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REVIEW

Emerging role of KLF4 in human gastrointestinal cancer

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Recent analyses revealed that Krüppel-like factors (KLFs) play important roles in both normal development and carcinogenesis. Of the 16 known KLFs, KLF4 has been shown to be involved in the regulation of proliferation, differentiation and tumorigenesis of gastrointestinal tract epithelium. Clinical, experimental and mechanistic findings indicate that KLF4 is a *bona fide* tumor suppressor for both gastric and colorectal cancers. In this review, we summarize how this growing area of research has formed and the challenging new frontiers for better understanding of the oncogenic potential of the KLFs.

Introduction

Cancers of the gastrointestinal (GI) tract account for 22% of all cancers and represent a major health threat (1). Although the incidence of gastric cancer declined in the West from the 1940s to the 1980s, it remains a major public health problem throughout the world (2). In Asia and parts of South America in particular, gastric cancer is the most common epithelial malignancy and leading cause of cancer-related death. Moreover, gastric cancer remains the fourth most frequently diagnosed malignancy worldwide and cause of 12% of all cancer-related deaths annually (2,3). In comparison, colorectal cancer (CRC) is the third most common cancer and second most common cause of cancer-related deaths in both men and women in the USA (2). Advances in the treatment of GI cancer are likely to come from a fuller understanding of its biology and behavior.

The aggressive nature of human GI cancer is related to a number of molecular abnormalities, including inactivation of various tumor suppressor genes, activation of various oncogenes, reactivation of telomerase, and abnormalities in several growth factors and their receptors (4–7). Investigators have made significant progress in understanding the molecular mechanisms that lead to CRC (8–10). For example, Fearon and Vogelstein (1990) (11) presented evidence of a multistep genetic model of colorectal carcinogenesis. This model is based on the understanding that CRC is the result of accumulation of changes in key genes, including inactivation of tumor suppressor genes and aberrant activation of proto-oncogenes, such as *TP53 and APC* (11–13). Numerous studies also have indicated the roles of altered oncogenes and tumor suppressor genes in gastric cancer development and progression, including *E-cadherin/CDH1*, *TP53*, *p16* (5–7,14–17) and runt-related genes (18,19). In addition, increasing evidence has indicated that Krüppel-like factor (KLF) 4 appears to be a putative tumor suppressor in both gastric cancer and CRC.

Abbreviations: BTE, basic transcription element; CRC, colorectal cancer; GI, gastrointestinal; HDAC, histone deacetylase; KLF, Krüppel-like factor; LOH, Loss of heterozygosity; ODC, ornithine decarboxylase; PanIN, pancreatic intraepithelial neoplasia.

KLF family and subfamilies

Krüppel is a zinc-finger-containing transcription factor in *Drosophila melanogaster* that is crucial for controlling embryogenesis (20). A large number of mammalian genes exhibit sequence homology with the DNA-binding domain of Krüppel. Among these, a group called the KLFs constitutes a particularly close family (21–24). The KLFs recently emerged as important contributors to the development of the mammalian embryo (23). The KLF family consists of at least 16 different members, which are in turn separated into a few structurally related subgroups (23). The largest of them includes at least seven proteins: erythroid KLF (EKLF or KLF1), lung KLF (LKLF or KLF2), basic KLF (BKLF or KLF3), gut KLF (GKLF/EZF or KLF4), intestinal KLF (IKLF/BTEB2 or KLF5), core promoter element-binding protein (COPEB/Zf9 or KLF6) and ubiquitous KLF (UKLF or KLF7). The prototype gene in this group is *KLF1* (25,26). The amino acid sequences of the zinc fingers (27) and the nuclear localization signals (28) of *KLF4* are closely related to those of *KLF1* (29) and *KLF2*, i.e. *KLF2* (29,30) and *KLF4* (27,31) are more closely related to *KLF1* than to any other members of the KLF family. In addition to their sequence homology, structural–functional studies cluster these three genes into a KLF subfamily (28). These nuclear factors are characterized by three highly conserved zinc fingers of the C_2H_2 type that bind to the consensus sequence 5′-NGGGNGNGG-3′ and by divergent N-termini that modulate gene transcription (23).

Of the KLFs discovered thus far, KLF1 and KLF2 have been the most extensively characterized. KLF1 is essential for expression of the β-*globin* gene and for erythropoiesis. It activates the β-*globin* gene by binding to a CACCC element in the β-globin promoter. Homologous gene targeting in mice has documented the importance of *Klf1* in β-*globin* gene activation and liver erythropoiesis, whereas *Klf2* is clearly involved in lymphopoiesis, vasculogenesis and lung development (25,26,32,33). Available evidence indicates that some members of the KLF family are potentially novel oncogenes or tumor suppressors. The major physiological and pathological functions of KLFs were summarized in recent published reviews (22,23,34). Collectively, these and other correlative lines of evidence support the notion that KLFs participate in mammalian morphogenesis by controlling the proliferation and/or differentiation of distinct cell lineages (24).

Physiological functions of KLF4

KLF4 gene structure and tissue distribution

Human *KLF4* (formerly known as gut-enriched KLF or epithelial zinc finger, EZF) was first identified from human umbilical vein endothelial cell cDNA library by using a DNA probe containing the zinc finger region of human erythroid Krüppel-like factor (*EKLF*, *KLF1*) in 1998 (35), shortly after the cloning of mouse *Klf4* in two independent laboratories (28,31). The human *KLF4* gene locus is located at chromosome 9q31, which covers a 6.3 kb region. *KLF4* has five exons, and the size of the KLF4 transcript is about 3.5 kb as detected by northern blot analysis in RNA from human umbilical vein endothelial and other cells (35–37). The cDNA of *KLF4* encodes a protein containing 470 amino acids with a predicted molecular mass of 50 kDa. Several functional domains have been characterized in the KLF4 protein, including an acidic transcriptional activation domain at the N-terminus; the carboxyl DNA-binding domain, which consists of 81 highly conserved amino acids that form three C_2H_2 zinc fingers that exhibit homology with the *D.melanogaster* segmentation gene product Krüppel (27); and nuclear localization signal and transcriptional repression domains at the N-terminus next to the three zinc fingers (35). In addition, there is a potential PEST sequence located between the transcriptional activation and transcriptional inhibitory domains, indicating that KLF4 may be degraded through ubiquitin-proteosome pathway. The structure of the *KLF4* gene and its protein functional domains are depicted in Figure 1. Because of the high degree of homology between the zinc finger regions of KLF4 and KLF1, KLF4 has been shown to bind to the same or similar elements (31,35,37,38). However, KLF4 also binds DNA sequences other than the CACCC element (38). For example, the basic transcription element (BTE), which is found in the promoter of a highly conserved family of genes encoding the cytochrome P-450 drug-metabolizing enzymes, including *CYP1A1* (39,40), is a high-affinity binding site for KLF4 (38,41). The presence of both activation and repression domains may allow KLF4 to alter their positive or negative transcriptional effect as the situation dictates.

In mice, *Klf4* is located on chromosome 4, and the size of the transcript of *Klf4* is 3.5 kb as detected by northern blot analysis. However, in tissues with high levels of expression, a minor mRNA of 1.9 kb has been reported (31). In addition, a comparison of the human and mouse KLF4 sequences revealed 91% similarity at the amino acid level, and the 103 amino acid residues of the C-terminus were completely identical. Since mouse *Klf4* was first described in 1996 (27,31), there have been a variety of reports on its distributions and functions in embryonic and adult tissues. *Klf4* transcripts can be detected from Embryonic day 4.5 on in the developing conceptus, and Klf4 expression before Embryonic day 10 is restricted to extraembryonic tissues. The embryo proper displays a highly dynamic and changing KLF4 signal from Embryonic day 10 of development on. In addition to being expressed in a stripe of mesenchymal cells extending rostrally from the forelimb bud over the branchial arches to the developing eye, *Klf4* is expressed in the mesenchyme surrounding the nasal pit at Embryonic day 11.5. In addition, Klf4 expression has been detected in the apical ectodermal ridge and adjacent mesenchymal cells in the limb buds and in mesenchymal cells of the developing body wall in trunk areas (42). These findings suggest that KLF4 plays an important role in regulating cellular proliferation, which underlies the morphogenetic changes that shape the developing embryo.

KLF4 is maximally expressed in the GI tract within a narrow window of time (Embryonic day 15.5) and in the developing layers of the skin starting from Embryonic day 16.5 during the late phase of development (31,33,43). In the thymus, *Klf4* is expressed during cortical differentiation, suggesting it has a role in T-lymphocyte differentiation (44). *Klf4* is also highly expressed in the testes, specifically, in the post-meiotic germ

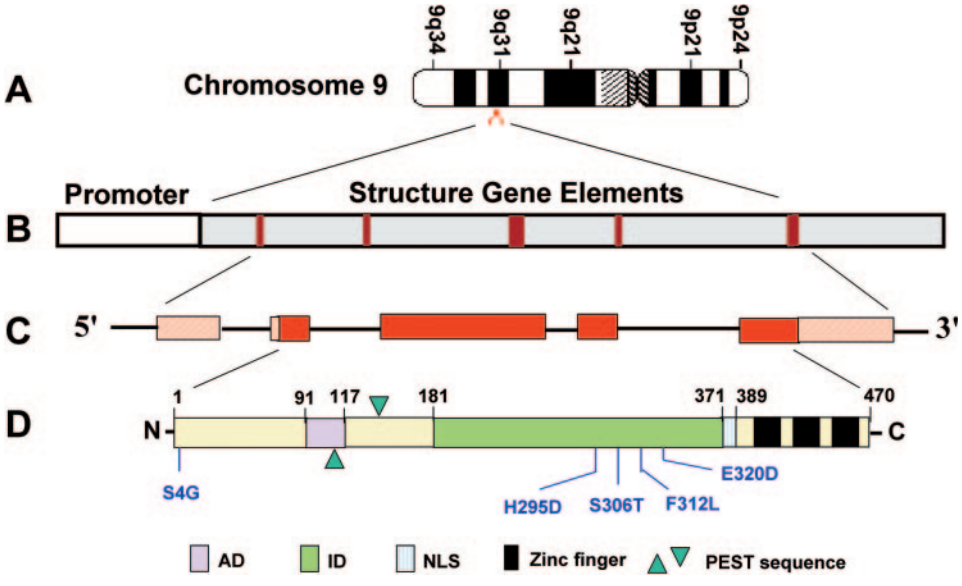

Fig. 1. Structural organization of the *klf4* gene and the corresponding protein. (**A**) The *Klf4* gene is located on chromosome 9q31, which covers a 6.3 kb region. (**B**) The dark red bars mark the locations of the five identified exons of the *klf4* gene. (**C**) Below the genomic map is the klf4 RNA transcript. The five boxes represent corresponding exons, whereas the solid red boxes show the *klf4* open reading frame within the 2639 bp cDNA. (**D**) The *Klf4* open reading frame encodes a protein of 470 amino acids with several functional domains, including the transcriptional activation domain (AD), transcriptional inhibitory domain (ID), zinc finger DNA-binding domain, nuclear localization signal (NLS) and potential PEST sequence. Several point mutations have been identified in tumor cells, which are shown in blue.

cells and somatic Sertoli's cells, suggesting an important role in testicular differentiation (45). *Klf4* is also expressed in the dorsal epithelium of the developing tongue, tooth bud and palate; in the mesenchymal cells of the skeletal primordia and metanephric kidney (31); other sites such as the respiratory tract, meninges and cartilaginous skeleton (46); and in conjunctival and corneal cells (47). However, $Klf4^{-/-}$ mice display subtle perturbations of late-stage differentiation structures in the upper layers of the skin and tongue (33,48), substantiating that *Klf4* is imperative for terminal differentiation of keratinocytes of the skin (33). Homologous gene targeting in mice has documented the importance of *Klf4* to skin integrity and function (33,48).

Among adult tissues, KLF4 is predominantly expressed in epithelial cells of the GI tract (27,31,36) and skin (31,33), vascular endothelial cells (35) and the thymus (27). In these organs, KLF4 expression is found primarily in post-mitotic terminally differentiated epithelial cells (27,31). For example, KLF4 expression has been reported in the middle to upper region of the crypt epithelium in the GI tract, suggesting that KLF4 plays important roles during the differentiation of gut epithelial cells (27,31). Consistent with its tissue distribution, KLF4 expression has been shown to be imperative for terminal differentiation of goblet cells of the intestines (49). Finally, in vascular endothelial cells, KLF4 expression can be upregulated by shear stress and endostatin or interleukin-4 treatment (50–52). KLF4 is not normally expressed in differentiated smooth muscle cells *in vivo* (53), but it is rapidly upregulated in smooth muscle cells in response to vascular injury (54). These findings suggest that KLF4 plays an important role in the development and homeostasis of the blood vascular system.

Regulation of cell proliferation

Several lines of evidence indicate that KLF4 is an important regulator of cell proliferation. In mice, *Klf4* transcripts begin to rise on Embryonic day 13 and peak at Embryonic day 17, which correlates with a critical period of gut epithelium morphogenesis (55). *In vivo*, KLF4 is highly expressed in post-mitotic terminally differentiated epithelial cells of the intestines (27) and skin (31,33). In cultured cells, expression of KLF4 is temporally associated with conditions that promote growth arrest, such as serum deprivation, contact inhibition and DNA damage (27,56), and constitutive production of KLF4 inhibits DNA synthesis (27). KLF4 activates the promoter of the negative cell-cycle-regulatory cyclin-dependent kinase inhibitor $p21^{WAF1/Cip1}$ gene in a p53-dependent manner (56,57). On the other hand, KLF4 represses several positive cell-cycle-regulatory gene promoters, such as *cyclin D1* (58,59) and ornithine decarboxylase (*ODC*) (59), and induces cell cycle arrest at the G_1/S boundary. The ability of p53 to activate the $p21^{WAF1/Cip1}$ promoter is dependent on KLF4, as the two proteins synergize to regulate $p21^{WAF1/Cip1}$ expression (56). Transcriptional repression of the *cyclin D1* gene by KLF4 is mediated in part by its interaction with the Sp1-binding domain on the *cyclin D1* promoter. Using a stably transfected cell line in which an inducible promoter controlled the expression of KLF4, investigators showed that induction of KLF4 results in cell cycle arrest at the transition from G_1 to S phase (57). More recently, using a combination of genetic and biochemical approaches, researchers showed that KLF4 is essential for mediating the G_1/S cell cycle effect of p53 as a consequence of DNA damage (57,60). These studies demonstrated an essential role for KLF4 in cell cycle control. These findings have been further substantiated by a microarray study showing that KLF4 is involved in the control of cell proliferation in that it elicits changes in the expression of numerous cell-cycle-regulatory genes in a concerted manner (61). In vascular smooth muscle cells, KLF4 inhibits proliferation of redox-sensitive growth and induces expression of several cell cycle negative regulatory genes such as *p21*, *p27*, *p53* and retinoblastoma (62). In *Klf4* mutant mice with gastric epithelial ablation of *Klf4* expression, investigators observed marked hypertrophy of gastric epithelia, a 4-fold increase in the number of proliferating cells in gastric unite and a 45% decrease in $p21^{WAF1/CIP1}$ mRNA expression in gastric epithelia (63). All of these results suggest that KLF4 is a negative regulator of cell growth.

Regulation of cell differentiation

In addition to its documented effect on cell proliferation, KLF4 has been shown to be important in regulating tissue differentiation, as indicated by gene knockout studies. For example, in *Klf4*-deficient mice, late-stage differentiation of the epidermis is disturbed, and the mice die shortly after birth because of an inability to maintain a skin barrier as measured by penetration of externally applied dyes and rapid loss of body fluids in newborn $Klf4^{-/-}$ mice (33). Interestingly, $Klf4^{-/-}$ mice have normal cell proliferation and cell death rates in the colon on post-natal day 1. However, $Klf4^{-/-}$ mice have a 90% decrease in the number of goblet cells in the colon, have abnormal expression of the goblet-cell-specific marker *Muc2* as determined by *in situ* hybridization, have abnormal Alcian blue staining of the colonic epithelium for acidic mucins, and lack normal goblet cell morphology as determined by ultrastructural analysis. All other epithelial cell types are present in the colon of $Klf4^{-/-}$ mice (49). Therefore, KLF4 plays a crucial role in colonic epithelial cell differentiation. In *Klf4* mutant mice with ablation of *Klf4* expression in gastric epithelia, investigators have found a >50% decrease in the number of parietal and mature zymogenic cells but a 4-fold increase in mucus neck cells per gland, indicating that differentiation of gastric epithelial precursor cells into mature cell lineages was perturbed (63). KLF4 has also been shown to be expressed in the esophageal squamous epithelium and pancreatic ductal cells, activating the promoters of the differentiation gene Epstein–Barr virus *ED-L2* and *keratin 4* in the esophagus and *keratin 19* in the pancreas (36,64). On the other hand, in *keratin 19* promoter-driven *lacZ* transgenic mice, transgene expression was shown to be restricted to ductal epithelial cells in the pancreas, surface colonocytes, small intestinal villi and gastric isthmus cells. Furthermore, transgene expression has been demonstrated to be correlated with K19 and KLF4 protein expression in the pancreas and stomach and to overlap this expression in the small and large intestine (65). Transcriptional profiling of *KLF4* has revealed that KLF4 regulates the expression of a group of epithelial-specific keratin genes in a manner consistent with a potential locus control region function (61). These studies demonstrate an important aspect of the role of KLF4 in controlling *in vivo* differentiation of specific epithelial functions.

Taken together, the studies described above suggest that KLF4 has a potentially important function in regulating the proliferation and differentiation of specific epithelial and endothelial tissues. Orchestrated expression and function of KLF4 may be critical to the homeostasis of these tissues,

whereas altered expression and function of KLF4 may contribute to tumor development and progression in these tissues.

KLF4 as a putative tumor suppressor in the GI tract

Clinical evidence

Consistent with the DNA-damage checkpoint function of KLF4, expression of KLF4 is altered in various models of intestinal tumorigenesis. For example, the level of KLF4 expression is significantly decreased in colonic adenomas in patients with familial adenomatous polyposis when compared with adjacent normal mucosa (55,66). Similarly, the *KLF4* mRNA level is decreased in sporadic colonic adenomas and carcinomas in humans when compared with normal colonic tissues (37). These studies suggest that downregulation of *KLF4* expression in the colon contributes to cellular hyperproliferation and malignant transformation (37).

Recently, we systematically analyzed KLF4 expression in primary gastric tumors and lymph node metastases. We found significantly reduced or lost KLF4 expression in both primary tumors and metastases as compared with that in the normal mucosa, with the lowest KLF4 expression levels occurring in the metastases. Significantly, patients had a clearly progressive loss of KLF4 expression as their disease advanced from American Joint Committee on Cancer stage I to stage IV, and loss of KLF4 expression was an independent predictor of poor survival (67). Our findings are further supported by a microarray analysis showing that *KLF4* expression is decreased or lost in gastric cancers (68) and by immunohistochemical and real-time quantitative PCR assays demonstrating that *KLF4* expression is decreased dramatically in human gastric cancers (63). All of these findings suggest that a decrease in or loss of KLF4 expression contributes to gastric cancer development and progression.

Reduced or lost KLF4 expression has also been found in human esophageal squamous cell carcinomas when compared with that in normal esophageal tissue (69,70). Furthermore, decreased expression of KLF4 in an esophageal cancer cell line by antisense *KLF4* transfection has been shown to increase cell proliferation and decrease cell-adhesion ability (69), whereas increased expression of KLF4 in the esophageal cancer cell line by sense *KLF4* transfection has been demonstrated to result in the upregulation of squamous cell differentiation-associated genes, such as *SPRR1A*, *SPRR2A* and *KRT4* (70).

In addition to tumors of the GI tract, decreased KLF4 expression has been found in other types of tumors. For example, KLF4 expression is decreased in the prostate in cases of prostate cancer and benign prostate hypertrophy (71,72). In addition, KLF4 is highly expressed in the normal bladder epithelium, whereas its expression is frequently downregulated in bladder cancer cell lines and tissue (73). KLF4 expression is also decreased in lung cancers (74). Compared with that in normal T cells, KLF4 expression is silenced in adult T-cell leukemia cells (75). Interestingly, expression of KLF4 is significantly repressed in human glioma-associated vascular endothelial cells as compared with that in non-neoplastic control vascular endothelial cells (76), suggesting that KLF4 is also involved in an antiangiogenic pathway.

Experimental evidence

The significance of the *in vivo* function of KLF4 as a putative tumor suppressor is heightened by the fact that expression of KLF4 has been shown to be both developmentally regulated and downregulated in a rodent model of intestinal tumorigenesis. For example, KLF4 expression has been demonstrated to be significantly decreased in intestinal adenomas in multiple intestinal neoplasia ($APC^{Min/+}$) mice (55). Because of increased proliferation and altered differentiation of their gastric epithelia, *Klf4* mutant mice with gastric epithelial ablation of *Klf4* expression display premalignant features of hypertrophy of gastric epithelia and aberrant expression of acidic mucins and TFF2/SP-positive cells (63). In addition, *in vitro* expression of KLF4 is temporally associated with a growth-arrested state, such as that induced by contact inhibition and serum deprivation (27). Constitutive expression of KLF4 suppresses cell proliferation (27,77) by blocking cell cycle progression from G_1 to S phase (57). Importantly, KLF4 expression is stimulated following DNA damage in a p53-dependent fashion (56), which leads to transcriptional upregulation of the gene encoding $p21^{WAF1/Cip1}$ (56). Recent genetic evidence suggests that induction of KLF4 expression is essential for mediating the G_1/S checkpoint function of p53 following DNA damage (60). In HT-29 human colon adenocarcinoma cells, overexpression of KLF4 significantly inhibits *cyclin D1* mRNA expression as well as *cyclin D1* gene promoter activity. These data suggest that KLF4 functions as a transcriptional repressor of the *cyclin D1* gene to control cell growth in the colon. Overexpression of KLF4 inhibits colony formation, migration and invasion *in vitro*, and has pronounced effects on tumorigenicity when grown as xenografts in nude mice. Also, we have observed reduced or lost KLF4 expression in various gastric cancer cell lines. Restored expression of KLF4 in human gastric cancer cells significantly inhibited their tumorigenicity and totally abrogated their ability to metastasize in orthotopic xenograft nude mouse models (67). KLF4 expression also induced both cell cycle arrest and apoptosis in gastric cancer cells. Consistently, KLF4 induces apoptosis in bladder cancer (73), colon cancer (59) and leukemia (75) cells. However, the mechanism by which KLF4 induces apoptosis is unknown.

Mechanistic evidence

A high frequency of loss of heterozygosity (LOH) on chromosome 9q31 (where *KLF4* is located) (31) has been found in human colorectal, gastric and esophageal cancers (78–81). Reduced or lost KLF4 expression may be caused by LOH in these cancers. Recently, direct evidence substantiated KLF4 as a tumor suppressor, including LOH in the *KLF4* locus in a subset of CRC specimens and cell lines (82). Similarly, among eight gastric cancer cell lines, hemizygous deletions of the *KLF4* gene were found in SK-GT5 and SNU-16 cells (67). In addition, several point mutations within the open reading frame of the *KLF4* gene have been identified in CRC cell lines (Figure 1). These mutations lead to restrained cytoplasmic distribution of the KLF4 protein and diminished ability to activate the $p21^{WAF1/Cip1}$ promoter (82). Increasing evidence suggests that aberrant DNA methylation of CpG islands around promoter regions can have the effect on the inactivation of tumor suppressor genes (83). Hypermethylation in the 5′-untranslated region of *KLF4* has been found in CRC and gastric cancer tissues and cell lines (67,82). Interestingly, blockade of gene hypermethylation reactivates *KLF4* expression in human gastric cancer cells. Therefore, both genetic and epigenetic alterations may contribute to reduced and lost KLF4 expression in GI cancers. All of these findings support the notion that *KLF4* is a tumor suppressor gene in GI cancers.

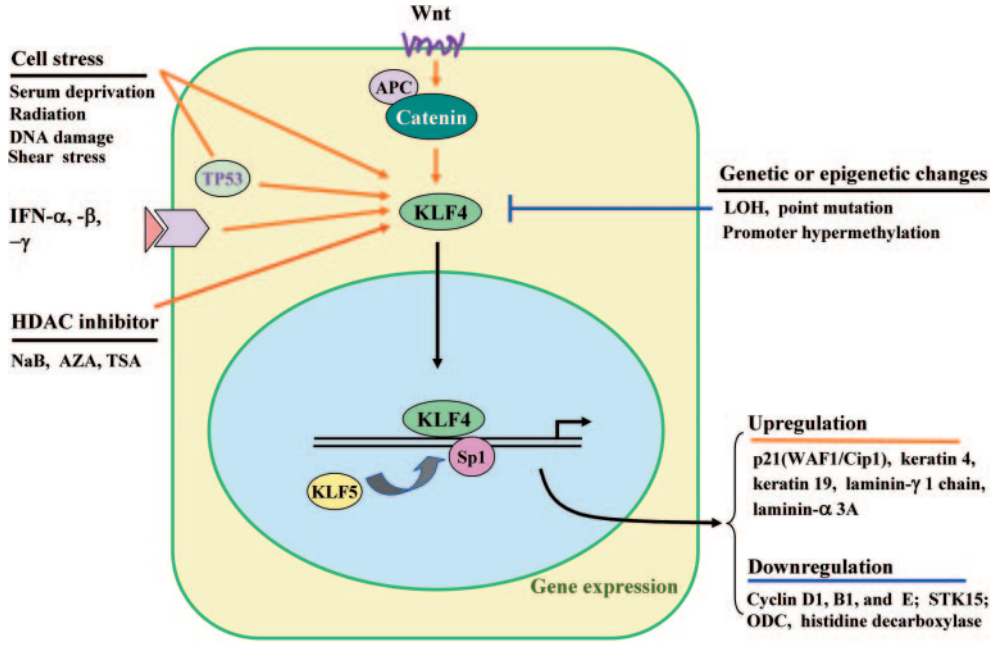

Fig. 2. Diagram of the major pathways of KLF4. Cell stress signal and interferon (IFN)-α, -β, and -γ can upregulate KLF4 expression. The Wnt/APC signal pathway also plays an important role in the regulation of KLF4 expression. LOH, point mutations in the coding region and promoter hypermethylation are the main causes of *klf4* gene silencing. Expressed KLF4 enters the nucleus and binds to the CACCC element or GC-rich sequence of the gene promoter region to regulate cell proliferation or differentiation-related gene expression. Altered expression and function of KLF4 may play important roles in GI carcinogenesis.

Moreover, recent studies indicated that KLF4 is a downstream target of the Wnt/APC/β-Catenin pathway (84,85), whereas Foxl1, a winged helix transcription factor, is an important regulator of the Wnt/APC/β-catenin pathway (86–88). Therefore, loss of APC expression, activation of β-catenin and alteration of Foxl1 expression may also contribute to the downregulation of KLF4 expression in GI cancers.

New challenging frontiers

The KLF family is expanding, and further characterization of this interesting protein family will yield more and more insights into the respective roles of its members in various physiological and pathological processes. The inconsistency in and discrepancy among KLF4 reports may reflect the pleiotropic functions of this protein and complexity of its tumorigenicity. The major pathways in which KLF4 may be involved are summarized in Figure 2. Recent studies have already pointed out some challenging but significant areas that warrant immediate and thorough investigation.

Dual regulation of cell growth

Apparently, KLF4 expression is associated with both inhibition and induction of proliferation. As described above, KLF4 expression is reduced in colorectal carcinoma (55,71,82), and enforced KLF4 expression has been shown to inhibit the tumorigenicity of a CRC cell line (89). In contrast with these studies, wild-type KLF4 has been identified to have transforming activity in cDNA libraries representing human squamous cell carcinoma of the head and neck (90). In fact, KLF4 expression is upregulated in human head and neck squamous cell carcinoma, particularly within the basal cell layer of adjacent dysplastic epithelium, and in breast cancer (90). Moreover, expression of KLF4 is increased in primary ductal carcinoma of the breast and oral squamous cancers, and overexpression of KLF4 inhibits expression of integrin and induces expression of the tumor marker clusterin, both changes that enhance tumor progression and metastasis (71). The same group that performed those studies recently reported that localization of KLF4 in the nucleus of breast cancer cells is a prognostic factor and identifies KLF4 as a marker of an aggressive phenotype in early-stage infiltrating ductal carcinoma (91). However, some of the evidence regarding the role of KLF4 in breast cancer biology is controversial. For example, expression of laminin-5, the major extracellular matrix protein produced by mammary epithelial cells, is markedly downregulated in breast cancer cells. Laminin-5 is composed of three chains designated alpha3A, beta3 and gamma2, each of which is encoded by a separate gene. Two KLF4-binding sites, which are required for the activity of the lama3a promoter, have been identified in the promoter region of the *lama3a* gene. Electrophoretic mobility shift assays have revealed the absence of KLF4-binding activity in extracts from five panels of breast cancer cell lines: T47D, MDA-MB 231, ZR75-1, MDA-MB 436 and MCF7. Additionally, transient transfection of a plasmid expressing KLF4 activates transcription from the LAMA3A promoter in breast cancer cells. A reporter vector containing duplicate KLF4-binding sites in its promoter is expressed at high levels in functionally normal MCF10A breast epithelial cells but at negligible levels in breast cancer cells. Thus, the absence of laminin alpha3A in breast cancer cells appears to be attributable to the lack of KLF4 activity (92), whereas promoter methylation may be an alternative explanation for the decreased and lost expression of LAMA3 in breast cancers (93).

In a transgenic mouse model, induction of KLF4 in basal keratinocytes induced outgrowth of dysplastic lesions resembling squamous cell carcinoma *in situ* (94). This study suggests that *KLF4* functions as either a tumor suppressor gene or an oncogene. Several issues remain to be addressed such as the

potential contributions to tumor phenotype by KLF4 somatic mutation and the expression and/or ratios of different isoforms of KLF4 proteins. Tumor suppressor/oncogenic function of KLF4 also may be dependent on the tumor type (e.g. genetic background). For example, colorectal carcinomas, which have reduced KLF4 expression, frequently exhibit activation of RAS and/or the APC/β-catenin-c-MYC pathway (8,95), whereas breast ductal carcinomas and oral squamous cell carcinomas, which have greatly increased KLF4 expression, do not frequently exhibit activation of APC/β-catenin, RAS and/or GLI.

Dual regulation of gene expression

A number of lines of evidence indicate that KLF4 can either activate or suppress gene expression. A recent examination of the relationship between KLF4 and promoter activity of the *CYP1A1* gene indicates that KLF4 is a suppressor of the *CYP1A1* promoter in a BTE-dependent fashion (40). It suppresses this promoter by competing with binding of Sp1 to the BTE and physically interacting with Sp1, which is a potent activator of *CYP1A1* (41). Both avenues of suppression seem to be mediated by the zinc fingers of KLF4. Another study showed that KLF4 possesses an intrinsic repression domain in a region of the protein preceding the zinc fingers (35). In different situations, however, KLF4 can be a potent activator of transcription (31,36,37). Moreover, the N-terminal region of KLF4 has been shown to confer the transcription-activating function (31,35). These observations indicate that KLF4 is a pleiotropic protein with dual activity in modulating transcription. The mechanisms by which KLF4 activates transcription are much more complex than initially thought. For example, full transactivating activity requires two clusters of acidic amino acid residues within KLF4. The same amino acid residues are indispensable for the interaction of KLF4 with the co-activator CBP and for KLF4 to suppress cell growth (77). Therefore, there is an important association between two primary biological activities of KLF4: transcription activation and growth suppression. Whether there is a similar mechanism by which KLF4 exerts its functions of transcription repression and growth stimulation is unclear. In addition to a report of microarray analysis (61), the other genes that are regulated by KLF4 are listed in Table I.

Table I. KLF4 regulated genes

Gene name/gene function/expression level	[PubMed ID][a]
Cyclin D1/ cell cycle/ ↓	[10908361]
Orinithine decarboxylase/ cell proliferation/ ↓	[12297499]
SM alpha-actin/ cell differentiation/ ↓	[12970361]
Laminin1/ tissue homeostasis/ ↓	[14634001]
Histidine decarboxylase/ enzyme/ ↓	[14670968]
CD11d/ cell adhesion/ ↓	[15561714]
CYP1A1/ drug-metabolizing enzymes/ ↓	[9651398]
p21(WAF1/Cip1)/ cyclin-dependent kinase inhibitor/ ↑	[10749849, 12087069]
Keratin 4/ cell differentiation/ ↑	[10802067]
Keratin 19/ stem cell marker/ ↑	[10859317]
Laminin alpha 3A/ cell differentiation/ ↑	[11551969]
Laminin gamma-1 chain/ cell differentiation/ ↑	[12034813]
Intestinal alkaline phosphatase/ cell differentiation/ ↑	[12919939]
Small praline-rich protein 1A/ cell differentiation/ ↑	[14647409]
Small praline-rich protein 2A/ cell differentiation/ ↑	[14647409]
Cytokeratin 4/ cell differentiation/ ↑	[14647409]
$P27^{KIP1}$/ cell cycle/ ↑	[12087069]
p53/ cell cycle, DNA damage/ ↑	[12087069]
Urokinase-type plasminogen activator receptor/ proteolysis/ ↑	[15031282]
A33 antigen/ intestinal epithelial cell marker/ ↑	[12853980]

[a]Major references are listed by PubMed identification numbers.
↑, upregulation; ↓, downregulation.

Crosstalk with known oncogenic pathways

Although induction of $p21^{WAF1/CIP1}$ expression has been shown to be a consequence of direct binding of p53 to its promoter, evidence implicates many other transcription factors in the regulation of $p21^{WAF1/CIP1}$ transcription (96). Among these is KLF4 (22,34,97). Constitutive expression of KLF4 inhibits DNA synthesis and reduces cell proliferation (27,77,89). This is in part a product of cell cycle arrest at the G_1/S boundary because of the ability of KLF4 to transcriptionally activate expression of $p21^{WAF1/CIP1}$ (56,57,61). In fact, $p21^{WAF1/CIP1}$ expression is activated in a p53-dependent fashion upon DNA damage by agents such as methyl methane sulfonate and γ-radiation and correlated with consequent G_1/S cell cycle arrest in cells with wild-type *p53*, supporting a checkpoint function for KLF4 (56,60). Importantly, inhibition of KLF4 expression in such cells after γ-irradiation results in abrogation of cell cycle arrest at G_1 in a manner similar to that in irradiated $p53^{-/-}$ cells (60). Conversely, conditional expression of KLF4 in irradiated $p53^{-/-}$ cells restores cell cycle arrest at G_1 as if they were wild-type for *p53* (60). These findings indicate that KLF4 is a necessary and sufficient mediator of p53 for cell cycle arrest at G_1/S resulting from DNA damage and mediates arrest by activating $p21^{WAF1/CIP1}$ expression.

In addition to G_1 arrest, $p21^{WAF1/CIP1}$ has been shown to be required for sustained cell cycle arrest at G_2 following γ-irradiation (98). Yang and co-workers sought to determine whether KLF4 is also involved in controlling the G_2/M checkpoint after DNA damage. They found sustained cell cycle arrest at the G_2 phase in HCT116 $p53^{+/+}$ cells after irradiation, which was accompanied by increased expression of p53, KLF4 and $p21^{WAF1/CIP1}$ but decreased expression of cyclin B1. However, the levels of cyclin B1 expression increased in irradiated $p53^{-/-}$ HCT116 cells, in which KLF4 expression did not increase because of an absence of p53. When KLF4 expression was inhibited by small interfering RNA, irradiated HCT116 cells exhibited increased mitotic indices and a rise in cyclin B1 levels. Conversely, irradiated HCT116 $p53^{-/-}$ cells that were infected with KLF4-expressing adenoviruses demonstrated a concurrent reduction in mitotic indices and cyclin B1 levels. In each case, Cdc2 kinase measurements showed an inverse correlation between Cdc2 kinase activities and KLF4 levels. In addition, co-transfection experiments showed that KLF4 repressed the cyclin B1 promoter through a specific GC-rich element and that both KLF4 and histone deacetylase (HDAC) were associated with the cyclin B1 promoter in irradiated HCT116 cells as demonstrated by chromatin immunoprecipitation analysis. These results suggest that KLF4 is essential for preventing mitotic entry following γ-irradiation in that it inhibits cyclin B1 expression (60). More recently, a report showed that in the hic5-PPAR-γ pathway KLF4 is also involved in the regulation of epithelial cell differentiation (99). In addition, KLF4 expression has been found to be upregulated in pancreatic intraepithelial neoplasia (PanIN) along with other extrapancreatic foregut markers, including *pepsinogen C*, *MUC6* and *TFF1*. Hedgehog pathway activation induced by transfection of immortalized human pancreatic ductal epithelial cells with *Gli1* resulting in upregulation of the

majority of foregut markers has been seen in early PanIN lesions. Whether the upregulation of KLF4 expression contributes to the development of PanIN lesions remains to be determined, whereas one of the explanations is that PanIN development may involve Hedgehog-mediated conversion to a gastric epithelial differentiation program (100).

Interaction with other members of the Sp/KLF family

The hallmark characteristics of the Sp/KLF family are a conserved zinc finger DNA-binding domain and its ability to recognize the similar *cis*-element (101). These characteristics provide the molecular basis for their physical interaction and functional overlap, synergism and antagonism. For example, Sp1 upregulates ODC expression by binding the GC-rich region of the gene promoter (102,103), whereas KLF4 represses *ODC* gene expression by interacting with the GC-rich region on the promoter (104). In fact, KLF4 inhibits HDAC promoter activity by competing with Sp1 at the upstream GC box and independently by binding the three downstream gastrin-responsive elements, indicating that KLF4 can repress *HDAC* gene expression by Sp1-dependent and Sp1-independent mechanisms (105). In contrast, KLF4 can synergistically activate the *laminin-γ1* gene promoter with Sp1; this synergistic effect is dependent on the promoter context (106). Interestingly, KLF4 and KLF5, two closely related KLF family members, are expressed in the intestinal epithelium with distinct patterns: KLF4 is primarily expressed in terminally differentiated villus cells, whereas KLF5 is primarily expressed in proliferating crypt cells. Functionally, KLF4 negatively regulates cell proliferation, whereas KLF5 positively regulates cell proliferation (107). A recent study demonstrated that KLF5 binds to a number of *cis*-DNA elements, which were previously shown to bind to KLF4, and that KLF4 and KLF5 antagonize each other (108), suggesting a basis for coordinated expression and regulation of the *KLF* gene in the intestinal epithelium. In addition, the expression of KLF4 is often subjected to the changes of cell environment. Therefore, further investigation is clearly required to determine how extracellular signaling stimuli and pathways affect KLF4 expression and function; how the detailed network of KLF4 signaling interacts with other transcription factors, and how those transcription factors corporately regulate the proliferation and differentiation of GI epithelium in a spatial and temporal manner; and how the altered expression of KLF4 leads to the carcinogenesis of GI epithelium.

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References

1. Parkin,D.M., Bray,F.I. and Devesa,S.S. (2001) Cancer burden in the year 2000. The global picture. *Eur. J. Cancer*, **37** (Suppl. 8), S4–S66.
2. Jemal,A., Thomas,A., Murray,T. and Thun,M. (2002) Cancer statistics, 2002. *CA Cancer J. Clin.*, **52**, 23–47.
3. Zheng,L., Wang,L., Ajani,J. and Xie,K. (2004) Molecular basis of gastric cancer development and progression. *Gastric Cancer*, **7**, 61–77.
4. Chan,A.O., Luk,J.M., Hui,W.M. and Lam,S.K. (1999) Molecular biology of gastric carcinoma: from laboratory to bedside. *J. Gastroenterol. Hepatol.*, **14**, 1150–1160.
5. Fenoglio-Preiser,C.M., Wang,J., Stemmermann,G.N. and Noffsinger,A. (2003) TP53 and gastric carcinoma: a review. *Hum. Mutat.*, **21**, 258–270.
6. Sud,R., Wells,D., Talbot,I.C. and Delhanty,J.D. (2001) Genetic alterations in gastric cancers from British patients. *Cancer Genet. Cytogenet.*, **126**, 111–119.
7. Carneiro,F., Huntsman,D.G., Smyrk,T.C., Owen,D.A., Seruca,R., Pharoah,P., Caldas,C. and Sobrinho-Simoes,M. (2004) Model of the early development of diffuse gastric cancer in E-cadherin mutation carriers and its implications for patient screening. *J. Pathol.*, **203**, 681–687.
8. Kinzler,K.W. and Vogelstein,B. (1996) Lessons from hereditary colorectal cancer. *Cell*, **87**, 159–170.
9. Ilyas,M., Straub,J., Tomlinson,I.P. and Bodmer,W.F. (1999) Genetic pathways in colorectal and other cancers. *Eur. J. Cancer*, **35**, 1986–2002.
10. Yang,V.W. (1999) The molecular genetics of colorectal cancer. *Curr. Gastroenterol. Rep.*, **1**, 449–454.
11. Fearon,E.R. and Vogelstein,B. (1990) A genetic model for colorectal tumorigenesis. *Cell*, **61**, 759–767.
12. Goel,A., Arnold,C.N., Niedzwiecki,D. *et al.* (2003) Characterization of sporadic colon cancer by patterns of genomic instability. *Cancer Res.*, **63**, 1608–1614.
13. Thiagalingam,S., Laken,S., Willson,J.K., Markowitz,S.D., Kinzler,K.W., Vogelstein,B. and Lengauer,C. (2001) Mechanisms underlying losses of heterozygosity in human colorectal cancers. *Proc. Natl Acad. Sci. USA*, **98**, 2698–2702.
14. Frebourg,T., Oliveira,C., Hochain,P. *et al.* (2005) Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer. *J. Med. Genet.* (in press).
15. Guilford,P., Hopkins,J., Harraway,J., McLeod,M., McLeod,N., Harawira,P., Taite,H., Scoular,R., Miller,A. and Reeve,A.E. (1998) E-cadherin germline mutations in familial gastric cancer. *Nature*, **392**, 402–405.
16. El-Rifai,W. and Powell,S.M. (2002) Molecular biology of gastric cancer. *Semin. Radiat. Oncol.*, **12**, 128–140.
17. Migliavacca,M., Ottini,L., Bazan,V. *et al.* (2004) TP53 in gastric cancer: mutations in the l3 loop and LSH motif DNA-binding domains of TP53 predict poor outcome. *J. Cell Physiol.*, **200**, 476–485.
18. Li,Q.L., Ito,K., Sakakura,C. *et al.* (2002) Causal relationship between the loss of RUNX3 expression and gastric cancer. *Cell.* **109**, 113–124.
19. Wei,D., Gong,W., Oh,S.C. *et al.* (2005) Loss of RUNX3 expression significantly affects the clinical outcome of gastric cancer patients and its restoration causes drastic suppression of tumor growth and metastasis. *Cancer Res.*, **65**, 4809–4816.
20. Schuh,R., Aicher,W., Gaul,U., Cote,S., Preiss,A., Maier,D., Seifert,E., Nauber,U., Schroder,C. and Kemler,R., (1986) A conserved family of nuclear proteins containing structural elements of the finger protein encoded by Krüppel, a *Drosophila* segmentation gene. *Cell.* **47**, 1025–1032.
21. Black,A.R., Black,J.D. and Azizkhan-Clifford,J. (2001) Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer. *J. Cell Physiol.*, **188**, 143–160.
22. Dang,D.T., Pevsner,J. and Yang,V.W. (2000) The biology of the mammalian Krüppel-like family of transcription factors. *Int. J. Biochem. Cell Biol.*, **32**, 1103–1121.
23. Turner,J. and Crossley,M. (1999) Mammalian Krüppel-like transcription factors: more than just a pretty finger. *Trends Biochem. Sci.*, **24**, 236–240.
24. Philipsen,S. and Suske,G. (1999) A tale of three fingers: the family of mammalian Sp/XKLF transcription factors. *Nucleic Acids Res.*, **27**, 2991–3000.
25. Perkins,A.C., Sharpe,A.H. and Orkin,S.H. (1995) Lethal beta-thalassaemia in mice lacking the erythroid CACCC-transcription factor EKLF. *Nature*, **375**, 318–322.
26. Nuez,B., Michalovich,D., Bygrave,A., Ploemacher,R. and Grosveld,F. (1995) Defective haematopoiesis in fetal liver resulting from inactivation of the *EKLF* gene. *Nature*, **375**, 316–318.
27. Shields,J.M., Christy,R.J. and Yang,V.W. (1996) Identification and characterization of a gene encoding a gut-enriched Krüppel-like factor expressed during growth arrest. *J. Biol. Chem.*, **271**, 20009–20017.
28. Shields,J.M. and Yang,V.W. (1997) Two potent nuclear localization signals in the gut-enriched Krüppel-like factor define a subfamily of closely related Krüppel proteins. *J. Biol. Chem.*, **272**, 18504–18507.

29. Anderson,K.P., Kern,C.B., Crable,S.C. and Lingrel,J.B. (1995) Isolation of a gene encoding a functional zinc finger protein homologous to erythroid Krüppel-like factor: identification of a new multigene family. *Mol. Cell Biol.*, **15**, 5957–5965.
30. Wani,M.A., Conkright,M.D., Jeffries,S., Hughes,M.J. and Lingrel,J.B. (1999) cDNA isolation, genomic structure, regulation, and chromosomal localization of human lung Krüppel-like factor. *Genomics,* **60**, 78–86.
31. Garrett-Sinha,L.A., Eberspaecher,H., Seldin,M.F. and de Crombrugghe,B. (1996) A gene for a novel zinc-finger protein expressed in differentiated epithelial cells and transiently in certain mesenchymal cells. *J. Biol. Chem.*, **271**, 31384–31390.
32. Wani,M.A., Wert,S.E. and Lingrel,J.B. (1999) Lung Krüppel-like factor, a zinc finger transcription factor, is essential for normal lung development. *J. Biol. Chem.*, **274**, 21180–21185.
33. Segre,J.A., Bauer,C. and Fuchs,E. (1999) Klf4 is a transcription factor required for establishing the barrier function of the skin. *Nat. Genet.*, **22**, 356–360.
34. Kaczynski,J., Cook,T. and Urrutia,R. (2003) Sp1- and Krüppel-like transcription factors. *Genome Biol.*, **4**, 206.
35. Yet,S.F., McA'Nulty,M.M., Folta,S.C. *et al.* (1998) a Krüppel-like zinc finger protein, is expressed in vascular endothelial cells and contains transcriptional activation and repression domains. *J. Biol. Chem.*, **273**, 1026–1031.
36. Jenkins,T.D., Opitz,O.G., Okano,J. and Rustgi,A.K. (1998) Transactivation of the human keratin 4 and Epstein-Barr virus ED-L2 promoters by gut-enriched Krüppel-like factor. *J. Biol. Chem.*, **273**, 10747–10754.
37. Shie,J.L., Chen,Z.Y., O'Brien,M.J., Pestell,R.G., Lee,M.E. and Tseng,C.C. (2000) Role of gut-enriched Krüppel-like factor in colonic cell growth and differentiation. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **279**, G806–G814.
38. Shields,J.M. and Yang,V.W. (1998) Identification of the DNA sequence that interacts with the gut-enriched Krüppel-like factor. *Nucleic Acids Res.*, **26**, 796–802.
39. Yanagida,A., Sogawa,K., Yasumoto,K.I. and Fujii-Kuriyama,Y. (1990) A novel *cis*-acting DNA element required for a high level of inducible expression of the rat *P-450c* gene. *Mol. Cell Biol.*, **10**, 1470–1475.
40. Fujii-Kuriyama,Y., Imataka,H., Sogawa,K., Yasumoto,K. and Kikuchi,Y. (1992) Regulation of CYP1A1 expression. *FASEB J.*, **6**, 706–710.
41. Zhang,W., Shields,J.M., Sogawa,K., Fujii-Kuriyama,Y. and Yang,V.W. (1998) The gut-enriched Krüppel-like factor suppresses the activity of the CYP1A1 promoter in an Sp1-dependent fashion. *J. Biol. Chem.*, **273**, 17917–17925.
42. Ehlermann,J., Pfisterer,P. and Schorle,H. (2003) Dynamic expression of Kruppel-like factor 4 (Klf4), a target of transcription factor AP-2alpha during murine mid-embryogenesis. *Anat. Rec. A. Discov. Mol. Cell Evol. Biol.*, **273**, 677–680.
43. Conkright,M.D., Wani,M.A., Anderson,K.P. and Lingrel,J.B. (1999) A gene encoding an intestinal-enriched member of the Krüppel-like factor family expressed in intestinal epithelial cells. *Nucleic Acids Res.*, **27**, 1263–1270.
44. Panigada,M., Porcellini,S., Sutti,F., Doneda,L., Pozzoli,O., Consalez,G.G., Guttinger,M. and Grassi,F. (1999) GKLF in thymus epithelium as a developmentally regulated element of thymocyte-stroma cross-talk. *Mech. Dev.*, **81**, 103–113.
45. Behr,R. and Kaestner,K.H. (2002) Developmental and cell type-specific expression of the zinc finger transcription factor Krüppel-like factor 4 (Klf4) in postnatal mouse testis. *Mech. Dev.*, **115**, 167–169.
46. Ohnishi,S., Laub,F., Matsumoto,N., Asaka,M., Ramirez,F., Yoshida,T. and Terada,M. (2000) Developmental expression of the mouse gene coding for the Krüppel-like transcription factor *KLF5*. *Dev. Dyn.*, **217**, 421–429.
47. Chiambaretta,F., De Graeve,F., Turet,G., Marceau,G., Gain,P., Dastugue,B., Rigal,D. and Sapin,V. (2004) Cell and tissue specific expression of human Krüppel-like transcription factors in human ocular surface. *Mol. Vis.*, **10**, 901–909.
48. Jaubert,J., Cheng,J. and Segre,J.A. (2003) Ectopic expression of krüppel like factor 4 (Klf4) accelerates formation of the epidermal permeability barrier. *Development*, **130**, 2767–2777.
49. Katz,J.P., Perreault,N., Goldstein,B.G., Lee,C.S., Labosky,P.A., Yang,V.W. and Kaestner,K.H. (2002) The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. *Development*, **129**, 2619–2628.
50. McCormick,S.M., Eskin,S.G., McIntire,L.V., Teng,C.L., Lu,C.M., Russell,C.G. and Chittur,K.K. (2001) DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells. *Proc. Natl Acad. Sci. USA*, **98**, 8955–8960.
51. Lee,Y.W., Eum,S.Y., Chen,K.C., Hennig,B. and Toborek,M. (2004) Gene expression profile in interleukin-4-stimulated human vascular endothelial cells. *Mol. Med.*, **10**, 19–27.
52. Mazzanti,C.M., Tandle,A., Lorang,D., Costouros,N., Roberts,D., Bevilacqua,G. and Libutti,S.K. (2004) Early genetic mechanisms underlying the inhibitory effects of endostatin and fumagillin on human endothelial cells. *Genome Res.*, **14**, 1585–1593.
53. Adam,P.J., Regan,C.P., Hautmann,M.B. and Owens,G.K. (2000) Positive- and negative-acting Krüppel-like transcription factors bind a transforming growth factor beta control element required for expression of the smooth muscle cell differentiation marker SM22alpha *in vivo*. *J. Biol. Chem.*, **275**, 37798–37806.
54. Liu,Y., Sinha,S., McDonald,O.G., Shang,Y., Hoofnagle,M.H. and Owens,G.K. (2005) Krüppel-like factor 4 abrogates myocardin-induced activation of smooth muscle gene expression. *J. Biol. Chem.*, **280**, 9719–9727.
55. Ton-That,H., Kaestner,K.H., Shields,J.M., Mahatanankoon,C.S. and Yang,V.W. (1997) Expression of the gut-enriched Krüppel-like factor gene during development and intestinal tumorigenesis. *FEBS Lett.*, **419**, 239–243.
56. Zhang,W., Geiman,D.E., Shields,J.M., Dang,D.T., Mahatan,C.S., Kaestner,K.H., Biggs,J.R., Kraft,A.S. and Yang,V.W. (2000) The gut-enriched Krüppel-like factor (Krüppel-like factor 4) mediates the transactivating effect of p53 on the p21WAF1/Cip1 promoter. *J. Biol. Chem.*, **275**, 18391–18398.
57. Chen,X., Johns,D.C., Geiman,D.E., Marban,E., Dang,D.T., Hamlin,G., Sun,R. and Yang,V.W. (2001) Krüppel-like factor 4 (gut-enriched Krüppel-like factor) inhibits cell proliferation by blocking G_1/S progression of the cell cycle. *J. Biol. Chem.*, **276**, 30423–30428.
58. Shie,J.L., Chen,Z.Y., Fu,M., Pestell,R.G. and Tseng,C.C. (2000) Gut-enriched Krüppel-like factor represses cyclin D1 promoter activity through Sp1 motif. *Nucleic Acids Res.*, **28**, 2969–2976.
59. Chen,Z.Y., Shie,J. and Tseng,C. (2000) Up-regulation of gut-enriched krüppel-like factor by interferon-gamma in human colon carcinoma cells. *FEBS Lett.*, **477**, 67–72.
60. Yoon,H.S., Chen,X. and Yang,V.W. (2003) Krüppel-like factor 4 mediates p53-dependent G_1/S cell cycle arrest in response to DNA damage. *J. Biol. Chem.*, **278**, 2101–2105.
61. Chen,X., Whitney,E.M., Gao,S.Y. and Yang,V.W. (2003) Transcriptional profiling of Krüppel-like factor 4 reveals a function in cell cycle regulation and epithelial differentiation. *J. Mol. Biol.*, **326**, 665–677.
62. Nickenig,G., Baudler,S., Muller,C., Werner,C., Werner,N., Welzel,H., Strehlow,K. and Bohm,M. (2002) Redox-sensitive vascular smooth muscle cell proliferation is mediated by GKLF and Id3 *in vitro* and *in vivo*. *FASEB J.*, **16**, 1077–1086.
63. Katz,J.P., Perreault,N., Goldstein,B.G., Actman,L., McNally,S.R., Silberg,D.G., Furth,E.E. and Kaestner,K.H. (2005) Loss of Klf4 in mice causes altered proliferation and differentiation and precancerous changes in the adult stomach. *Gastroenterology*, **128**, 935–945.
64. Brembeck,F.H. and Rustgi,A.K. (2000) The tissue-dependent *keratin 19* gene transcription is regulated by GKLF/KLF4 and Sp1. *J. Biol. Chem.*, **275**, 28230–28239.
65. Brembeck,F.H., Moffett,J., Wang,T.C. and Rustgi,A.K. (2001) The keratin 19 promoter is potent for cell-specific targeting of genes in transgenic mice. *Gastroenterology*, **120**, 1720–1728.
66. Dang,D.T., Bachman,K.E., Mahatan,C.S., Dang,L.H., Giardiello,F.M. and Yang,V.W. (2000) Decreased expression of the gut-enriched Krüppel-like factor gene in intestinal adenomas of multiple intestinal neoplasia mice and in colonic adenomas of familial adenomatous polyposis patients. *FEBS Lett.*, **476**, 203–207.
67. Wei,D., Gong,W., Kanai,M., Schlunk,C., Wang,L., Yao,J.C., Wu,T.T., Huang,S. and Xie,K. (2005) Drastic down-regulation of Kruppel-like factor 4 expression is critical in human gastric cancer development and progression. *Cancer Res.*, **65**, 2746–2754.
68. Kim,B., Bang,S., Lee,S. *et al.* (2003) Expression profiling and subtype-specific expression of stomach cancer. *Cancer Res.*, **63**, 8248–8255.
69. Wang,N., Liu,Z.H., Ding,F., Wang,X.Q., Zhou,C.N. and Wu,M. (2002) Down-regulation of gut-enriched Krüppel-like factor expression in esophageal cancer. *World J. Gastroenterol.*, **8**, 966–970.
70. Luo,A., Kong,J., Hu,G., Liew,C.C., Xiong,M., Wang,X., Ji,J., Wang,T., Zhi,H., Wu,M. and Liu,Z. (2004) Discovery of $Ca2^{+}$-relevant and differentiation-associated genes downregulated in esophageal squamous cell carcinoma using cDNA microarray. *Oncogene*, **23**, 1291–1299.

71. Foster,K.W., Frost,A.R., McKie-Bell,P., Lin,C.Y., Engler,J.A., Grizzle,W.E. and Ruppert,J.M. (2000) Increase of GKLF messenger RNA and protein expression during progression of breast cancer. *Cancer Res.*, **60**, 6488–6495.
72. Luo,J., Dunn,T., Ewing,C., Sauvageot,J., Chen,Y., Trent,J. and Isaacs,W. (2002) Gene expression signature of benign prostatic hyperplasia revealed by cDNA microarray analysis. *Prostate*. **51**, 189–200.
73. Ohnishi,S., Ohnami,S., Laub,F., Aoki,K., Suzuki,K., Kanai,Y., Haga,K., Asaka,M., Ramirez,F. and Yoshida,T. (2003) Downregulation and growth inhibitory effect of epithelial-type Krüppel-like transcription factor KLF4, but not KLF5, in bladder cancer. *Biochem. Biophys. Res. Commun.*, **308**, 251–256.
74. Bianchi,F., Hu,J., Pelosi,G., Cirincione,R., Ferguson,M., Ratcliffe,C., Di Fiore,P.P., Gatter,K., Pezzella,F. and Pastorino,U. (2004) Lung cancers detected by screening with spiral computed tomography have a malignant phenotype when analyzed by cDNA microarray. *Clin. Cancer Res.*, **15**, 6023–6028.
75. Yasunaga,J., Taniguchi,Y., Nosaka,K., Yoshida,M., Satou,Y., Sakai,T., Mitsuya,H. and Matsuoka,M. (2004) Identification of aberrantly methylated genes in association with adult T-cell leukemia. *Cancer Res.*, **64**, 6002–6009.
76. Madden,S.L., Cook,B.P., Nacht,M. *et al.* (2004) Vascular gene expression in nonneoplastic and malignant brain. *Am. J. Pathol.*, **165**, 601–608.
77. Geiman,D.E., Ton-That,H., Johnson,J.M. and Yang,V.W. (2000) Transactivation and growth suppression by the gut-enriched Krüppel-like factor (Krüppel-like factor 4) are dependent on acidic amino acid residues and protein-protein interaction. *Nucleic Acids Res.*, **28**, 1106–1113.
78. Kakinuma,N., Kohu,K., Sato,M., Yamada,T., Nakajima,M., Akiyama,T., Ohwada,S. and Shibanaka,Y. (2004) Candidate regions of tumor suppressor locus on chromosome 9q31.1 in gastric cancer. *Int. J. Cancer*, **109**, 71–75.
79. Miura,K., Suzuki,K., Tokino,T., Isomura,M., Inazawa,J., Matsuno,S. and Nakamura,Y. (1996) Detailed deletion mapping in squamous cell carcinomas of the esophagus narrows a region containing a putative tumor suppressor gene to about 200 kilobases on distal chromosome 9q. *Cancer Res.*, **56**, 1629–1634.
80. Miura,K., Okita,K., Furukawa,Y., Matsuno,S. and Nakamura,Y. (1995) Deletion mapping in squamous cell carcinomas of the esophagus defines a region containing a tumor suppressor gene within a 4-centimorgan interval of the distal long arm of chromosome 9. *Cancer Res.*, **55**, 1828–1830.
81. Roth,M.J., Hu,N., Emmert-Buck,M.R., Wang,Q.H., Dawsey,S.M., Li,G., Guo,W.J., Zhang,Y.Z. and Taylor,P.R. (2001) Genetic progression and heterogeneity associated with the development of esophageal squamous cell carcinoma. *Cancer Res.*, **61**, 4098–4104.
82. Zhao,W., Hisamuddin,I.M., Nandan,M.O., Babbin,B.A., Lamb,N.E. and Yang,V.W. (2004) Identification of *Krüppel-like factor 4* as a potential tumor suppressor gene in colorectal cancer. *Oncogene*, **23**, 395–402.
83. Baylin,S.B., Esteller,M., Rountree,M.R., Bachman,K.E., Schuebel,K. and Herman,J.G. (2001) Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. *Hum. Mol. Genet.*, **10**, 687–692.
84. Dang,D.T., Mahatan,C.S., Dang,L.H., Agboola,I.A. and Yang,V.W. (2001) Expression of the gut-enriched Krüppel-like factor (*Krüppel-like factor 4*) gene in the human colon cancer cell line RKO is dependent on CDX2. *Oncogene*, **20**, 4884–4890.
85. Stone,C.D., Chen,Z.Y. and Tseng,C.C. (2002) Gut-enriched Krüppel-like factor regulates colonic cell growth through APC/beta-catenin pathway. *FEBS Lett.*, **530**, 147–152.
86. Perreault,N., Katz,J.P., Sackett,S.D. and Kaestner,K.H. (2001) Foxl1 controls the Wnt/beta-catenin pathway by modulating the expression of proteoglycans in the gut. *J. Biol. Chem.*, **276**, 43328–43333.
87. Perreault,N., Sackett,S.D., Katz,J.P., Furth,E.E. and Kaestner,K.H. (2005) Foxl1 is a mesenchymal modifier of Min in carcinogenesis of stomach and colon. *Genes Dev.*, **19**, 311–315.
88. Kaestner,K.H., Silberg,D.G., Traber,P.G. and Schutz,G. (1997) The mesenchymal winged helix transcription factor Fkh6 is required for the control of gastrointestinal proliferation and differentiation. *Genes Dev.*, **11**, 1583–1595.
89. Dang,D.T., Chen,X., Feng,J., Torbenson,M., Dang,L.H. and Yang,V.W. (2003) Overexpression of Krüppel-like factor 4 in the human colon cancer cell line RKO leads to reduced tumorigenicity. *Oncogene*, **22**, 3424–3430.
90. Foster,K.W., Ren,S., Louro,I.D., Lobo-Ruppert,S.M., McKie-Bell,P., Grizzle,W., Hayes,M.R., Broker,T.R., Chow,L.T. and Ruppert,J.M. (1999) Oncogene expression cloning by retroviral transduction of adenovirus E1A-immortalized rat kidney RK3E cells: transformation of a host with epithelial features by c-MYC and the zinc finger protein GKLF. *Cell Growth Differ.*, **10**, 423–434.
91. Pandya,A.Y., Talley,L.I., Frost,A.R. *et al.* (2004) Nuclear localization of KLF4 is associated with an aggressive phenotype in early-stage breast cancer. *Clin. Cancer Res.*, **10**, 2709–2719.
92. Miller,K.A., Eklund,E.A., Peddinghaus,M.L., Cao,Z., Fernandes,N., Turk,P.W., Thimmapaya,B. and Weitzman,S.A. (2001) Krüppel-like factor 4 regulates laminin alpha 3A expression in mammary epithelial cells. *J. Biol. Chem.*, **276**, 42863–42868.
93. Sathyanarayana,U.G., Padar,A., Huang,C.X., Suzuki,M., Shigematsu,H., Bekele,B.N. and Gazdar,A.F. (2003) Aberrant promoter methylation and silencing of laminin-5-encoding genes in breast carcinoma. *Clin. Cancer Res.*, **9**, 6389–6394.
94. Foster,K.W., Liu,Z., Nail,C.D. *et al.* (2005) Induction of KLF4 in basal keratinocytes blocks the proliferation-differentiation switch and initiates squamous epithelial dysplasia. *Oncogene*, **24**, 1491–1500.
95. Su,L.K., Vogelstein,B. and Kinzler,K.W. (1993) Association of the APC tumor suppressor protein with catenins. *Science*, **262**, 1734–1737.
96. Gartel,A.L. and Tyner,A.L. (1999) Transcriptional regulation of the *p21((WAF1CIP1))* gene. *Exp. Cell Res.*, **246**, 280–289.
97. Bieker,J.J. (2001) Krüppel-like factors: three fingers in many pies. *J. Biol. Chem.*, **276**, 34355–34358.
98. Bunz,F., Dutriaux,A., Lengauer,C., Waldman,T., Zhou,S., Brown,J.P., Sedivy,J.M., Kinzler,K.W. and Vogelstein,B. (1998) Requirement for p53 and p21 to sustain G_2 arrest after DNA damage. *Science*, **282**, 1497–1501.
99. Drori,S., Girnun,G.D., Tou,L., Szwaya,J.D., Mueller,E., Kia,X., Shivdasani,R.A. and Spiegelman,B.M. (2005) Hic-5 regulates an epithelial program mediated by PPARgamma. *Genes Dev.*, **19**, 362–375.
100. Prasad,N.B., Biankin,A.V., Fukushima,N., Maitra,A., Dhara,S., Elkahloun,A.G., Hruban,R.H., Goggins,M. and Leach,S.D. (2005) Gene expression profiles in pancreatic intraepithelial neoplasia reflect the effects of Hedgehog signaling on pancreatic ductal epithelial cells. *Cancer Res.*, **65**, 1619–1626.
101. Suske,G., Bruford,E. and Philipsen,S. (2005) Mammalian SP/KLF transcription factors: bring in the family. *Genomics*, **85**, 551–556.
102. Kumar,A.P., Mar,P.K., Zhao,B., Montgomery,R.L., Kang,D.C. and Butler,A.P. (1995) Regulation of rat ornithine decarboxylase promoter activity by binding of transcription factor Sp1. *J. Biol. Chem.*, **270**, 4341–4348.
103. Kumar,A.P. and Butler,A.P. (1999) Enhanced Sp1 DNA-binding activity in murine keratinocyte cell lines and epidermal tumors. *Cancer Lett.*, **137**, 159–165.
104. Chen,Z.Y., Shie,J.L. and Tseng,C.C. (2002) Gut-enriched Krüppel-like factor represses ornithine decarboxylase gene expression and functions as checkpoint regulator in colonic cancer cells. *J. Biol. Chem.*, **277**, 46831–46839.
105. Ai,W., Liu,Y., Langlois,M. and Wang,T.C. (2004) Krüppel-like factor 4 (KLF4) represses histidine decarboxylase gene expression through an upstream Sp1 site and downstream gastrin responsive elements. *J. Biol. Chem.*, **279**, 8684–8693.
106. Higaki,Y., Schullery,D., Kawata,Y., Shnyreva,M., Abrass,C. and Bomsztyk,K. (2002) Synergistic activation of the rat laminin gamma1 chain promoter by the gut-enriched Krüppel-like factor (GKLF/KLF4) and Sp1. *Nucleic Acids Res.*, **30**, 2270–2279.
107. Ghaleb,A.M., Nandan,M.O., Chanchevalap,S., Dalton,W.B., Hisamuddin,I.M. and Yang,V.W. (2005) Krüppel-like factors 4 and 5: the yin and yang regulators of cellular proliferation. *Cell Res.*, **15**, 92–96.
108. Dang,D.T., Zhao,W., Mahatan,C.S., Geiman,D.E. and Yang,V.W. (2002) Opposing effects of Krüppel-like factor 4 (gut-enriched Krüppel-like factor) and Krüppel-like factor 5 (intestinal-enriched Krüppel-like factor) on the promoter of the *Krüppel-like factor 4* gene. *Nucleic Acids Res.*, **30**, 2736–2741.

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