Kruppel-like factor 4 (KLF4) 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: Kruppel-like factor 4 (KLF4) is a member of the KLF family of transcription factors and regulates proliferation, differentiation, apoptosis and somatic cell reprogramming. KLF4 is also a tumor suppressor in certain cancers. KLF4 can be an indicator of stem-like capacity in embryonic stem cells (ESCs) and mesenchymal stem cells (MSCs), etc. The KLF4 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog, etc. Kruppel-like factor 4 (KLF4) is highly expressed in more than 70% of breast cancers and functions as an oncogene. KLF4 inhibits lung cancer cell invasion by suppressing SPARC gene expression.
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1. Introduction

Kruppel-like factor 4 (KLF4) is a member of the KLF family of transcription factors and regulates proliferation, differentiation, apoptosis and somatic cell reprogramming. KLF4 is also a tumor suppressor in certain cancers. KLF4 can be an indicator of stem-like capacity in embryonic stem cells (ESCs) and mesenchymal stem cells (MSCs), etc. The KLF4 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog, etc.

Kruppel-like factor 4 (KLF4) is highly expressed in more than 70% of breast cancers and functions as an oncogene. KLF4 inhibits lung cancer cell invasion by suppressing SPARC gene expression.

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 KLF4 and cancer.

Akaogi, K., Y. Nakajima, et al. "KLF4 suppresses estrogen-dependent breast cancer growth by inhibiting the transcriptional activity of ERalpha." Oncogene. 2009 Aug 13;28(32):2894-902. doi: 10.1038/onc.2009.151. Epub 2009 Jun 8.

Kruppel-like factor 4 (KLF4) is a transcription factor that participates in both tumor suppression and oncogenesis. To determine the association of KLF4 with tumorigenesis, we integrated data assembled in the Oncomine database and discovered a decrease in KLF4 gene transcripts in breast cancers. Further analysis of the database also showed a correlation between KLF4 expression and estrogen receptor-alpha (ERalpha) positivity. Knockdown of KLF4 in MCF-7 cells elevated the growth rate of these cells in the presence of estrogen. Therefore, we examined the interaction between KLF4 and ERalpha, and found that KLF4 bound to the DNA-binding region of ERalpha. KLF4 thus inhibits the binding of ERalpha to estrogen response elements in promoter regions, resulting in a reduction in ERalpha target gene transcription. Earlier studies have reported that KLF4 is transcriptionally activated by p53 following DNA damage. We also showed that activation of p53 decreased the transcriptional activity of ERalpha by elevating KLF4 expression. Our studies discovered a novel molecular network between p53, KLF4 and ERalpha. As both p53 and ERalpha are involved in cell growth and apoptosis, these results may explain why KLF4 possesses both tumor suppressive and oncogenic functions in breast cancers.

Bellance, N., L. Pabst, et al. "Oncosecretomics coupled to bioenergetics identifies alpha-amino adipic acid, isoleucine and GABA as potential biomarkers of cancer: Differential expression of c-Myc, Oct1 and KLF4 coordinates metabolic changes." Biochim Biophys Acta. 2012 Nov;1817(11):2060-71. doi: 10.1016/j.bbabio.2012.07.004. Epub 2012 Jul 25.

Bioenergetic profiling of tumors is a new challenge of cancer research and medicine as therapies

are currently being developed. Meanwhile, methodological means must be proposed to gather information on tumor metabolism in order to adapt these potential therapies to the bioenergetic specificities of tumors. Studies performed on tumors and cancer cell lines have shown that cancer cells bioenergetics is highly variable. This profile changes with microenvironmental conditions (eg. substrate availability), the oncogenes activated (and the tumor suppressors inactivated) and the interaction with the stroma (i.e. reverse Warburg effect). Here, we assessed the power of metabolic footprinting (MFP) to unravel the bioenergetics and associated anabolic changes induced by three oncogenes, c-Myc, KLF4 and Oct1. The MFP approach provides a quantitative analysis of the metabolites secreted and consumed by cancer cells. We used ultra performance liquid chromatography for quantifying the amino acid uptake and secretion. To investigate the potential oncogene-mediated alterations in mitochondrial metabolism, we measured oxygen consumption rate and ATP production as well as the glucose uptake and lactate release. Our findings show that c-Myc deficiency initiates the Warburg effect along with a reduction of mitochondrial respiration. KLF4 deficiency also stimulated glycolysis, albeit without cellular respiration impairment. In contrast, Oct1 deficiency reduced glycolysis and enhanced oxidative phosphorylation efficiency. MFP revealed that c-Myc, KLF4 and Oct1 altered amino acid metabolism with specific patterns. We identified isoleucine, alpha-aminoadipic acid and GABA (gamma-aminoisobutyric acid) as biomarkers related. Our findings establish the impact of Oct1, KLF4 and c-Myc on cancer bioenergetics and evidence a link between oncosecretomics and cellular bioenergetics profile.

Chen, H. Y., Y. M. Lin, et al. "miR-103/107 promote metastasis of colorectal cancer by targeting the metastasis suppressors DAPK and KLF4." Cancer Res. 2012 Jul 15;72(14):3631-41. doi: 10.1158/0008-5472.CAN-12-0667. Epub 2012 May 16.

Metastasis is the major cause of poor prognosis in colorectal cancer (CRC), and increasing evidence supports the contribution of miRNAs to cancer progression. Here, we found that high expression of miR-103 and miR-107 (miR-103/107) was associated with metastasis potential of CRC cell lines and poor prognosis in patients with CRC. We showed that miR-103/107 targeted the known metastasis suppressors death-associated protein kinase (DAPK) and Kruppel-like factor 4 (KLF4) in CRC cells, resulting in increased cell motility and cell-matrix adhesion and decreased cell-cell adhesion and epithelial marker expression. miR-103/107 expression was increased in the presence of hypoxia, thereby potentiating DAPK and KLF4 downregulation and hypoxia-induced motility and invasiveness. In mouse models of CRC, miR-103/107 overexpression potentiated local invasion and liver metastasis effects, which were suppressed by reexpression of DAPK or KLF4. miR-103/107-mediated downregulation of DAPK and KLF4 also enabled the colonization of CRC cells at a metastatic site. Clinically, the signature of a miR-103/107 high, DAPK low, and KLF4 low expression profile correlated with the extent of lymph node and distant metastasis in patients with CRC and served as a prognostic marker for metastasis recurrence and poor survival. Our findings therefore indicate that miR-103/107-mediated repression of DAPK and KLF4 promotes metastasis in CRC, and this regulatory circuit may contribute in part to hypoxia-stimulated tumor metastasis. Strategies that disrupt this regulation might be developed to block CRC metastasis.

Cho, Y. G., J. H. Song, et al. "Genetic and epigenetic analysis of the KLF4 gene in gastric cancer." APMIS. 2007 Jul;115(7):802-8.

KLF4, which is also known as the gut-enriched Kruppel-like factor, plays important roles during the proliferation and differentiation of gastrointestinal epithelial cells. A loss of KLF4 expression has been observed in human tumors, particularly in the gastrointestinal tract. In this study, the molecular basis of the KLF4 inactivation in gastric cancer was investigated by analyzing the somatic mutation, the allelic loss with two microsatellite markers, D9S53 and D9S105, and hypermethylation of the KLF4 gene in 47 gastric adenomas and 81 gastric adenocarcinomas. Mutational analysis revealed one mutation of the KLF4 gene in a diffuse-type advanced gastric adenocarcinoma, but not in the gastric adenoma. This mutation was a somatic missense mutation, GGG-->AGG (Gly-->Arg) at codon 107 in exon 3, which encodes a transcriptional activation domain of the protein. An allelic loss was found in 7 (22.6%) of the 31 informative gastric adenoma cases and 15 (31.3%) of the 48 informative cancer cases at one or both markers. In addition, promoter hypermethylation of the KLF4 gene was observed in only two gastric cancers. These results suggest that genetic and epigenetic alterations of the KLF4 gene might play a minor role in gastric carcinogenesis.

Cittelly, D. M., J. Finlay-Schultz, et al. "Progestin suppression of miR-29 potentiates dedifferentiation of breast cancer cells via KLF4." Oncogene. 2013 May 16;32(20):2555-64. doi: 10.1038/onc.2012.275. Epub 2012 Jul 2.

The female hormone progesterone (P4) promotes the expansion of stem-like cancer cells in estrogen receptor (ER)- and progesterone receptor (PR)-positive breast tumors. The expanded tumor cells lose expression of ER and PR, express the tumor-initiating marker CD44, the progenitor marker cytokeratin 5 (CK5) and are more resistant to standard endocrine and chemotherapies. The mechanisms underlying this hormone-stimulated reprogramming have remained largely unknown. In the present study, we investigated the role of microRNAs in progestin-mediated expansion of this dedifferentiated tumor cell population. We demonstrate that P4 rapidly downregulates miR-29 family members, particularly in the CD44(+) cell population. Downregulation of miR-29 members potentiates the expansion of CK5(+) and CD44(+) cells in response to progestins, and results in increased stem-like properties in vitro and in vivo. We demonstrate that miR-29 directly targets Kruppel-like factor 4 (KLF4), a transcription factor required for the reprogramming of differentiated cells to pluripotent stem cells, and for the maintenance of breast cancer stem cells. These results reveal a novel mechanism, whereby progestins increase the stem cell-like population in hormone-responsive breast cancers, by decreasing miR-29 to augment PR-mediated upregulation of KLF4. Elucidating the mechanisms whereby hormones mediate the expansion of stem-like cells furthers our understanding of the progression of hormone-responsive breast cancers.

He, H., S. Li, et al. "12-O-tetradecanoylphorbol-13-acetate promotes breast cancer cell motility by increasing S100A14 level in a Kruppel-like transcription factor 4 (KLF4)-dependent manner." J Biol Chem. 2014 Mar 28;289(13):9089-99. doi: 10.1074/jbc.M113.534271. Epub 2014 Feb 14.

The S100 protein family represents the largest subgroup of calcium binding EF-hand type proteins. These proteins have been reported to be involved in a wide range of biological functions that are related to normal cell development and tumorigenesis. S100A14 is a recently identified member of the S100 protein family and differentially expressed in a number of different human malignancies. However, the transcriptional regulation of S100A14 and its role in breast cancer needs to be further investigated. Here, we determined that 12-O-tetradecanoylphorbol-13-acetate (TPA) up-regulated the expression of KLF4 and facilitated its binding directly to two conserved GC-rich DNA segments within the S100A14 promoter, which is essential for the transactivation of KLF4 induced S100A14 expression. Furthermore, stable silencing of KLF4 significantly suppressed breast cancer cell migration induced by TPA. Collectively, these results offer insights into the fact that TPA provokes cell motility through regulating the expression and function of S100A14 in a KLF4-dependent manner.

Hu, R., Y. Zuo, et al. "KLF4 Expression Correlates with the Degree of Differentiation in Colorectal Cancer." Gut Liver. 2011 Jun;5(2):154-9. doi: 10.5009/gnl.2011.5.2.154. Epub 2011 Jun 23.

BACKGROUND/AIMS: Kruppel-like factor 4 (KLF4) is an epithelial-specific transcription factor primarily expressed in the gastrointestinal tract that mediates growth arrest in the colonic epithelium. We tried to find whether KLF4 expression is associated with the progression and differentiation of colorectal cancer. METHODS: We detected KLF4 expression in 109 colorectal specimens (40 normal appearing mucosa, 7 adenomas, and 62 carcinomas) by immunohistochemistry using a tissue microarray. Western blot and RT-PCR analyses were also performed. RESULTS: The upregulation of KLF4 expression in carcinoma tissue was statistically significant ($p<0.05$) when compared to normal appearing mucosa. The negative and weak positive staining rates in normal appearing mucosa, adenoma, and carcinoma were 42.5%, 71.4%, and 82.3%, respectively, indicating a decreased degree of KLF4 expression over the course of progressive transformation of normal cells into malignant derivatives. KLF4 protein levels showed no correlation with sex, age, or metastatic state ($p>0.05$), while KLF4 protein expression correlated with the diagnostic stage ($p<0.05$). Furthermore, strong KLF4 staining was detected in 22.9% (11/48) and 0% (0/14) of well/moderately and poorly differentiated colorectal cancers, respectively. Our results clearly indicate that KLF4 protein expression significantly correlates with the degree of differentiation in colorectal cancers ($p<0.05$). KLF4 expression in RKO cells is also upregulated by butyrate, an inducer of differentiation. CONCLUSIONS: Downregulation of KLF4 expression may lead to more poorly differentiated tumors.

Krstic, M., S. Stojnev, et al. "KLF4 expression and apoptosis-related markers in gastric cancer." J BUON. 2013 Jul-Sep;18(3):695-702.

PURPOSE: To correlate the expression of Kruppel-like factor 4 (KLF4) with clinicopathological properties of gastric cancer (GC) and to evaluate any possible correlation between KLF4 expression and the expression of apoptosis-related markers p53, Fas, Bcl-2, survivin and FLICE inhibitory protein (Flip-l). METHODS: Formalin-fixed, paraffin-embedded tissue specimens obtained from 96 patients with GC who had undergone gastric surgery were analyzed for

pathological parameters, while KLF4, p53, Fas, Bcl-2, survivin and Flip-l expression was assessed by immunohistochemistry. RESULTS: TKLF4 immunohistochemical staining was noted in 78.1% of the cases. Strong positivity was found in 15.6% and weak in 62.5% of the samples. Positive expression of p53, Fas, Bcl-2, survivin, Flip-l was found in 56.2%, 44.8%, 15.6%, 41.7% and 38.5% of the samples, respectively. KLF4 expression was significantly associated with p53 nuclear staining and Fas immunoreactivity. p53-positive tumors demonstrated more often high KLF4 staining compared to p53-negative tumors. Fas-positive tumors were associated with decreased KLF4 expression. Logistic regression analysis of apoptosis-related markers to KLF4 expression revealed that Fas positivity significantly decreased the probability of strong KLF4 expression, and inversely, Bcl-2 expression improved the prediction of KLF4 staining. When all 5 predictive variables were considered together (p53, Fas, survivin, Bcl-2, Flip-l) they significantly predicted the type of KLF4 expression in GC cells (p=0.019). CONCLUSION: Our results suggest that the decrease or loss of KLF4 expression correlates with diffuse-type GC and immunoreactivity to Fas, and are inversely linked with p53 nuclear accumulation. The significance of KLF4 in GC requires further studies and should be more thoroughly investigated for potential use in the evaluation and better stratification of GC patients.

Lambertini, C., S. Pantano, et al. "Differential control of Notch1 gene transcription by Klf4 and Sp3 transcription factors in normal versus cancer-derived keratinocytes." PLoS One. 2010 Apr 28;5(4):e10369. doi: 10.1371/journal.pone.0010369.

In specific cell types like keratinocytes, Notch signaling plays an important pro-differentiation and tumor suppressing function, with down-modulation of the Notch1 gene being associated with cancer development. Besides being controlled by p53, little else is known on regulation of Notch1 gene expression in this context. We report here that transcription of this gene is driven by a TATA-less "sharp peak" promoter and that the minimal functional region of this promoter, which extends from the -342 bp position to the initiation codon, is differentially active in normal versus cancer cells. This GC rich region lacks p53 binding sites, but binds Klf4 and Sp3. This finding is likely to be of biological significance, as Klf4 and, to a lesser extent, Sp3 are up-regulated in a number of cancer cells where Notch1 expression is down-modulated, and Klf4 over-expression in normal cells is sufficient to down-modulate Notch1 gene transcription. The combined knock-down of Klf4 and Sp3 was necessary for the reverse effect of increasing Notch1 transcription, consistent with the two factors exerting an overlapping repressor function through their binding to the Notch1 promoter.

Le Magnen, C., L. Bubendorf, et al. "Klf4 transcription factor is expressed in the cytoplasm of prostate cancer cells." Eur J Cancer. 2013 Mar;49(4):955-63. doi: 10.1016/j.ejca.2012.09.023. Epub 2012 Oct 22.

BACKGROUND: Cancer initiation and progression might be driven by small populations of cells endowed with stem cell-like properties. Here we comparatively addressed the expression of genes encoding putative stemness regulators including c-Myc, Klf4, Nanog, Oct4A and Sox2 genes in benign prostatic hyperplasia (BPH) and prostate cancer (PCA). METHODS: Fifty-eight PCA and thirty-nine BPH tissues samples were used for gene expression analysis, as evaluated by quantitative real-time polymerase chain reaction (qRT-PCR). The expression of specific Klf4 isoforms was tested by conventional PCR. Klf4 specific antibodies were used for protein detection in a tissue microarray including 404 prostate samples. RESULTS: Nanog, Oct4A and Sox2 genes were comparably expressed in BPH and PCA samples, whereas c-Myc and Klf4 genes were expressed to significantly higher extents in PCA than in BPH specimens. Immunohistochemical studies revealed that Klf4 protein is detectable in a large majority of epithelial prostatic cells, irrespective of malignant transformation. However, in PCA, a predominantly cytoplasmic location was observed, consistent with the expression of a differentially spliced Klf4alpha isoform. CONCLUSION: Klf4 is highly expressed at gene and protein level in BPH and PCA tissues but a cytoplasmic location of the specific gene product is predominantly detectable in malignant cells. Klf4 location might be of critical relevance to steer its functions during oncogenesis.

Lee, H. Y., J. B. Ahn, et al. "High KLF4 level in normal tissue predicts poor survival in colorectal cancer patients." World J Surg Oncol. 2014 Jul 24;12:232. doi: 10.1186/1477-7819-12-232.

BACKGROUND: Kruppel-like factor 4 (KLF4) is involved in many important cellular processes such as growth, development, differentiation, proliferation, and apoptosis. The purpose of this study was to determine the significance of KLF4 in both tumors and normal tissues of patients with colorectal cancer (CRC). METHODS: Between January 2003 and June 2005, 125 patients with CRC receiving treatment at the Yonsei Cancer Center were selected. We examined the mRNA level of the KLF4 gene in primary CRC

specimens and matched normal colon tissues using real-time RT-PCR. Correlation of survival with clinicopathological parameters, including KLF4 level, was investigated with univariate and multivariate analyses. RESULTS: CRC tissue had a significantly lower KLF4 level when compared with matched normal tissues (KLF4 in tumors: 2007 +/- 1531 copies/mul, KLF4 in normal tissues: 6586 +/- 2834 copies/mul; P < 0.0001). However, there was a correlation between the KLF4 level in tumors and normal tissues. Patients with a high KLF4 level in matched normal tissues were more likely than those with a low KLF4 level to develop recurrence and had poorer overall survival (P = 0.005). Therefore, the KLF4 level in the normal tissue of individuals was associated with prognosis of individuals. CONCLUSIONS: Our data suggest that KLF4 mRNA expression level in normal tissues and tumors may be a useful prognostic marker in patients with CRC.

Li, D., Z. Peng, et al. "KLF4-mediated negative regulation of IFITM3 expression plays a critical role in colon cancer pathogenesis." Clin Cancer Res. 2011 Jun 1;17(11):3558-68. doi: 10.1158/1078-0432.CCR-10-2729. Epub 2011 Apr 29.

PURPOSE: IFITM3, an IFN-inducible gene, is overexpressed in human colorectal cancer. In this study, we sought to determine the clinical significance and underlying mechanisms of its dysregulated expression in human colon tumor specimens and murine models of this disease. EXPERIMENTAL DESIGN: IFITM3 expression in a tissue microarray of tumor and matched normal colon tissue specimens and lymph node metastasis specimens obtained from 203 patients with colon cancer was measured immunohistochemically. RESULTS: IFITM3 was expressed at higher levels in colon tumors and, particularly, nodal metastases than in normal colon tissue. A Cox proportional hazards model showed that IFITM3 expression was an independent prognostic factor for disease-free survival in patients with colon cancer. Knockdown of IFITM3 expression by a specific siRNA significantly suppressed the proliferation, colony formation, migration, and invasion of colon cancer cells in vitro and tumor growth and metastasis in a xenograft model. Restored expression of KLF4, a putative tumor suppressor, downregulated IFITM3 expression in colon cancer cells in vitro. Two KLF4-binding sites in the IFITM3 promoter bound specifically to KLF4 protein in a chromatin immunoprecipitation assay and promoter mutagenesis analyses. Specific deletion of KLF4 led to IFITM3 overexpression in colon mucosa in Villin-Cre(+);Klf4(fl/fl) mice. An inverse correlation between loss of KLF4 expression and IFITM3 overexpression was evident in human colon tumors. CONCLUSION: These clinical and mechanistic findings indicate that IFITM3 is a direct transcriptional target of KLF4 and that dysregulated KLF4 expression leads to aberrant IFITM3 expression, thus contributing to colon cancer progression and metastasis.

Li, H., J. Wang, et al. "Epigenetic inactivation of KLF4 is associated with urothelial cancer progression and early recurrence." J Urol. 2014 Feb;191(2):493-501. doi: 10.1016/j.juro.2013.08.087. Epub 2013 Sep 7.

PURPOSE: KLF4 is a transcription factor with divergent functions in different malignancies. We analyzed KLF4 expression and DNA methylation, and their clinical relevance and biological function in urothelial cancer. MATERIALS AND METHODS: Immunohistochemistry and Sequenom MassARRAY(R) were done to detect the expression and promoter methylation of KLF4 in urothelial cancer tissues. The association of the recurrence-free survival rate and decreased KLF4 or KLF4 methylation status was analyzed by the Kaplan-Meier method, Cox regression analysis and ROC assay. Lentivirus based KLF4 over expression and dsRNA mediated knockdown were used to detect KLF4 functions in urothelial cancer in vitro and in vivo. RESULTS: KLF4 was down-regulated in urothelial cancer due to promoter hypermethylation. Each correlated with recurrence-free survival in patients with nonmuscle invasive bladder cancer after transurethral resection of bladder cancer, which potentiates them as valuable predictive biomarkers for early recurrence. Moreover, in and ex vivo experiments showed that KLF4 suppressed urothelial cancer cell growth, migration and invasion inhibited the epithelial-to-mesenchymal transition. CONCLUSIONS: KLF4 may function as a tumor suppressor gene in urothelial cancer since down-regulation of KLF4 by promoter hypermethylation would promote cancer progression. In addition, decreased expression of KLF4 or its promoter hypermethylation may have predictive value for early recurrence in patients with nonmuscle invasive bladder cancer.

Liu, Y. N., W. Abou-Kheir, et al. "Critical and reciprocal regulation of KLF4 and SLUG in transforming growth factor beta-initiated prostate cancer epithelial-mesenchymal transition." Mol Cell Biol. 2012 Mar;32(5):941-53. doi: 10.1128/MCB.06306-11. Epub 2011 Dec 27.

Epithelial-mesenchymal transition (EMT) is implicated in various pathological processes within the prostate, including benign prostate hyperplasia (BPH) and prostate cancer progression. However, an

ordered sequence of signaling events initiating carcinoma-associated EMT has not been established. In a model of transforming growth factor beta (TGFbeta)-induced prostatic EMT, SLUG is the dominant regulator of EMT initiation in vitro and in vivo, as demonstrated by the inhibition of EMT following Slug depletion. In contrast, SNAIL depletion was significantly less rate limiting. TGFbeta-stimulated KLF4 degradation is required for SLUG induction. Expression of a degradation-resistant KLF4 mutant inhibited EMT, and furthermore, depletion of Klf4 was sufficient to initiate SLUG-dependent EMT. We show that KLF4 and another epithelial determinant, FOXA1, are direct transcriptional inhibitors of SLUG expression in mouse and human prostate cancer cells. Furthermore, self-reinforcing regulatory loops for SLUG-KLF4 and SLUG-FOXA1 lead to SLUG-dependent binding of polycomb repressive complexes to the Klf4 and Foxa1 promoters, silencing transcription and consolidating mesenchymal commitment. Analysis of tissue arrays demonstrated decreased KLF4 and increased SLUG expression in advanced-stage primary prostate cancer, substantiating the involvement of the EMT signaling events described in model systems.

Min, K. W., X. Zhang, et al. "A peroxisome proliferator-activated receptor ligand MCC-555 imparts anti-proliferative response in pancreatic cancer cells by PPARgamma-independent up-regulation of KLF4." Toxicol Appl Pharmacol. 2012 Sep 1;263(2):225-32. doi: 10.1016/j.taap.2012.06.014. Epub 2012 Jun 30.

MCC-555 is a novel PPARalpha/gamma dual ligand of the thiazolidinedione class and was recently developed as an anti-diabetic drug with unique properties. MCC-555 also has anti-proliferative activity through growth inhibition and apoptosis induction in several cancer cell types. Our group has shown that MCC-555 targets several proteins in colorectal tumorigenesis including nonsteroidal anti-inflammatory drug (NSAID)-activated gene (NAG-1) which plays an important role in chemoprevention responsible for chemopreventive compounds. NAG-1 is a member of the TGF-beta superfamily and is involved in tumor progression and development; however, NAG-1's roles in pancreatic cancer have not been studied. In this report, we found that MCC-555 alters not only NAG-1 expression, but also p21 and cyclin D1 expression. NAG-1 and p21 expression was not blocked by PPARgamma-specific antagonist GW9662, suggesting that MCC-555-induced NAG-1 and p21 expression is independent of PPARgamma activation. However, decreasing cyclin D1 by MCC-555 seems to be affected by PPARgamma activation. Further, we found that the GC box located in the NAG-1 promoter play an important role in NAG-1 transactivation by MCC-555. Subsequently, we screened several transcription factors that may bind to the GC box region in the NAG-1 promoter and found that KLF4 potentially binds to this region. Expression of KLF4 precedes NAG-1 and p21 expression in the presence of MCC-555, whereas blocking KLF4 expression using specific KLF4 siRNA showed that both NAG-1 and p21 expression by MCC-555 was blocked. In conclusion, MCC-555's actions on anti-proliferation involve both PPARgamma-dependent and -independent pathways, thereby enhancing anti-tumorigenesis in pancreatic cancer cells.

Moon, J. S., H. E. Kim, et al. "Kruppel-like factor 4 (KLF4) activates the transcription of the gene for the platelet isoform of phosphofructokinase (PFKP) in breast cancer." J Biol Chem. 2011 Jul 8;286(27):23808-16. doi: 10.1074/jbc.M111.236737. Epub 2011 May 17.

Kruppel-like factor 4 (KLF4) is a transcription factor that plays an important role in cell differentiation, proliferation, and survival, especially in the context of cancers. This study revealed that KLF4 activates glycolytic metabolism in breast cancer cells by up-regulating the platelet isoform of phosphofructokinase (PFKP). KLF4 activated the transcription of the PFKP gene by directly binding to the PFKP promoter. Whereas glucose uptake and lactate production were inhibited by the knockdown of KLF4, they were activated by the overexpression of KLF4. Unlike PFKP, the expressions of the other isoforms of phosphofructokinase and glycolytic genes were unaffected by KLF4. The human breast cancer tissues showed a close correlation between KLF4 and PFKP expression. This study also showed that PFKP plays a critical role in cell proliferation in breast cancer cells. In conclusion, it is suggested that KLF4 plays a role in maintenance of high glycolytic metabolism by transcriptional activation of the PFKP gene in breast cancer cells.

Nagata, T., Y. Shimada, et al. "Prognostic significance of NANOG and KLF4 for breast cancer." Breast Cancer. 2014 Jan;21(1):96-101. doi: 10.1007/s12282-012-0357-y. Epub 2012 Apr 17.

BACKGROUND: Some of the induced pluripotent stem cell (iPS cell)-inducing factors have been reported to be expressed in breast cancer. The aim of the present study was to examine the relationship between the expression of iPS cell-inducing factors and the prognosis of breast cancer patients. METHODS: In 100 breast cancer patients, the expression of c-MYC, KLF4, NANOG, OCT4, and SOX2 was determined by immunohistochemistry using a tissue microarray analysis. RESULTS:

Patients with strong expression of NANOG had significantly lower disease-free survival (DFS) and overall survival rates than those with weak expression of NANOG (P = 0.004 and 0.033, respectively). In contrast, patients with strong expression of KLF4 had better DFS (P = 0.014). CONCLUSIONS: Strong expression of NANOG is an indicator of a poor prognosis for breast cancer patients, whereas KLF4 is a favorable prognostic indicator. Our results suggest that NANOG stimulates the growth and metastasis of breast cancer cells, whereas KLF4 inhibits these processes.

Ohnishi, S., S. Ohnami, et al. "Downregulation and growth inhibitory effect of epithelial-type Kruppel-like transcription factor KLF4, but not KLF5, in bladder cancer." Biochem Biophys Res Commun. 2003 Aug 22;308(2):251-6.

Kruppel-like factors (KLFs) are key transcriptional regulators of cell differentiation and proliferation. Among the KLF family, the expression of KLF4 (GKLF) and KLF5 (IKLF) is highly restricted in the epithelial cells of several organs such as the gut and skin, and it has been reported that these epithelial-type KLF genes may be involved in colon carcinogenesis. Recently we found that Klf4 and Klf5 genes were significantly expressed in the developmental bladder epithelium of mice as well. Therefore, in this report we studied the involvement of the KLF4 and KLF5 genes in bladder carcinogenesis. First, we analyzed the expression of KLF4 and KLF5 in a variety of human bladder cancer cell lines and surgical specimens by RNA blot and in situ hybridization analyses. Both genes were highly expressed in the normal bladder epithelium, whereas KLF4, but not KLF5, was frequently downregulated in bladder cancer cell lines and cancer tissues. We then transduced the KLF4 and KLF5 genes into the bladder cancer cell lines using adenoviral vectors to examine the biological activities of the genes on those cells. The transduction of KLF4, but not KLF5, suppressed cell growth and induced apoptosis. Our study suggests that inactivation of KLF4 is one of the frequent steps towards bladder carcinogenesis.

Okuda, H., F. Xing, et al. "miR-7 suppresses brain metastasis of breast cancer stem-like cells by modulating KLF4." Cancer Res. 2013 Feb 15;73(4):1434-44. doi: 10.1158/0008-5472.CAN-12-2037. Epub 2013 Feb 5.

Despite significant improvement in survival rates of patients with breast cancer, prognosis of metastatic disease is still dismal. Cancer stem-like cells (CSC) are considered to play a role in metastatic progression of breast cancer; however, the exact pathologic role of CSCs is yet to be elucidated. In this report, we found that CSCs (CD24(-)/CD44(+)/ESA(+)) isolated from metastatic breast cell lines are significantly more metastatic than non-CSC populations in an organ-specific manner. The results of our microRNA (miRNA) profile analysis for these cells revealed that CSCs that are highly metastatic to bone and brain expressed significantly lower level of miR-7 and that this miRNA was capable of modulating one of the essential genes for induced pluripotent stem cell, KLF4. Interestingly, high expression of KLF4 was significantly and inversely correlated to brain but not bone metastasis-free survival of patients with breast cancer, and we indeed found that the expression of miR-7 significantly suppressed the ability of CSCs to metastasize to brain but not to bone in our animal model. We also examined the expression of miR-7 and KLF4 in brain-metastatic lesions and found that these genes were significantly down- or upregulated, respectively, in the tumor cells in brain. Furthermore, the results of our in vitro experiments indicate that miR-7 attenuates the abilities of invasion and self-renewal of CSCs by modulating KLF4 expression. These results suggest that miR-7 and KLF4 may serve as biomarkers or therapeutic targets for brain metastasis of breast cancer.

Pandya, A. Y., L. I. Talley, et al. "Nuclear localization of KLF4 is associated with an aggressive phenotype in early-stage breast cancer." Clin Cancer Res. 2004 Apr 15;10(8):2709-19.

PURPOSE: The Kruppel-like transcription factor KLF4/GKLF induces both malignant transformation and a slow-growth phenotype in vitro. Although KLF4 expression is increased in most cases of breast cancer, it was unknown whether these cases represent a distinct subtype with a different clinical outcome. EXPERIMENTAL DESIGN: We examined expression of KLF4 by immunostaining 146 cases of human primary infiltrating ductal carcinoma of the breast. Staining patterns were correlated with clinical outcome and with established prognostic factors. RESULTS: Subcellular localization exhibited case-to-case variation. Tumors with high nuclear staining and low cytoplasmic staining were termed type 1. For patients with early-stage disease (i.e., stage I or IIA), type 1 staining was associated with eventual death because of breast cancer (hazard ratio, 2.8; 95% confidence interval, 1.23-6.58; P = 0.011). The association was stronger in patients with early-stage cancer and small primary tumors (i.e., < or =2.0 cm in diameter; hazard ratio, 4.3; 95% confidence interval, 1.75-10.62; P < 0.001). For patients with early-stage disease, multivariate analysis indicated that type 1 staining was independently associated with outcome (adjusted hazard ratio 2.6; 95% confidence interval,

1.10-6.05; P = 0.029). Type 1 staining was also associated with high histological grade (P = 0.032), increased expression of Ki67 (P = 0.016), and reduced expression of BCL2 (P = 0.032). In vitro, KLF4 was localized within the nucleus of transformed RK3E epithelial cells, consistent with a nuclear function of this transcription factor during induction of malignant transformation. CONCLUSIONS: The results suggest that localization of KLF4 in the nucleus of breast cancer cells is a prognostic factor and identify KLF4 as a marker of an aggressive phenotype in early-stage infiltrating ductal carcinoma.

Parasramka, M. A., W. M. Dashwood, et al. "A role for low-abundance miRNAs in colon cancer: the miR-206/Kruppel-like factor 4 (KLF4) axis." Clin Epigenetics. 2012 Sep 24;4(1):16. doi: 10.1186/1868-7083-4-16.

BACKGROUND: MicroRNAs (miRNAs or miRs) are short non-coding RNAs that affect the expression of genes involved in normal physiology, but that also become dysregulated in cancer development. In the latter context, studies to date have focused on high-abundance miRNAs and their targets. We hypothesized that among the pool of low-abundance miRNAs are some with the potential to impact crucial oncogenic signaling networks in colon cancer. RESULTS: Unbiased screening of over 650 miRNAs identified miR-206, a low-abundance miRNA, as the most significantly altered miRNA in carcinogen-induced rat colon tumors. Computational modeling highlighted the stem-cell marker Kruppel-like factor 4 (KLF4) as a potential target of miR-206. In a panel of primary human colon cancers, target validation at the mRNA and protein level confirmed a significant inverse relationship between miR-206 and KLF4, which was further supported by miR-206 knockdown and ectopic upregulation in human colon cancer cells. Forced expression of miR-206 resulted in significantly increased cell proliferation kinetics, as revealed by real-time monitoring using HCT116 cells. CONCLUSIONS: Evolutionarily conserved high-abundance miRNAs are becoming established as key players in the etiology of human cancers. However, low-abundance miRNAs, such as miR-206, are often among the most significantly upregulated miRNAs relative to their expression in normal non-transformed tissues. Low-abundance miRNAs are worthy of further investigation, because their targets include KLF4 and other pluripotency and cancer stem-cell factors.

Rageul, J., S. Mottier, et al. "KLF4-dependent, PPARgamma-induced expression of GPA33 in colon cancer cell lines." Int J Cancer. 2009 Dec 15;125(12):2802-9. doi: 10.1002/ijc.24683.

The glycoprotein A33 (GPA33) is a colon cancer antigen. Phase I trials with 131I and 125I monoclonal antibody A33 in colon carcinoma patients showed excellent localization to colorectal cancer and some evidence of tumor response. Using DNA microarrays, we have identified the GPA33 gene as a target of PPARgamma in HT29-Cl.16E colon cancer cells. Treatment of HT29-Cl.16E, Caco2, SW1116 and LS174T colon cancer cells with the PPARgamma agonist GW7845 induced a 2- to 6-fold increase in GPA33 mRNA as determined by real-time PCR. This induction was also found in HT29-Cl.16E cells treated with rosiglitazone and ciglitazone and was prevented by cotreatment with the PPARgamma antagonist GW9662, indicating that this regulation was PPARgamma dependent. No canonical PPAR responsive element was found in the GPA33 promoter. We therefore analyzed the expression of transcription factors involved in GPA33 expression. CDXl, CDX2 and KLF5 expression was not modified by PPARgamma activation. By contrast, a significant increase in KLF4 was seen, both at mRNA and protein levels. Furthermore, chromatin immunoprecipitation studies demonstrated that an increased amount of KLF4 protein was bound to the GPA33 promoter in cells treated with rosiglitazone. Finally, downregulation of KLF4 expression by siRNA reduced rosiglitazone-induced GPA33 expression. This indicates that PPARgamma activation induces KLF4 expression, which in turn increases GPA33 expression. We also demonstrate that PPARgamma activation leads to increased (p21WAF1/Cip1 and keratin 19) or decreased (cyclin D1) expression of known KLF4 targets, suggesting that KLF4 is a nodal player in a network of PPARgamma-regulated genes.

Sellak, H., S. Wu, et al. "KLF4 and SOX9 transcription factors antagonize beta-catenin and inhibit TCF-activity in cancer cells." Biochim Biophys Acta. 2012 Oct;1823(10):1666-75. doi: 10.1016/j.bbamcr.2012.06.027. Epub 2012 Jul 2.

The transcriptional activator beta-catenin is a key mediator of the canonical Wnt signaling pathway. beta-catenin itself does not bind DNA but functions via interaction with T-cell factor (TCF)/lymphoid-enhancing factor (LEF) transcription factors. Thus, in the case of active Wnt signaling, beta-catenin, in cooperation with TCF/LEF proteins family, activates the expression of a wide variety of genes. To date, the list of established beta-catenin interacting targets is far from complete. In this study, we aimed to establish the interaction between beta-catenin and transcription factors that might affect TCF activity. We took advantage of EMSA, using TCF as a probe, to screen oligonucleotides known to bind specific transcription factors that might dislodge or antagonize beta-

catenin/TCF binding. We found that Sox9 and KLF4 antagonize beta-catenin/TCF binding in HEK293, A549, SW480, and T47D cells. This inhibition of TCF binding was concentration-dependent and correlated to the in vitro TCF-luciferase functional assays. Overexpression of Sox9 and KLF4 transcription factors in cancer cells shows a concentration-dependent reduction of TCF-luciferase as well as the TCF-binding activities. In addition, we demonstrated that both Sox9 and KLF4 interact with beta-catenin in an immunoprecipitation assay and reduce its binding to TCF4. Together, these results demonstrate that Sox9 and KLF4 transcription factors antagonize beta-catenin/TCF in cancer cells.

Tang, W., Y. Zhu, et al. "MicroRNA-29a promotes colorectal cancer metastasis by regulating matrix metalloproteinase 2 and E-cadherin via KLF4." Br J Cancer. 2014 Jan 21;110(2):450-8. doi: 10.1038/bjc.2013.724. Epub 2013 Nov 26.

BACKGROUND: Growing evidence suggests that miR-29a has an important role in regulating tumourigenesis and development of various types of cancer. However, the role and the underlying mechanism of miR-29a in colorectal cancer (CRC) remain largely unknown. METHODS: MiR-29a targeted gene was identified by the luciferase assay and western blot. MiR-29a function was analysed by invasion assays and the orthotopic transplantation mouse model. The miR-29a pathway was assayed by real-time PCR, western blot and chip analysis. RESULTS: KLF4 was identified as a direct target gene of miR-29a. MiR-29a promoted CRC cell invasion, which was blocked by re-expression of KLF4. In addition, MMP2 was identified as a novel direct target of KLF4. Both miR-29a overexpression and KLF4 knockdown promoted MMP2 expression but inhibited E-cadherin expression. Furthermore, clinical data indicated that both miR-29a high expression and KLF4 mRNA low expression were associated with metastasis and poor prognosis in CRC patients, and KLF4 protein expression was inversely correlated with MMP2 but positively correlated with E-cad protein expression. CONCLUSION: Increased expression of miR-29a promoted CRC metastasis by regulating MMP2/E-cad through direct targeting KLF4, which highlights the potential of the miR-29a inhibitor as a novel agent against CRC metastasis.

Tetreault, M. P., M. L. Wang, et al. "Klf4 overexpression activates epithelial cytokines and inflammation-mediated esophageal squamous cell cancer in mice." Gastroenterology. 2010 Dec;139(6):2124-2134.e9. doi: 10.1053/j.gastro.2010.08.048.

BACKGROUND & AIMS: Esophageal squamous cell cancer accounts for more than 90% of cases of esophageal cancers. Its pathogenesis involves chronic epithelial irritation, although the factors involved in the inflammatory process and the mechanisms of carcinogenesis are unknown. We sought to develop a mouse model of this cancer. METHODS: We used the ED-L2 promoter of Epstein-Barr virus to overexpress the transcriptional regulator Kruppel-like factor 4 (Klf4) in esophageal epithelia of mice; we used mouse primary esophageal keratinocytes to examine the mechanisms by which KLF4 induces cytokine production. RESULTS: KLF4 was an epithelial-specific mediator of inflammation; we developed a new mouse model of esophageal squamous dysplasia and inflammation-mediated squamous cell cancer. KLF4 activated a number of proinflammatory cytokines, including TNF-alpha, CXCL5, G-CSF and IL-1alpha, within keratinocytes in an NF-kappaB-dependent manner. KLF4 was not detected in proliferating or cancer cells, indicating a non-cell autonomous effect of KLF4 on proliferation and carcinogenesis. CONCLUSIONS: KLF4 has distinct functions in carcinogenesis; upregulation of Klf4 specifically in esophageal epithelial cells induces inflammation. This mouse model might be used to determine the molecular mechanisms of esophageal squamous cell cancer and inflammation-mediated carcinogenesis.

Tian, Y., A. Luo, et al. "MicroRNA-10b promotes migration and invasion through KLF4 in human esophageal cancer cell lines." J Biol Chem. 2010 Mar 12;285(11):7986-94. doi: 10.1074/jbc.M109.062877. Epub 2010 Jan 14.

Recently, microRNAs have emerged as regulators of cancer metastasis through acting on multiple signaling pathways involved in metastasis. In this study, we have analyzed the level of miR-10b and cell motility and invasiveness in several human esophageal squamous cell carcinoma cell lines. Our results reveal a significant correlation of miR-10b level with cell motility and invasiveness. Overexpression of miR-10b in KYSE140 cells increased cell motility and invasiveness, whereas inhibition of miR-10b in EC9706 cells reduced cell invasiveness, although it did not alter cell motility. Additionally, we identified KLF4, a known tumor suppressor gene that has been reported to suppress esophageal cancer cell migration and invasion, as a direct target of miR-10b. Furthermore, overexpression of miR-10b in KYSE140 and KYSE450 cells led to a reduction of endogenous KLF4 protein, whereas silencing of miR-10b in EC9706 cells caused up-regulation of KLF4 protein. Coexpression of miR-10b and KLF4 in KYSE140 cells and coexpression of

small interfering RNA for KLF4 mRNA and miR-10b-AS in EC9706 cells partially abrogated the effect of miR-10b on cell migration and invasion. Finally, analyses of the miR-10b level in 40 human esophageal cancer samples and their paired normal adjacent tissues revealed an elevated expression of miR-10b in 95% (38 of 40) of cancer tissues, although no significant correlation of the miR-10b level with clinical metastasis status was observed in these samples.

Vaira, V., A. Faversani, et al. "Regulation of lung cancer metastasis by Klf4-Numb-like signaling." Cancer Res. 2013 Apr 15;73(8):2695-705. doi: 10.1158/0008-5472.CAN-12-4232. Epub 2013 Feb 25.

Metastatic traits seem to be acquired by transformed cells with progenitor-like cancer-initiating properties, but there remains little mechanistic insight into this linkage. In this report, we show that the polarity protein Numbl, which is expressed normally in neuronal progenitors, becomes overexpressed and mislocalized in cancer cells from a variety of human tumors. Numbl overexpression relies on loss of the tumor suppressor miRNA-296-5p (miR-296), which actively represses translation of Numbl in normal cells. In turn, deregulated expression of Numbl mediates random tumor cell migration and invasion, blocking anoikis and promoting metastatic dissemination. In clinical specimens of non-small cell lung cancer, we found that Numbl overexpression correlated with a reduction in overall patient survival. Mechanistically, Numbl-mediated tumorigenesis involved suppression of a "stemness" transcriptional program driven by the stem cell programming transcription factor Klf4, thereby preserving a pool of progenitor-like cells in lung cancer. Our results reveal that Numbl-Klf4 signaling is critical to maintain multiple nodes of metastatic progression, including persistence of cancer-initiating cells, rationalizing its therapeutic exploitation to improve the treatment of advanced lung cancer.

Wang, J., R. F. Place, et al. "Prognostic value and function of KLF4 in prostate cancer: RNAa and vector-mediated overexpression identify KLF4 as an inhibitor of tumor cell growth and migration." Cancer Res. 2010 Dec 15;70(24):10182-91. doi: 10.1158/0008-5472.CAN-10-2414.

KLF4/GLKF4 is a transcription factor that can have divergent functions in different malignancies. The role of KLF4 in prostate cancer etiology remains unclear. We have recently reported that small double-stranded RNA can induce gene expression by targeting promoter sequence in a phenomenon referred to as RNA activation (RNAa). In this study, we examine KLF4 levels in prostate cancer tissue and utilize RNAa as a tool for gene overexpression to investigate its function. Expression analysis indicated that KLF4 is significantly downregulated in prostate cancer cell lines compared with nontumorigenic prostate cells. Meta-analysis of existing cDNA microarray data also revealed that KLF4 is frequently depleted in prostate cancer tissue with more pronounced reduction in metastases. In support, tissue microarray analysis of tumors and patient-matched controls indicated downregulation of KLF4 in metastatic tumor samples. Logistic regression analysis found that tumors with a KLF4 staining score less than 5 had a 15-fold higher risk for developing metastatic prostate cancer ($P = 0.001$; 95% confidence interval, 3.0-79.0). In vitro analysis indicated that RNAa-mediated overexpression of KLF4 inhibited prostate cancer cell proliferation and survival and altered the expression of several downstream cell-cycle-related genes. Ectopic expression of KLF4 via viral transduction recapitulated the RNAa results, validating its inhibitory effects on cancer growth. Reactivation of KLF4 also suppressed migration and invasion of prostate cancer cells. These results suggest that KLF4 functions as an inhibitor of tumor cell growth and migration in prostate cancer and decreased expression has prognostic value for predicting prostate cancer metastasis.

Xiao, H., H. Li, et al. "MicroRNA-10b promotes migration and invasion through KLF4 and HOXD10 in human bladder cancer." Oncol Rep. 2014 Apr;31(4):1832-8. doi: 10.3892/or.2014.3048. Epub 2014 Feb 24.

The present study was performed to investigate the effect of microRNA-10b (miR-10b) on cell migration and invasion in human bladder cancer (BC). Real-time PCR was performed to detect the expression of miR-10b in BC cell lines. miR-10b mimics, the negative control for mimics, miR-10b inhibitor and the negative control for inhibitor were transfected into BC cell lines and the effects of miR-10b on the migration and invasion of cells were investigated through Transwell assay. Meanwhile, protein levels of KLF4, HOXD10, E-cadherin and MMP14 were measured. Luciferase assays were also performed to validate KLF4 and HOXD10 as miR-10b targets. In vivo metastasis assay was performed to validate if miR-10b can promote BC cell line metastasis in vivo. miR-10b is significantly upregulated in BC cell lines and metastatic tissues. Increased miR-10b expression significantly enhanced BC cell migration and invasion, while decreased miR-10b expression reduced cell migration and invasion. In vivo metastasis assay demonstrated that

overexpression of miR-10b markedly promoted BC metastasis. Moreover, KLF4 and HOXD10 were identified as direct targets of miR-10b in BC cells. Silencing of KLF4 or HOXD10 recapitulated the pro-metastatic function. Furthermore, we found that E-cadherin and MMP14 may be the downstream factors of KLF4 and HOXD10 in the suppression of BC metastasis by miR-10b. These data suggest that miR-10b may function as oncogenes in BC cells. Targeting these novel strategies, inhibition of miR-10b/KLF4/E-cadherin axis and miR-10b/HOXD10/MMP14 axis may be helpful as a therapeutic approach to block BC cell metastasis.

Yang, Y., B. G. Goldstein, et al. "KLF4 and KLF5 regulate proliferation, apoptosis and invasion in esophageal cancer cells." Cancer Biol Ther. 2005 Nov;4(11):1216-21. Epub 2005 Nov 11.

KLF4 and KLF5, members of the KLF family of transcription factors, play key roles in proliferation, differentiation, and carcinogenesis in a number of gastrointestinal tissues. While KLF4 is expressed in differentiating epithelial cells, KLF5 is found in proliferating cells of the gastrointestinal tract, including the esophagus. KLF4 regulates a number of genes vital for esophageal epithelial differentiation, and decreased expression of KLF4 is seen in esophageal squamous cancers. Nonetheless, the roles of KLF4 and KLF5 in esophageal tumor progression are not known. Here, using TE2 cells stably infected with retroviral vectors to express KLF4 or KLF5, we demonstrate that KLF4 and KLF5 are key players in a number of cellular processes critical for esophageal carcinogenesis. TE2 cells, derived from a patient with poorly differentiated esophageal squamous cancer, normally lack KLF4 and KLF5. Expression of KLF5 in TE2 cells inhibits proliferation, and both KLF4 and KLF5 decrease viability after treatment with hydrogen peroxide and increase anoikis. In response to DNA damage from UV irradiation, viability is decreased in KLF5 but not KLF4 infected cells. Both KLF4 and KLF5 upregulate the cdk inhibitor p21(waf1/cip1) following UV irradiation, but the pro-apoptotic protein BAX is markedly induced only by KLF5. Thus KLF4 may preferentially activate DNA repair pathways while KLF5 induces both DNA repair and apoptosis after UV irradiation. Expression of KLF4 or KLF5 in TE2 cells also inhibits invasion, consistent with a role for each in preventing tumor metastasis. In summary, KLF4 and KLF5 regulate esophageal carcinogenesis by affecting proliferation, apoptosis, and invasion.

Yoon, O. and J. Roh "Downregulation of KLF4 and the Bcl-2/Bax ratio in advanced epithelial ovarian cancer." Oncol Lett. 2012 Nov;4(5):1033-1036. Epub 2012 Jul 30.

Kruppel-like factor 4 (KLF4) is a key transcriptional regulator of cell differentiation and proliferation and an altered expression of KLF4 has been reported in a number of human malignancies. In the present study, we investigated KLF4 expression and its role in cell proliferation in advanced epithelial ovarian cancer (EOC). We compared KLF4, Bcl-2 and Bax transcript levels in ovaries isolated from advanced EOC and normal control ovaries. In addition, the KLF4 gene was transduced into ovarian cancer cells and transcript levels of Bcl-2 and Bax and cell proliferation were analyzed by real-time RT-PCR and MTT assays, respectively. Ovarian KLF4 expression and Bcl-2/Bax ratios were downregulated in most cases of advanced EOC. In addition, KLF4 overexpression in ovarian cancer cells increased the Bcl-2/Bax ratio. However, MTT analysis indicated that the overexpression of KLF4 had no effect on the proliferation of ovarian cancer cells. The inactivation of KLF4 is frequently observed in ovarian cancers and a reduced expression of KLF4 in the ovarian cancers may lead to a reduction in the Bcl-2/Bax ratio. The latter has a role in predicting cancer grade, although its exact role in ovarian carcinogenesis requires clarification.

Yu, F., J. Li, et al. "Kruppel-like factor 4 (KLF4) is required for maintenance of breast cancer stem cells and for cell migration and invasion." Oncogene. 2011 May 5;30(18):2161-72. doi: 10.1038/onc.2010.591. Epub 2011 Jan 17.

Kruppel-like factor 4 (KLF4) is highly expressed in more than 70% of breast cancers and functions as an oncogene. However, an exact mechanism by which KLF4 enhances tumorigenesis of breast cancer remains unknown. In this study, we show that KLF4 was highly expressed in cancer stem cell (CSC)-enriched populations in mouse primary mammary tumor and breast cancer cell lines. Knockdown of KLF4 in breast cancer cells (MCF-7 and MDA-MB-231) decreased the proportion of stem/progenitor cells as demonstrated by expression of stem cell surface markers such as aldehyde dehydrogenase 1, side population and by in vitro mammosphere assay. Consistently KLF4 overexpression led to an increase of the cancer stem cell population. KLF4 knockdown also suppressed cell migration and invasion in MCF-7 and MDA-MB-231 cells. Furthermore, knockdown of KLF4 reduced colony formation in vitro and inhibited tumorigenesis in immunocompromised non-obese diabetic/severe combined immunodeficiency mice, supporting an oncogenic role for KLF4 in breast cancer development. Further mechanistic studies revealed

that the Notch signaling pathway was required for KLF4-mediated cell migration and invasion, but not for CSC maintenance. Taken together, our study provides evidence that KLF4 has a potent oncogenic role in mammary tumorigenesis likely by maintaining stem cell-like features and by promoting cell migration and invasion. Thus, targeting KLF4 may provide an effective therapeutic approach to suppress tumorigenicity in breast cancer.

Yu, T., X. Chen, et al. "Regulation of the potential marker for intestinal cells, Bmi1, by beta-catenin and the zinc finger protein KLF4: implications for colon cancer." J Biol Chem. 2012 Feb 3;287(6):3760-8. doi: 10.1074/jbc.M111.316349. Epub 2011 Dec 14.

B lymphoma Mo-MLV insertion region 1 (Bmi1) is a Polycomb Group (PcG) protein important in gene silencing. It is a component of Polycomb Repressive Complex 1 (PRC1), which is required to maintain the transcriptionally repressive state of many genes. Bmi1 was initially identified as an oncogene that regulates cell proliferation and transformation, and is important in hematopoiesis and the development of nervous systems. Recently, it was reported that Bmi1 is a potential marker for intestinal stem cells. Because Wnt signaling plays a key role in intestinal stem cells, we analyzed the effects of Wnt signaling on Bmi1 expression. We found that Wnt signaling indeed regulates the expression of Bmi1 in colon cancer cells. In addition, the expression of Bmi1 in human colon cancers is significantly associated with nuclear beta-catenin, a hallmark for the activated Wnt signaling. Kruppel-like factor 4 (KLF4) is a zinc finger protein highly expressed in the gut and skin. We recently found that KLF4 cross-talks with Wnt/beta-catenin in regulating intestinal homeostasis. We demonstrated that KLF4 directly inhibits the expression of Bmi1 in colon cancer cells. We also found that Bmi1 regulates histone ubiquitination and is required for colon cancer proliferation in vitro and in vivo. Our findings further suggest that Bmi1 is an attractive target for cancer therapeutics.

Zhou, Y., W. L. Hofstetter, et al. "KLF4 inhibition of lung cancer cell invasion by suppression of SPARC expression." Cancer Biol Ther. 2010 Apr 1;9(7):507-13. Epub 2010 Apr 1.

Kruppel-Like Factor 4 (KLF4) functions as a tumor suppressor in some cancers, but its molecular mechanism is not clear. Our recent study also showed that the expression of KLF4 is dramatically reduced in primary lung cancer tissues. To investigate the possible role of KLF4 in lung cancer, we stably transfected KLF4 into cells from lung cancer cell lines H322 and A549 to determine the cells' invasion ability. Our results showed that ectopic expression of KLF4 extensively suppressed lung cancer cell invasion in Matrigel. This effect was independent of KLF4-mediated p21 up-regulation because ectopic expression of p21 had minimal effect on cell invasion. Our analysis of the expression of 12 genes associated with cell invasion in parental, vector-transfected, and KLF4-transfected cells showed that ectopic expression of KLF4 resulted in extensively repressed expression of secreted protein acidic and rich in cysteine (SPARC), an extracellular matrix protein that plays a role in tumor development and metastasis. Knockdown of SPARC expression in H322 and A549 cells led to suppression of cell invasion, comparable to that observed in KLF4-transfected cells. Moreover, retrovirus-mediated restoration of SPARC expression in KLF4-transfected cells abrogated KLF4-induced anti-invasion activity. Together, our results indicate that KLF4 inhibits lung cancer cell invasion by suppressing SPARC gene expression.

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transcription factor 4 (KLF4)-dependent manner." J Biol Chem. 2014 Mar 28;289(13):9089-99. doi: 10.1074/jbc.M113.534271. Epub 2014 Feb 14.
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9/15/2014