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Kruppel like factor 4 (KLF4): a transcription factor with diverse context-dependent functions

Review Article

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Summary

Kruppel like factor 4 (KLF4) is a zinc finger containing transcription factor, which is expressed in a variety of tissues and regulates numerous biological processes including proliferation, differentiation, development, inflammation, and apoptosis. Thereby, it helps in maintaining homeostasis. As a transcription factor KLF4 both activates and represses the transcription of different genes depending on the cellular context. Physiologically, KLF4 can function both as a tumor suppressor and an oncogene. A recent study reported that p21 status may be a switch that determines the tumor suppressive or oncogenic function of KLF4. Over expression of KLF4 has been found to promote embryonic stem cell renewal. In addition, KLF4 plays an important role in reprogramming both mouse and human differentiated fibroblasts into induced pluripotent stem cells (iPS cells), which highly resemble embryonic stem cells, along with other three transcription factors including Oct4, Sox2, and c-Myc. This review discusses the function of KLF4 in cancer development and the underlying mechanisms. The role of KLF4 in stem cell biology and the future directions of KLF4 studies in this area have also been speculated.

I. Introduction

Kruppel like factor 4 (KLF4) is a zinc finger containing transcription factor expressed in a wide range of tissues in mammals, which plays a critical role in regulating diverse array of cellular processes like proliferation, differentiation, development, maintenance of normal tissue homeostasis and apoptosis. It is reported to have a dual function as a tumor suppressor and as an oncogene depending on the type of tissue in which it is expressed. KLF4 is a member of Kruppel like factor family. The KLF family members were named so because of the strong homology with the Drosophila gene product Kruppel ('cripple' in German), which plays an important role in the segmentation of developing embryo. The deletion of this gene caused a "crippled" phenotype (Wieschaus et al., 1984). The feature that distinguishes the KLF family from other zinc finger containing transcriptional factors, such as Sp1 and Krox, is the presence of three highly conserved zinc finger motifs at their C-terminus (Bieker, 2001; Philipsen and Suske, 1999; Turner and Crossley, 1999). Zinc fingers 1 and 2 contain 23 amino acid residues, while the third finger has only 21 residues. These fingers enable KLFs to bind to the related GC – and CACCC- boxes of DNA (Kaczynski et al., 2003). The fingers are connected by the characteristic Kruppel-link, a seven amino acid spacer TGEKP (Y/F) X sequence, highly conserved among family members (Dang et al., 2000b). Each zinc finger in KLF4 chelates a zinc ion, coordinated by 2 cysteine and 2 histidine residues. The critical residues that determine sequence specificity are largely conserved, so that while individual KLFs have slightly varying specificities they all recognize related sequences (Pearson et al., 2008).

KLF4 was first identified and characterized in mice in the year 1996 independently by two groups followed by identification of human KLF4 from human umbilical vein endothelial cell cDNA library (Yet et al., 1998). It was given two different names - GKLF (gut enriched kruppel like factor) due to the fact that it was found to be highly expressed in the intestine (Shields et al., 1996) and EZF (epithelial zinc finger) because of its high expression in differentiated epithelial cells of the skin (Garrett-Sinha et al., 1996). It was later renamed as KLF4 as it was found to be expressed also in lung, testis (Garrett-Sinha et al., 1996; Shields et al., 1996), thymus (Panigada et al., 1999), cornea (Chiambaretta et al., 2004), cardiac myocytes (Cullingford et al., 2008) and lymphocytes (Fruman et al.,

2002). The human KLF4 gene locus is located on chromosome 9q31 whereas mouse KLF4 is located on chromosome 4B3. KLF4 has 5 exons, and the size of KLF4 transcript is ~3.5kb as detected by northern blot analysis of RNA in human umbilical vein endothelial and other cells (Shie et al., 2000b; Yet et al., 1998). It has been shown that adult mouse testis has multiple KLF4 transcripts (Godmann et al., 2005; Shields et al., 1996). The human and mice KLF4 sequences are shown to have 91% sequence similarity at the amino acid level. The human KLF4 cDNA encodes a protein containing 470 amino acids with a predicted molecular mass 50 kDa. Several functional domains have been characterized in mouse KLF4 protein, which include an acidic N-terminal transcriptional activation domain (1-157 a.a), a repressor domain next to it (158-385) followed by an NLS (nuclear localization signal) sequence (386-401 a.a) having PKRGRR repeats and the carboxyl DNA binding domain which comprises 81 highly conserved a.a (402-483) (**Figure 1**). The activation domain is found to interact with p300/CBP (Evans et al., 2007). The repressor domain is reported to have two lysine residues (K225, K229), which are acetylated by p300/CBP (Dang et al., 2002). The DNA binding domain has three zinc fingers, each of which contains an anti- parallel β-pleated sheet, followed by an α helix. The Cys2/His2 residues are located within the α-helix that bind a zinc ion and help in stabilizing the fold. In addition to these domains, a PEST sequence is reported to be located between the activation and inhibitory domains. This suggests KLF4 may be subjected to ubiquitin-mediated proteosomal degradation. Thus the presence of both activation and repressor domains might allow KLF4 to switch between its positive and negative transcriptional effects on its targets depending upon the type of tissue in which it is expressed (Evans and Liu, 2008; Garrett-Sinha et al., 1996; Geiman et al., 2000; McConnell et al., 2007; Shie et al., 2000b; Wei et al., 2006; Yet et al., 1998; Zheng et al., 2009).

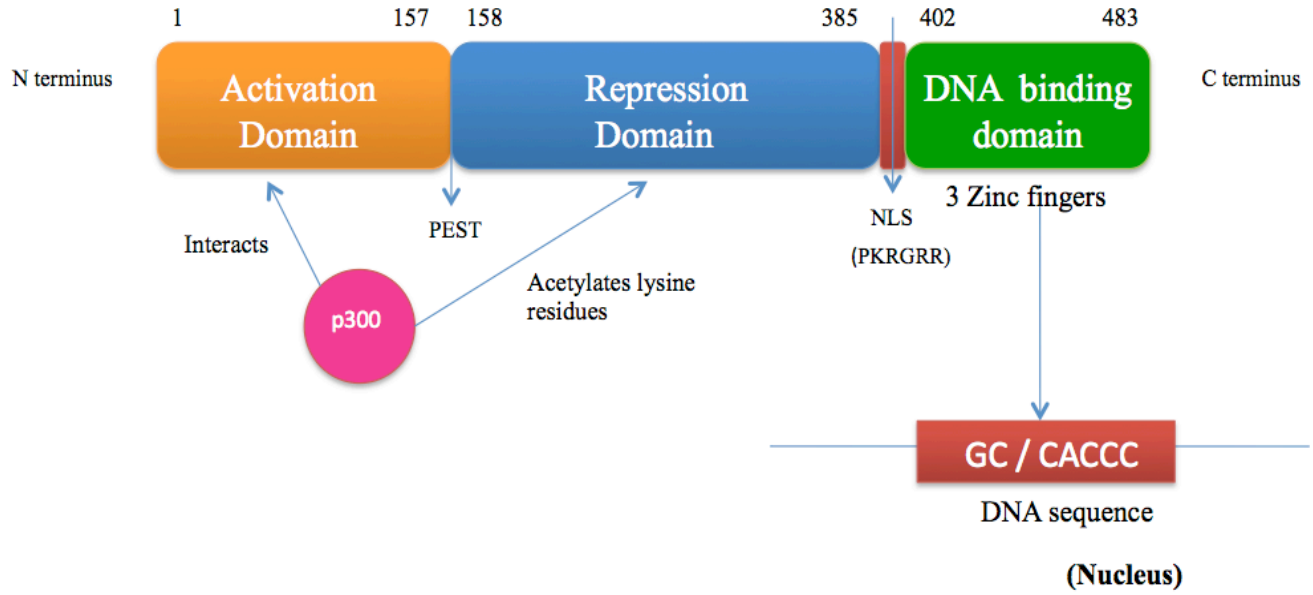

Figure 1: Structure of KLF4. KLF4 has an N-terminal transcriptional activation domain, followed by a transcriptional repression domain, containing two lysine residues K225 and K229. These residues are acetylated by p300/CBP, which interacts with the activation domain. There lies a potential PEST sequence between the activation and repression domains. The C-terminus has a DNA binding domain containing three zinc fingers, which recognize and bind to the GC/CACCC target sequences in the nucleus. A Nuclear Localization Signal (NLS) (6 a.a.) is placed between the DNA binding and repression domains, which facilitates the transport of KLF4 into the nucleus. Please note that the size of each domain is not exactly proportional.

II. Expression and functions

A. Expression

KLF4 is highly expressed in the differentiated, post mitotic cells of the gut and skin epithelium (Dang et al., 2000b). In the intestinal epithelium, KLF4 is expressed in terminally differentiated epithelial cells at the villus borders of the mucosa (McConnell et al., 2007). Actively proliferating cells, which are situated at the base of the crypts, migrate toward the luminal surface as they differentiate to be sloughed off. KLF4 expression is found to be highest near the luminal surface as compared to the base of the crypts. From this observation, it can be inferred that KLF4 promotes differentiation. In KLF4 knockout mice, the numbers of goblet cells considerably decreased, suggesting that KLF4 may be required for goblet cell differentiation (Katz et al., 2002). The KLF4 knockout mice die from dehydration soon after birth due to the failure of late stage differentiation in the epidermis of the skin, resulting in a loss of normal barrier formation (Katz et al., 2002; Segre et al., 1999). This implies the role of KLF4 as an important factor in formation of skin epithelium. In the thymus, KLF4 is expressed during cortical differentiation (Panigada et al., 1999). KLF4 is also expressed in the corneal epithelium of the eye. Deletion of KLF4 in the eye leads to corneal fragility,

stromal edema and lack of goblet cells in the conjunctiva (Swamynathan et al., 2007). KLF4 has also been reported to be expressed in testis, specifically in the post mitotic germ cells and somatic Sertoli's cells, indicating its importance in testicular differentiation (Behr and Kaestner, 2002). KLF4's role has been implied in development of vasculature - studies in zebra fish and mammals showed that KLF4 is important in blood vessel development in addition to hematopoiesis and epidermal development. It is induced by shear stress (McCormick et al., 2001; Oates et al., 2001). KLF4 is expressed in the dorsal epithelium of the developing tongue, tooth bud and palate and in the mesenchymal cells of the skeletal primordia and metanephric kidney (Garrett-Sinha et al., 1996). It has also been reported to be expressed in the respiratory tract, meninges and cartilaginous skeleton (Ohnishi et al., 2000).

B. Major functions

1. Inhibition of cell proliferation

KLF4 is known to induce growth arrest, inhibiting cell proliferation by regulating the expression of key cell cycle genes **(Figure 2).** In actively proliferating NIH3T3 fibroblast cells, KLF4 is little expressed. When these cells are subjected to serum starvation, KLF4 levels are found to be significantly expressed and the ectopic expression of KLF4 in NIH3T3 cells resulted in inhibition of DNA synthesis (Shields et al., 1996). Over expression of KLF4 in COS-1 has also been shown to inhibit DNA synthesis in those cells.

Progression through cell cycle is driven by Cyclins and their dependent kinases - Cdks which phosphorylate and inactivate the cell cycle inhibitors like p16, p21, p27 and p57 (Matsuoka et al., 1995; Sherr and Roberts, 1999; Vidal and Koff, 2000) and allow the cells to go through the cell cycle. Microarray analysis confirms that KLF4 activates a number of genes, which function as negative regulators of cell cycle as well as suppresses genes that promote cell cycle progression. KLF4 has been shown to inhibit cell proliferation by blocking G1/S progression in cell cycle and to mediate p53 dependent G1/S cell cycle arrest in response to DNA damage (Bunz et al., 1998; Yoon et al., 2003; Yoon and Yang, 2004). While wild type HCT 116 colon cancer cells on UV irradiation were arrested at the G2/M phase checkpoint, p53 knockout cells were able to continue the cell cycle by entering into M phase even on irradiation. It was observed that upon over expression of KLF4 in these knockout mice, the mitotic indices were considerably reduced and the Cyclin B levels were also reinstated (Yoon et al., 2003). These studies suggest that KLF4 is a critical factor in regulating entry of the cells into the mitotic phase. The CDKN1A gene encoding the cyclin dependent kinase inhibitor p21 is a transcriptional target for tumor suppressor signaling pathways, which include p53, TGFβ and APC. A number of evidence supports that KLF4 plays a vital role in regulating p21 gene expression. When p21 is activated, it down regulates the expression of cyclin D and cyclin B, thereby, restricting the entry of the cells from G1 to S and G2 to M respectively (Chen et al., 2001a; Chen et al., 2001b; Nandan et al., 2004; Shie et al., 2000a; Yoon and Yang, 2004). KLF4 mediates p53 transactivation activity on the p21 promoter upon DNA damage and in turn p53 up regulates KLF4 promoter activity in Chinese hamster ovary cells. It has been shown that the levels of KLF4 mRNA increased in a p53 dependent manner, which coincides with the increased expression of p21, when treated with menthanosulfate or when subjected to γ radiation (Zhang et al., 2000). KLF4 also mediates selenium (Liu et al., 2008b) or butyrate (Chen et al., 2004) induced growth arrest.

2. Promotion of cell differentiation

As previously discussed, KLF4 plays a vital role in goblet cell differentiation in the intestine (Katz et al., 2005; Katz et al., 2002), conjunctiva (Swamynathan et al., 2008) and also in the formation of the epithelial barrier of the skin (Katz et al., 2002; Segre et al., 1999). Its expression has been detected to be more in well-differentiated cells than in actively proliferating cells. The knockout mice phenotypes provided this evidence. Microarray analysis showed that many keratin genes were up regulated on KLF4 induction, an observation suggestive of its role in epithelial differentiation. KLF4 has been reported to transactivate promoters of epithelial genes like CYP1A1 (Zhang et al., 1998), laminin α 3A (Miller et al., 2001), laminin 1 (Higaki et al., 2002), keratin 4 (Brembeck and Rustgi, 2000) in the esophagus, keratin 19 (Okano et al., 2000) in the pancreas and Keratin 12 and Aqp5 in the cornea (Swamynathan et al., 2008).

KLF4 has been shown to repress TGFβ dependent increase of smooth muscle cell differentiation marker genes which includes α-smooth actin and SM22α (Adam et al., 2000), which somewhat contradicts its well established role in the differentiation of cells. A recent study has shown that KLF4, which is normally not expressed in differentiated SMC *in vivo*, was rapidly up-regulated in response to vascular injury (Yan et al., 2008). Taken together, these results indicated that KLF4 represses SMC genes both by down-regulating myocardin expression and preventing myocardin from associating with SMC gene promoters, and suggested that KLF4 may be a key effector of PDGF-BB and injury-induced phenotypic switching of SMC (Liu et al., 2005). A recent study suggests that the expression of KLF4 is closely correlated to the growth-arrest and the first step of odontoblast and ameloblast differentiation in murine tooth (Chen et al., 2009). This study shows that both KLF4 and KLF5, a closely related Kruppel like factor, are important for murine tooth development.

C. Other functions

KLF4 is essential for differentiation of mouse inflammatory monocytes, and is involved in the differentiation of resident monocytes (Alder et al., 2008; Feinberg et al., 2007). KLF4 was previously shown to mediate proinflammatory signaling in human macrophages *in vitro* (Feinberg et al., 2005; Liu et al., 2008a). KLF4 was also shown to regulate the expression of interleukin-10 in RAW264.7 macrophages (Chung et al., 2007; Liu et al., 2007). The bone marrow monocytes from KLF4 (-/-) chimeras expressed lower levels of key trafficking molecules and were more apoptotic and show

that KLF4 is necessary for differentiation of monocytes (Alder et al., 2008). In addition, inducible expression of KLF4 in the HL60 (human acute myeloid leukemia cell line) stimulated monocyte differentiation and enhanced 12-O-tetradecanoylphorbol 13-acetate induced macrophage differentiations, but blocked all-trans-retinoic acid induced granulocytic differentiation of HL60 cells. The inflammation-selective effects of loss-of-KLF4 and gain-of-KLF4-induced monocytic differentiation in HL60 cells identify KLF4 as a key regulator of monocytic differentiation and a potential target for translational immune modulation (Alder et al., 2008). KLF4 positively regulates human ghrelin expression (Lee et al., 2009). Ghrelin is expressed in the gastrointestinal tract, predominantly the stomach, as is KLF4. Treatment with butyrate, an inducer of KLF4 expression, stimulates ghrelin expression and fasting, which induces ghrelin expression, also increased KLF4 expression, suggesting that ghrelin expression is associated with KLF4. In AGS stomach cancer cells, KLF4 expression specifically stimulated human ghrelin promoter activity in a dose-dependent manner. In addition, it was found that KLF4 is an immediate early gene for Nerve Growth Factor (Dijkmans et al., 2009). A recent study showed that glutamatergic stimulation can trigger rapid elevation of KLF4 mRNA and protein levels, and that the over expression of KLF4 can regulate neuronal cell cycle proteins and sensitize neurons to NMDA-induced caspase-3 activity (Zhu et al., 2009). Another study demonstrated that KLF4 is involved in regulating the proliferation of CD8+ cells (Yamada et al., 2009). The transcription factor ELF4 directly activated the tumor suppressor KLF4 'downstream' of T cell antigen receptor signaling to induce cell cycle arrest in naive CD8 (+) T cells (Yamada et al., 2009).

KLF4 has been implicated in the regulation of apoptosis (Ghaleb et al., 2007; Wei et al., 2005). During DNA damage, cells can take two routes - either passes into the next phase overcoming the checkpoint or gets arrested at the checkpoint and activates the repair machinery. As discussed previously, over expression of KLF4 in RKO colon cancer cells, when subjected to UV radiation, reduced the percentage of apoptotic cells (Dang et al., 2003). In esophageal cancer cell lines, KLF4 has been shown to bind to the promoter and repress the activity of surviving gene *in vivo* (Zhang et al., 2009), which is necessary for caspase inactivation and therefore acts as a negative regulator of apoptosis.

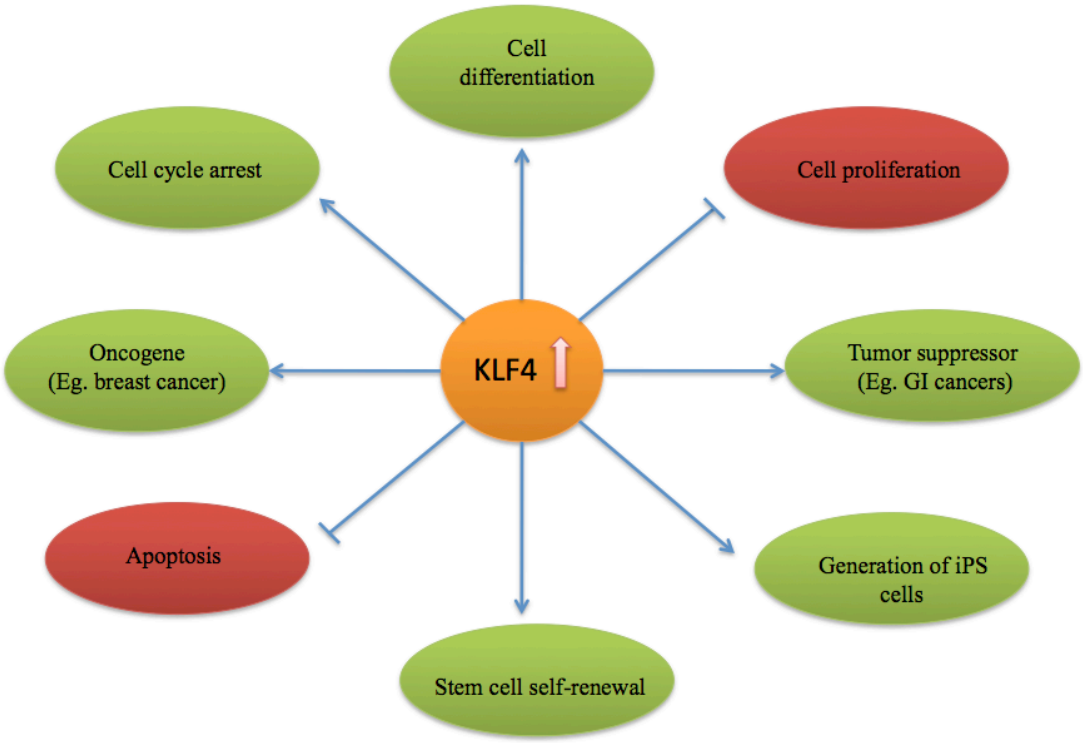

Figure 2: Multiple context-dependent functions of KLF4. Up regulation or over expression of KLF4 promotes cell differentiation, inhibits cell proliferation, and induces cell cycle arrest, down-regulates apoptosis for maintaining tissue homeostasis. It functions as a tumor suppressor in cancers of some tissues and as an oncogene in some tissues like those of breast. KLF4 is instrumental in stem cell renewal and generation of induced pluripotent stem cells (iPS cells).

III. Role and regulation of KLF4 in cancer

KLF4 plays a critical role as transcription factor in regulating cell proliferation. Since cancers display uncontrolled cell growth, KLF4 is thought to play a key role in cancer progression and development. KLF4 is proved to induce growth arrest, so it can be assumed to have an anti cancerous activity. KLF4 expression is shown to be down- regulated in a number of cancers and it was said to have tumor suppressive activity. However its role

in cancers is not fully conclusive as it is also identified to act as an oncogene in some specific cancers in tissues.

A. Evidence of its tumor suppressor activity

1. Observations

Many studies have revealed the role of KLF4 as a tumor suppressor in various human cancers. KLF4 expression was significantly down regulated in the cancer of the bladder epithelium (Ohnishi et al., 2003). The transduction of KLF4 into the bladder cancer cell lines using adenoviral vectors suppressed growth and induced apoptosis. This study suggests that inactivation of KLF4 is one of the frequent steps taken toward bladder carcinogenesis. In human colorectal carcinoma, KLF4 expression is down regulated due to hypermethylation and loss of heterozygosity (Choi et al., 2006; Xu et al., 2008). Mutational analysis revealed that the KLF4 gene is silenced by being subjected to deletion, mutation and methylation in significant number of colon and gastric cancers (Wei et al., 2006; Zhao et al., 2004). KLF4 expression has drastically decreased colonic adenomas in patients with familial adenomatous polyposis when compared with adjacent normal mucosa (Dang et al., 2000a; Ton-That et al., 1997). The KLF4 mRNA levels are decreased in colonic carcinoma and adenomatous polyps when compared to normal colon (Choi et al., 2006). KLF4 expression in mouse models of colorectal cancer yielded similar observations. The $APC^{min/+}$ mice developed a number of intestinal adenomas at an early stage (Moser et al., 1990; Xu et al., 2008). Reverse transcription-PCR showed an inverse correlation between adenoma size and KLF4 mRNA levels in both KLF4 (+/-)/ $Apc^{(Min/+)}$ and $Apc^{(Min/+)}$ mice (Ghaleb et al., 2007b). This study indicated the role of KLF4 as a tumor suppressor *in vivo*. In studies using a KLF4 (-/-) mice specific for gastric epithelium, loss of KLF4 resulted in increased proliferation and altered differentiation of epithelial cells in the stomach, leading to precancerous lesions (Katz et al., 2005; Wei et al., 2005). The $APC^{min/+}$ mice expressing a truncated form of the APC protein deregulated the Wnt signaling pathway in their intestine (Korinek et al., 1997; Morin et al., 1997).

Induction of KLF4 mRNA and protein expression by interferon-gamma treatment showed a marked reduction of ornithine decarboxylase (ODC) gene expression and enzyme activity in colon cancer HT-29 cells (Chen et al., 2002). Over expression of KLF4 in HT-29 cells significantly reduced ODC mRNA and protein levels as well as enzyme activity and resulted in growth arrest, indicating that ODC might be a downstream target of KLF4. This conclusion was further supported by observation that KLF4 mRNA and protein concentrations were the highest at the G1/S boundary of the cell cycle, where ODC mRNA and protein levels were the lowest and that over expression of KLF4 resulted in cell arrested at the G1 phase (Chen et al., 2002).

KLF4 expression was reduced in human esophageal squamous cell carcinomas, when compared to the levels of expression in the normal esophageal tissue (Luo et al., 2004; Wang et al., 2002). Decreased expression of KLF4 in an esophageal cancer cell line by antisense KLF4 transfection has reportedly shown to increase cell proliferation and decrease cell adhesion stability (Ohnishi et al., 2003). Functional analysis of the alteration in the expression of KLF4 suggested that KLF4 might be able to regulate the expression of squamous cell differentiation associated genes like SPRR1A, SPRR2A and KRT4 in ESCC (Choi et al., 2006). In the tumors of the gastrointestinal tract, KLF4 expression was found to be decreased in the prostate gland in case of prostate cancer and benign prostate hypertrophy (Foster et al., 2000; Luo et al., 2002). KLF4 expression has also been reported to be reduced in lung cancers (Bianchi et al., 2004). KLF4 is a candidate tumor suppressor gene in B-lymphocytes. The RNA and protein expression of murine KLF4 has been shown to decrease following B cell activation. Forced expression of KLF4 in proliferating B cells caused a G1 cell cycle arrest (Kharas et al., 2007; Yusuf et al., 2008). This effect requires the DNA binding and transactivation domains of KLF4 and correlates with changes in the expression of known KLF4 target genes. KLF4 is a target gene for FOXO transcription factors, which also suppress B cell proliferation (Yusuf et al., 2008). KLF4 (-/-) mice with B cell-specific deletion of the KLF4 gene exhibited normal B cell development and function with no evidence of a lowered activation threshold. This indicates that KLF4 has growth-suppressive properties in B cells but, might be redundant with other members of the KLF family in maintaining B cell quiescence (Yusuf et al., 2008). KLF4 expression is silenced in adult T-cell leukemia cells, when compared with normal T cells (Yasunaga et al., 2004). The expression of KLF4 is also significantly repressed in human glioma associated vascular endothelial cells when compared to non neoplastic control vascular endothelial cells, indicating that KLF4 may also be involved in antiangiogenic pathway (Madden et al., 2004). In all the above-discussed tumors, KLF4 expression is significantly reduced, consistent with its activity of cell cycle checkpoint and growth arrest. Thus, it can be said that KLF4 plays an active role as a tumor suppressor.

2. Underlying mechanisms

2.1. Wnt signaling and KLF4

APC (adenomatous polyposis coli) protein plays an important role in many cellular processes that determine whether a cell develops into a tumor. It is a critical component of the Wnt/β-catenin signaling pathway (Nathke, 1999), which plays an important role in normal intestinal homeostasis and colorectal cancers (Giles et al., 2003; Moon et al., 2004). KLF4 binds β-catenin and inhibits its function, thereby inhibiting β-catenin mediated tumor genesis (Zhang et al., 2006). It has also been postulated that a cross talk between KLF4 and β-catenin regulates intestinal homeostasis (Zhang et al., 2006). This might be another possible way of KLF4 regulation in intestinal crypts, at the bottom, where KLF4 expression is low and β-catenin signaling is active, which results in proliferation of stem cells and progenitor cells. With cells migrating upward, cells become more differentiated. This might be due to the interaction between KLF4 and β-catenin, resulting in the inhibition of expression of downstream Wnt-responsive genes (Zhang et al., 2006;

Zheng et al., 2009). A recent study showed that lower levels of KLF4 expression in the proliferative compartment of the intestinal epithelium are regulated by the transcription factors TCF4 and SOX9, an effector and a target, respectively, of Wnt/β-catenin (Flandez et al., 2008). This substantiates the finding that reduced levels of KLF4 tumor suppressor activity in colon tumors may be driven by elevated Wnt/β-catenin signaling.

2.2. Notch signaling and KLF4

Goblet cell differentiation is regulated by the Notch signaling pathway (Jensen et al., 2000). The Notch signaling pathway suppresses goblet cell formation and is up regulated in tumors (Ghaleb et al., 2008). Notch genes encode evolutionarily conserved transmembrane receptors that control a broad range of cell fate decisions in development (Artavanis-Tsakonas et al., 1999; Baron, 2003). KLF4 is proposed as the downstream target of Notch signaling pathway and the former's promoter activity is inhibited by Notch (Ghaleb et al., 2008; Zheng et al., 2009). These studies also showed that Notch inhibition increases KLF4 gene expression. A constitutively active version of Notch, ICN1 (a COOH-terminal region of human Notch1) was used to assess the inhibition of KLF4 promoter activity *in vitro* (Zheng et al., 2009). An ICN1 responsive element was mapped between -151 to -122 of the hKLF4 promoter (Zheng et al., 2009). The inhibition of KLF4 promoter is also thought to occur indirectly through a transcription factor that binds to the ICN1 responsive element. Nevertheless KLF4 inhibition by Notch will be consistent with the tumor suppressor role of KLF4 and up regulation of Notch in tumors. In addition, cross talk between KLF4 and β-catenin and inhibition of KLF4 gene expression by Notch provide another link between the Wnt and Notch signaling pathways that play a critical role in maintaining homeostasis of the normal intestine and in tumor genesis of colorectal cancers (Nakamura et al., 2007).

B. Evidence of its oncogenic activity

Though KLF4 was initially described for its role as a tumor suppressor, three different studies identified KLF4 as an oncogene by unbiased genetic screens. E1A immortalized rat kidney epithelial cells were used to screen for KLF4, which induces transformation in those cells (Foster et al., 1999). In oral squamous epithelium of RK3E cells, KLF4 expression was detected in the upper, differentiating cell layers. In dysplastic epithelium, expression was prominently increased and was detected diffusely throughout the entire epithelium, indicating that KLF4 is misexpressed in the basal compartment early during tumor progression. KLF4-transformed rat kidney epithelial cells produced tumors in xenografted mice. Increased expression of KLF4 was also reported in the ductal carcinoma of the breast cancer cells. In situ hybridization, Northern blot analysis, and immunohistochemistry techniques were used to detect KLF4 at various stages of tumor progression in the breast, prostate, and colon. Overall, the expression of KLF4 mRNA was detected by in situ hybridization in 68% of carcinoma of the breast, whereas low-level expression of KLF4 mRNA was observed in morphologically normal breast epithelium adjacent to tumor cells. Ductal carcinoma in situ exhibited similar expression as invasive carcinoma, suggesting that KLF4 is activated prior to invasion through the basement membrane (Zhao et al., 2004). Recent evidence suggests that the role of KLF4 may depend on the genetic context it is functioning (Rowland et al., 2005). The functioning of KLF4 as an oncogene was discovered by the mechanism in which KLF4 allows the overrides of oncogenic RAS^{V12}-induced senescence in primary fibroblasts, through suppressing the expression of p53 by directly acting on its promoter. This would allow RAS^{V12}- mediated transformation and provide resistance to DNA damage induced apoptosis. To test this hypothesis, KLF4 was knocked down which resulted in an increase in the levels of p53 and induced apoptosis. These results also present KLF4 as a regulator of p53 that oncogenically transforms cells as a function of $p21^{Cip1}$ status.

p21 protein has been reported to function as a switch in KLF4-mediated tumor genesis. p21 is a CDK inhibitory protein and is a transcriptional target of p53, which results in cell cycle arrest (Gu et al., 1993; Harper et al., 1993; Sherr and Roberts, 1999; Xiong et al., 1993). It was found that the inactivation of cyclin D1 or cell cycle inhibitor p21 not only abolishes the cytostatic effect of KLF4, but also mediates KLF4 in oncogenic transformation of cells (Rowland et al., 2005). Over expression of KLF4 alone increases expression of $p21^{Cip1/WAF1}$ and results in cell cycle arrest. However, the addition of Ras^{V12} resulted in inhibition of $p21^{Cip1/WAF1}$ expression, allowing KLF4's ability to repress p53 to predominate. Thus, KLF4 has multiple, context-dependent roles in cancer development. These data support a model in which KLF4 can switch from a tumor suppressor to an oncogene, depending on the status of p21 (Evans and Liu, 2008; Rowland and Peeper, 2006). KLF4 and p21 may utilize the same signaling pathway to function as a tumor suppressor or an oncogene. However, the oncogenic function of KLF4 apparently is independent of p21, as KLF4 can transform primary cells in the absence of p21 at least *in vitro* (Rowland et al., 2005). It is likely that p21 level is under a tight control so that growth arrest and transformation is well balanced. In the absence of p21, p21-mediated growth arrest by KLF4 is abolished as well as p21-mediated transformation. Under this condition, KLF4 may reveal its transformation activity by inhibition of p53 that is activated by oncogenic Ras^{V12}. KLF4 has been reported to suppress apoptosis through down regulation of the Bax promoter (Ghaleb et al., 2007). Overall, KLF4 might act either as a tumor suppressor or as an oncogene depending on the cellular context, expression patterns of other genes and the chromatin environment of individual cells.

IV. KLF4 in stem cell biology

Stem cells are characterized by the ability to renew themselves through mitotic cell division and to differentiate into a diverse range of specialized cell types. The two key characteristic properties of stem cells are self-renewal and pluripotency. These properties can be utilized to cure a number of human diseases (Audet, 2004;

Constantinescu, 2003; Thomson et al., 1998). ES cells are considered to be pluripotent, so the factors thought to be responsible for the pluripotency of ES cells have been screened in mouse embryonic fibroblasts (Takahashi and Yamanaka, 2006; Yamanaka and Takahashi, 2006). While twenty four factors were screened, they concluded that mouse embryonic fibroblasts can be reprogrammed to a pluripotent state similar to that observed in ES cells, by retroviral transduction of four genes Oct4, Sox2, c-Myc, and KLF4. This discovery opened exciting new vistas in the field of stem cell biology. The discovery of these 'induced pluripotent stem (iPS) cells' was regarded as a major development in stem cell research and gave new insights into the pathways involved in the maintenance of pluripotency.

iPS cells are similar to ES cells in morphology, proliferation, and pluripotency, considering teratoma formation and chimera contribution (Yamanaka, 2008). Out of the four genes mentioned, c-Myc and KLF4 are oncogenes, which are thought to contribute to the increased proliferative capacity of iPS cells. Later, the generation of iPS cells from human dermal fibroblasts with the same four factors was demonstrated (Takahashi et al., 2007). Human iPS cells are a potential source of patient-specific pluripotent stem cells that would bypass immune rejection. iPS cells can also be used to study diseases for which there are no adequate human *in vitro* or animal models. A recent study using murine embryonic stem cells showed that ES cells over expressing KLF4 have a greater capacity to self-renew based on secondary embryoid body (EB) formation. ES cells overexposing SOCS-3 showed an increased capacity to differentiate to hematopoietic progenitors, rather than to self-renew. KLF4-transduced d6 EBs expressed higher levels of Oct-4, consistent with the notion that KLF4 promotes ES cell self-renewal. These findings reveal that murine embryonic stem cell differentiation is promoted by SOCS-3 and inhibited by KLF4 (Li et al., 2005). Mechanistically KLF4 has also been found to activate Lefty1 gene expression along with Oct3/4 and Sox2, and is proposed to act as a mediating factor that specifically binds to the proximal element of the Lefty1 promoter (Nakatake et al., 2006). But, recent discoveries have shown that, KLF4 in reprogramming the cells to a pluripotent state to be dispensable. Valproic acid, a histone deacetylase inhibitor, enables reprogramming of primary human fibroblasts with only two factors, Oct4 and Sox2, without the need for the oncogenes c-Myc or KLF4 (Huangfu et al., 2008). Very recently, a group described the use of Kenpaullone, a molecule identified to activate Nanog expression, replacing KLF4 in producing iPS cells similar to murine ES cells (Lyssiotis et al., 2009). However, the iPS cells are different from the ES cells with regards to gene expression, DNA methylation patterns, and failure to produce adult chimaeras (Okita et al., 2007). Selection for Nanog expression resulted in a germline-competent iPS cells with increased ES-cell-like gene expression and DNA methylation patterns compared with Fbx15 iPS (Okita et al., 2007).

The use of oncogenes and retrovirus in the current iPS cell establishment protocol raised safety concerns. For example, reactivation of the c-Myc retrovirus, increased tumorigenicity in the chimeras and progeny mice, hindering clinical applications (Nakagawa et al., 2008). To overcome this drawback, plasmids are used to transfect mouse embryonic fibroblasts in place of retroviruses (Kaji et al., 2009; Okita et al., 2008). Another problem is that iPS cells are refractory to differentiation and thereby increase the risk of immature teratoma formation after directed differentiation and transplantation into patients. Even if only a small portion of cells within each iPS cell clone shows impaired differentiation, then those cells might be sufficient to produce immature teratomas (Yamanaka, 2009). If somatic stem or progenitor cells can directly generated from fibroblasts or other types of somatic cells, then there would not be a need of iPS cells. Nevertheless, the iPS cell technology potentially can overcome two important obstacles associated with human ES cells: immune rejection after transplantation and ethical concerns regarding the use of human embryos (Yamanaka, 2009). The advantage of iPS cell technology is that iPS cells can be generated using a few programming factors in any laboratory using standard techniques and equipment. To generate clinical quality iPS cells, the development of novel reprogramming methods that avoid permanent genetic modification is highly desired. But, the molecular mechanisms that mediate reprogramming are essentially unknown. Establishment of a stable and self-sustainable ES-specific transcriptional regulatory network is essential for reprogramming (Zhao and Daley, 2008). iPS cells still have the scope for clinical applications provided that proper ways are established to precisely evaluate each iPS cell clone and to select appropriate sub clones prior to clinical application (Yamanaka, 2009).

V. Future directions

Though much have been found out about KLF4, the exact molecular mechanisms by which KLF4 works is yet to be discovered. So far it has been described that KLF4 functions as a transcriptional activator, repressor, tumor suppressor and an oncogene depending on the genetic context. However, there is not much direct evidence to show how KLF4, as a transcription factor, interacts with its downstream targets to carry out the formerly mentioned functions. Its position in the signaling cascade is also ambiguous. For example, KLF4 is sometimes placed downstream of p53 and sometimes upstream. Also, whether it represses the activity of p21 through p53 or along with p53 is not clearly defined. p21 is proposed to contribute to the switching of KLF4 function between a tumor suppressor or an oncogene *in vitro* (Rowland et al., 2005). However, there is no experimental evidence to support this notion *in vivo*. We may possibly determine this by knocking out p21 in the setting of KLF4 knockout mice and examine whether KLF4 functions as an oncogene. Furthermore, why KLF4 has a dual-role in different tissues in cancer development, either as a tumor suppressor or oncogene, is still unknown. Identification of it binding partners, effectors and downstream targets to some extent might reason its behavior in different tissues (p21 knockout mice).

KLF4 as discussed is thought to play a significant role in ES cell self-renewal and generating iPS cells. A lot of questions need to be answered before moving ahead with reprogramming. What is the exact role of KLF4 in epigenetic reprogramming process and how does it collaborate with other transcription factors including Oct4, Sox2 and c-Myc in generation of iPS cells? A more comprehensive analysis of the underlying molecular mechanisms will provide a further insight into its function in stem cell biology. As KLF4 is one of the major transcription factors responsible for transformation of mouse embryonic fibroblasts to stem cell like cells, KLF4 can be speculated to have similar functions in cancer stem cells (Schoenhals et al., 2009). Since KLF4 has been reported to function as an oncogene, we might want to investigate if KLF4 contributes to the tumorigencity of the cancer stem cells. If it is so, the deregulation of KLF4 in cancer stem cell population may provide a way to destroy cancer stem cells and to some extent eradicate cancer. Mechanistically, Notch signaling pathway has been shown to control stem cell self-renewal and pluripotency (Calvi et al., 2003; Hansen et al., 2004). The ability of Notch to promote self-renewal is probably due to its ability to inhibit cell differentiation under some circumstances (Henrique et al., 1997). Therefore it is critical to determine the relative functioning of Notch and KLF4 in stem cells. From the studies of goblet cell differentiation in the mouse gastrointestinal tract (Ghaleb et al., 2008; Zheng et al., 2009), KLF4 and Notch work antagonistically, but studies on mammary stem/progenitor cells show that Notch promotes self renewal of mammary stem cells (Dontu et al., 2004), which is line with the function of KLF4 in stem cells. This becomes another major concern regarding KLF4 in stem cell biology, and we need to address through which pathway KLF4 promotes stem cell renewal and how it exerts its action - through a simple pathway or by integration of different pathways. Therefore it is expected that KLF4 functions and the potential mechanisms in stem cell biology will be under active investigation in the next few years to come.

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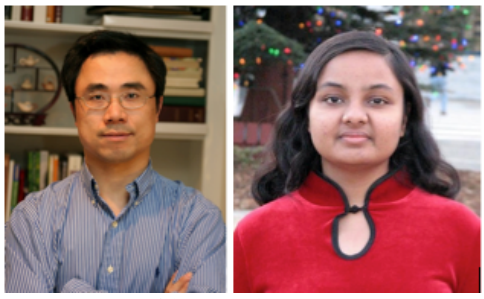

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