in hypopharyngeal and laryngeal SCC (38%, 39/101) and (42%, 28/67), respectively, compared to sinonasal cancer SCC (14%, 7/51). SOX2 expression did not correlate to disease stage, T or N classification, lymph node metastasis, recurrence or clinical outcome in any of the three sublocalizations. These results indicate that SOX2 expression is a common event in hypopharynx and larynx, but not in sinonasal SCC. The absence of correlation to clinical outcome, may suggest a role for SOX2 in tumor initiation, but not in tumor progression.

Hoffmann, S. A., D. Hos, et al. "Stem cell factor Sox2 and its close relative Sox3 have differentiation functions in oligodendrocytes." Development **141**(1): 39-50.

Neural precursor cells of the ventricular zone give rise to all neurons and glia of the central nervous system and rely for maintenance of their precursor characteristics on the closely related SoxB1 transcription factors Sox1, Sox2 and Sox3. We show in mouse spinal cord that, whereas SoxB1 proteins are usually downregulated upon neuronal specification, they continue to be expressed in glial precursors. In the oligodendrocyte lineage, Sox2 and Sox3 remain present into the early phases of terminal differentiation. Surprisingly, their deletion does not alter precursor characteristics but interferes with proper differentiation. Although a direct influence on myelin gene expression may be part of their function, we provide evidence for another mode of action. SoxB1 proteins promote oligodendrocyte differentiation in part by negatively controlling miR145 and thereby preventing this microRNA from inhibiting several pro-differentiation factors. This study presents one of the few cases in which SoxB1 proteins, including the stem cell factor Sox2, are associated with differentiation rather than precursor functions.

Honing, J., K. V. Pavlov, et al. "Loss of CD44 and SOX2 Expression is Correlated with a Poor Prognosis in Esophageal Adenocarcinoma Patients." Ann Surg Oncol.

BACKGROUND: It has been suggested that markers associated with cancer stem cells (CSC) may play a role in esophageal cancer. Our aim was to investigate the expression pattern of proposed CSC markers ALDH1, Axin2, BMI1, CD44, and SOX2 in esophageal adenocarcinoma (EAC) and to relate their

expression to survival. METHODS: In this study we included 94 EAC patients and examined the expression of the above-mentioned markers by using immunohistochemistry on tissue microarrays. Expression was scored as positive or negative or categorized as low or high in terms of an immunoreactivity score (IRS). Expression rates were related to clinicopathologic characteristics and overall and disease-free survival (DFS). RESULTS: In a multivariate analysis, negative expression of CD44 and of SOX2 were both significant prognostic factors for DFS [hazard ratio (HR), 1.73; 95 % confidence interval (CI), 1.00-2.96; P = 0.046 and HR, 2.06; 95 % CI 1.14-3.70 P = 0.016). When CD44 and SOX2 expression were analyzed together, negative SOX2 expression was an independent prognostic factor for DFS (HR, 1.91; 95 % CI 1.05-3.46; P = 0.034). Low IRS scores for ALDH1 or Axin2 were associated with a reduced median survival (12.8 vs. 28.7 and 12.1 vs. 25.5 months, respectively). However, these markers and BMI1 were not prognostic factors for survival. CONCLUSIONS: Loss of CD44 expression and loss of SOX2 expression are prognostic factors of poor survival in EAC patients. This suggests a role of these proteins in EAC that requires further investigation.

Hutz, K., R. Mejias-Luque, et al. "The stem cell factor SOX2 regulates the tumorigenic potential in human gastric cancer cells." Carcinogenesis **35**(4): 942-50.

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.

Ilieva, M. and M. Dufva "SOX2 and OCT4 mRNA-expressing cells, detected by molecular beacons, localize to the center of neurospheres during differentiation." PLoS One **8**(8): e73669.

Neurospheres are used as in vitro assay to measure the properties of neural stem cells. To investigate the molecular and phenotypic heterogeneity of neurospheres, molecular beacons (MBs) targeted against the stem cell markers OCT4 and SOX2 were designed, and synthesized with a 2'-O-methyl RNA backbone. OCT4 and SOX2 MBs were transfected into human embryonic mesencephalon derived cells, which spontaneously form neurospheres when grown on poly-L-ornitine/fibronectin matrix and medium complemented with bFGF. OCT4 and SOX2 gene expression were tracked in individual cell using the MBs. Quantitative image analysis every day for seven days showed that the OCT4 and SOX2 mRNA-expressing cells clustered in the centre of the neurospheres cultured in differentiation medium. By contrast, cells at the periphery of the differentiating spheres developed neurite outgrowths and expressed the tyrosine hydroxylase protein, indicating terminal differentiation. Neurospheres cultured in growth medium contained OCT4 and SOX2-positive cells distributed throughout the entire sphere, and no differentiating neurones. Gene expression of SOX2 and OCT4 mRNA detected by MBs correlated well with gene and protein expression measured by qRT-PCR and immunostaining, respectively. These experimental data support the theoretical model that stem cells cluster in the centre of neurospheres, and demonstrate the use of MBs for the spatial localization of specific gene-expressing cells within heterogeneous cell populations.

Justilien, V., M. P. Walsh, et al. "The PRKCI and SOX2 oncogenes are coamplified and cooperate to activate Hedgehog signaling in lung squamous cell carcinoma." Cancer Cell **25**(2): 139-51.

We report that two oncogenes coamplified on chromosome 3q26, PRKCI and SOX2, cooperate to drive a stem-like phenotype in lung squamous cell carcinoma (LSCC). Protein kinase Ciota (PKCiota) phosphorylates SOX2, a master transcriptional regulator of stemness, and recruits it to the promoter of Hedgehog (Hh) acyltransferase (HHAT) that catalyzes the rate-limiting step in Hh ligand production. PKCiota-mediated SOX2 phosphorylation is required for HHAT promoter occupancy, HHAT expression, and maintenance of a stem-like phenotype. Primary LSCC tumors coordinately overexpress PKCiota, SOX2, and HHAT and require PKCiota-SOX2-HHAT signaling to maintain a stem-like phenotype. Thus, PKCiota and SOX2 are genetically, biochemically, and functionally linked in LSCC, and together they drive tumorigenesis by establishing a cell-autonomous Hh signaling axis.

Kallas, A., M. Pook, et al. "SOX2 Is Regulated Differently from NANOG and OCT4 in Human Embryonic Stem Cells during Early Differentiation Initiated with Sodium Butyrate." Stem Cells Int **2014**: 298163.

Transcription factors NANOG, OCT4, and SOX2 regulate self-renewal and pluripotency in human embryonic stem (hES) cells; however, their expression profiles during early differentiation of hES cells are unclear. In this study, we used multiparameter flow cytometric assay to detect all three transcription factors (NANOG, OCT4, and SOX2) simultaneously at single cell level and monitored the changes in their expression during early differentiation towards endodermal lineage (induced by sodium butyrate). We observed at least four distinct populations of hES cells, characterized by specific expression patterns of NANOG, OCT4, and SOX2 and differentiation markers. Our results show that a single cell can express both differentiation and pluripotency markers at the same time, indicating a gradual mode of developmental transition in these cells. Notably, distinct regulation of SOX2 during early differentiation events was detected, highlighting the potential importance of this transcription factor for self-renewal of hES cells during differentiation.

Koike, T., T. Wakabayashi, et al. "Sox2 in the adult rat sensory nervous system." Histochem Cell Biol **141**(3): 301-9.

Sex-determining region Y (SRY)-box 2 (Sox2) is a member of the Sox family transcription factors. In the central nervous system, Sox2 is expressed in neural stem cells from neurogenic regions, and regulates stem cell proliferation and differentiation. In the peripheral nervous system, Sox2 is found only in the immature and dedifferentiated Schwann cells, and is involved in myelination inhibition or N-cadherin redistribution. In the present immunohistochemical study, we found that Sox2 is also expressed in other cells of the adult rat peripheral nervous system. Nuclear Sox2 was observed in all satellite glial cells, non-myelinating Schwann cells, and the majority of terminal Schwann cells that form lamellar corpuscles and longitudinal lanceolate endings. Sox2 was not found in myelinating Schwann cells and terminal Schwann cells of subepidermal free nerve endings. Satellite glial cells exhibit strong Sox2 immunoreactivity, whereas non-myelinating Schwann cells show weak immunoreactivity. RT-PCR confirmed the presence of Sox2 mRNA, indicating that the cells are likely Sox2 expressors. Our findings suggest that the role of Sox2 in the peripheral nervous system may be cell-type-dependent.

Kokalj Vokac, N., B. Cizmarevic, et al. "An evaluation of SOX2 and hTERC gene amplifications as screening markers in oral and oropharyngeal squamous cell carcinomas." Mol Cytogenet **7**(1): 5.

BACKGROUND: Oral and oropharyngeal squamous cell carcinomas (OSCC) are among the most common cancers. The poor survival rate among oral cancer patients can be attributed to several factors, one of them being lack of early detection. A key approach to this problem would be to detect potentially malignant lesion at their early stage. Using the FISH technique, oral brush cytology slides can be an easy and rapid screening approach for malignant cell detection. The present study was designed to detect hTERC and SOX2 amplifications in OSSC exfoliative tumor cells and evaluate whether those two gene amplifications might serve as a supportive biomarker in early detection and diagnosis of oral and oropharyngeal SCC. RESULTS: Brush biopsies were collected from exophytic and exulcerated oral and oropharyngeal lesions of the oral cavity of 71 patients and 22 healthy controls. FISH techniques using a TERC-specific DNA probe and a SOX2 DNA specific probe both combined with a centromere 3-specific control probe was performed on the cytology slides. A 100 squamous epithelial cell nuclei of the smears per slide were analysed. As abnormal FISH pattern were considered amplified and polyploid patterns.From 71 brush biopsies of oropharynx and other locations in oral cavity analysed by FISH 49 were considered to be abnormal (69%). The over representation of polyploidy and/or TERC/SOX2 amplification in tumour samples was statistically significant when compared to controls ($p = 0.01$). CONCLUSION: SOX2 and TERC gene amplifications are common in all squamous cell carcinomas and their detection in early stages could be crucial for early detection and more accurate prognosis. Our study strongly suggests that early detection by FISH on cytobrushed samples could be a possible non-invasive screening method even before a tissue biopsy is performed.

Kregel, S., K. J. Kiriluk, et al. "Sox2 is an androgen receptor-repressed gene that promotes castration-resistant prostate cancer." PLoS One **8**(1): e53701.

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 [SRY (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.

Langer, L., K. Sulik, et al. "Cleft Palate in a Mouse Model of SOX2 Haploinsufficiency." Cleft Palate Craniofac J **51**(1): 110-4.

Objective: While SEX-determining region Y-Box 2 (SOX2) mutations are typically recognized as yielding ocular and central nervous system abnormalities, they have also been associated with other craniofacial defects. To elucidate the genesis of the latter, Sox2 hypomorphic (Sox2(HYP)) mice were examined, with particular attention to secondary palatal development. Results: Clefts of the secondary palate were found to be highly penetrant in Sox2(HYP) mice. The palatal clefting occurred in the absence of mandibular hypoplasia and resulted from delayed or failed shelf elevation. Conclusions: Sox2 hypomorphism can result in clefting of the secondary palate, an effect that appears to be independent of mandibular hypoplasia and is thus expected to result from an abnormality that is inherent to the palatal shelves and/or their progenitor tissues. Further clinical attention relative to SOX2 mutations as a basis for secondary palatal clefts appears warranted.

Ma, K., X. Pan, et al. "Loss of miR-638 in vitro promotes cell invasion and a mesenchymal-like transition by influencing SOX2 expression in colorectal carcinoma cells." Mol Cancer **13**: 118.

BACKGROUND: Colorectal carcinoma (CRC) is a major cause of cancer mortality. The aberrant expression of several microRNAs is associated with CRC progression; however, the molecular mechanisms underlying this phenomenon are unclear. METHODS: miR-638 and SRY-box 2 (SOX2) expression levels were detected in 36 tumor samples and their adjacent, non-tumor tissues from patients with CRC, as well as in 4 CRC cell lines, using real-time quantitative RT-PCR (qRT-PCR). SOX2 expression levels were detected in 90 tumor samples and their adjacent tissue using immunohistochemistry. Luciferase reporter and Western blot assays were used to validate SOX2 as a target gene of miR-638. The regulation of SOX2 expression by miR-638 was assessed using qRT-PCR and Western blot assays, and the effects of exogenous miR-638 and SOX2 on cell invasion and migration were evaluated in vitro using the HCT-116 and SW1116 CRC cell lines. RESULTS: We found that miR-638 expression was differentially impaired in CRC specimens and dependent on tumor grade. The inhibition of miR-638 by an antagomiR promoted cell invasion and a mesenchymal-like transition (lamellipodium stretching increased and cell-cell contacts decreased, which was accompanied by the suppression of the epithelial cell marker ZO-1/E-cadherin and the upregulation of the mesenchymal cell marker vimentin). A reporter assay revealed that miR-638 repressed the luciferase activity of a reporter gene coupled to the 3'-untranslated region of SOX2. miR-638 overexpression downregulated SOX2 expression, and miR-638 inhibition upregulated SOX2 expression. Moreover, miR-638 expression levels were correlated inversely with SOX2 mRNA levels in human CRC tissues. The RNAi-mediated knockdown of SOX2 phenocopied the invasion-inhibiting effect of miR-638; furthermore, SOX2 overexpression blocked the miR-638-induced CRC cell transition to epithelial-like cells. CONCLUSIONS: These results demonstrate that the loss of miR-638 promotes invasion and a mesenchymal-like transition by directly targeting SOX2 in vitro. These findings define miR-638 as a new, invasion-associated tumor suppressor of CRC.

Macchiaroli, A., D. Kelberman, et al. "A novel heterozygous SOX2 mutation causing congenital bilateral anophthalmia, hypogonadotropic hypogonadism and growth hormone deficiency." Gene **534**(2): 282-5.

Heterozygous de novo mutations in SOX2 have been reported in approximately 10-20% of patients with unilateral or bilateral anophthalmia or microphthalmia. An additional phenotype of hypopituitarism, with anterior pituitary hypoplasia and hypogonadotropic hypogonadism, has been reported in patients carrying SOX2 alterations. We report a novel heterozygous mutation in the SOX2 gene in a male affected with congenital bilateral anophthalmia, hypogonadotrophic hypogonadism and growth hormone deficiency. The mutation we describe is a cytosine deletion in position 905 (c905delC) which causes frameshift and an aberrant C-terminal domain. Our report highlights the fact that

subjects affected with eye anomalies and harboring SOX2 mutations are at high risk for gonadotropin deficiency, which has important implications for their clinical management.

Malecki, M., X. Tombokan, et al. "TRA-1-60, SSEA-4, POU5F1, SOX2, NANOG Clones of Pluripotent Stem Cells in the Embryonal Carcinomas of the Testes." J Stem Cell Res Ther **3**(1).

Cancer of the testes is currently the most frequent neoplasm and a leading cause of morbidity in men 15-35 years of age. Its incidence is increasing. Embryonal carcinoma is its most malignant form, which either may be resistant or may develop resistance to therapies, which results in relapses. Cancer stem cells are hypothesized to be drivers of these phenomena. The specific aim of this work was identification and isolation of spectra of single, living cancer stem cells, which were acquired directly from the patients' biopsies, followed by testing of their pluripotency. Biopsies were obtained from the patients with the clinical and histological diagnoses of the primary, pure embryonal carcinomas of the testes. The magnetic and fluorescent antibodies were genetically engineered. The SSEA-4 and TRA-1-60 cell surface display was analyzed by multiphoton fluorescence spectroscopy (MPFS), flow cytometry (FCM), immunoblotting (IB), nuclear magnetic resonance spectroscopy (NMRS), energy dispersive x-ray spectroscopy (EDXS), and total reflection x-ray spectroscopy (TRXFS). The single, living cells were isolated by magnetic or fluorescent sorting followed by their clonal expansion. The OCT4A, SOX2, and NANOG genes' transcripts were analyzed by qRTPCR and the products by IB and MPFS. The clones of cells, with the strong surface display of TRA-1-60 and SSEA-4, were identified and isolated directly from the biopsies acquired from the patients diagnosed with the pure embryonal carcinomas of the testes. These cells demonstrated high levels of transcription and translation of the pluripotency genes: OCT4A, SOX2, and NANOG. They formed embryoid bodies, which differentiated into ectoderm, mesoderm, and endoderm. In the pure embryonal carcinomas of the testes, acquired directly from the patients, we identified, isolated with high viability and selectivity, and profiled the clones of the pluripotent stem cells. These results may help in explaining therapy-resistance and relapses of these neoplasms, as well as, in designing targeted, personalized therapy.

Mukhopadhyay, A., K. C. Berrett, et al. "Sox2 cooperates with Lkb1 loss in a mouse model of squamous cell lung cancer." Cell Rep **8**(1): 40-9.

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.

Ochieng, J. K., K. Schilders, et al. "Sox2 regulates the emergence of lung Basal cells by directly activating the transcription of trp63." Am J Respir Cell Mol Biol **51**(2): 311-22.

Lung development is determined by the coordinated expression of several key genes. Previously, we and others have shown the importance of the sex determining region Y-box 2 (Sox2) gene in lung development. Transgenic expression of Sox2 during lung development resulted in cystic airways, and here we show that modulating the timing of ectopic Sox2 expression in the branching regions of the developing lung results in variable cystic lesions resembling the spectrum of the human congenital disorder congenital cystic adenomatoid malformation (CCAM). Sox2 dominantly differentiated naive epithelial cells into the proximal lineage irrespective of the presence of Fgf10. Sox2 directly induced the expression of Trp63, the master switch toward the basal cell lineage and induced the expression of Gata6, a factor involved in the emergence of bronchoalveolar stem cells. We showed that SOX2 and TRP63 are coexpressed in the lungs of human patients with type II CCAM. The combination of premature differentiation toward the proximal cell lineage and the induction of proliferation resulted in the cyst-like structures. Thus, we show that Sox2 is directly responsible for the emergence of two lung progenitor cells: basal cells by regulating the master gene Trp63 and bronchoalveolar stem cells by regulating Gata6.

Ogai, K., K. Nakatani, et al. "Function of Sox2 in ependymal cells of lesioned spinal cords in adult zebrafish." Neurosci Res.

The sex-determining region Y-box 2 (Sox2) is related not only to pluripotency, but also to cell proliferation. Zebrafish can regain their motor function after spinal cord injury (SCI). Following SCI, new motor neurons are produced from proliferating ependymal cells. Here, we investigated the expression and function of Sox2 after SCI in zebrafish. Sox2 was upregulated as early as 1 day post-lesion (dpl) in ependymal cells, which was followed by cell proliferation. Sox2 knockdown significantly decreased the number of proliferating cells at 5dpl. The results of this study suggest a role of Sox2 as one of the proliferation initiators in ependymal cells after SCI.

Ogony, J. W., E. Malahias, et al. "Ethanol alters the balance of Sox2, Oct4, and Nanog expression in distinct subpopulations during differentiation of embryonic stem cells." Stem Cells Dev **22**(15): 2196-210.

The transcription factors Sox2, Oct4, and Nanog regulate within a narrow dose-range embryonic stem (ES) cell pluripotency and cell lineage commitment. Excess of Oct4 relative to Sox2 guides cells to mesoendoderm (ME), while abundance of Sox2 promotes neuroectoderm (NE) formation. Literature does not address whether ethanol interferes with these regulatory interactions during neural development. We hypothesized that ethanol exposure of ES cells in early differentiation causes an imbalance of Oct4 and Sox2 that diverts cells away from NE to ME lineage, consistent with the teratogenesis effects caused by prenatal alcohol exposure. Mouse ES cells were exposed to ethanol (0, 25, 50, and 100 mM) during retinoic acid (10 nM)-directed differentiation to NE for 0-6 days, and the expression of Sox2, Oct4, and Nanog was measured in single live cells by multiparametric flow cytometry, and the cellular phenotype was characterized by immunocytochemistry. Our data showed an ethanol dose- and time-dependent asymmetric modulation of Oct4 and Sox2 expression, as early as after 2 days of exposure. Single-cell analysis of the correlated expression of Sox2, Oct4, and Nanog revealed that ethanol promoted distinct subpopulations with a high Oct4/Sox2 ratio. Ethanol-exposed cells differentiated to fewer beta-III tubulin-immunoreactive cells with an immature neuronal phenotype by 4 days. We interpret these data as suggesting that ethanol diverted cells in early differentiation from the NE fate toward the ME lineage. Our results provide a novel insight into the mode of ethanol action and opportunities for discovery of prenatal biomarkers at early stages.

Oliver-De La Cruz, J., J. Carrion-Navarro, et al. "SOX2+ cell population from normal human brain white matter is able to generate mature oligodendrocytes." PLoS One **9**(6): e99253.

OBJECTIVES: A number of neurodegenerative diseases progress with a loss of myelin, which makes them candidate diseases for the development of cell-replacement therapies based on mobilisation or isolation of the endogenous neural/glial progenitor cells, in vitro expansion, and further implantation. Cells expressing A2B5 or PDGFRA/CNP have been isolated within the pool of glial

progenitor cells in the subcortical white matter of the normal adult human brain, all of which demonstrate glial progenitor features. However, the heterogeneity and differentiation potential of this pool of cells is not yet well established. METHODS: We used diffusion tensor images, histopathology, and immunostaining analysis to demonstrate normal cytoarchitecture and the absence of abnormalities in human temporal lobe samples from patients with mesial temporal sclerosis. These samples were used to isolate and enrich glial progenitor cells in vitro, and later to detect such cells in vivo. RESULTS: We have identified a subpopulation of SOX2+ cells, most of them co-localising with OLIG2, in the white matter of the normal adult human brain in vivo. These cells can be isolated and enriched in vitro, where they proliferate and generate immature (O4+) and mature (MBP+) oligodendrocytes and, to a lesser extent, astrocytes (GFAP+). CONCLUSION: Our results demonstrate the existence of a new glial progenitor cell subpopulation that expresses SOX2 in the white matter of the normal adult human brain. These cells might be of use for tissue regeneration procedures.

Ormsbee Golden, B. D., E. L. Wuebben, et al. "Sox2 expression is regulated by a negative feedback loop in embryonic stem cells that involves AKT signaling and FoxO1." PLoS One **8**(10): e76345.

The self-renewal and pluripotency of embryonic stem cells (ESC) is regulated by a highly integrated network of essential transcription factors, which includes Sox2. Previous studies have shown that elevating Sox2 on its own in mouse ESC induces differentiation and inhibits the expression of endogenous Sox2 at the protein and mRNA level. These findings led us to hypothesize that increases in Sox2 activate a negative feedback loop that inhibits the transcription of the endogenous Sox2 gene. To test this hypothesis, we used i-OSKM-ESC, which elevate Sox2 in conjunction with Oct4, Klf4, and c-Myc when treated with doxycycline (Dox). Elevating the expression of these four transcription factors in i-OSKM-ESC does not induce differentiation, but it represses expression of endogenous Sox2. We determined that increases of Sox2 in i-OSKM-ESC lead to increases in activated AKT and inactivation of FoxO1 (an activator of Sox2), as well as decreases in binding of FoxO1 to the 5'flanking region of Sox2. Importantly, we determined that inhibition of AKT in Dox-treated i-OSKM-ESC leads to re-expression of endogenous Sox2 at the mRNA and protein level and reactivation of FoxO1. These findings argue that AKT signaling is part of the negative feedback loop that helps carefully control the transcription of Sox2 in ESC by modulating the binding of FoxO1 to the Sox2 gene. Collectively, our findings provide new insights into the mechanisms that enable ESC to carefully regulate the levels of Sox2 and retain their stem cell properties.

Popowski, M., T. D. Templeton, et al. "Bright/Arid3A Acts as a Barrier to Somatic Cell Reprogramming through Direct Regulation of Oct4, Sox2, and Nanog." Stem Cell Reports **2**(1): 26-35.

We show here that singular loss of the Bright/Arid3A transcription factor leads to reprograming of mouse embryonic fibroblasts (MEFs) and enhancement of standard four-factor (4F) reprogramming. Bright-deficient MEFs bypass senescence and, under standard embryonic stem cell (ESC) culture conditions, spontaneously form clones that in vitro express pluripotency markers, differentiate to all germ lineages, and in vivo form teratomas and chimeric mice. We demonstrate that BRIGHT binds directly to the promoter/enhancer regions of Oct4, Sox2, and Nanog to contribute to their repression in both MEFs and ESCs. Thus, elimination of the BRIGHT barrier may provide an approach for somatic cell reprogramming.

Raghoebir, L., K. Biermann, et al. "Disturbed balance between SOX2 and CDX2 in human vitelline duct anomalies and intestinal duplications." Virchows Arch **462**(5): 515-22.

Congenital gastric-type heteroplasia is common in intestinal duplications and anomalies, which originate from incomplete resorption of the omphalomesenteric duct during development. Two transcription factors determine the proximodistal specification of the gastrointestinal tract, SOX2, expressed exclusively in the proximal part of the primitive gut, and CDX2, expressed solely in the distal part. Aberrant expression of these factors may result in abnormal development and congenital abnormalities. Therefore, we analyzed the expression of SOX2 and CDX2 in a number of pediatric intestinal anomalies. We investigated the expression pattern of SOX2 and CDX2 in three congenital intestinal anomalies in which ectopic gastric tissue may be present, Meckel's diverticulum (N = 8), persistent ductus omphalomesentericus (N = 14), and intestinal duplications (N = 8). CDX2, but not SOX2, was detected in intestinal epithelial cells in tissue lacking gastric heteroplasia. In gastric-type heteroplasia, a reciprocal expression pattern existed between SOX2 and CDX2 in the gastric and intestinal tissues, respectively. Interestingly, patches of CDX2-positive cells were present within the gastric mucosa in a subset of Meckel's diverticula and intestinal duplications, suggesting that it is not the absence of CDX2, but rather the ectopic expression of SOX2 that leads to gastric tissue in the prospective intestinal tissue. This is in concordance with our previous mouse studies. Collectively, our data indicate that a fine balance between SOX2 and CDX2 expression in the gastrointestinal tract is essential for proper development and that ectopic expression of SOX2 may lead to malformations of the gut.

Raghoebir, L., K. Biermann, et al. "Aberrant SOX2 expression in colorectal cancers does not correlate with mucinous differentiation and gastric mucin MUC5AC expression." Virchows Arch.

Colorectal cancer (CRC) can be divided into non-mucinous and mucinous subtypes, of which the latter portends to have a worse clinical prognosis. A previous study suggested a putative link between SOX2 expression observed selectively in mucinous CRC and the induction of the gastric mucin MUC5AC. In this study, we re-evaluated the expression behavior of SOX2, MUC5AC, and CDX2 in both types of CRC. We performed immunohistochemical analysis on 90 cases of non-mucinous CRCs, 57 cases of mucinous CRCs, and 15 case-matched normal intestinal mucosa. In contrast to the previously suggested link between SOX2 and mucinous CRC, we observe aberrant expression of SOX2 at equal levels in both subtypes. Fluorescence in situ hybridization (FISH) analysis shows that expression is not attributed to genomic amplification. While SOX2 and CDX2 are normally expressed in a reciprocal manner, SOX2-positive tumor cells co-express CDX2. Furthermore, we show that MUC5AC is expressed independently of SOX2. In conclusion, we show that aberrant SOX2 expression is specifically linked neither to mucinous CRCs nor to the induction of MUC5AC, in contrast to previous suggestions.

Remaud, S., S. A. Lopez-Juarez, et al. "Inhibition of Sox2 Expression in the Adult Neural Stem Cell Niche In Vivo by Monocationic-based siRNA Delivery." Mol Ther Nucleic Acids **2**: e89.

RNA interference (RNAi) is a major tool for basic and applied investigations. However, obtaining RNAi data that have physiological significance requires investigation of regulations and therapeutic strategies in appropriate in vivo settings. To examine in vivo gene regulation and protein function in the adult neural stem cell (NSC) niche, we optimized a new non-viral vector for delivery of siRNA into the subventricular zone (SVZ). This brain region contains the neural stem and progenitor cells populations that express the stem cell marker, SOX2. Temporally and spatially controlled Sox2 knockdown was achieved using the monocationic lipid vector, IC10. siRNA/IC10 complexes were stable over time and smaller (<40 nm) than jetSi complexes (approximately 400 nm). Immunocytochemistry showed that siRNA/IC10 complexes efficiently target both the progenitor and stem cell populations in the adult SVZ. Injection of the complexes into the lateral brain ventricle resulted in specific knockdown of Sox2 in the SVZ. Furthermore, IC10-mediated transient in vivo knockdown of Sox2-modulated expression of several genes implicated in NSC

maintenance. Taken together, these data show that IC10 cationic lipid formulation can efficiently vectorize siRNA in a specific area of the adult mouse brain, achieving spatially and temporally defined loss of function.Molecular Therapy-Nucleic Acids (2013) 2, e89; doi:10.1038/mtna.2013.8; published online 23 April 2013.

Rizzino, A. "Concise review: The Sox2-Oct4 connection: critical players in a much larger interdependent network integrated at multiple levels." Stem Cells **31**(6): 1033-9.

The transcription factors Sox2 and Oct4 have been a major focus of stem cell biology since the discovery, more than 10 years ago, that they play critical roles during embryogenesis. Early work established that these two transcription factors work together to regulate genes required for the self-renewal and pluripotency of embryonic stem cells (ESC). Surprisingly, small changes (approximately twofold) in the levels of either Oct4 or Sox2 induce the differentiation of ESC. Consequently, ESC must maintain the levels of these two transcription factors within narrow limits. Genome-wide binding studies and unbiased proteomic screens have been conducted to decipher the complex roles played by Oct4 and Sox2 in the transcriptional circuitry of ESC. Together, these and other studies provide a comprehensive understanding of the molecular machinery that sustains the self-renewal of ESC and restrains their differentiation. Importantly, these studies paint a landscape in which Oct4 and Sox2 are part of a much larger interdependent network composed of many transcription factors that are interconnected at multiple levels of function.

Ruan, J., B. Wei, et al. "Predictive value of Sox2 expression in transurethral resection specimens in patients with T1 bladder cancer." Med Oncol **30**(1): 445.

Sox2 is thought to be an important regulator of self-renewal in embryonic stem cell. According to the cancer stem cell (CSC) theory, the overexpression of Sox2 is potentially involved in carcinogenesis and could affect tumor recurrence and metastasis. Previous study proved Sox2 might be prognostic marker for multiple human malignancies. The purpose of this study was to investigate the clinicopathological significance of Sox2 expression in human non-muscle-invasive bladder cancer. We examined Sox2 expression in 32 paired non-muscle-invasive bladder cancer tissues and adjacent non-cancerous tissues by quantitative real-time RT-PCR (qrtRT-PCR). In addition, we analyzed Sox2 and Ki-67 expression in 126 non-muscle-invasive bladder cancer samples and bladder cancer cell line T24 by immunohistochemistry and immunofluorescence assays. The recurrence-free survival was determined by Kaplan-Meier method and log-rank test. Cox regression was adopted for univariate and multivariate analyses of prognostic factors. The expression of Sox2 was significantly increased in non-muscle-invasive bladder cancer tissues. Sox2 expression was significantly correlated with that of Ki-67 ($P < 0.001$). The expression of Sox2 was significantly associated with tumor size ($P = 0.006$), tumor number ($P = 0.037$), and tumor grade ($P < 0.001$). Patients with high Sox2 expression had significantly poorer recurrence-free survival ($P = 0.0002$) when compared with patients with the low expression of Sox2. On multivariate analysis, Sox2 expression and tumor grade were found to be independent prognostic factors for recurrence-free survival ($P < 0.05$). Our data suggested for the first time that the high expression of Sox2 may contribute to the development of non-muscle-invasive bladder cancer and serve as a novel prognostic marker in patients with T1 bladder cancer.

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 **25**(12): 2734-42.

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.

Salem, N. J., M. Hempel, et al. "Anal atresia, coloboma, microphthalmia, and nasal skin tag in a female patient with 3.5 Mb deletion of 3q26 encompassing SOX2." Am J Med Genet A **161A**(6): 1421-4.

A full term female newborn presented with prominent forehead, bilateral microphthalmia, iris coloboma and cataract, wide intercanthal distance, large, low-set and protruding ears, skin tag at the left nasal nostril, imperforate anus with rectovestibular fistula, and postnatal growth delay with brachymicrocephaly. A marker chromosome was not detectable and the copy number of 22q11 was normal. However, array CGH revealed a 3.5 Mb microdeletion of chromosome region 3q26.32-3q26.33 (chr. 3: 178,598,162-182,114,483; hg19) which comprised the SOX2 gene. While SOX2 haploinsufficiency is known to cause microphthalmia and coloboma, it has not been described before in patients with anal atresia.

Santini, R., S. Pietrobono, et al. "SOX2 regulates self-renewal and tumorigenicity of human melanoma-initiating cells." Oncogene **0**.

Melanoma is one of the most aggressive types of human cancer, characterized by enhanced heterogeneity and resistance to conventional therapy at advanced stages. We and others have previously shown that HEDGEHOG-GLI (HH-GLI) signaling is required for melanoma growth and for survival and expansion of melanoma-initiating cells (MICs). Recent reports indicate that HH-GLI signaling regulates a set of genes typically expressed in embryonic stem cells, including SOX2 (sex-determining region Y (SRY)-Box2). Here we address the function of SOX2 in human melanomas and MICs and its interaction with HH-GLI signaling. We find that SOX2 is highly expressed in melanoma stem cells. Knockdown of SOX2 sharply decreases self-renewal in melanoma spheres and in putative melanoma stem cells with high aldehyde dehydrogenase activity (ALDHhigh). Conversely, ectopic expression of SOX2 in melanoma cells enhances their self-renewal in vitro. SOX2 silencing also inhibits cell growth and induces apoptosis in melanoma cells. In addition, depletion of SOX2 progressively abrogates tumor growth and leads to a significant decrease in tumor-initiating capability of ALDHhigh MICs upon xenotransplantation, suggesting that SOX2 is required for tumor initiation and for continuous tumor growth. We show that SOX2 is regulated by HH signaling and that the transcription factors GLI1 and GLI2, the downstream effectors of HH-GLI signaling, bind to the proximal promoter region of SOX2 in primary melanoma cells. In functional studies, we find that SOX2 function is required for HH-induced melanoma cell growth and MIC self-renewal in vitro. Thus SOX2 is a critical factor for self-renewal and tumorigenicity of MICs and an important mediator of HH-GLI signaling in melanoma. These findings could provide the basis for novel therapeutic strategies based on the inhibition of SOX2 for the

treatment of a subset of human melanomas.Oncogene advance online publication, 31 March 2014; doi:10.1038/onc.2014.71.

Schonitzer, V., R. Wirtz, et al. "Sox2 Is a Potent Inhibitor of Osteogenic and Adipogenic Differentiation in Human Mesenchymal Stem Cells." Cell Reprogram.

Abstract Human mesenchymal stem cells (hMSCs) are a promising target for cell-based bone regeneration. However, their application for clinical use is limited because hMSCs lose their ability for cell division and differentiation during longer in vitro cultivation. The osteogenic differentiation is regulated through a complex network of molecular signal transduction pathways where the canonical Wnt pathway plays an important role. Sox2, a known key factor for maintenance of cellular pluripotency in stem cells, is supposed to influence the Wnt pathway in osteoblasts. In this study, we overexpressed Sox2 in immortalized hMSCs by lentiviral gene transfer. Sox2 overexpression significantly reduced the osteogenic and adipogenic differentiation potentials. This effect was abolished by knockdown of Sox2 overexpression. In addition, Oct4 and Nanog, other key transcription factors for pluripotency, are strongly upregulated when Sox2 is overexpressed. Furthermore, Dkk1, a target gene of the Sox2-Oct4 heterodimer and a Wnt antagonist, is downregulated. Sox2 overexpression causes higher expression levels of beta-catenin, the central transcription factor of the canonical Wnt pathway. These results suggest that Sox2 keeps hMSCs in an undifferentiated state by influencing the canonical Wnt pathway. Regulated expression of Sox2 may be a promising tool to cultivate hMSCs in sufficient quantities for cell and gene therapy applications.

Schrock, A., M. Bode, et al. "Expression and role of the embryonic protein SOX2 in head and neck squamous cell carcinoma." Carcinogenesis **35**(7): 1636-42.

Recently, SOX2 has been identified as a potential lineage-specific oncogene in lung squamous cell carcinomas. Since head and neck squamous cell carcinomas (HNSCC) are morphologically and clinically highly related to lung squamous cell carcinomas, we hypothesized that SOX2 also plays an oncogenic role in this tumor entity. We assembled a cohort of 496 patients with HNSCC, including 253 metastases and 135 recurrences. SOX2 amplification (FISH) and SOX2 protein expression (immunohistochemistry) were correlated with molecular and clinicopathological parameters. In order to investigate the functional role of SOX2 in human HNSCC, SOX2 knockdown and overexpression in SCC-25 cells were generated by lentiviral constructs and subjected to cell cycle analysis, proliferation and apoptosis assays. Furthermore, SOX2 expression was correlated with the expression of proliferation and apoptosis-related proteins in primary HNSCC samples. SOX2 amplification was detected in 21% of primary HNSCC and mostly observed in a concordant manner between primary tumors and corresponding metastatic tissues. Overall, SOX2 amplification resulted in protein overexpression and was mutually exclusive with human papillomavirus infection. SOX2 protein overexpression was associated with clinicopathological parameters of worse outcome. Functionally, SOX2 induced the expression of the antiapoptotic protein BCL-2 and enhanced resistance to apoptosis-inducing agents including cisplatin, indicating SOX2 as a mediator of therapy resistance in human HNSCC. Targeting SOX2 and related molecular downstream pathways such as BCL-2 may enhance therapy efficacy in SOX2-expressing HNSCC.

Suzuki, J., N. Azuma, et al. "Mutation spectrum and phenotypic variation in nine patients with SOX2 abnormalities." J Hum Genet **59**(6): 353-6.

Multiple mutations in SOX2 have been identified in patients with ocular anomalies and/or pituitary dysfunction. Here, we identified SOX2 abnormalities in nine patients. The molecular defects included one missense, one nonsense and four frameshift mutations, and three submicroscopic deletions involving SOX2. Three of the six mutations and all deletions were hitherto unreported. The breakpoints determined in one deletion were located within Alu repeats and accompanied by an overlap of 11 bp. Three of the six mutations encoded SOX2 proteins that lacked in vitro transactivation activity for the HESX1 promoter, whereas the remaining three generated proteins with approximately 15-approximately 20% of transactivation activity. All cases manifested ocular anomalies of various severities, together with several complications including arachnoid cyst and hamartoma. There was no apparent correlation between the residual activity and clinical severity. The results indicate that molecular defects in SOX2 are highly variable and include Alu repeat-mediated genomic rearrangements. Our data provide further evidence for wide phenotypic variation of SOX2 abnormalities and the lack of genotype-phenotype correlation in patients carrying SOX2 lesions.

Takagi, M., S. Narumi, et al. "A novel mutation in SOX2 causes hypogonadotropic hypogonadism with mild ocular malformation." Horm Res Paediatr **81**(2): 133-8.

BACKGROUND: Heterozygous SOX2 mutations have been reported to cause isolated hypogonadotropic hypogonadism (HH) in addition to ocular and brain abnormalities. OBJECTIVE: We report a novel missense SOX2 (Y110C) mutation in an HH patient with mild ocular malformation. PATIENTS: The 20-year-old male was referred because of typical signs of complete hypogonadism, with small intrascrotal testes (2 ml), no pubic hair (P1), and a micropenis. Hormone assays revealed very low plasma testosterone levels and very low levels of plasma gonadotropin. He was found to have retinal detachment in his right eye and surgery was performed at the age of 14 years. RESULTS: Using a next-generation sequencing strategy, we identified a novel heterozygous SOX2 mutation, c.329A>G (p.Y110C). Y110C SOX2 had reduced transactivation and no dominant negative effect. Subcellular localization revealed no significant difference between wild-type and mutant SOX2. EMSA experiments showed that the Y110C SOX2 abrogated DNA-binding ability. CONCLUSION: The Y110C mutation affects a critical residue in the SOX2 protein. This study extends our understanding of the phenotypic features, molecular mechanism, and developmental course associated with mutations in SOX2. When multiple genes need to be analyzed for mutations simultaneously, targeted sequence analysis of interesting genomic regions is an attractive approach.

Toschi, L., G. Finocchiaro, et al. "Increased SOX2 gene copy number is associated with FGFR1 and PIK3CA gene gain in non-small cell lung cancer and predicts improved survival in early stage disease." PLoS One **9**(4): e95303.

BACKGROUND: We aimed to investigate prevalence and prognostic role of SOX2, PIK3CA, FGFR1 and BRF2 gene gain in patients with surgically resected non-small cell lung cancer (NSCLC). METHODS: SOX2, PIK3CA, FGFR1 and BRF2 gene copy number was assessed by fluorescence in situ hybridization (FISH) in arrayed tissue cores from 447 resected NSCLCs. RESULTS: Increased gene copy number (FISH+) for SOX2, PIK3CA, FGFR1 and BRF2 was observed in 23.6%, 29.2%, 16.6% and 14.9% of cases, respectively. FISH+ status for each gene was significantly associated with smoking history, squamous cell carcinoma (SCC) histology, and increased copy number of the other studied genes. Multivariate analysis of overall survival indicated increased SOX2 gene copy number ($P = 0.008$), stage I-II ($P<0.001$), and adenocarcinoma or SCC histology ($P = 0.016$) as independent, favorable prognostic factors. A statistically significant interaction was observed between stage and SOX2 gene status ($P = 0.021$), indicating that the prognostic impact of SOX2 gene gain differs across stages and is limited to patients with stage I-II disease (HR 0.44, 95% CI: 0.25-0.77; $P = 0.004$, adjusted for histology). CONCLUSIONS: Increased SOX2 gene copy number is an independent and favorable prognostic factor in surgically resected, early stage NSCLC, regardless of histology. SOX2, PIK3CA, FGFR1 and BRF2 gene gains are likely to occur concurrently, with

potentially relevant implications for the development of new therapeutic strategies.

Trohatou, O., D. Zagoura, et al. "Sox2 suppression by miR-21 governs human mesenchymal stem cell properties." Stem Cells Transl Med **3**(1): 54-68.

MicroRNAs (miRNAs) have recently been shown to act as regulatory signals for maintaining stemness and for determining the fate of adult and fetal stem cells, such as human mesenchymal stem cells (hMSCs). hMSCs constitute a population of multipotent stem cells that can be expanded easily in culture and are able to differentiate into many lineages. We have isolated two subpopulations of fetal mesenchymal stem cells (MSCs) from amniotic fluid (AF) known as spindle-shaped (SS) and round-shaped (RS) cells and characterized them on the basis of their phenotypes, pluripotency, proliferation rates, and differentiation potentials. In this study, we analyzed the miRNA profile of MSCs derived from AF, bone marrow (BM), and umbilical cord blood (UCB). We initially identified 67 different miRNAs that were expressed in all three types of MSCs but at different levels, depending on the source. A more detailed analysis revealed that miR-21 was expressed at higher levels in RS-AF-MSCs and BM-MSCs compared with SS-AF-MSCs. We further demonstrated for the first time a direct interaction between miR-21 and the pluripotency marker Sox2. The induction of miR-21 strongly inhibited Sox2 expression in SS-AF-MSCs, resulting in reduced clonogenic and proliferative potential and cell cycle arrest. Strikingly, the opposite effect was observed upon miR-21 inhibition in RS-AF-MSCs and BM-MSCs, which led to an enhanced proliferation rate. Finally, miR-21 induction accelerated osteogenesis and impaired adipogenesis and chondrogenesis in SS-AF-MSCs. Therefore, these findings suggest that miR-21 might specifically function by regulating Sox2 expression in human MSCs and might also act as a key molecule determining MSC proliferation and differentiation.

Trowe, M. O., L. Zhao, et al. "Inhibition of Sox2-dependent activation of Shh in the ventral diencephalon by Tbx3 is required for formation of the neurohypophysis." Development **140**(11): 2299-309.

Tbx2 and Tbx3 are two highly related members of the T-box transcription factor gene family that regulate patterning and differentiation of a number of tissue rudiments in the mouse. Both genes are partially co-expressed in the ventral diencephalon and the infundibulum; however, a functional requirement in murine pituitary development has not been reported. Here, we show by genetic lineage tracing that Tbx2(+) cells constitute the precursor population of the neurohypophysis. However, Tbx2 is dispensable for neurohypophysis development as revealed by normal formation of this organ in Tbx2-deficient mice. By contrast, loss of Tbx3 from the ventral diencephalon results in a failure to establish the Tbx2(+) domain in this region, and a lack of evagination of the infundibulum and formation of the neurohypophysis. Rathke's pouch is severely hypoplastic, exhibits defects in dorsoventral patterning, and degenerates after E12.5. In Tbx3-deficient embryos, the ventral diencephalon is hyperproliferative and displays an abnormal cellular architecture, probably resulting from a failure to repress transcription of Shh. We further show that Tbx3 and Tbx2 repress Shh by sequestering the SRY box-containing transcription factor Sox2 away from a Shh forebrain enhancer (SBE2), thus preventing its activation. These data suggest that Tbx3 is required in the ventral diencephalon to establish a Shh(-) domain to allow formation of the infundibulum.

Vanner, R. J., M. Remke, et al. "Quiescent sox2(+) cells drive hierarchical growth and relapse in sonic hedgehog subgroup medulloblastoma." Cancer Cell **26**(1): 33-47.

Functional heterogeneity within tumors presents a significant therapeutic challenge. Here we show that quiescent, therapy-resistant Sox2(+) cells propagate sonic hedgehog subgroup medulloblastoma by a mechanism that mirrors a neurogenic program. Rare Sox2(+) cells produce rapidly cycling doublecortin(+) progenitors that, together with their postmitotic progeny expressing NeuN, comprise tumor bulk. Sox2(+) cells are enriched following anti-mitotic chemotherapy and Smoothened inhibition, creating a reservoir for tumor regrowth. Lineage traces from Sox2(+) cells increase following treatment, suggesting that this population is responsible for relapse. Targeting Sox2(+) cells with the antineoplastic mithramycin abrogated tumor growth. Addressing functional heterogeneity and eliminating Sox2(+) cells presents a promising therapeutic paradigm for treatment of sonic hedgehog subgroup medulloblastoma.

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 **12**(22): 3471-7.

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 E 2/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 E 2/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 E 2 signaling that leads to nuclear phospho-Ser118-ERalpha, which ultimately exacerbates genomic ER signaling in response to E 2. Because E 2 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 E 2 to increase breast CSC activity may have profound implications for the development of new CSC-directed strategies for breast cancer prevention and therapy.

Velcheti, V., K. Schalper, et al. "High SOX2 levels predict better outcome in non-small cell lung carcinomas." PLoS One **8**(4): e61427.

BACKGROUND: SOX2 is an embryonic developmental transcription factor, which is important in the development of the respiratory tract. SOX2 overexpression is associated with aggressive disease in several tumor types. However, SOX2 overexpression and gene amplification associates with favorable outcome in lung squamous cell carcinomas (SCC) and dissimilar results have been reported in lung adenocarcinomas (ADC). The aim of the present study was to evaluate SOX2 expression in NSCLC and determine

the relationship with clinico-pathological variables and outcome. METHODS: SOX2 protein levels were measured in tissue microarrays (TMAs) containing FFPE samples from two independent lung cancer cohorts (n = 340 & 307) using automated quantitative immunofluorescence (QIF). Assay validation was performed using FFPE preparations of cell lines with known SOX2 expression. Associations of SOX2 levels with main clinico-pathological characteristics and with overall survival were studied using uni-and multivariate analysis. RESULTS: SOX2 levels were higher in patients with SCC than in ADC in both cohorts (p value<0.0001). In the training cohort, NSCLC patients whose tumors showed high SOX2 (n = 245) had longer survival than those with low SOX2 levels (log rank p = 0.0002).

Vencken, S. F., P. Sethupathy, et al. "An integrated analysis of the SOX2 microRNA response program in human pluripotent and nullipotent stem cell lines." BMC Genomics **15**(1): 711.

BACKGROUND: SOX2 is a core component of the transcriptional network responsible for maintaining embryonal carcinoma cells (ECCs) in a pluripotent, undifferentiated state of self-renewal. As such, SOX2 is an oncogenic transcription factor and crucial cancer stem cell (CSC) biomarker in embryonal carcinoma and, as more recently found, in the stem-like cancer cell component of many other malignancies. SOX2 is furthermore a crucial factor in the maintenance of adult stem cell phenotypes and has additional roles in cell fate determination. The SOX2-linked microRNA (miRNA) transcriptome and regulome has not yet been fully defined in human pluripotent cells or CSCs. To improve our understanding of the SOX2-linked miRNA regulatory network as a contribution to the phenotype of these cell types, we used high-throughput differential miRNA and gene expression analysis combined with existing genome-wide SOX2 chromatin immunoprecipitation (ChIP) data to map the SOX2 miRNA transcriptome in two human embryonal carcinoma cell (hECC) lines. RESULTS: Whole-microRNAome and genome analysis of SOX2-silenced hECCs revealed many miRNAs regulated by SOX2, including several with highly characterised functions in both cancer and embryonic stem cell (ESC) biology. We subsequently performed genome-wide differential expression analysis and applied a Monte Carlo simulation algorithm and target prediction to identify a SOX2-linked miRNA regulome, which was strongly enriched with epithelial-to-mesenchymal transition (EMT) markers. Additionally, several deregulated miRNAs important to EMT processes had SOX2 binding sites in their promoter regions. CONCLUSION: In ESC-like CSCs, SOX2 regulates a large miRNA network that regulates and interlinks the expression of crucial genes involved in EMT.

Weber, F. A., G. Bartolomei, et al. "Artd1/Parp1 regulates reprogramming by transcriptional regulation of Fgf4 via Sox2 ADP-ribosylation." Stem Cells **31**(11): 2364-73.

The recently established reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) by Takahashi and Yamanaka represents a valuable tool for future therapeutic applications. To date, the mechanisms underlying this process are still largely unknown. In particular, the mechanisms how the Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc) directly drive reprogramming and which additional components are involved are still not yet understood. In this study, we aimed at analyzing the role of ADP-ribosyltransferase diphtheria toxin-like one (Artd1; formerly called poly(ADP-ribose) polymerase 1 [Parp1]) during reprogramming. We found that poly(ADP-ribosylation) (PARylation) of the reprogramming factor Sox2 by Artd1 plays an important role during the first days upon transduction with the reprogramming factors. A process that happens before Artd1 in conjunction with 10-11 translocation-2 (Tet2) mediates the histone modifications necessary for the establishment of an activated chromatin state at pluripotency loci (e.g., Nanog and Essrb) [Nature 2012;488:652-655]. Wild-type (WT) fibroblasts treated with an Artd1 inhibitor as well as fibroblasts deficient for Artd1 (Artd1-/-) show strongly decreased reprogramming capacity. Our data indicate that Artd1-mediated PARylation of Sox2 favors its binding to the fibroblast growth factor 4 (Fgf4) enhancer, thereby activating Fgf4 expression. The importance of Fgf4 during the first 4 days upon initiation of reprogramming was also highlighted by the observation that exogenous addition of Fgf4 was sufficient to restore the reprogramming capacity of Artd1-/- fibroblast to WT levels. In conclusion, our data clearly show that the interaction between Artd1 and Sox2 is crucial for the first steps of the reprogramming process and that early expression of Fgf4 (day 2 to day 4) is an essential component for the successful generation of iPSCs.

Weina, K. and J. Utikal "SOX2 and cancer: current research and its implications in the clinic." Clin Transl Med **3**: 19.

SOX2 is a gene that encodes for a transcription factor belonging to the SOX gene family and contains a high-mobility group (HMG) domain, which permits highly specific DNA binding. Consequently, SOX2 functions as an activator or suppressor of gene transcription. SOX2 has been described as an essential embryonic stem cell gene and moreover, a necessary factor for induced cellular reprogramming. SOX2 research has only recently switched focus from embryogenesis and development to SOX2's function in disease. Particularly, the role of SOX2 in cancer pathogenesis has become of interest in the field. To date, studies have shown SOX2 to be amplified in various cancer types and affect cancer cell physiology via involvement in complicated cell signaling and protein-protein interactions. Recent reviews in this field have highlighted SOX2 in mammalian physiology, development and pathology. In this review, we comprehensively compile what is known to date about SOX2's involvement in cancer biology, focusing on the most recent findings in the fields of cellular signaling and cancer stem cells. Lastly, we underscore the role of SOX2 in the clinic and highlight new findings, which may provide novel clinical applications for SOX2 as a prognostic marker, indicator of metastasis, biomarker or potential therapeutic target in some cancer types.

Whitney, I. E., P. W. Keeley, et al. "Sox2 regulates cholinergic amacrine cell positioning and dendritic stratification in the retina." J Neurosci **34**(30): 10109-21.

The retina contains two populations of cholinergic amacrine cells, one positioned in the ganglion cell layer (GCL) and the other in the inner nuclear layer (INL), that together comprise approximately 1/2 of a percent of all retinal neurons. The present study examined the genetic control of cholinergic amacrine cell number and distribution between these two layers. The total number of cholinergic amacrine cells was quantified in the C57BL/6J and A/J inbred mouse strains, and in 25 recombinant inbred strains derived from them, and variations in their number and ratio (GCL/INL) across these strains were mapped to genomic loci. The total cholinergic amacrine cell number was found to vary across the strains, from 27,000 to 40,000 cells, despite little variation within individual strains. The number of cells was always lower within the GCL relative to the INL, and the sizes of the two populations were strongly correlated, yet there was variation in their ratio between the strains. Approximately 1/3 of that variation in cell ratio was mapped to a locus on chromosome 3, where Sex determining region Y box 2 (Sox2) was identified as a candidate gene due to the presence of a 6-nucleotide insertion in the protein-coding sequence in C57BL/6J and because of robust and selective expression in cholinergic amacrine cells. Conditionally deleting Sox2 from the population of nascent cholinergic amacrine cells perturbed the normal ratio of cells situated in the GCL versus the INL and induced a bistratifying morphology, with dendrites distributed to both ON and OFF strata within the inner plexiform layer.

Zimmerman, D. L., C. S. Boddy, et al. "Oct4/Sox2 binding sites contribute to maintaining hypomethylation of the maternal igf2/h19 imprinting control region." PLoS One **8**(12): e81962.

A central question in genomic imprinting is how parental-specific DNA methylation of imprinting control regions (ICR) is established during gametogenesis and maintained after fertilization. At the imprinted Igf2/H19 locus, CTCF binding maintains the unmethylated state of the maternal ICR after the blastocyst stage. In addition, evidence from Beckwith-Wiedemann patients and cultured mouse cells suggests that two Sox-Oct binding motifs within the Igf2/H19 ICR also participate in maintaining hypomethylation of the maternal allele. We found that the Sox and octamer elements from both Sox-Oct motifs were required to drive hypomethylation of integrated transgenes in mouse embryonic carcinoma cells. Oct4 and Sox2 showed cooperative binding to the Sox-Oct motifs, and both were present at the endogenous ICR. Using a mouse with mutations in the Oct4 binding sites, we found that maternally transmitted mutant ICRs acquired partial methylation in somatic tissues, but there was little effect on imprinted expression of H19 and Igf2. A subset of mature oocytes also showed partial methylation of the mutant ICR, which suggested that the Sox-Oct motifs provide some protection from methylation during oogenesis. The Sox-Oct motifs, however, were not required for erasure of paternal methylation in primordial germ cells, which indicated that the oocyte methylation was acquired post-natally. Maternally inherited mutant ICRs were unmethylated in blastocysts, which suggested that at least a portion of the methylation in somatic tissues occurred after implantation. These findings provide evidence that Sox-Oct motifs contribute to ICR hypomethylation in post-implantation embryos and maturing oocytes and link imprinted DNA methylation with key stem cell/germline transcription factors.

References

1. National Center for Biotechnology Information, U.S. National Library of Medicine**.** http://www.ncbi.nlm.nih.gov/pubmed. 2014.
2. Wikipedia. The free encyclopedia. http://en.wikipedia.org. 2014.
3. Adachi, K., I. Nikaido, et al. "Context-dependent wiring of Sox2 regulatory networks for self-renewal of embryonic and trophoblast stem cells." Mol Cell **52**(3): 380-92.
4. Ahlfeld, J., R. Favaro, et al. "Sox2 requirement in sonic hedgehog-associated medulloblastoma." Cancer Res **73**(12): 3796-807.
5. Aksoy, I., R. Jauch, et al. "Oct4 switches partnering from Sox2 to Sox17 to reinterpret the enhancer code and specify endoderm." Embo J **32**(7): 938-53.
6. Aktug, H., A. Uysal, et al. "The detrimental effects of diabetes on pluripotency determined by KLF4, SOX2, C-MYC and OCT4 immunoreactivity in rat testes." Adv Clin Exp Med **22**(3): 327-35.
7. Albayrak, C., W. C. Yang, et al. "Pluripotency transcription factor Sox2 is strongly adsorbed by heparin but requires a protein transduction domain for cell internalization." Biochem Biophys Res Commun **431**(3): 641-5.
8. Amini, S., F. Fathi, et al. "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.
9. Andoniadou, C. L., D. Matsushima, et al. "Sox2(+) stem/progenitor cells in the adult mouse pituitary support organ homeostasis and have tumor-inducing potential." Cell Stem Cell **13**(4): 433-45.
10. Asadi, M. H., A. Derakhshani, et al. "Concomitant upregulation of nucleostemin and downregulation of Sox2 and Klf4 in gastric adenocarcinoma." Tumour Biol **35**(7): 7177-85.
11. Askarian-Amiri, M. E., V. Seyfoddin, et al. "Emerging role of long non-coding RNA SOX2OT in SOX2 regulation in breast cancer." PLoS One **9**(7): e102140.
12. Berezovsky, A. D., L. M. Poisson, et al. "Sox2 promotes malignancy in glioblastoma by regulating plasticity and astrocytic differentiation." Neoplasia **16**(3): 193-206, 206 e19-25.
13. 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 **66**(9): 819-22.
14. Brafman, D. A., N. Moya, et al. "Analysis of SOX2-Expressing Cell Populations Derived from Human Pluripotent Stem Cells." Stem Cell Reports **1**(5): 464-78.
15. Brustmann, H. and A. Brunner "Immunohistochemical expression of SOX2 in vulvar intraepithelial neoplasia and squamous cell carcinoma." Int J Gynecol Pathol **32**(3): 323-8.
16. Campolo, F., M. Gori, et al. "Essential role of Sox2 for the establishment and maintenance of the germ cell line." Stem Cells **31**(7): 1408-21.
17. Carina, V., G. Zito, et al. "Multiple pluripotent stem cell markers in human anaplastic thyroid cancer: the putative upstream role of SOX2." Thyroid **23**(7): 829-37.
18. Corominas-Faja, B., S. Cufí, 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 **12**(18): 3109-24.
19. Dhodapkar, K. M., S. N. Gettinger, et al. "SOX2-specific adaptive immunity and response to immunotherapy in non-small cell lung cancer." Oncoimmunology **2**(7): e25205.
20. 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 **85**(1): 1-6.
21. Eini, R., H. Stoop, et al. "Role of SOX2 in the etiology of embryonal carcinoma, based on analysis of the NCCIT and NT2 cell lines." PLoS One **9**(1): e83585.
22. Evsen, L., S. Sugahara, et al. "Progression of neurogenesis in the inner ear requires inhibition of Sox2 transcription by neurogenin1 and neurod1." J Neurosci **33**(9): 3879-90.
23. Forghanifard, M. M., S. Ardalan Khales, et al. "Stemness state regulators SALL4 and SOX2 are involved in progression and invasiveness of esophageal squamous cell carcinoma." Med Oncol **31**(4): 922.
24. Gagliardi, A., N. P. Mullin, et al. "A direct physical interaction between Nanog and Sox2 regulates embryonic stem cell self-renewal." Embo J **32**(16): 2231-47.
25. Galatro, T. F., M. Uno, et al. "Differential expression of ID4 and its association with TP53 mutation, SOX2, SOX4 and OCT-4 expression levels." PLoS One **8**(4): e61605.
26. Gonzalez-Marquez, R., J. L. Llorente, et al. "SOX2 expression in hypopharyngeal, laryngeal, and sinonasal squamous cell carcinoma." Hum Pathol **45**(4): 851-7.
27. Hoffmann, S. A., D. Hos, et al. "Stem cell factor Sox2 and its close relative Sox3 have differentiation functions in oligodendrocytes." Development **141**(1): 39-50.
28. Honing, J., K. V. Pavlov, et al. "Loss of CD44 and SOX2 Expression is Correlated with a Poor Prognosis in Esophageal Adenocarcinoma Patients." Ann Surg Oncol.
29. Hutz, K., R. Mejias-Luque, et al. "The stem cell factor SOX2 regulates the tumorigenic potential in human gastric cancer cells." Carcinogenesis **35**(4): 942-50.
30. Ilieva, M. and M. Dufva "SOX2 and OCT4 mRNA-expressing cells, detected by molecular beacons, localize to the center of neurospheres during differentiation." PLoS One **8**(8): e73669.
31. Justilien, V., M. P. Walsh, et al. "The PRKCI and SOX2 oncogenes are coamplified and cooperate to activate Hedgehog signaling in lung squamous cell carcinoma." Cancer Cell **25**(2): 139-51.
32. Kallas, A., M. Pook, et al. "SOX2 Is Regulated Differently from NANOG and OCT4 in Human Embryonic Stem Cells during Early Differentiation Initiated with Sodium Butyrate." Stem Cells Int **2014**: 298163.
33. Koike, T., T. Wakabayashi, et al. "Sox2 in the adult rat sensory nervous system." Histochem Cell Biol **141**(3): 301-9.

34. Kokalj Vokac, N., B. Cizmarevic, et al. "An evaluation of SOX2 and hTERC gene amplifications as screening markers in oral and oropharyngeal squamous cell carcinomas." Mol Cytogenet **7**(1): 5.
35. Kregel, S., K. J. Kiriluk, et al. "Sox2 is an androgen receptor-repressed gene that promotes castration-resistant prostate cancer." PLoS One **8**(1): e53701.
36. Langer, L., K. Sulik, et al. "Cleft Palate in a Mouse Model of SOX2 Haploinsufficiency." Cleft Palate Craniofac J **51**(1): 110-4.
37. Ma, K., X. Pan, et al. "Loss of miR-638 in vitro promotes cell invasion and a mesenchymal-like transition by influencing SOX2 expression in colorectal carcinoma cells." Mol Cancer **13**: 118.
38. Macchiaroli, A., D. Kelberman, et al. "A novel heterozygous SOX2 mutation causing congenital bilateral anophthalmia, hypogonadotropic hypogonadism and growth hormone deficiency." Gene **534**(2): 282-5.
39. Malecki, M., X. Tombokan, et al. "TRA-1-60, SSEA-4, POU5F1, SOX2, NANOG Clones of Pluripotent Stem Cells in the Embryonal Carcinomas of the Testes." J Stem Cell Res Ther **3**(1).
40. Mukhopadhyay, A., K. C. Berrett, et al. "Sox2 cooperates with Lkb1 loss in a mouse model of squamous cell lung cancer." Cell Rep **8**(1): 40-9.
41. Ochieng, J. K., K. Schilders, et al. "Sox2 regulates the emergence of lung Basal cells by directly activating the transcription of trp63." Am J Respir Cell Mol Biol **51**(2): 311-22.
42. Ogai, K., K. Nakatani, et al. "Function of Sox2 in ependymal cells of lesioned spinal cords in adult zebrafish." Neurosci Res.
43. Ogony, J. W., E. Malahias, et al. "Ethanol alters the balance of Sox2, Oct4, and Nanog expression in distinct subpopulations during differentiation of embryonic stem cells." Stem Cells Dev **22**(15): 2196-210.
44. Oliver-De La Cruz, J., J. Carrion-Navarro, et al. "SOX2+ cell population from normal human brain white matter is able to generate mature oligodendrocytes." PLoS One **9**(6): e99253.
45. Ormsbee Golden, B. D., E. L. Wuebben, et al. "Sox2 expression is regulated by a negative feedback loop in embryonic stem cells that involves AKT signaling and FoxO1." PLoS One **8**(10): e76345.
46. Popowski, M., T. D. Templeton, et al. "Bright/Arid3A Acts as a Barrier to Somatic Cell Reprogramming through Direct Regulation of Oct4, Sox2, and Nanog." Stem Cell Reports **2**(1): 26-35.
47. Raghoebir, L., K. Biermann, et al. "Aberrant SOX2 expression in colorectal cancers does not correlate with mucinous differentiation and gastric mucin MUC5AC expression." Virchows Arch.
48. Raghoebir, L., K. Biermann, et al. "Disturbed balance between SOX2 and CDX2 in human vitelline duct anomalies and intestinal duplications." Virchows Arch **462**(5): 515-22.
49. Remaud, S., S. A. Lopez-Juarez, et al. "Inhibition of Sox2 Expression in the Adult Neural Stem Cell Niche In Vivo by Monocationic-based siRNA Delivery." Mol Ther Nucleic Acids **2**: e89.
50. Rizzino, A. "Concise review: The Sox2-Oct4 connection: critical players in a much larger interdependent network integrated at multiple levels." Stem Cells **31**(6): 1033-9.
51. Ruan, J., B. Wei, et al. "Predictive value of Sox2 expression in transurethral resection specimens in patients with T1 bladder cancer." Med Oncol **30**(1): 445.
52. 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 **25**(12): 2734-42.
53. Salem, N. J., M. Hempel, et al. "Anal atresia, coloboma, microphthalmia, and nasal skin tag in a female patient with 3.5 Mb deletion of 3q26 encompassing SOX2." Am J Med Genet A **161A**(6): 1421-4.
54. Santini, R., S. Pietrobono, et al. "SOX2 regulates self-renewal and tumorigenicity of human melanoma-initiating cells." Oncogene **0**.
55. Schonitzer, V., R. Wirtz, et al. "Sox2 Is a Potent Inhibitor of Osteogenic and Adipogenic Differentiation in Human Mesenchymal Stem Cells." Cell Reprogram.
56. Schrock, A., M. Bode, et al. "Expression and role of the embryonic protein SOX2 in head and neck squamous cell carcinoma." Carcinogenesis **35**(7): 1636-42.
57. Suzuki, J., N. Azuma, et al. "Mutation spectrum and phenotypic variation in nine patients with SOX2 abnormalities." J Hum Genet **59**(6): 353-6.
58. Takagi, M., S. Narumi, et al. "A novel mutation in SOX2 causes hypogonadotropic hypogonadism with mild ocular malformation." Horm Res Paediatr **81**(2): 133-8.
59. Toschi, L., G. Finocchiaro, et al. "Increased SOX2 gene copy number is associated with FGFR1 and PIK3CA gene gain in non-small cell lung cancer and predicts improved survival in early stage disease." PLoS One **9**(4): e95303.
60. Trohatou, O., D. Zagoura, et al. "Sox2 suppression by miR-21 governs human mesenchymal stem cell properties." Stem Cells Transl Med **3**(1): 54-68.
61. Trowe, M. O., L. Zhao, et al. "Inhibition of Sox2-dependent activation of Shh in the ventral diencephalon by Tbx3 is required for formation of the neurohypophysis." Development **140**(11): 2299-309.
62. Vanner, R. J., M. Remke, et al. "Quiescent sox2(+) cells drive hierarchical growth and relapse in sonic hedgehog subgroup medulloblastoma." Cancer Cell **26**(1): 33-47.
63. 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 **12**(22): 3471-7.
64. Velcheti, V., K. Schalper, et al. "High SOX2 levels predict better outcome in non-small cell lung carcinomas." PLoS One **8**(4): e61427.
65. Vencken, S. F., P. Sethupathy, et al. "An integrated analysis of the SOX2 microRNA response program in human pluripotent and nullipotent stem cell lines." BMC Genomics **15**(1): 711.
66. Weber, F. A., G. Bartolomei, et al. "Artd1/Parp1 regulates reprogramming by transcriptional regulation of Fgf4 via Sox2 ADP-ribosylation." Stem Cells **31**(11): 2364-73.
67. Weina, K. and J. Utikal "SOX2 and cancer: current research and its implications in the clinic." Clin Transl Med **3**: 19.
68. Whitney, I. E., P. W. Keeley, et al. "Sox2 regulates cholinergic amacrine cell positioning and dendritic stratification in the retina." J Neurosci **34**(30): 10109-21.
69. Zimmerman, D. L., C. S. Boddy, et al. "Oct4/Sox2 binding sites contribute to maintaining hypomethylation of the maternal igf2/h19 imprinting control region." PLoS One **8**(12): e81962.