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Krüppel-like factor 4 (KLF4): What we currently know

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Abstract

Krüppel-like factor 4 (KLF4) is an evolutionarily conserved zinc finger-containing transcription factor that regulates diverse cellular processes such as cell growth, proliferation, and differentiation. Since its discovery in 1996, KLF4 has been gaining a lot of attention, after it was shown in 2006 as one of four factors required for the induction of pluripotent stem cells (iPSCs). Here we review the current knowledge on the different functions and roles of KLF4 in various tissue and organ systems.

Keywords

KLF4; gene expression; transcription; tissues; organs; diseases; proliferation; differentiation; cell cycle; stem cells; inflammation; cancer; epithelial-to-mesenchymal transition

1. Introduction

The Krüppel-like factor 4 (KLF4; also called gut-enriched Krüppel-like factor or GKLF) was first isolated by Shields *et al.*, from a NIH3T3 cDNA library (1). The gene is conserved among vertebrate species from zebrafish to human (Table 1). In recent years, KLF4 has gained notoriety not only due its diverse functions in physiology and diseases but due to its role as one of four key factors required for inducing pluripotent stem cells. In this article, we review the current understanding of the functions of KLF4.

2. Biochemical activities

2.1. Structure

KLF4 belongs to the family of SP/KLF factors that are characterized by three zinc finger motifs within their carboxyl terminal sequences (2). Within its amino terminus, KLF4

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Conflict of interest

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possess a transactivation domain (TAD) and adjacent to it, a repression domain, together of which determine the specificity of KLF4's transcriptional regulating activity by interacting with other factors and modulating DNA binding efficiency (Figure 1) (3,4). Two nuclear localization signals (NLS) have also been identified in mouse KLF4 (5). The first is directly adjacent to the most amino terminal zinc finger motif and the second spans the first and half of the second zinc finger domains (5). Mouse KLF4 contains 483 amino acids with a predicted molecular weight of 53 kDa, and is 91% identical to that of human KLF4 (4). Several splicing variants of the human *KLF4* gene have been identified in normal and cancer cells (e.g. human pancreatic cancer cells; Figure 2) (6–8).

2.2. Regulation of KLF4

Expression of *KLF4* is regulated at both transcriptional and post-transcriptional levels. Studies show that hypermethylation of CpG islands in the *KLF4* promoter and methylation of histones modulate its activity in cancer and stem cells (9–12). Micro-RNAs (miR) are another mechanism that is important in modulating *KLF4* expression in stem/progenitors cells, cardiovascular remodeling and during tumorigenesis (13–16). Multiple signaling pathways regulate the expression pattern of KLF4 via their effectors. Several examples are listed in Table 2. Additional information with references included can be found in supplementary materials (Supplementary Table 1).

At the post-translational level, KLF4 activity has been shown to be negatively regulated by ERK1 and ERK2 phosphorylation at serine 132, which results in induction of embryonic stem cell differentiation (17). This phosphorylation has the secondary effect of recruiting the F-box proteins β TrCP1 or β TrCP2 to the KLF4's N-terminal domain, which in turn results in ubiquitination and degradation of KLF4 (17). Acetylation of KLF4 at lysine residues 225 and 229 mediated by the p300/CBP complex inhibits the ability of KLF4 to activate downstream targets (18). In urinary bladder cancer cells, p21/CK2 interaction enhances HDAC2 phosphorylation and restricts KLF4 deacetylation and subsequent tumor promotion (19). In contrast, overexpression of p300/CBP causes KLF4 acetylation and switches its activity to a tumor suppressor. Du and colleagues demonstrated that KLF4 interacts with SUMO-1 via the SUMO-interacting motif (SIM) of acidic and hydrophobic residue-rich region in KLF4, which is required for KLF4-dependent transactivation of target promoters (20). On the other hand, in embryonic stem cells sumoylation of KLF4 at lysine 275 reduces its transcriptional potential as shown by the decrease in the *Nanog* promoter activity and inhibition of induced pluripotent stem cells (iPSCs) induction (21). Furthermore, sumoylation of human KLF4 mediated by PIAS1 promotes its degradation although the exact mechanism is unknown (22). Multiple lysine residues are implicated in facilitating KLF4 ubiquitination and proteasomal degradation (lysine residues 32, 52, 232, and 252 of murine KLF4) (23). PRMT5, a protein arginine methyltransferase, directly interacts with human KLF4 and catalyzes the methylation of arginine residues 374, 376 and 377, and subsequently stabilizes and increases the transcriptional activity of KLF4 (24). Finally, putative casein kinase II (CKII) recognition motifs have been identified within KLF4 activation domain (THQE) (25).

2.3. Regulation by KLF4

KLF4 is a versatile transcription factor involved in regulating numerous cellular processes (see Table 2 and Supplementary Table 1 for a summary). KLF4-mediated genes transactivation is regulated on multiple levels by modulating KLF4's status through phosphorylation, acetylation, methylation, and ubiquitination in a context-dependent manner.

2.4. Effects on biological processes

KLF4 was initially identified as a factor associated with growth arrest (1). In actively proliferating NIH3T3 cells, the levels of KLF4 are infinitesimal but are significantly elevated in growth-arrested cells caused by either serum starvation or contact inhibition (26). Consistently overexpression of *KLF4* induces cell cycle arrest in several cell lines (27,28). A primary mechanism by which KLF4 regulates the cell cycle is by inducing the expression of *CDKN1A* (the gene encoding p21^{CIP1/WAF1}, a CDK1 inhibitor) (29). This was elucidated by studies investigating the role of KLF4 in modulating cell cycle progression following DNA damage. Following treatment of cultured cells with DNA-damaging agents, it was determined that KLF4 transactivates the *CDKN1A* promoter by binding to a specific SP1-like *cis*-element in the proximal region of the promoter, upon which KLF4 recruits p53 to the *CDKN1A* promoter, allowing p53 to drive transcription of the *CDKN1A* gene (26,30). Activation of p21^{CIP1/WAF1} expression following DNA damage causes cell cycle arrest at both the G₁/S and G₂/M transition points. Moreover, KLF4 has been reported to inhibit expression of *CCND1* and *CCNB1*, which promotes progression through the G₁/S and G₂/M boundary in the cell cycle (31)(32), respectively. Also, KLF4 has been shown to suppress transcription of *CCNE* to prevent centrosome amplification following DNA damage by γ -irradiation (33). Not only does KLF4 play a role in regulating centrosome duplication following DNA damage, but it regulates both centrosome duplication and chromosome number (genetic stability) both *in vitro* and *in vivo* (34,35).

One of the main roles of KLF4 in the cell is promoting survival by suppressing apoptosis (36–41). It was found that KLF4 suppresses the p53-dependent apoptotic pathway by directly inhibiting *TP53* and by directly suppressing *BAX* expression (36,38). Another study showed that following HDAC inhibitor-induced caspase activation, KLF4 impedes apoptosis by suppressing the SAPK pathway by targeting *CDKN1C* (42). However, it was later revealed that under certain conditions KLF4 may switch its role from anti-apoptotic to pro-apoptotic (43–45). KLF4 thus possesses a context-dependent activity.

3. Physiological functions in tissues and organs

3.1. Intestine

KLF4 was originally identified as a gut-enriched transcription factor in the intestine (1). Further studies on intestinal tissue localize its expression to the post-mitotic, terminally differentiated columnar intestinal epithelial cells (46–48). In the intestinal epithelium, KLF4 plays several important roles in regulating intestinal epithelial homeostasis. For example, KLF4 has a critical role in the development and terminal differentiation of goblet cells (49). Using mutant mice with intestine-specific deletion of *Klf4* it was shown that KLF4 is also

required for the terminal differentiation of enterocytes, where deletion of *Klf4* results in a substantial reduction in the expression of the brush-border intestinal alkaline phosphatase and carbonic anhydrase in the small intestine and colon, respectively (46). KLF4 also regulates cellular positioning. Intestine-specific deletion of *Klf4* leads to the mispositioning of Paneth cells to the upper crypt region of the small intestine instead of at the bottom of the crypts and this is attributed to suppressed *Efnb1* expression (46). In line with its *in vitro* role as an inhibitor of cell cycle progression, *in vivo* studies show that mice haploinsufficient for *Klf4* have increased *Ccnd1* expression in the intestinal crypts. In another study mice with intestine-specific deletion of *Klf4* have a statistically significant increase in both the number and migratory rate of epithelial cells (46). This study also shows that deletion of *Klf4* from the intestinal epithelium results in a general activation of genes in the WNT pathway and a global reduction in expression of genes encoding regulators of differentiation. Taken together, the studies provided new insights into the function of KLF4 in regulating postnatal maturation, differentiation, proliferation, migration, and positioning of intestinal epithelial cells, thus establishing a crucial role for KLF4 in maintaining normal intestinal epithelial homeostasis.

3.2. Eye

Serial analysis of gene expression (SAGE) identified *Klf4* as one of the most highly expressed transcription factors in the mouse cornea (50). Its function in the cornea was investigated by conditionally deleting the *Klf4* gene from the surface ectoderm-derived structures of the eye, including cornea, lens, and conjunctiva, where its deletion results in multiple ocular defects, including corneal epithelial fragility, stromal edema, defective lens, and loss of conjunctival goblet cells (51). Mechanistically KLF4 is a critical regulator of a genetic network of factors regulating corneal homeostasis, such as aquaporin-3, crystallins, Aldh3A1 and TKT, and epidermal keratinocyte differentiation markers (52). Additionally, it was shown that KLF4 contributes to corneal epithelial barrier function by upregulating the expression of functionally related subsets of cell junctional proteins and basement membrane components (53). KLF4 is also required for lens maturation, but not for retinal cell differentiation (54,55).

3.3. Skin

KLF4 is highly expressed in the epidermis of the skin. Its function in the skin was first reported by studying mice with whole-body homozygous *Klf4* deletion which die shortly after birth and suffer from a loss of the skin barrier function (56). Further analysis revealed significant mRNA upregulation of three cornified envelope proteins: small proline rich protein 2a (*Sprr2a*), repetin (*Rptn*) and plasminogen activating inhibitor 2 (*Planh2*), of which only *Sprr2a* promoter has a functional KLF4 binding site (56). The role of KLF4 in skin barrier function was confirmed when it was shown that its ectopic expression accelerates the formation of the skin permeability barrier (57). Additionally, it was found that together with corticosteroids, KLF4 activates a set of transcriptional targets that accelerate epidermal barrier acquisition *in utero* (58). Further studies elucidated additional mechanisms of the KLF4's role in the skin. Induction of *KLF4* was shown to be in response to ZNF750 (a p63 target gene) to drive terminal epidermal differentiation, while KLF4 deficiency correlates with increased cell proliferation and enhanced skin tumorigenesis (59,60). Also, KLF4

promotes wound healing by driving generation of fibrocytes from myeloid-derived suppressor cells (61).

3.4. Bone and teeth

During early embryonic development, KLF4 is expressed in mesenchymal cells of the skeletal primordia but its expression is diminished postnatally (62). Ectopic expression of *Klf4* in mice results in severe skeletal deformities and mice die soon after birth (63). Investigation of the role of KLF4 during skeletal development demonstrated that it regulates normal skeletal development through coordinating the differentiation and migration of osteoblasts, chondrocytes, vascular endothelial cells, and osteoclasts (64,65).

KLF4 is specifically expressed in both polarizing odontoblasts and ameloblasts, and is closely correlated to their growth arrest and differentiation (66). During odontoblast differentiation, *Klf4* expression is upregulated by nuclear factor I-C (NFIC), where KLF4 in turn enhances the expression of dentin sialophosphoprotein (*Dspp*) and dentin matrix protein 1 (*Dmp1*), resulting in enhanced dentin mineralization, and promoting differentiation by directly upregulating *Cdh1* expression. Furthermore, it has been shown that miR-143/145 control odontoblast differentiation through regulation of KLF4 and osterix (OSX – SP7) (67).

3.5. Testis

KLF4 plays an important role during spermatogenesis. In mouse testis, *Klf4* is strongly expressed in the postmeiotic germ cells undergoing differentiation into sperm cells, and in the somatic Sertoli cells (68). In human testis, KLF4 is also found to be expressed in differentiating spermatids and in most Leydig cells, but unlike in mouse, it is not expressed in Sertoli cells (69). In mice, it was found that KLF4, together with transcription factors GATA1, GATA6, SP1, and SP3, differentially activate tight junction transmembrane protein CXADR like membrane protein (CLMP) in Sertoli cells, and thus regulate germ cell translocation across the blood-testis-barrier during spermatogenesis (70). In mouse Sertoli cells, *Klf4* expression is strongly induced by follicle stimulation hormone, and its specific deletion from Sertoli cells results in perturbed germinal epithelium and Sertoli cell morphology during puberty, though the mice remain fertile and have normal testicular morphology (71). KLF4 was later found to be widely expressed in the epithelial cells of the mouse male and female reproductive tracts, although its deletion does not inhibit spermatogenesis (72,73).

3.6. Endothelial cells

KLF4 is constitutively expressed in endothelial cells (ECs) where its expression is induced by proinflammatory stimuli and shear stress (74,75). It has been shown that inhibition of KLF4 results in an increase of NF- κ B transcriptional activity and the expression of downstream prothrombotic, proinflammatory genes (76). KLF4 is a positive regulator of proliferation, migration, and tube formation in human retinal microvascular endothelial cells via upregulation of vascular endothelial growth factor (VEGF) levels (77).

3.7. Vascular smooth muscle cells

KLF4 is not normally expressed in differentiated vascular smooth muscle cells (VSMC) *in vivo*, but it is transiently induced in VSMC after vascular injury (74,78,79). In VSMC, *KLF4* was identified as a transcriptional target of both bone morphogenetic proteins (BMP-2, -4 and -6) and transforming growth factor- β 1 (TGF- β 1) to modulate VSMC differentiation (80). Its transcriptional induction in VSMC in response to BMP-4 and TGF- β 1 is regulated by miRNA-143/145 (81). Functionally, KLF4 has a critical role in maintaining the integrity of adherens junctions (AJs) and in preventing vascular leakage in response to inflammatory stimuli by regulating vascular endothelial (VE)-cadherin expression at the AJs and in the acquisition of VE-cadherin-mediated endothelial barrier function (82). Additionally, KLF4 interacts with the closely related KLF2 for the proper maintenance of vascular integrity during mouse embryonic vasculogenesis (83). KLF4 also plays a key role in the regulation of gene transcription in the cardiovascular system. In cardiomyofibroblasts, KLF4 is pivotal to their differentiation and to collagen synthesis by transcriptionally upregulating *Tgfb1* (84). Following injury, KLF4 represses VSMC differentiation markers and arterial inflammation by regulating neointimal formation in non-VSMCs such as VECs (85). KLF4 plays an important role in several vascular diseases. It is expressed by different cell types involved in the development of vascular disease, such as monocyte-derived macrophages, endothelial and vascular smooth muscle cells (86). Moreover, it was shown that KLF4 regulates vascular inflammation and monocyte differentiation (87). Its role in atherosclerosis appears to be cell-type dependent. It was reported that its deletion in the myeloid cells promotes macrophage accumulation in the plaque. In macrophages, it was recently shown that KLF4 up-regulates apolipoprotein E, which is an essential antiatherosclerotic factor (88).

In contrast, KLF4 expression in peripheral blood mononuclear cells (PBMCs) may promote atherosclerosis. KLF4 expression in PBMCs positively correlates with cellular markers of immune activation (89). KLF4 is also induced by high-density lipoproteins and can promote atherosclerosis through the upregulation of scavenger receptor class B type 1 in PBMCs and human THP-1 monocytes (90). The upregulation of KLF4 in the response to vascular injury is an important effector of injury-induced phenotypic switching of SMC (91). KLF4 has also been shown to repress TGF- β 1-dependent increase of SMC differentiation marker genes, including α SM actin and SM22 α (22). However, in response to TGF- β 1 in VSMCs, KLF4 activates SMAD2/3 and p38 MAPK signaling which increases its interaction with SMAD2 and cooperatively increase *TbRI* expression to facilitate positive feedback loop during phenotype switching (92). Further studies demonstrated that ATRA and TGF- β 1 induce phosphorylation of KLF4, which in turn positively regulates its interaction with p300 and increases expression of TGF- β -regulated genes (93,94). Additionally, it has been shown that KLF4 and miR-146a form a feedback loop that regulates VSMCs proliferation and vascular neointimal hyperplasia (95). Recent *in vitro* and *in vivo* studies showed that PDGF-BB stimulated sumoylation of KLF4 inhibits p21 transcription by recruiting transcriptional co-repressors to p21 promoter and thus enhances VSMCs' proliferation (96). Taken together these studies show that KLF4 is an important mediator of TGF- β 1- induced phenotypic switch of VSMCs and could be considered as a potential therapeutic target.

3.8. Blood cells

KLF4, together with seven other KLFs, are present in human erythroid cell lines, and are expressed in mouse erythroid tissues throughout development (97). In macrophages, KLF4 controls their activation in response to lipopolysaccharide (LPS) stimulation by regulating key inflammatory signaling pathways such as NF κ B, TGF- β 1, and inhibiting IL1 β , and promoting the expression, translocation, and release of high-mobility group box 1 (HMGB1), which is an important late inflammatory mediator (98–100). KLF4 is also a critical regulator of monocyte differentiation, and of B-cell number and proliferation, in addition to having a global role in embryonic erythropoiesis, at least in zebrafish (101–105). During monocyte/macrophage differentiation, KLF4 is transcriptionally regulated by demethylation of its promoter in an activation induced cytidine deaminase-dependent manner (106).

KLF4 is also important for a number of other types of white blood cells: it regulates thymocyte development and proliferation, and IL-17 expression during Th17 differentiation; is required for lineage commitment of T-cells; and has differential roles in the differentiation vs development and homeostasis of CD8+ T-cell; it maintains the number of dendritic cells in the spleen and promotes the survival of natural killer cells; and is required for Th2 cell responses in dendritic cells (107–110). It was recently shown that KLF4 enhances memory B-cells differentiation into early plasma cell and long lived plasma cells (111–113).

3.9. Kidney

The role of KLF4 in the kidney has been only recently appreciated. It was initially shown that KLF4 is involved in the differentiation of murine embryonic stem and induced pluripotent stem cells into renal lineage *in vitro* and that it is epigenetically silenced by promoter CpG methylation in renal cell carcinoma (114,115). KLF4 is expressed in normal glomerular podocytes, where its expression is decreased in diseased glomeruli in both animal models and humans with proteinuria, and was found to epigenetically modulate podocyte phenotype and function (116). KLF4 also plays a role in some pathological conditions. Attenuation of proteinuria by renin-angiotensin blockage was found to be in part via KLF4 suppression (116). In contrast to the proinflammatory role of KLF4 in other systems, KLF4 was shown to reduce inflammation (triggered by TGF- β 1) and fibrotic responses, in renal tubular cells during diabetic nephropathy (117). Also, it was recently shown that KLF4 is involved in the regulation of renal physiological functions and the progression of fibrosis (118).

4. Roles in diseases

4.1. Development and progression of cancer/EMT

KLF4 expression is frequently lost in various human cancer types, such as colorectal cancer, gastric cancer, esophageal squamous cell carcinoma, intestinal cancer, prostate cancer, and bladder cancer (119–124). KLF4 was shown to undergo promoter methylation and loss of heterozygosity in gastrointestinal cancer (119). Consistent with its tumor-suppressive function, overexpression of *KLF4* reduces the tumorigenicity of colonic and gastric cancer cells *in vivo* (28,125). In colon cancer tissues, KLF4 levels are lower as compared with

adjacent noncancer tissues and its expression is inversely correlated with the stage of tumors and survival (126). In lung cancer, it was demonstrated that *KLF4* also functions as a tumor suppressor gene. In one study, the expression of *KLF4* is downregulated in the majority of primary lung cancers examined, and ectopic expression of *KLF4* suppresses lung cancer cell proliferation and clonogenic formation *in vitro*, and suppresses tumor growth *in vivo* (127,128). A recent study showed differential expression of *KLF4* in lung cancer, where a significant decrease in *KLF4* expression is observed in non-small-cell lung cancer compared with that in normal tissue, while a significant overexpression is detected in small-cell lung cancer (129).

Conversely, *KLF4* may function as an oncogene in a context-dependent manner. High *KLF4* expression has been found in primary breast ductal carcinoma (130,131). In breast cancer cells, the oncogenic role of *KLF4* was found to be dependent on the status of p21^{CIP1/WAF1} (132). *KLF4*-transformed rat kidney epithelial cells exhibit morphological transformation and increased tumorigenicity in athymic mice (133). Increased *KLF4* expression has been reported in human head and neck squamous cell carcinoma (134). Moreover, *KLF4* expression has been demonstrated to be a poor prognostic factor for early breast cancer and skin cancer, corroborating its oncogenic role (135,136). In the skin, overexpression of *KLF4* results in hyperplasia and dysplasia, eventually leading to the development of squamous cell carcinoma (137). It was also reported that in oral carcinogenesis *KLF4* can act either as a tumor suppressor or an oncogene (138). Whether *KLF4* acts as a tumor suppressor or an oncogene is likely determined by differences in cellular context, expression patterns of other genes and the chromatin environment of individual cells. However, for the most part, the mechanism(s) underlying these differences remains undefined.

Touching on its context-dependent function, the role of *KLF4* in epithelial-to-mesenchymal transition (EMT) seems controversial, where in some systems it promotes EMT while in other systems it suppresses EMT. Several lines of evidence showed a direct link between EMT and stemness of cancer cells (139,140). Among the first reports of the involvement of *KLF4* in regulating EMT through stemness was a report by Wellner *et al* (2009) (141). They showed that in pancreatic cancer *KLF4* promotes EMT by functioning as a stem cell factor that is required to maintain stemness to promote mobile, migrating cancer stem cells (142). This notion was later confirmed and found to be enhanced in a p53-dependent manner, or regulated by doublecortin like kinase 1 (DCLK1) in pancreatic ductal adenocarcinoma (PDAC) (143). Similar finding on the role of *KLF4* as a stem cell factor promoting EMT was reported in endometrial cancer, human nasopharyngeal carcinoma, non-small cell lung cancer and via interaction between TWIST1-JAGGED1-*KLF4* in angiogenesis in head and neck cancer (144–146).

The anti-EMT role of *KLF4* was shown in other systems (147). In prostate cancer, three reports showed an opposite role for *KLF4* in EMT. *KLF4* can promote EMT in prostate cancer cells by maintaining stemness in a p53-dependent fashion, or following *CDH2* overexpression (148,149). However, in TGFβ1-induced EMT of prostate cancer cells, *KLF4* suppresses EMT by downregulating *SNAI2*, which was also shown to downregulate *KLF4* expression to promote EMT (150). Others reported that following TGFβ1 induction, *KLF4* suppresses EMT in pancreatic cancer (151). In epidermal cells, *SNAI2* was shown to

specifically suppress *KLF4* and grainyhead like transcription factor 3 (*GRHL3*) expression leading to de-differentiation and decreased cell adhesion of epidermal cells (152). In an animal model of breast cancer xenograft, *KLF4* overexpression suppresses the tumorigenic progression of SK-BR-3 cell line (45). Additionally, overexpression of *KLF4* in lung cancer cells inhibits cell migration and invasion increases the expression of *CDH1* and suppresses *SNAI2*, *TWIST1*, and vimentin (*VIM*) expression (153). Also, in cisplatin-resistant nasopharyngeal carcinoma cells, *KLF4* was shown to inhibit EMT by suppressing NOTCH1 expression which promotes cell migration, invasion, and EMT, while in hepatocellular carcinoma cells, *KLF4* suppresses EMT by inducing specific miRNA species that inhibited *SNAI1*, *SNAI2* and *ZEB1* expression (154,155). Additionally, overexpression of *KLF4* in endothelial cells was shown to suppress angiogenesis, in part by mediating the expression and activity of NOTCH1 (156). Conversely, *KLF4* overexpression in vascular endothelial cells of the microvasculature within the central nervous system leads to vascular dysplasia (Cerebral cavernous malformation) (157). These findings are in line with what is becoming to be known as the context-dependent function *KLF4*.

4.2. Inflammation

The role of *KLF4* in regulating inflammation was first reported by Feinberg *et al* 2005, where they showed that in response IFN- γ and LPS, *KLF4* physically interacts with the NF- κ B p65 subunit to induce the *NOS2* promoter (98). The involvement of *KLF4* in NF- κ B-mediated inflammation was further illustrated by two independent studies. In the esophageal epithelium, overexpression of *Klf4* was shown to activate several proinflammatory cytokines within the keratinocytes in an NF- κ B-dependent manner, and that which eventually lead to esophageal squamous cell cancer (158). Recently, it was shown that *KLF4* plays a role in promoting acute colitis in mice in response to chemically-induced inflammatory trigger (159). In this study, specific deletion of *Klf4* from the intestinal epithelium renders the mice resistant to dextran sulfate-sodium (DSS)-induced colitis, a phenotype that is accompanied by inhibited NF- κ B signaling, together with suppressed *Il1b* and *Il6* production and inflammatory infiltrate, as compared to DSS-treated wild type mice (159).

Conversely, *KLF4* suppresses the activation of inflammatory signaling. Its overexpression in endothelial cells induces the expression of multiple anti-inflammatory and anti-thrombotic factors including endothelial nitric-oxide synthase and thrombomodulin, whereas its knockdown enhances TNF α -induced vascular cell adhesion molecule (*VCAM1*) (160).

4.3. Radiation injury

The role of *KLF4* in radiation-induced DNA damage was first reported by Yoon *et al* (2003), when they showed that *KLF4* is induced by p53 following γ -irradiation injury and that it acts as an essential mediator of p53-dependent G₁/S cell cycle arrest following γ -irradiation-induced DNA damage (30). Additionally, it was shown that following γ -irradiation-induced DNA damage, *KLF4* is required for preventing cell cycle entry into mitosis and it does so by suppressing *CCNB1* expression, and that it prevents centrosome amplification by suppressing *CCNE* expression (32,33). In colon cancer cell lines and mouse embryonic fibroblasts (MEFs), *KLF4* plays a critical role in decision-making in favor of inhibiting apoptosis by suppressing p53-dependent apoptotic pathway, and simultaneously preventing

cell cycle progression by inducing *CDKN1A* expression (30,38). This property of KLF4 was also corroborated in breast cancer cell line MCF-7. Here following radiation-induced DNA damage, p53 induces *KLF4* expression which in turn suppresses the expression of estrogen receptor- α , and thus abrogating estrogen-dependent breast cancer cell growth (161). In MEFs it was shown that KLF4 plays an essential role in modulating the DNA damage response and repair mechanisms (34,35). *In vivo*, KLF4 plays a role as a radio-protective factor against gastrointestinal syndrome in mice following total body γ -irradiation, does so both by inhibiting apoptosis in the acute response to irradiation and by contributing to crypt regeneration (39,41). In this model however, KLF4 is not essential for *CDKN1A* expression in the intestinal epithelium following total body γ -irradiation. In contrast to the role of KLF4 following γ -irradiation exposure, exposure of murine astrocytes to X-ray radiation upregulates KLF4 expression, which induces more double-strand DNA breaks (DSB), less single-strand breaks (SSB) and increases apoptosis (162). This is intriguing because there is evidence that under certain conditions repairable single-strand breaks may not result in cell death, while irreparable DSB are deemed lethal events, and where it was also shown that in some systems, the ratio of SSB to DSB are crucial in determining which death pathway the cell may undertake (163–165). The above findings again emphasize the context-dependent function of KLF4 which seems to be a common theme for the different roles of KLF4. Here, the type of radiation, the dose and cell/tissue type are factors to be considered.

5. Stem cells

The importance of KLF4 as a stemness factor first came in the spotlight from the work of Takahashi and Yamanaka (2006), where they demonstrated for the first time that, under specific culture conditions, mouse embryonic and adult fibroblasts can be induced into pluripotent stem cells by overexpressing four specific factors: *Oct3/4*, *Sox2*, *Myc*, and *Klf4* (166). This was later replicated using adult human fibroblasts (167). These initial publications opened the door for a widely expanding and clinically relevant studies in stem cell research, cementing the pivotal role of KLF4 (together with the other three factors) in stem cells. A PubMed search for “induced pluripotent stem cells” retrieved 60 hits from year 1900 to 2005, compared to more than 10,000 results for this topic in the past 10 years. Recent studies demonstrated that KLF4 plays a dual role during induction of pluripotency, initially repressing markers of differentiation and facilitating the expression of pluripotency genes at the later stage (168). By itself, it was shown to be directly regulated by leukemia inhibitory factor (129)/STAT3 pathway (169). The role and regulation of KLF4 in the induction of pluripotency have been previously presented and discussed (170–173).

In line with the importance of KLF4 in stem cells, a recent work from our lab have investigated the *in vivo* role of KLF4 in intestinal stem cells and intestinal epithelium regeneration following γ -irradiation-induced gut injury (41). In mice, the intestinal epithelium is renewed every 3–4 days through intestinal stem cells (ISCs) which reside at the base of the intestinal crypt (174). The current consensus is that there are two types of ISCs. One population is termed the crypt base columnar (CBC) cells at the base of the intestinal crypt, and which can be identified by their expression of leucine-rich G-protein-coupled receptor 5 (*LGR5*) (174). The second type is identified by their expression of B-cell-specific Moloney murine leukemia virus integration site 1 (*BMI1*), and they reside at the

+4 position of the intestinal crypt (175). Functional studies *in vivo* and *ex vivo* have demonstrated that LGR5⁺ ISC are actively proliferating and can lineage trace under normal homeostasis, however they are unable to lineage trace post-radiation injury as they are susceptible to radiation injury (176,177). In contrast, BMI1⁺ ISCs are normally quiescent, yet they are resistant to radiation injury and do contribute to the regenerative response to ionizing radiation injury (177). Our investigation showed that under normal homeostasis, deletion of *Klf4* from the BMI1⁺ cells increased their proliferative ability and increase lineage expansion (41). On the other hand, following irradiation, *Bmi1*-specific *Klf4* deletion lead to decreased expansion of the BMI1⁺ lineage as consequence of reduced proliferation and increased apoptosis. Our results support an essential role for KLF4 in modulating BMI1⁺ intestinal stem cell fate both in homeostasis and during the regenerative response to radiation injury. Furthermore, it has been shown that KLF4 play important role in hair follicle stem cells by augmenting cutaneous wound healing post-injury (178).

6. Concluding remarks

Recent studies on KLF4 are expanding our knowledge of its diverse functions and essential roles in numerous tissues/organs and cellular processes. A common theme is that KLF4 has a context-dependent function, under certain conditions KLF4 would undergo a certain role, while under different conditions it may play a completely opposite role. This highlights the critical role of different factors that interact with KLF4 and those which dictate what role it plays, and when. Thus, we believe that future research on KLF4 function should not lose sight of the other co-players, and which will help immensely in the effort to fully understand the role of KLF4, and ultimately in identifying novel targets for therapy.

Supplementary Material

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HIGHLIGHTS

GENE-D-16-02319

Krüppel-like factor 4 (KLF4): What we currently know

- KLF4 is a member of the zinc finger-containing Krüppel-like factor family.
- KLF4 regulates diverse physiological functions and cellular processes, including somatic cell reprogramming.
- KLF4 plays important roles in the pathogenesis of diseases including cancer and vascular diseases.

Figure 1.

The schematic representation of transcriptional and post-transcriptional regulation of KLF4 activity. For details please see text.

SP 043474 KLF4_HUMAN	MRQPPGESDMAVSDALLPSFSTFASGPAGREKTLRQAGAPNNRWREELSHMKRLPPVLP	60
SP 043474-1 KLF4_HUMAN	MRQPPGESDMAVSDALLPSFSTFASGPAGREKTLRQAGAPNNRWREELSHMKRLPPVLP	60
SP 043474-4 KLF4_HUMAN	-----MKRLPPVLP	10
SP 043474-5 KLF4_HUMAN	MRQPPGESDMAVSDALLPSFSTFASGPAGREKTLRQAGAPNNSSCHVPACQSPSPQRGE	60
SP 043474-6 KLF4_HUMAN	MRQPPGESDMAVSDALLPSFSTFASGPAGREKTLRQAGAPNN-----	42
SP 043474 KLF4_HUMAN	RPYDLAAATVATDLESGGAGAACGGSNLAPLPRETEEFNDLLDLDFILNSLTHPPESV	120
SP 043474-1 KLF4_HUMAN	RPYDLAAATVATDLESGGAGAACGGSNLAPLPRETEEFNDLLDLDFILNSLTHPPESV	120
SP 043474-4 KLF4_HUMAN	RPYDLAAATVATDLESGGAGAACGGSNLAPLPRETEEFNDLLDLDFILNSLTHPPESV	70
SP 043474-5 KLF4_HUMAN	DDRGPGKGPPPT-LVIT--RAAAKPTQRPVPISRHTCE-PTQVRNLTTVTG-----	106
SP 043474-6 KLF4_HUMAN	-----VRNLTTVTG-----	51
SP 043474 KLF4_HUMAN	AATVSSASASSSSSPSSSGPASAPSTCSFTYPIRAGNDPGVAPGGTGGGLLYGRESAPP	180
SP 043474-1 KLF4_HUMAN	AATVSSASASSSSSPSSSGPASAPSTCSFTYPIRAGNDPGVAPGGTGGGLLYGRESAPP	180
SP 043474-4 KLF4_HUMAN	AATVSSASASSSSSPSSSGPASAPSTCSFTYPIRAGNDPGVAPGGTGGGLLYGRESAPP	130
SP 043474-5 KLF4_HUMAN	-----TAVDGNSPAQMN-----	118
SP 043474-6 KLF4_HUMAN	-----TAVDGNSPAQMN-----	63
SP 043474 KLF4_HUMAN	PTAPFNLADINDVSPSGGFVAELLRPELDPVYIPQPPQPPGGGLMGKFLKASLSAPGS	240
SP 043474-1 KLF4_HUMAN	PTAPFNLADINDVSPSGGFVAELLRPELDPVYIPQPPQPPGGGLMGKFLKASLSAPGS	240
SP 043474-4 KLF4_HUMAN	PTAPFNLADINDVSPSGGFVAELLRPELDPVYIPQPPQPPGGGLMGKFLKASLSAPGS	190
SP 043474-5 KLF4_HUMAN	-----	
SP 043474-6 KLF4_HUMAN	-----	
SP 043474 KLF4_HUMAN	EYGSPSVISVSKGSPDGSHPVVAPYNGGPPRTCPKIKQEAUVSSCTHLGAGPPLSNHRP	300
SP 043474-1 KLF4_HUMAN	EYGSPSVISVSKGSPDGSHPVVAPYNGGPPRTCPKIKQEAUVSSCTHLGAGPPLSNHRP	300
SP 043474-4 KLF4_HUMAN	EYGSPSVISVSKGSPDGSHPVVAPYNGGPPRTCPKIKQEAUVSSCTHLGAGPPLSNHRP	250
SP 043474-5 KLF4_HUMAN	-----	
SP 043474-6 KLF4_HUMAN	-----	
SP 043474 KLF4_HUMAN	AAHDFPLGRQLPSRTTPTLGLLEVLSSRDCHPALPLPPGFHHPGPNYPSFLPDQMPPQV	360
SP 043474-1 KLF4_HUMAN	AAHDFPLGRQLPSRTTPTLGLLEVLSSRDCHPALPLPPGFHHPGPNYPSFLPDQMPPQV	360
SP 043474-4 KLF4_HUMAN	AAHDFPLGRQLPSRTTPTLGLLEVLSSRDCHPALPLPPGFHHPGPNYPSFLPDQMPPQV	310
SP 043474-5 KLF4_HUMAN	-----	
SP 043474-6 KLF4_HUMAN	-----	
SP 043474 KLF4_HUMAN	PPLHYQGQSRGFVARAGEPCVCWPHFGTHGMMLTPPSSPLELMPGSCMPEEPKPKRGRR	420
SP 043474-1 KLF4_HUMAN	PPLHYQ-----ELMPGSCMPEEPKPKRGRR	386
SP 043474-4 KLF4_HUMAN	PPLHYQ-----ELMPGSCMPEEPKPKRGRR	336
SP 043474-5 KLF4_HUMAN	-----	
SP 043474-6 KLF4_HUMAN	-----	
SP 043474 KLF4_HUMAN	SWPRKRTATHTCDYAGCGKTYTKSSHLKAHLRTHTEGKPYHCDWDGCGWKFARSDELTRH	480
SP 043474-1 KLF4_HUMAN	SWPRKRTATHTCDYAGCGKTYTKSSHLKAHLRTHTEGKPYHCDWDGCGWKFARSDELTRH	446
SP 043474-4 KLF4_HUMAN	SWPRKRTATHTCDYAGCGKTYTKSSHLKAHLRTHTEGKPYHCDWDGCGWKFARSDELTRH	396
SP 043474-5 KLF4_HUMAN	-----	
SP 043474-6 KLF4_HUMAN	-----	
SP 043474 KLF4_HUMAN	YRKHTGHRPFQCCQCDRAFSRSDHLALHMKRHF	513
SP 043474-1 KLF4_HUMAN	YRKHTGHRPFQCCQCDRAFSRSDHLALHMKRHF	479
SP 043474-4 KLF4_HUMAN	YRKHTGHRPFQCCQCDRAFSRSDHLALHMKRHF	429
SP 043474-5 KLF4_HUMAN	-----	
SP 043474-6 KLF4_HUMAN	-----	

Figure 2.

Comparison of amino acid sequence in splice variants of human KLF4 identified in pancreatic cancer cell lines. Alignment was done using Uniprot. Asterisk (*) indicates positions which have a single, fully conserved residue. Colon (:) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. Period (.) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.

Table 1

Length and mass of human, mouse, rat and zebra fish KLF4.

Species	<i>Homo sapiens</i> (human)	<i>Mus musculus</i> (house mouse)	<i>Rattus norvegicus</i> (Norway rat)	<i>Danio rerio</i> (zebrafish)
Description	Krüppel like factor 4	Kruppel-like factor 4	Krüppel like factor 4	Krüppel-like factor 4
Location	Chromosome 9, NC_000009.12 (107484852..10748 9720, complement)	Chromosome 4, NC_000070.6 (55527137..5553247 5, complement)	Chromosome 5, NC_005104.4 (72283311..72287669, complement)	Chromosome 21, NC_007132.6 (437845..485915, complement)
Aliases	EZF, GKLF	EZF, Gklf, Zie	GKLF	klf4a
Ensembl	ENSG00000136826	ENSMUSG00000003032	ENSRNOG00000016299	ENSDARG00000079922
UniProt	O43474	Q60793	Q923V7	A9X6Q5
RefSeq (mRNA)	NM_001314052.1 NM_004235	NM_010637	NM_053713.1	NM_001113483.1
RefSeq (protein)	NP_001300981.1N P_004226.3	NP_034767.2	NP_446165.1	NP_001106955.1
Gene type	Protein coding	Protein coding	Protein coding	Protein coding
Length (a.a.)^a	513	483	482	396
Mass (Da)^a	54,671	51,880	51,722	43,591

^aBased on Uniprot database.

Table 2

A summary of some factors/pathways/genes that play a role in regulation of KLF4 and those that KLF4 plays a role in their regulation.

Factors that regulate KLF4	Pathways/genes regulated by KLF4
TGF- β 1	IL-10
IFN- γ	TGF- β 1
IL-10	IL-6
IL-17	Slurp1
IL-1 β	NF- κ B pathway
Erk5	Notch1
Notch1	HNF-6
Shh	Apolipoprotein E
C/EBP α	Keratin 4 & 19
SUMOylation	Laminin a 3A
KLF4	Ornithine decarboxylase
KLF5	Laminin a 1
AP-2 α	Histidine decarboxylase
Butyrate	p53
p53	Wnt signaling and b-Catenin
Prostaglandin	PSG-5
PPAR γ receptor	cGMP-dependent protein kinase
Polyadenylation	Epidermal barrier transcriptional targets
Heat stress	ABL oncogenes
Tip60	Tight junction protein CLMP
FOXO	Steroidogenic genes LDLR and CYP11A
HMGB1	Adeopogenesis
All-trans retinoic acid	HSC70
AICAR	Zip4
Sp1	Ghrelin
ELF4	Estrogen receptor α
Cyclosporin	Platelet-derived growth factor receptor β
Platelet factor 4	p57
HDL	GPA33
p63	Nitric oxide synthase
Estrogen	HSP90
PKC	Mitofusin 2
Trichostatin	p21
Acetylbritanilactone	cyclin D1
Lim mineralization protein 3	cyclin D2
pVHL	cyclin E

Factors that regulate KLF4	Pathways/genes regulated by KLF4
ZNF750	cyclin B
LIF	Thyrotropin-releasing hormone
CDX2	SM22a
Oxygen	Conjunctival forniceal gene expression
TLR9	HBG
Src	Phosphofructokinase platelet isoform
Hypermethylation	Bmi1
Change in chromatin structure	DLK2
DNA damage	Alkaline phosphatase
Nerve growth factor	Proton-coupled folate transporter (PCFT)
Hydrogen peroxide	E-cadherin
Sheer stress	DMP1
Leptin	Myocardin
Honokiol	epigenetic remodeling
Dnmt1	Tight junction proteins ZO-1, occludin and claudin-5
AGPAT9	EMT promoting factors
Ubiquitylation	Epidermal progenitor genes and differentiation genes
Renin-angiotensin	Pax6
APC	MET
p300	Elk3
CtBPs	T-cell associated genes
Contact inhibition	Keratin 13
Serum starvation	TIMP-1 and -2
cAMP	Hepatocyte nuclear factor-6
Gastrin	Histone acetylation
Dextran sulfate sodium	mTOR pathway
Oxidative stress	DNA repair mechanisms
Matrix stiffness in ex vivo cultures of mouse proximal tubular epithelial cells	Ephrin-B/EphB signaling
Anaphase promoting complex	Autophagy markers LC3II and Atg7
Bone morphogenetic protein (BMP)-2, -4 & -6	C/EBP β
SLUG	p27
miRNA	Apoptosis