The octamer-binding transcription factor 4 (OCT4) and cancer literatures

Ma Hongbao [1], Margaret Young [2]

[1] Brookdale Hospital, Brooklyn, NY 11212, USA; [2] Cambridge, MA 02138, USA
ma8080@gmail.com

Abstract: Octamer-binding transcription factor 4 (*OCT4*) is one of the key regulatory genes that maintains the pluripotency and self-renewal properties of stem cells, and it can function as oncogene in cancers. There is negative correlation between levels of BAK1 and OCT4, and positive between OCT4 and miR-125b in primary cervical cancers. 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.
[Ma H, Young M. **The octamer-binding transcription factor 4 (OCT4) and cancer literatures.** *Cancer Biology* 2014;4(3):56-65]. (ISSN: 2150-1041). http://www.cancerbio.net. 7

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

1. Introduction

Octamer-binding transcription factor 4 (*OCT4*) is one of the key regulatory genes that maintains the pluripotency and self-renewal properties of stem cells, and it can function as oncogene in cancers. There is negative correlation between levels of BAK1 and OCT4, and positive between OCT4 and miR-125b in primary cervical cancers.

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 the octamer-binding transcription factor 4 (OCT4) literatures.

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. 2014 Mar;47(1):1-11. doi: 10.5115/acb.2014.47.1.1. Epub 2014 Mar 13.

Uncontrolled self-renewal plays a direct function in the progression of different types of carcinomas. The same molecular pathway that manages self-renewal in normal stem cells also seems to manage cancer stem cells. Here, we examine the expressions of self-renewal regulatory factors Oct4, Nanog, Sox2, nucleostemin, Zfx, Esrrb, 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.

Asadi, M. H., S. J. Mowla, et al. "OCT4B1, a novel spliced variant of OCT4, is highly expressed in gastric cancer and acts as an antiapoptotic factor." Int J Cancer. 2011 Jun 1;128(11):2645-52. doi: 10.1002/ijc.25643. Epub 2010 Nov 3.

The octamer-binding transcription factor 4 (OCT4) is involved in regulating pluripotency and self-renewal maintenance of embryonic stem cells. Recently, misexpression of OCT4 has been also reported in some adult stem as well as cancer cells; a finding which is still controversial. In addition to the previously described spliced variants of the gene (e.g., OCT4A and OCT4B), we have recently identified a novel variant of the gene, designated as OCT4-B1. In this study, we investigated a potential expression and function of OCT4B1 in a series of gastric cancer tissues and a gastric adenocarcinoma cell line, AGS. Using the Taqman real-time PCR approach, we have detected the expression of OCT4B1 in tumors with no or much lower expression in marginal samples of the same patients ($p < 0.002$). We have also analyzed the effects of OCT4B1 knock-down in AGS cell line treated with specific siRNA directed toward OCT4B1. Our data revealed that interfering with the expression of OCT4B1 caused profound changes in the morphology and cell cycle distribution of the cells. Furthermore, down-regulation of OCT4B1 significantly elevated the relative activity of caspase-3/caspase-7 and the rate of apoptosis in the cells (more than 30%). All together, our findings suggest that OCT4B1 has a potential role in tumorigenesis of gastric cancer and candidates the variant as a new tumor marker with potential value in diagnosis and treatment of gastric cancer.

Bourguignon, L. Y., G. Wong, et al. "Hyaluronan-CD44v3 interaction with Oct4-Sox2-Nanog promotes miR-302 expression leading to self-renewal, clonal formation, and cisplatin resistance in cancer stem cells from head and neck squamous cell carcinoma." J Biol Chem. 2012 Sep 21;287(39):32800-24. Epub 2012 Jul 30.

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

Chinnathambi, S., S. Wiechert, et al. "Treatment with the cancer drugs decitabine and doxorubicin induces human skin keratinocytes to express Oct4 and the OCT4 regulator mir-145." J Dermatol. 2012 Jul;39(7):617-24. doi: 10.1111/j.1346-8138.2012.01553.x. Epub 2012 Apr 9.

Previously, we showed that transient transfection with OCT4 not only produced high expression of Oct4 in skin keratinocytes, but also caused a generalized demethylation of keratinocyte DNA. We hypothesized that DNA demethylation alone might allow expression of endogenous OCT4. Here, we report that treatment with the cancer drug decitabine results in generalized DNA demethylation in skin keratinocytes, and by 48 h after treatment, 96% of keratinocytes show expression of the endogenous Oct4 protein and the OCT4 repressor mir-145. This is true for keratinocytes only, as skin fibroblasts treated similarly show no OCT4 or mir-145 expression. Decitabine-treated keratinocytes also show increased mir-302c and proliferation similar to other Oct4(+) cells. Treatment with doxorubicin, another cancer drug, induces expression of mir-145 only in cells that already express OCT4, suggesting that Oct4 regulates its own repressor. Co-treatment with decitabine and

doxorubicin results first in increased OCT4 and mir-145, then a decrease in both, suggesting that OCT4 and mir-145 regulate each other. The novel strategy presented here provides a regulatable system to produce Oct4(+) cells for transformation studies and provides a unique method to study the effects of endogenous Oct4 in cancer cells and the surrounding somatic cells.

Chiou, S. H., M. L. Wang, et al. "Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation." Cancer Res. 2010 Dec 15;70(24):10433-44. doi: 10.1158/0008-5472.CAN-10-2638.

Epithelial-mesenchymal transition (EMT), a critical process of cancer invasion and metastasis, is associated with stemness property of cancer cells. Though Oct4 and Nanog are homebox transcription factors essential to the self-renewal of stem cells and are expressed in several cancers, the role of Oct4/Nanog signaling in tumorigenesis is still elusive. Here microarray and quantitative real-time PCR analysis showed a parallel, elevated expression of Oct4 and Nanog in lung adenocarcinoma (LAC). Ectopic expressions of Oct4 and Nanog in LACs increased the percentage of CD133-expressing subpopulation and sphere formation, enhanced drug resistance, and promoted EMT. Ectopic expressions of Oct4 and Nanog activated Slug and enhanced the tumor-initiating capability of LAC. Furthermore, double knockdown of Oct4 and Nanog suppressed the expression of Slug, reversed the EMT process, blocked the tumorigenic and metastatic ability, and greatly improved the mean survival time of transplanted immunocompromised mice. The immunohistochemical analysis demonstrated that expressions of Oct4, Nanog, and Slug were present in high-grade LAC, and triple positivity of Oct4/Nanog/Slug indicated a worse prognostic value of LAC patients. Our results support the notion that the Oct4/Nanog signaling controls epithelial-mesenchymal transdifferentiation, regulates tumor-initiating ability, and promotes metastasis of LAC.

de Resende, M. F., L. T. Chinen, et al. "Prognostication of OCT4 isoform expression in prostate cancer." Tumour Biol. 2013 Oct;34(5):2665-73. doi: 10.1007/s13277-013-0817-9. Epub 2013 May 1.

Cancer stem cells (CSCs) refer to a subset of tumor cells that self-renew and affect tumor heterogeneity. This model has attracted considerable interest in recent years due to its implications in the prognosis and clinical management of cancer because CSCs mediate the occurrence, growth, and recurrence of tumors. OCT4 is central to embryonic stem cell self-renewal and differentiation into specific lineages and encodes two chief isoforms that are generated by alternative splicing--OCT4A and OCT4B. Their function in prostate cancer (PCa) is unknown. The prognostic function of OCT4 isoforms in PCa samples was examined by immunohistochemistry (IHC) and sensitivity and specificity of the antibodies used were evaluated by molecular biology techniques. Biochemical and pathological data and specimens from 193 patients with PCa were evaluated retrospectively. IHC, western blot, immunofluorescence, and automated image analysis were also performed. IHC was performed on a tissue microarray, and western blot and immunofluorescence were performed using the PCa cell line DU-145. IHC expression of OCT4 isoforms correlated with biochemical and pathological parameters, particularly biochemical recurrence-free survival (BCRFS). Patients with higher levels of OCT4B had lower Gleason scores and decreased likelihood of experiencing biochemical recurrence (BR). OCT4A(+) OCT4B(-) patients had the shortest BCRFS, and positivity for OCT4B expression was an independent prognostic factor for BCRFS in the multivariate analysis. We conclude that the expression of OCT4B is a strong marker of good prognosis, and its presence is associated with a decreased likelihood of BR. Thus, OCT4B might represent a powerful clinical prognostic biomarker for PCa patients.

Emhemmed, F., S. Ali Azouaou, et al. "Selective anticancer effects of a synthetic flavagline on human Oct4-expressing cancer stem-like cells via a p38 MAPK-dependent caspase-3-dependent pathway." Biochem Pharmacol. 2014 May 15;89(2):185-96. doi: 10.1016/j.bcp.2014.02.020. Epub 2014 Mar 4.

Cancer stem cells (CSCs) are considered as the initiators of the carcinogenic process and are therefore emerging targets for innovative anticancer therapies. In order to evaluate the anticancer chemopreventive activity of flavagline derivatives, we used the pluripotent teratocarcinomal cell as a model of Oct4-expressing cancer stem-like cell and determined the underlying cellular and molecular mechanisms induced by a synthetic flavagline. We precisely investigated the effects of the flavagline derivative FL3 on the human embryonal carcinoma (EC) cell line NT2/D1 and compared the responses to those of a normal more restrictive pluripotent stem cell line (i.e. BJ fibroblast cell line). FL3 selectively inhibited the proliferation of NT2/D1 cells by inducing G1 phase cell cycle arrest in a dose-dependent manner. Moreover, FL3 treatment specifically triggered apoptosis in association with an

induction of the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and caspase-3 activation followed by a drastic downregulation of the master regulator of stemness Oct4. Forced inhibition of p38 MAPK activity by the specific pharmacological inhibitor SB203580 or by p38 MAPK gene knockdown using small-interfering RNA (siRNA) counteracted the effects of FL3, demonstrating that its chemopreventive action is related to growth inhibition and a p38-dependent caspase-3-dependent induction of apoptosis in Oct4-expressing CSCs. This study also shows that FL3 selectively kills poorly differentiated and highly aggressive carcinomal cells, but has little effect on normal stem-like cells. Thus FL3 offers great promise for cancer treatment since it is able to target the carcinogenic process without affecting normal cells.

Gazouli, M., M. G. Roubelakis, et al. "OCT4 spliced variant OCT4B1 is expressed in human colorectal cancer." Mol Carcinog. 2012 Feb;51(2):165-73. doi: 10.1002/mc.20773. Epub 2011 Apr 7.

OCT4, a POU-domain transcription factor is considered to be a key factor in maintaining the pluripotency of stem cells. Several OCT4 isoforms are differentially expressed in human pluripotent and non-pluripotent cells. Reactivation of OCT4 expression is postulated to occur in differentiated cells that have undergone tumorigenesis. To examine OCT4 expression in colorectal cancer (CRC) tissues, and to assess the efficacy of OCT4 as a potential biomarker for CRC, in this study, we investigated its expression in CRC tissues, evaluated its relationship to various clinicopathological parameters and defined the isoform of OCT4 that was found to be expressed in CRC cases. Primary tumor tissues and matching adjacent non-cancerous tissues were obtained from 84 CRC patients. OCT4 expression and isoform determination were documented by reverse transcription-PCR and real-time PCR. OCT4, Sox-2, and NANOG localization were performed using immunohistochemistry. The isoforms expressed in the studied cases were confirmed by sequencing. Twenty biopsy specimens representing healthy tissues, retrieved from colonoscopy were studied in parallel as controls. OCT4 expression levels were higher in CRC tissues compared to matching, adjacent non-cancerous tissues, and healthy controls. Additionally, the levels of OCT4 expression in CRC tissues correlated with tumor stage. OCT4 and Sox-2 were localized in the nuclei and the cytoplasm of CRC cells. In all CRC cases, we found that the OCT4B1 isoform is expressed. Over-expression of OCT4B1 was found in poorly and moderately differentiated CRC tissues. In conclusion, the data imply that OCT4B1 isoform may represent a potential biomarker for the initiation, progression, and differentiation of CRC.

Hatefi, N., N. Nouraee, et al. "Evaluating the expression of oct4 as a prognostic tumor marker in bladder cancer." Iran J Basic Med Sci. 2012 Nov;15(6):1154-61.

OBJECTIVES: The key transcriptional regulator Oct4 is one of the self-renewal and differentiation-related factors in cancer stem cells, where it maintains "stemness" state. Cancer stem cells have been identified in a variety of solid malignancies. They are a small population of tumor cells with stem cell characteristics, which are a likely cause of relapse in cancer patients. Due to high incidence, mortality, and recurrence rates of bladder cancer and the necessity of accurate prediction of malignant behavior of the tumors, we evaluated the prognostic value of Oct4 expression in formalin-fixed paraffin-embedded (FFPE) tissues of bladder cancer. MATERIALS AND METHODS: In this study, Oct4 expression was evaluated in 52 (FFPE) tissues of bladder cancer. RNA extraction from samples of 30 patients from the archive of Labbafi-Nejad Medical Centre in Tehran was performed and Oct4 expression levels were examined by semi-quantitative RT-PCR. The intracellular distribution of Oct4 protein was also determined by immunohistochemistry (IHC). RESULTS: The results revealed a significant correlation between the expression level of Oct4 and the tumors' grade and stage. A mostly cytoplasmic distribution of Oct4 protein was also confirmed by IHC. CONCLUSION: All together, our data indicate that the expression level of Oct4 gene is correlated with the clinical and histopathological prognostic indexes of tumors and thus can be considered as a potential prognostic tumor marker.

Hayashi, H., T. Arao, et al. "The OCT4 pseudogene POU5F1B is amplified and promotes an aggressive phenotype in gastric cancer." Oncogene. 2013 Dec 23. doi: 10.1038/onc.2013.547.

POU5F1B (POU domain class 5 transcription factor 1B), a processed pseudogene that is highly homologous to OCT4, was recently shown to be transcribed in cancer cells, but its clinical relevance and biological function have remained unclear. We now show that POU5F1B, which is located adjacent to MYC on human chromosome 8q24, is frequently amplified in gastric cancer (GC) cell lines. POU5F1B, but not OCT4, was also found to be expressed at a high level in GC cell lines and clinical specimens. In addition, the DNA copy number and mRNA abundance for POU5F1B showed a positive correlation in both cancer cell lines and GC specimens. Overexpression of POU5F1B in GC cells

promoted colony formation in vitro as well as both tumorigenicity and tumor growth in vivo, and these effects were enhanced in the additional presence of MYC overexpression. Furthermore, knockdown of POU5F1B expression with a short hairpin RNA confirmed a role for the endogenous pseudogene in the promotion of cancer cell growth in vitro and tumor growth in vivo. POU5F1B overexpression induced upregulation of various growth factors in GC cells as well as exhibited mitogenic, angiogenic and antiapoptotic effects in GC xenografts. Finally, amplification of POU5F1B was detected in 17 (12%) of 145 cases of GC and was a significant predictor of poor prognosis in patients with stage IV disease. In conclusion, we found that the POU5F1B pseudogene is amplified and expressed at a high level in, as well as confers an aggressive phenotype on, GC, and that POU5F1B amplification is associated with a poor prognosis in GC patients.Oncogene advance online publication, 23 December 2013; doi:10.1038/onc.2013.547.

Iki, K. and P. M. Pour "Expression of Oct4, a stem cell marker, in the hamster pancreatic cancer model." Pancreatology. 2006;6(4):406-13. Epub 2006 Jun 29.

BACKGROUND: Oct4 has been shown to present a stem cell marker that is expressed in embryonic cells and in germ cell tumors. Recently, its expression in a few human tissues and cancer cells has been reported. Because in the hamster pancreatic cancer model most tumors develop from within islets presumably from stem cells, we investigated the expression of Oct4 in this model. METHODS: Two normal pancreases and 15 pancreatic cancers induced by N-nitrosobis(2-oxypropyl)amine (BOP) were processed for immunohistochemistry using a monoclonal Oct4 antibody at a concentration of 1:500. RESULTS: In the normal pancreas, Oct4 was expressed only in islet cells in a diffuse cytoplasmic pattern. No nuclear staining was found in any cells. In 14 of the pancreatic cancers, nuclear staining was detected in many cells or in small foci. Diffuse cytoplasmic but no nuclear staining was found in one tumor and a mixed Golgi type and nuclear staining in two cases. Nuclear staining was also identified in early intrainsular ductular and in Ca in situ lesions. CONCLUSIONS: BOP reactivates the Oct4 gene and can be considered an early tumor marker in this model.

Karoubi, G., M. Gugger, et al. "OCT4 expression in human non-small cell lung cancer: implications for therapeutic intervention." Interact Cardiovasc Thorac Surg. 2009 Apr;8(4):393-7. doi: 10.1510/icvts.2008.193995. Epub 2009 Jan 5.

Here we investigate the expression of OCT4 human lung adenocarcinoma and bronchioloalveolar carcinoma (BAC) tumor biopsies and tumor-derived primary cell cultures. OCT4 has been detected in several human tumors suggesting a potentially critical role in tumorigenesis. We assessed the presence of OCT4 in clinical tumor samples of both adenocarcinoma and BAC at the cellular and transcriptional levels, respectively. Furthermore, we evaluated tumor-derived cell cultures for potential differences in OCT4 expression. Immunohistochemical analysis depicted OCT4 in 2 of 8 adenocarcinoma tumor samples and 3 of 5 BAC tumor samples, with no apparent difference in the degree of expression among the sections examined. These results were validated by transcript analysis. Flow cytometric assessment of 11 adenocarcinoma-derived cell cultures and 3 BAC-derived cell cultures revealed significantly higher OCT4 expression in adenocarcinoma tumors compared to their normal counterparts. This, however, was not observed in the BAC cultures. Comparative studies of OCT4 in adenocarcinoma and BAC tumor cell cultures demonstrated a dramatically higher expression in the former. The expression of OCT4 may represent a specific and effective target for therapeutic intervention in adenocarcinoma and BAC. In addition, the aberrant expression and distribution of OCT4 may indicate important parameters concerning the differences between adenocarcinoma and BAC.

Kim, R. J. and J. S. Nam "OCT4 Expression Enhances Features of Cancer Stem Cells in a Mouse Model of Breast Cancer." Lab Anim Res. 2011 Jun;27(2):147-52. doi: 10.5625/lar.2011.27.2.147. Epub 2011 Jun 22.

The cancer stem cell (CSC) hypothesis proposes that CSCs are responsible for metastasis and disease recurrence. Therefore, targeting CSCs has the potential to significantly improve outcomes for cancer patients. The OCT4 transcription factor gene is a master gene that plays a key role in the self-renewal and pluripotency of stem cells. In this study, we introduced an OCT4 reporting vector into 4T1 mouse breast cancer cells and sorted OCT4 high and OCT4 low cell populations. We then determined whether OCT4 expression is associated with maintenance and expansion of CSCs. We found that OCT4(high) 4T1 cells have an increased ability to form tumorsphere and a high expression of stem cell markers such as Sca-1, CD133, CD34, and ALDH1, when compared with OCT4(low) 4T1 cells. In addition, OCT4(high) 4T1 cells have greater tumorigenic potential in vivo. These findings suggest that OCT4 expression may be a useful target for stem cell-specific cancer therapy.

Kong, D., G. Su, et al. "Coexpression of HMGA2 and Oct4 predicts an unfavorable prognosis in human gastric cancer." Med Oncol. 2014 Aug;31(8):130. doi: 10.1007/s12032-014-0130-5. Epub 2014 Jul 19.

High mobility group protein A2 (HMGA2) and octamer-binding transcription factor 4 (Oct4) are transcription factors that play major roles in the acquisition of cancer stemness phenotypes and tumorigenicity of malignant neoplasms. The aim of this study was to analyze the association between HMGA2 and Oct4 expression and various clinicopathologic features in gastric cancer patients including invasion, metastasis, and clinical prognosis, in addition to overall survival. Immunohistochemistry was performed to explore the expression of HMGA2 and Oct4 in 158 gastric cancer and surrounding non-tumor tissues. Moreover, HMGA2 and Oct4 mRNA and protein levels were also detected by qRT-PCR and Western blotting, respectively, in 86 clinical tissue specimens and various gastric epithelial cell lines (GES-1, SGC7901, MKN45, and MKN27). Finally, associations between HMGA2 and Oct4 expression and clinicopathological features were analyzed by Pearson correlation coefficient. Survival analysis was performed by univariate and multivariate analyses. Taken together, we found that HMGA2 and Oct4 expression was significantly higher in gastric cancer tissues compared with non-cancerous tissues ($P < 0.01$), and HMGA2 and Oct4 protein levels were significantly higher in poorly differentiated gastric cancer cell lines (MKN45), moderately differentiated cell lines (SGC7901), and well-differentiated cell lines (MKN28) compared with human immortalized gastric epithelial cell lines (GES-1) ($P < 0.01$). Elevated HMGA2 and Oct4 levels were significantly associated with poor clinical prognosis ($P < 0.05$). Further conclusion showed that coexpression of HMGA2 and Oct4 in gastric cancer correlated with tumor invasion, metastasis, and clinical prognosis and predicted an unfavorable clinical outcome. These transcription factors may represent useful biomarkers to identify patients at high risk of postoperative recurrence.

Kumar, S. M., S. Liu, et al. "Acquired cancer stem cell phenotypes through Oct4-mediated dedifferentiation." Oncogene. 2012 Nov 22;31(47):4898-911. doi: 10.1038/onc.2011.656. Epub 2012 Jan 30.

There is enormous interest to target cancer stem cells (CSCs) for clinical treatment because these cells are highly tumorigenic and resistant to chemotherapy. Oct4 is expressed by CSC-like cells in different types of cancer. However, function of Oct4 in tumor cells is unclear. In this study, we showed that expression of Oct4 gene or transmembrane delivery of Oct4 protein promoted dedifferentiation of melanoma cells to CSC-like cells. The dedifferentiated melanoma cells showed significantly decreased expression of melanocytic markers and acquired the ability to form tumor spheroids. They showed markedly increased resistance to chemotherapeutic agents and hypoxic injury. In the subcutaneous xenograft and tail vein injection assays, these cells had significantly increased tumorigenic capacity. The dedifferentiated melanoma cells acquired features associated with CSCs such as multipotent differentiation capacity and expression of melanoma CSC markers such as ABCB5 and CD271. Mechanistically, Oct4-induced dedifferentiation was associated with increased expression of endogenous Oct4, Nanog and Klf4, and global gene expression changes that enriched for transcription factors. RNAi-mediated knockdown of Oct4 in dedifferentiated cells led to diminished CSC phenotypes. Oct4 expression in melanoma was regulated by hypoxia and its expression was detected in a sub-population of melanoma cells in clinical samples. Our data indicate that Oct4 is a positive regulator of tumor dedifferentiation. The results suggest that CSC phenotype is dynamic and may be acquired through dedifferentiation. Oct4-mediated tumor cell dedifferentiation may have an important role during tumor progression.

Langenfeld, E., M. Deen, et al. "Small molecule antagonist of the bone morphogenetic protein type I receptors suppresses growth and expression of Id1 and Id3 in lung cancer cells expressing Oct4 or nestin." Mol Cancer. 2013 Oct 26;12(1):129. doi: 10.1186/1476-4598-12-129.

BACKGROUND: Bone morphogenetic proteins (BMP) are embryonic morphogens that are aberrantly expressed in lung cancer. BMPs mediate cell fate decisions and self-renewal of stem cells, through transcription regulation of inhibitor of differentiation protein/DNA binding proteins (Id1-3). Inhibition of BMP signaling decreases growth and induces cell death of lung cancer cells lines by downregulating the expression of Id proteins. It is not known whether the BMP signaling cascade regulates growth and the expression of Id proteins of lung cancer cells expressing the stem cell markers Oct4 and/or nestin. METHODS: Lung cancer cells expressing Oct4 or nestin were isolated from lung cancer cell lines by stably transfecting the Oct4 promoter or nestin promoter expression vectors that induce expression of the green fluorescent protein reporter. RESULTS: Our studies suggest that lung cancer cells expressing Oct4 or nestin are different cell populations. Microarray and quantitative RT-PCR demonstrated that the expression of specific stem cell markers were different between isolated Oct4 and nestin cells. Both the Oct4 and nestin populations

were more tumorigenic than controls but histologically they were quite different. The isolated Oct4 and nestin cells also responded differently to inhibition of BMP signaling. Blockade of BMP signaling with the BMP receptor antagonist DMH2 caused significant growth inhibition of both the Oct4 and nestin cell populations but only increased cell death in the nestin population. DMH2 also induced the expression of nestin in the Oct4 population but not in the nestin cells. We also show that BMP signaling is an important regulator of Id1 and Id3 in both the Oct4 and nestin cell populations. Furthermore, we show that NeuN is frequently expressed in NSCLC and provide evidence suggesting that Oct4 cells give rise to cancer cells expressing nestin and/or NeuN. CONCLUSION: These studies show that although biologically different, BMP signaling is growth promoting in cancer cells expressing Oct4 or nestin. Inhibition of BMP signaling decreases expression of Id proteins and suppresses growth of cancer cells expressing Oct4 or Nestin. Small molecule antagonists of the BMP type I receptors represent potential novel drugs to target the population of cancer cells expressing stem cell markers.

Monk, M., M. Hitchins, et al. "Differential expression of the embryo/cancer gene ECSA(DPPA2), the cancer/testis gene BORIS and the pluripotency structural gene OCT4, in human preimplantation development." Mol Hum Reprod. 2008 Jun;14(6):347-55. doi: 10.1093/molehr/gan025. Epub 2008 May 8.

In this paper, we examine the expression profiles of two new putative pluripotent stem cell genes, the embryo/cancer sequence A gene (ECSA) and the cancer/testis gene Brother Of the Regulator of Imprinted Sites (BORIS), in human oocytes, preimplantation embryos, primordial germ cells (PGCs) and embryo stem (ES) cells. Their expression profiles are compared with that of the well-known pluripotency gene, OCT4, using a primer design that avoids amplification of the multiple OCT4 pseudogenes. As expected, OCT4 is high in human oocytes, down-regulated in early cleavage stages and then expressed de novo in human blastocysts and PGCs. BORIS and ECSA show distinct profiles of expression in that BORIS is predominantly expressed in the early stages of preimplantation development, in oocytes and 4-cell embryos, whereas ECSA is predominantly expressed in the later stages, blastocysts and PGCs. BORIS is not detected in blastocysts, PGCs or other fetal and adult somatic tissue tested. Thus, BORIS and ECSA may be involved in two different aspects of reprogramming in development, viz., in late gametogenesis, and at the time of formation of the ES cells (inner cell mass (ICM) and PGC), respectively. However, in human ES cells, where a deprogrammed stem cell state is stably established in culture, an immunofluoresence study shows that all three genes are co-expressed at the protein level. Thus, following their derivation from ICM cells, ES cells may undergo further transformation in culture to express a number of embryo and germ line stem cell functions, which, in normal development, show different temporal and spatial specificity of expression.

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

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

Samardzija, C., M. Quinn, et al. "Attributes of Oct4 in stem cell biology: perspectives on cancer stem cells of

the ovary." J Ovarian Res. 2012 Nov 21;5(1):37. doi: 10.1186/1757-2215-5-37.

Epithelial ovarian cancer (EOC) remains the most lethal of all the gynaecological malignancies with drug resistance and recurrence remaining the major therapeutic barrier in the management of the disease. Although several studies have been undertaken to understand the mechanisms responsible for chemoresistance and subsequent recurrence in EOC, the exact mechanisms associated with chemoresistance/recurrence continue to remain elusive. Recent studies have shown that the parallel characteristics commonly seen between embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSC) are also shared by a relatively rare population of cells within tumors that display stem cell-like features. These cells, termed 'cancer initiating cells' or 'cancer stem cells (CSCs)' have been shown not only to display increased self renewal and pluripotent abilities as seen in ESCs and iPSCs, but are also highly tumorigenic in in vivo mouse models. Additionally, these CSCs have been implicated in tumor recurrence and chemoresistance, and when isolated have consistently shown to express the master pluripotency and embryonic stem cell regulating gene Oct4. This article reviews the involvement of Oct4 in cancer progression and chemoresistance, with emphasis on ovarian cancer. Overall, we highlight why ovarian cancer patients, who initially respond to conventional chemotherapy subsequently relapse with recurrent chemoresistant disease that is essentially incurable.

Wezel, F., J. Pearson, et al. "Differential expression of Oct4 variants and pseudogenes in normal urothelium and urothelial cancer." Am J Pathol. 2013 Oct;183(4):1128-36. doi: 10.1016/j.ajpath.2013.06.025. Epub 2013 Aug 8.

The transcription factor octamer-binding protein 4 (Oct4; encoded by POU5F1) has a key role in maintaining embryonic stem cell pluripotency during early embryonic development and it is required for generation of induced pluripotent stem cells. Controversy exists concerning Oct4 expression in somatic tissues, with reports that Oct4 is expressed in normal and in neoplastic urothelium carrying implications for a bladder cancer stem cell phenotype. Here, we show that the pluripotency-associated Oct4A transcript was absent from cultures of highly regenerative normal human urothelial cells and from low-grade to high-grade urothelial carcinoma cell lines, whereas alternatively spliced variants and transcribed pseudogenes were expressed in abundance. Immunolabeling and immunoblotting studies confirmed the absence of Oct4A in normal and neoplastic urothelial cells and tissues, but indicated the presence of alternative isoforms or potentially translated pseudogenes. The stable forced expression of Oct4A in normal human urothelial cells in vitro profoundly inhibited growth and affected morphology, but protein expression was rapidly down-regulated. Our findings demonstrate that pluripotency-associated isoform Oct4A is not expressed by normal or malignant human urothelium and therefore is unlikely to play a role in a cancer stem cell phenotype. However, our findings also indicate that urothelium expresses a variety of other Oct4 splice-variant isoforms and transcribed pseudogenes that warrant further study.

Yasuda, H., K. Tanaka, et al. "CD133, OCT4, and NANOG in ulcerative colitis-associated colorectal cancer." Oncol Lett. 2011 Nov;2(6):1065-1071. Epub 2011 Sep 6.

Stem cells are thought to contribute to tissue regeneration as well as carcinogenesis. Ulcerative colitis-associated colorectal cancer (UC-CRC) has shown distinct characteristics compared with those of sporadic CRC. The aim of this study was to evaluate the expression of stem cell markers CD133, OCT4 and NANOG in UC-CRC and the inflamed colonic epithelium of UC patients. Total RNAs of UC-CRC (n=6), inflamed colonic epithelium (n=24), sporadic CRC (n=37) and adjacent normal colonic epithelium (n=37) were isolated from formalin-fixed, paraffin-embedded specimens using microdissection techniques in order to purify colonic epithelial cells. Relative mRNA levels of CD133 (PROM), OCT4 (POU5F1) and NANOG were measured using real-time reverse transcription polymerase chain reaction. Three stem cell markers were also investigated immunohistochemically. PROM, POU5F1 and NANOG levels were found to be significantly lower in UC-CRC than in inflamed colonic epithelium of UC patients. By contrast, sporadic CRC showed a significantly higher expression of PROM, POU5F1 and NANOG compared with adjacent normal colonic epithelium. POU5F1 and NANOG levels were significantly lower in UC-CRC than in sporadic CRC. PROM and NANOG levels in inflamed colonic epithelium were significantly higher among younger UC patients ($P<0.05$). Longer disease duration was significantly associated with lower PROM expression ($P=0.0117$). No significant difference was found in PROM levels between UC-CRC and inflamed colonic epithelium in patients with longer disease duration. UC-CRC showed different expression profiles of stem cell markers compared with sporadic CRC. Decreases in PROM expression of inflamed colonic epithelium may identify UC patients at high risk for the development of UC-CRC.

Zhang, Y., X. Zhang, et al. "Inhibition of LDH-A by lentivirus-mediated small interfering RNA suppresses intestinal-type gastric cancer tumorigenicity through the downregulation of Oct4." Cancer Lett. 2012 Aug 1;321(1):45-54. doi: 10.1016/j.canlet.2012.03.013. Epub 2012 Mar 16.

Many tumors metabolise the majority of the glucose that they take up through glycolysis even in the presence of an adequate oxygen supply. Lactate dehydrogenase A (LDH-A) is the critical enzyme that catalyses the transformation of pyruvate to lactate. We demonstrate that LDH-A reduction can suppress the tumorigenicity of intestinal-type gastric cancer (ITGC) cells by downregulating Oct4 both in vitro and in vivo. A statistical analysis of 661 ITGC specimens showed a significant correlation between LDH-A and Oct4 expression. Moreover, patients with low LDH-A/negative Oct4 expression exhibited better overall survival than patients with other combinations. We conclude that the close correlation of LDH-A and Oct4 may offer a promising therapeutic strategy for ITGC.

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. 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. 2014 Mar;47(1):1-11. doi: 10.5115/acb.2014.47.1.1. Epub 2014 Mar 13.
4. Asadi, M. H., S. J. Mowla, et al. "OCT4B1, a novel spliced variant of OCT4, is highly expressed in gastric cancer and acts as an antiapoptotic factor." Int J Cancer. 2011 Jun 1;128(11):2645-52. doi: 10.1002/ijc.25643. Epub 2010 Nov 3.
5. Bourguignon, L. Y., G. Wong, et al. "Hyaluronan-CD44v3 interaction with Oct4-Sox2-Nanog promotes miR-302 expression leading to self-renewal, clonal formation, and cisplatin resistance in cancer stem cells from head and neck squamous cell carcinoma." J Biol Chem. 2012 Sep 21;287(39):32800-24. Epub 2012 Jul 30.
6. Chinnathambi, S., S. Wiechert, et al. "Treatment with the cancer drugs decitabine and doxorubicin induces human skin keratinocytes to express Oct4 and the OCT4 regulator mir-145." J Dermatol. 2012 Jul;39(7):617-24. doi: 10.1111/j.1346-8138.2012.01553.x. Epub 2012 Apr 9.
7. Chiou, S. H., M. L. Wang, et al. "Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation." Cancer Res. 2010 Dec 15;70(24):10433-44. doi: 10.1158/0008-5472.CAN-10-2638.
8. de Resende, M. F., L. T. Chinen, et al. "Prognostication of OCT4 isoform expression in prostate cancer." Tumour Biol. 2013 Oct;34(5):2665-73. doi: 10.1007/s13277-013-0817-9. Epub 2013 May 1.
9. Emhemmed, F., S. Ali Azouaou, et al. "Selective anticancer effects of a synthetic flavagline on human Oct4-expressing cancer stem-like cells via a p38 MAPK-dependent caspase-3-dependent pathway." Biochem Pharmacol. 2014 May 15;89(2):185-96. doi: 10.1016/j.bcp.2014.02.020. Epub 2014 Mar 4.
10. Gazouli, M., M. G. Roubelakis, et al. "OCT4 spliced variant OCT4B1 is expressed in human colorectal cancer." Mol Carcinog. 2012 Feb;51(2):165-73. doi: 10.1002/mc.20773. Epub 2011 Apr 7.
11. Hatefi, N., N. Nouraee, et al. "Evaluating the expression of oct4 as a prognostic tumor marker in bladder cancer." Iran J Basic Med Sci. 2012 Nov;15(6):1154-61.
12. Hayashi, H., T. Arao, et al. "The OCT4 pseudogene POU5F1B is amplified and promotes an aggressive phenotype in gastric cancer." Oncogene. 2013 Dec 23. doi: 10.1038/onc.2013.547.
13. Iki, K. and P. M. Pour "Expression of Oct4, a stem cell marker, in the hamster pancreatic cancer model." Pancreatology. 2006;6(4):406-13. Epub 2006 Jun 29.
14. Karoubi, G., M. Gugger, et al. "OCT4 expression in human non-small cell lung cancer: implications for therapeutic intervention." Interact Cardiovasc Thorac Surg. 2009 Apr;8(4):393-7. doi: 10.1510/icvts.2008.193995. Epub 2009 Jan 5.
15. Kim, R. J. and J. S. Nam "OCT4 Expression Enhances Features of Cancer Stem Cells in a Mouse Model of Breast Cancer." Lab Anim Res. 2011 Jun;27(2):147-52. doi: 10.5625/lar.2011.27.2.147. Epub 2011 Jun 22.
16. Kong, D., G. Su, et al. "Coexpression of HMGA2 and Oct4 predicts an unfavorable prognosis in human gastric cancer." Med Oncol. 2014 Aug;31(8):130. doi: 10.1007/s12032-014-0130-5. Epub 2014 Jul 19.
17. Kumar, S. M., S. Liu, et al. "Acquired cancer stem cell phenotypes through Oct4-mediated dedifferentiation." Oncogene. 2012 Nov

22;31(47):4898-911. doi: 10.1038/onc.2011.656. Epub 2012 Jan 30.
18. Langenfeld, E., M. Deen, et al. "Small molecule antagonist of the bone morphogenetic protein type I receptors suppresses growth and expression of Id1 and Id3 in lung cancer cells expressing Oct4 or nestin." Mol Cancer. 2013 Oct 26;12(1):129. doi: 10.1186/1476-4598-12-129.
19. Monk, M., M. Hitchins, et al. "Differential expression of the embryo/cancer gene ECSA(DPPA2), the cancer/testis gene BORIS and the pluripotency structural gene OCT4, in human preimplantation development." Mol Hum Reprod. 2008 Jun;14(6):347-55. doi: 10.1093/molehr/gan025. Epub 2008 May 8.
20. Saigusa, S., K. Tanaka, et al. "Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy." Ann Surg Oncol. 2009 Dec;16(12):3488-98. doi: 10.1245/s10434-009-0617-z.
21. Samardzija, C., M. Quinn, et al. "Attributes of Oct4 in stem cell biology: perspectives on cancer stem cells of the ovary." J Ovarian Res. 2012 Nov 21;5(1):37. doi: 10.1186/1757-2215-5-37.
22. Wezel, F., J. Pearson, et al. "Differential expression of Oct4 variants and pseudogenes in normal urothelium and urothelial cancer." Am J Pathol. 2013 Oct;183(4):1128-36. doi: 10.1016/j.ajpath.2013.06.025. Epub 2013 Aug 8.
23. Yasuda, H., K. Tanaka, et al. "CD133, OCT4, and NANOG in ulcerative colitis-associated colorectal cancer." Oncol Lett. 2011 Nov;2(6):1065-1071. Epub 2011 Sep 6.
24. Zhang, Y., X. Zhang, et al. "Inhibition of LDH-A by lentivirus-mediated small interfering RNA suppresses intestinal-type gastric cancer tumorigenicity through the downregulation of Oct4." Cancer Lett. 2012 Aug 1;321(1):45-54. doi: 10.1016/j.canlet.2012.03.013. Epub 2012 Mar 16.

9/15/2014