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REVIEW ARTICLE

Krüppel-like factor 10 (KLF10) as a critical signaling mediator: Versatile functions in physiological and pathophysiological processes

Hong-Yang Luo [a,b], Jie-Ying Zhu [a,b], Min Chen [a,b], Wang-Jing Mu [a,b], Liang Guo [a,b,*]

[a] *Shanghai Frontiers Science Research Base of Exercise and Metabolic Health, Shanghai University of Sport, Shanghai 200438, China*
[b] *School of Kinesiology, Shanghai University of Sport, Shanghai 200438, China*

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Abstract Krüppel-like factor 10 (KLF10), also known as TGFβ-inducible early gene-1 (TIEG1), was first found in human osteoblasts. Early studies show that KLF10 plays an important role in osteogenic differentiation. Through decades of research, KLF10 has been found to have complex functions in many different cell types, and its expression and function is regulated in multiple ways. As a downstream factor of transforming growth factor β (TGFβ)/SMAD signaling, KLF10 is involved in various biological functions, including glucose and lipid metabolism in liver and adipose tissue, the maintenance of mitochondrial structure and function of the skeletal muscle, cell proliferation and apoptosis, and plays roles in multiple disease processes, such as nonalcoholic steatohepatitis (NASH) and tumor. Besides, KLF10 shows gender-dependent difference of regulation and function in many aspects. In this review, the biological functions of KLF10 and its roles in disease states is updated and discussed, which would provide new insights into the functional roles of KLF10 and a clearer view of potential therapeutic strategies by targeting KLF10.

* Corresponding author. School of Kinesiology, Shanghai University of Sport, Shanghai 200438, China.
E-mail address: guoliang@sus.edu.cn (L. Guo).
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Abbreviations

Acc 1	acetyl-CoA carboxylase 1
Acly	ATP citrate lyase
Akt	AKT serine/threonine kinase 1
ALT	alanine aminotransferase
AMPK	protein kinase AMP-activated catalytic subunit alpha 1
AST	aspartate aminotransferase
ATP	adenosine triphosphate
Axvr1	activin A receptor type 1
BAT	brown adipose tissue
Bax	BCL2 associated X
Bcl 2	BCL2 apoptosis regulator
BI-1	BAX inhibitor 1
Bmal1	aryl hydrocarbon receptor nuclear translocator like
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BMP	bone morphogenetic protein
C/EBPα	CCAAT/enhancer-binding protein alpha
C/EBPβ	CCAAT/enhancer-binding protein beta
ChIP	chromatin immunoprecipitation
ChREBP	carbohydrate-responsive element-binding protein
Col1a1	collagen type I alpha 1 chain
CS	citrate synthase
CVB3	coxsackievirus B3
DEN	diethylnitrosamine
DIO	diet-induced obesity
DMBA	7,12-dimethylbenz[a]anthracene
Dnmt1	DNA methyltransferase 1
ECM	extracellular matrix
EDL	extensor digitorum longus
Elovl6	ELOVL fatty acid elongase 6
EMT	epithelial-to-mesenchymal transition
Fasn	fatty acid synthase
FBW7	F-box and WD repeat domain containing 7
Foxp3	forkhead box P3
G6pc	glucose-6-phosphatase, catalytic
HCC	hepatocellular carcinoma
HDAC1	histone deacetylase 1
HFSD	high fat sucrose diet
HGF	high glucose fructose
HSC	hepatic stellate cell
HSD	high sucrose diet
Inhbb	inhibin subunit beta B
ITCH	itchy E3 ubiquitin protein ligase
IVDD	intervertebral disc degeneration
JARID1B	Jumonji AT-rich interactive domain 1 B
KDM6A	lysine demethylase 6 A
KLF10	Krüppel-like factor 10
Mat1a	methionine adenosyltransferase 1 A
MCD	methionine and choline deficient
MCE	mitotic clonal expansion
MCP1	monocyte chemoattractant protein 1
Mlxipl	MLX interacting protein like
MM	multiple myeloma
MSC	mesenchymal stem cell
mTORC1	CREB-regulated transcription coactivator
NAFL	non-alcoholic fatty liver
NAFLD	non-alcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NHF	normal human fibroblasts
NP	nucleus pulposus
OA	osteoarthritis
OVX	ovariectomy
Pck1	phosphoenolpyruvate carboxykinase 1
Pgc-1α	PPARG coactivator 1 alpha
PH	primary hepatocytes
PI3K	phosphatidylinositol 3-kinase
Pklr	pyruvate kinase L/R
Pnpla3	patatin like phospholipase domain containing 3
PPARγ	peroxisome proliferator-activated receptor gamma
PTEN	phosphatase and tensin homolog
PTTG1	PTTG1 regulator of sister chromatid separation, securin
RhoB	ras homolog family member B
SH3	Src homology-3
SIAH1	seven in absentia homologue-1
Slc2a4	solute carrier family 2 member 4
α-SMA	alpha-smooth muscle actin
SNAI2	snail family transcriptional repressor 2
Sol	Soleus
SREBP-1C	sterol regulatory element binding transcription factor 1
TG	triglycerides
TGFβ	transforming growth factor β
TGF-βRI	transforming growth factor β receptor I
Thr	threonine
TIEG1	TGFβ-inducible early gene-1
Timp1	tissue inhibitor of metalloproteinase 1
UV-B	ultraviolet radiation-B
VLDL	very low density lipoprotein
VMC	viral myocarditis
WAT	white adipose tissue

Introduction

KLF10 is a member of the SP (specificity proteins)/KLF (Krüppel-like factors) family of transcription factors.[1–3] KLF10 was initially identified in normal human fetal osteoblasts (hFOB) as a downstream factor of TGFβ and acts as a positive regulator of bone growth.[4] In the last decades, KLF10 was also found to be expressed in many other tissues, such as liver, adipose tissue and skeletal muscle. KFL10 is recognized as an effector of TGFβ/SMAD pathway. However, increasing number of studies indicate that KLF10 is also involved many other pathways, such as Wnt signaling pathway and estrogen pathway.[5,6] And there has been growing recognition of the important link between KLF10 and cell proliferation, apoptosis, glucose and lipid metabolism and so on. A large amount of evidence suggests that

KLF10 is an important regulator in the physiological processes of organisms and its underlying mechanisms are also explored. In addition, the role of KLF10 has also been investigated in pathophysiological processes such as tumorigenesis, intervertebral disc degeneration (IVDD), osteoarthritis, cardiovascular diseases and diabetic nephropathy.[2,7–12] TGFβ has been reported to play an essential role in liver fibrosis, a feature of nonalcoholic steatohepatitis (NASH).[13] As the downstream factor of TGFβ signaling and a regulator of lipid metabolism, the role of KLF10 in NASH has attracted much attention. This review aims to update and discuss the functional roles and mechanisms of KLF10 in various physiological and pathophysiological processes, which would be instructive to further understanding of KLF10 biological feature and shed light on the KLF10-targeted therapeutic potentials for diseases.

Molecule feature and the multi-level regulation of KLF10

As shown in Figure 1A, KLF10 protein structure contains a DNA-binding domain, several proline-rich Src homology-3 (SH3) binding domains and three transcriptional repression domains (R1-R3).[1,14] The DNA-binding domain contains three tandem Cys_2His_2 zinc-finger motifs, which bind to GC-rich promotor elements.[3] The SH3 binding domain could bind with some other transcriptional factors or co-factors, such as Sp1, to regulate transcription.[3] The repression domains contain 10 amino acids for R1, 12 amino acids for *r*2 and approximately 80 amino acids for R3.[14] KLF10 could interact with some co-repressors, such as mSin3A, through the repression domains, thereby suppressing the transcription of target genes. The KLF10 protein homology among human, *Mus musculus*, *Bos taurus* and *Lacerta agilis* is 81.28% (Fig. 1B). The high homology of KLF10 protein among species suggests a critical role of KLF10 in biological processes.

The most famous signaling pathway that KLF10 is involved in is transforming growth factor β (TGFβ)/SMAD signaling pathway. TGFβ binds to the type I receptor on the membrane of cells, triggering the phosphorylation of SMAD2/SMAD3 (Fig. 2A).[15] Then, the activated SMAD2 and SMAD3 form oligomeric complexes with SMAD4, which will then translocate into the nucleus and bind to the promoters of their target genes, among which is *Klf10* (Fig. 2A, B).[15] After being transactivated by the TGFβ/SMAD signaling, KLF10 can further enhance the transcription of *Smad 2*, which will form a positive feedback loop (Fig. 2A).[16] SMAD7 is an inhibitor of TGFβ/SMAD signaling pathway,[17,18] and KLF10 can bind to the promoter of *Smad 7* and inhibit its transcription, thereby facilitating the activation of TGFβ/SMAD signaling pathway (Fig. 2A).[19]

KLF10 is also involved in estrogen signaling. In human breast cancer cells, estrogen signaling could stimulate the expression of *Klf10* gene (Fig. 2A, B), which then inhibited the transcription of anti-apoptotic gene *BAX inhibitor 1* (*BI-1*) (Fig. 2A).[20] The reduced expression of BI-1 induced the release of Ca^{2+} and consequently increased cell apoptosis in breast cancer (Fig. 2A).[20] Moreover, in the ovariectomy (OVX) mice, *Klf10* deficiency blunted the role of exogenous estrogen supplement in increasing bone mineral density (BMD) and bone mineral content (BMC) (Fig. 2A).[5] These studies suggest an important interplay between KLF10 and estrogen signaling.

JunB binding site on the proximal promoter region of *KLF10* gene, which is highly conserved in human and mouse, is indispensable for promoting *Klf10* transcription.[21] JunB could bind with JunB binding site on *Klf10* gene promoter (−101 bp to −44 bp from transcription start site) and promote the transcriptional expression of *Klf10* (Fig. 2B). Mutation of this JunB binding site completely abolished the transcription of *Klf10*. In this way, JunB functions as a positive basic factor for the transactivation of *KLF10* gene.[21] Lysine demethylase 6 A (KDM6A) can also facilitate the transcription of *Klf10* to exacerbate diabetic nephropathy (Fig. 2B).[12]

The protein stability of KLF10 can be regulated by ubiquitination. After binding with seven in absentia homologue-1 (SIAH1), a kind of E3 ubiquitin ligase, KLF10 protein can be degraded (Fig. 2B).[22] SIAH1 can bind to the amino acids 1—210 (Fig. 1A) of KLF10 protein and amino acids 211—350 of KLF10 can enhance this binding.[22] By promoting the proteasomal degradation of KLF10, SIAH1 may limit the duration and/or magnitude of TGFβ signaling.[22] Besides SIAH1, FBW7, another E3 ubiquitin ligase, can directly bind to the amino acids 82—86 (Fig. 1A) in KLF10 and promote the degradation of KLF10 protein (Fig. 2B).[23] In this way, FBW7 blunted the role of KLF10 in inhibiting *Smad 7* expression.[23] The binding of KLF10 with some ubiquitin ligases may promote the function of KLF10. By binding with itchy E3 ubiquitin ligase (ITCH), the function of KLF10 in protecting against airway inflammation can be enhanced (Fig. 2B).[24] In T cells, it was found that KLF10 could bind with the 'WW' domains in ITCH, which has two highly conserved tryptophan residues. This interaction existed even in the absence of TGFβ stimulation. In the presence of an active form of TGFβ receptor, ITCH induced-ubiquitination of KLF10 could be enhanced.[24] Further studies showed that ITCH-mediated ubiquitination of KLF10 facilitated TGFβ signaling-induced binding of KLF10 on *Foxp3* gene promoter to enhance the transcription of *Foxp3* in regulatory T cells (Treg cells), thereby controlling of allergic responses.[24] In another paper, it was also found that ITCH could mediate SMAD7 ubiquitination and promote its subsequent degradation.[25] In this way, ITCH might promote TGFβ signaling to increase KLF10 expression, which could also facilitate the transcription of *Foxp3* in Treg cells. Further studies are needed to investigate whether the ITCH/SMAD7/KLF10 pathway described above is also involved in regulating *Foxp3* gene expression in Treg cells. It is interesting that the ubiquitination of KLF10 can bring about different effects, degradation of KLF10 protein or enhancement of KLF10 function. Different conjugate models between ubiquitin and the substrates could lead to different effects of the ubiquitination. For example, homotypic Lys11-and Lys48-linked ubiquitin chains can drive proteasomal degradation of many proteins.[26,27] Moreover, Lys 63-or Met1-linked ubiquitin chains can enhance the function of some proteins, such as NF-κB.[26] The different effects on KLF10 mediated by SIAH1/FBW7 and ITCH may be explained by the different linkage types of ubiquitin chains during the ubiquitination of KLF10 protein, which merits further studies.

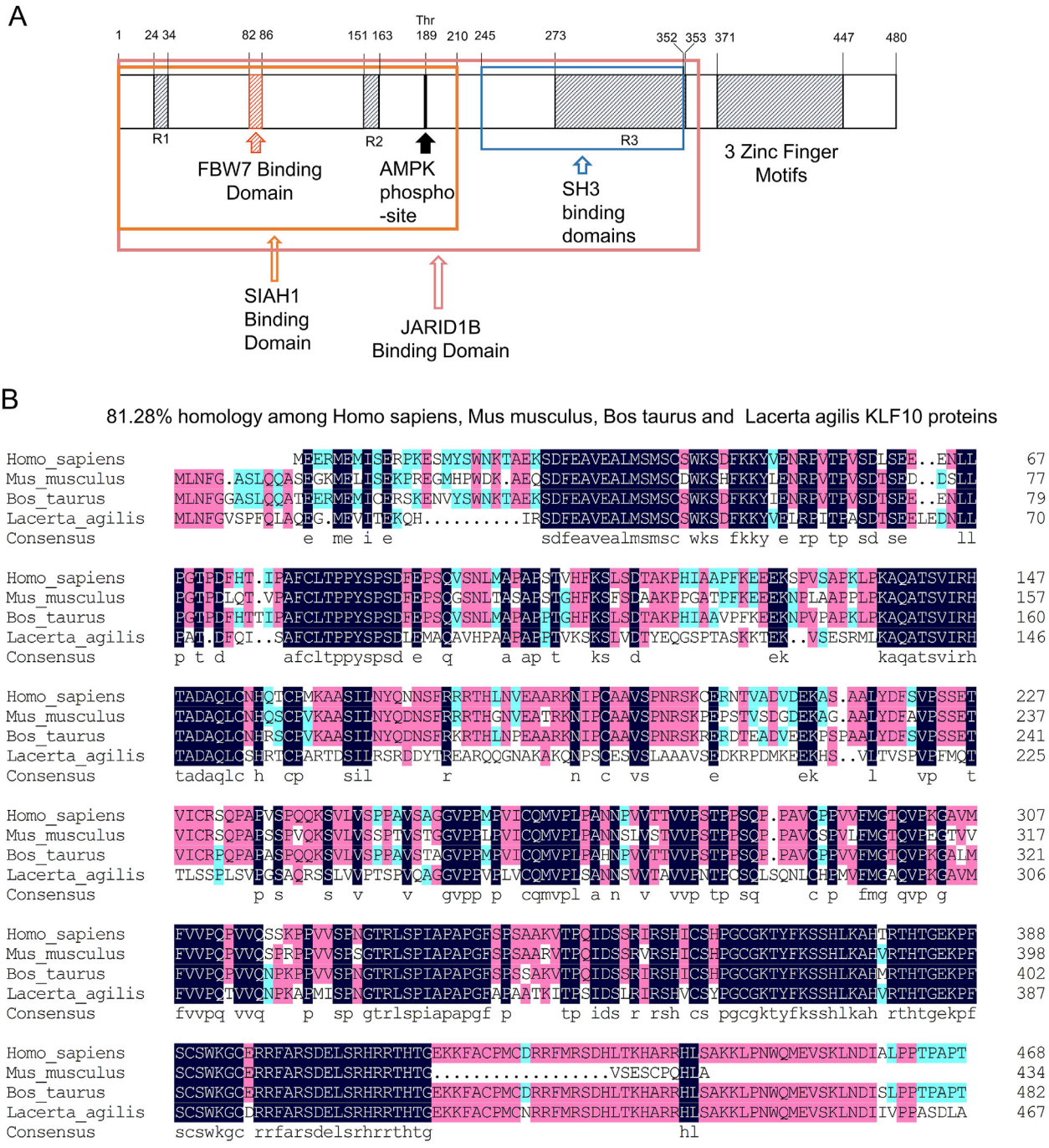

Figure 1 The protein structure of KLF10 with various protein and DNA binding domains and sequence alignment of KLF10 proteins among species. **(A)** Illustration of the protein structure of human KLF10 with various protein and DNA binding domains. The protein structure of KLF10 contains a DNA binding domain with three zinc finger motifs at the C-terminal, several SH3 binding domains and three transcriptional repression domains (*r*1-R3) at the N-terminal. KLF10 may interact with Sp1 via the SH3 binding domain. The repression domains may mediate the binding between KLF10 and some co-repressors, such as mSin3A. The regions that mediate the binding of KLF10 with FBW7, SIAH1 and JARID1B are also indicated, respectively. AMPK can phosphorylate KLF10 on the site of Thr189 **(B)** Comparison and alignment of KLF10 protein sequences of human, mouse, bos taurus, and lacerta agilis. Among the 4 sequences, 2 identical residues are represented in blue; 3 identical residues are represented in red; 4 identical residues are represented in black.

It was found that KLF10 could be phosphorylated at Thr189 (Fig. 1A) by AMP-activated protein kinase (AMPK) in liver.[28] The phosphorylated KLF10 could bind to the SP/KLF binding site (−13 bp to −23 bp of transcription start site) of *Srebf1* promoter and suppressed its transcription. *Srebf1* is the gene encoding sterol regulatory element-binding

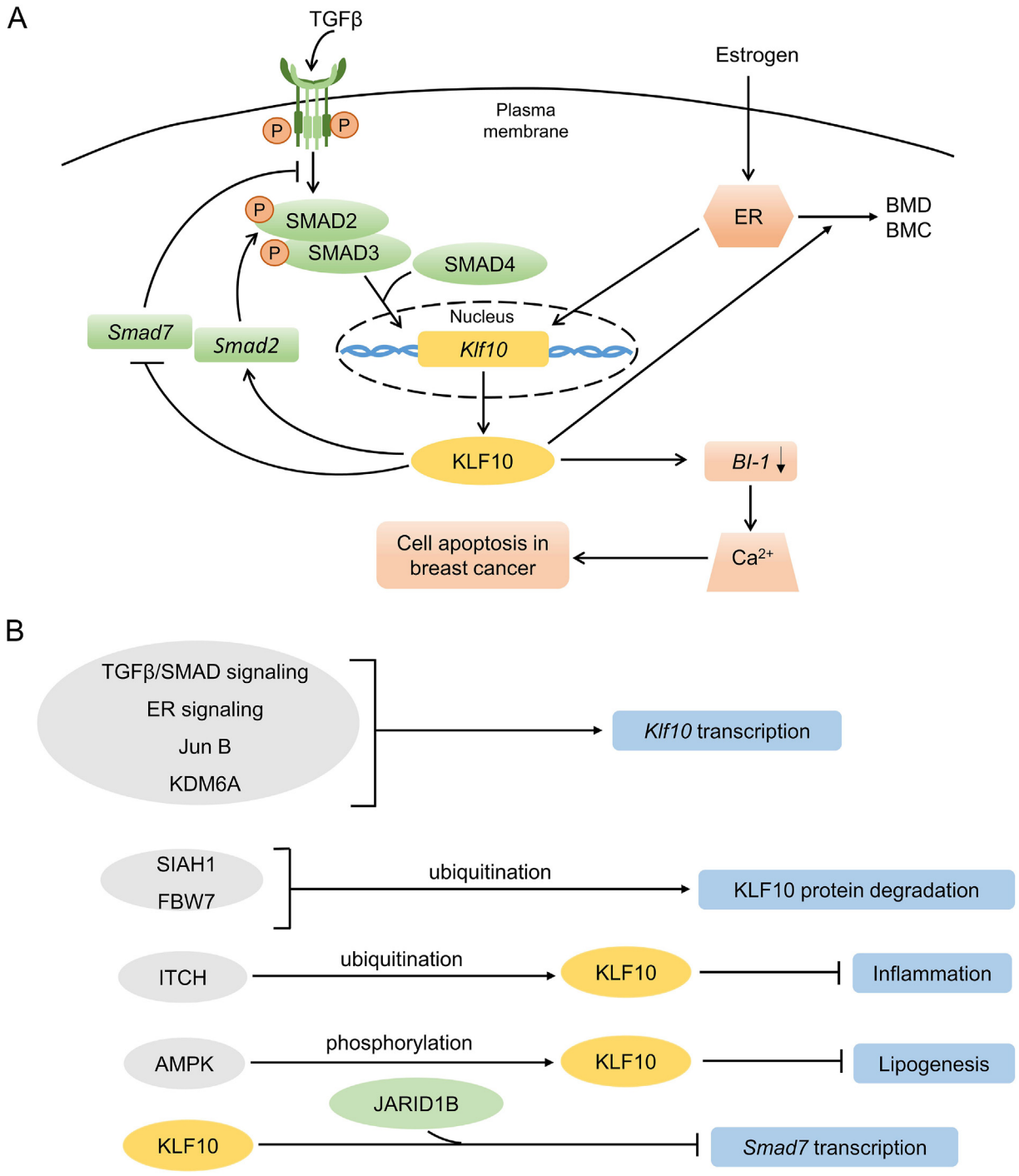

Figure 2 The involvement of KLF10 in TGFβ/SMAD signaling pathway and estrogen signaling pathway, and the regulation of KLF10 expression and function at multiple levels **(A)** TGFβ binds to its membrane receptor, triggering the phosphorylation of SMAD2/SMAD3. The activated SMAD2 and SMAD3 form oligomeric complexes with SMAD4, which will then translocate into the nucleus to increase the expression of KLF10. KLF10 can further enhance the transcription of *Smad 2* and inhibit the transcription of *Smad 7*, which further activates the TGFβ/SMAD signaling pathway. Estrogen binds to the intracellular estrogen receptor (ER) and then stimulates the expression of KLF10, which inhibits the transcription of *BI-1* gene. The reduced expression of BI-1 induced the release of Ca^{2+} and consequently increased cell apoptosis in breast cancer. In addition, KLF10 can facilitate estrogen signaling-mediated increase of bone mineral density (BMD) and bone mineral content (BMC) **(B)** The expression and function of KLF10 can be regulated at multiple levels. TGFβ/SMAD signaling pathway and estrogen signaling can promote the transcription of *Klf10*. JunB and KDM6A can bind to the *Klf10* gene promoter to facilitate its transcription. SIAH1 and FBW7, two E3 ubiquitin ligases, can bind to KLF10 protein and promote its degradation through the ubiquitin proteasome pathway. ITCH can induce the ubiquitination of KLF10, which would enhance the function of KLF10 to transactivate *Foxp3* gene expression in regulatory T cells (Treg cells), thereby protecting against airway inflammation. AMPK-mediated phosphorylation of KLF10 can facilitate the effect of KLF10 on inhibiting lipogenic genes transcription. KLF10 and JARID1B can inhibit the transcription of *Smad7* in a synergistic manner.

protein (SREBP-1C).[28] Therefore, the downregulation of *Srebf1* inhibited the expression of SREBP-1C and its target genes involved in lipogenesis (Fig. 2B, 3).[28] This study demonstrates that AMPK-mediated phosphorylation of KLF10 can facilitate the inhibitory effect of KLF10 on the transcription of *Srebf1*, thereby suppressing lipogenesis in hepatocytes.

As a transcriptional suppressor, KLF10 can repress the transcription of *Smad 7* along with its corepressor histone demethylase Jumonji AT-rich interactive domain 1 B (JARID1B).[29] The amino acids 801—1,150 and amino acids 1,151—1,544 of JARID1B can bind with the amino acids 1—360 (Fig. 1A) of KLF10 which contains three repression domains *r*1, *r*2 and *r*3. Thus, JARID1B can be recruited by KLF10 and decrease the level of trimethylated lysine 4 on histone H3 (H3K4me3) of *SMAD7* gene promoter to repress its transcription.[29] KLF10 and JARID1B can inhibit the transcription of *Smad7* in a synergistic manner (Fig. 2B). In melanomas, the expression levels of KLF10 and JARID1B were both decreased compared to normal skin cells, suggesting that KLF10 may cooperate with JARID1B to repress skin tumor by inhibiting *Smad7* expression.[29] Further studies are needed to investigate whether KLF10 may also suppress other tumors along with JARID1B.

In general, KLF10 expression and function is regulated in multiple ways. TGFβ/SMAD signaling and estrogen signaling can increase the expression of *Klf10* gene. The transcription of *Klf10* gene is also activated by JunB and KDM6A. KLF10 protein stability is impaired by SIAH1 and FBW7-mediated ubiquitination. The transcriptional role of KLF10 in regulating the expression of its target genes can be regulated by its ubiquitination by ITCH, its interaction with JARID1B, and its phosphorylation by AMPK. Targeting the above regulatory mechanisms may help to regulate KLF10-mediated biological functions better.

Roles of KLF10 in biological processes

KLF10 is clock-controlled and regulates glucose and lipid metabolism in the liver

Liver is an important organ regulating metabolic homeostasis and its function in metabolism exhibits circadian rhythm. KFL10 has been shown to display a robust rhythmic expression pattern in mouse liver, which disappears when the core clock gene *Bmal1* is knocked out.[30] Furthermore, it was found that among genes showing altered expression in the liver of *Klf10* knockout (KO) mice, more than half of them were clock-controlled and were also enriched in pathways regulating glucose and lipid metabolism, highlighting the close association of KLF10 with hepatic metabolism and circadian rhythm.[30] Leclèreet et al studied the rhythmic expression changes of core clock genes and *Klf10* by comparing the livers of mice fed with methionine and choline deficient (MCD) diet and the livers of mice fed with chow diet. After MCD diet feeding, expression of core clock genes exhibited changes in phase and amplitude, and *Klf10*

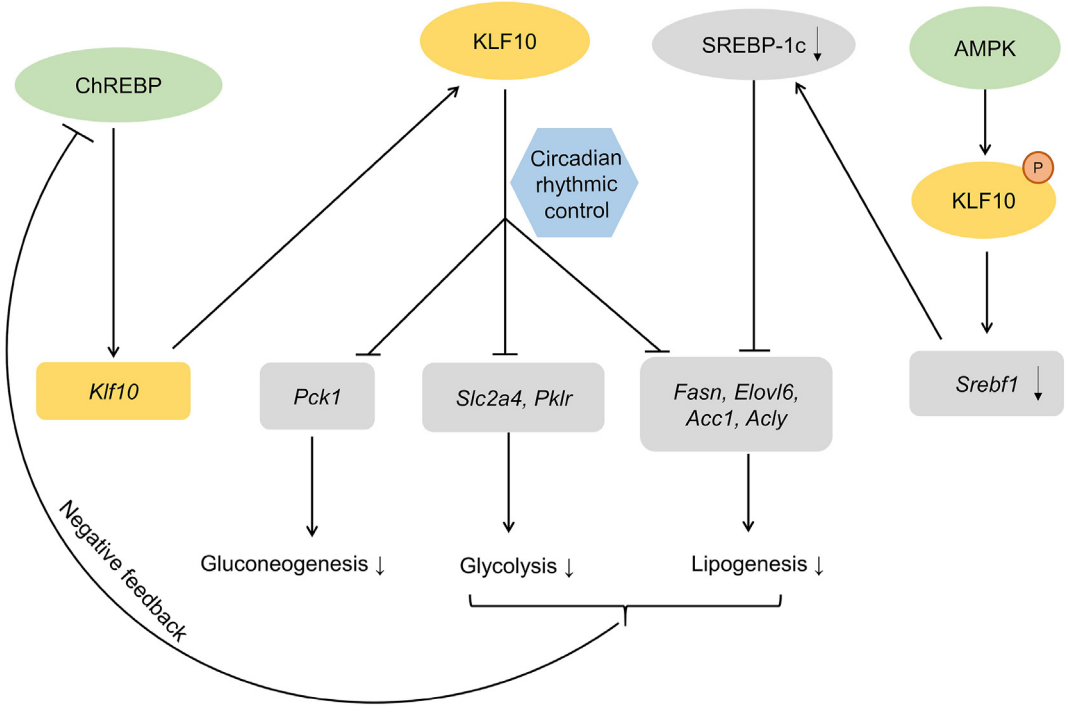

Figure 3 The role of KLF10 in hepatic glucose and lipid metabolism. In liver, KLF10 suppresses the expressions of gluconeogenesis gene (*Pck1*), glycolysis genes (*Slc2a4* and *Pklr*) and lipogenesis genes (*Fasn*, *Elovl6*, *Acc 1*, and *Acly*), in a circadian rhythmic manner. As a downstream target gene of ChREBP, *Klf10* can be induced by ChREBP. And in turn, KLF10 inhibits the expression of ChREBP target genes, which would form a negative feedback loop. AMPK mediated phosphorylation of KLF10 can facilitate the role of KLF10 in inhibiting the transcriptional expression of *Srebf1*, thereby suppressing the expression of SREBP-1C and its target genes, including *Fasn*, *Acly* and *Acc1* in the liver.

also lost its normal rhythmic expression.[31] Ruberto et al found that hepatic *Klf10* deficiency in mice (hepKO) led to extensive reprogramming of liver circadian transcriptome.[32] Most transcripts that displayed a dampened rhythmicity in hepKO mice livers were involved in biological processes of oxidative phosphorylation, fatty acid metabolism and mTORC1 signaling.[32] While transcripts that displayed enhanced rhythmicity in hepKO mice were involved in glycolysis, inflammatory response and apoptosis.[32] These results demonstrate a high correlation between hepatic metabolism and circadian rhythm and that *Klf10*, as a circadian-controlled gene, plays an important role in maintaining liver circadian rhythms at the transcriptional level.

Studies have shown that KLF10 is involved in hepatic glucose and lipid metabolism. The metabolic phenotypes of *Klf10* deficiency in mice showed sex-dependent difference. When *Klf10* gene was deficient, male mice showed postprandial and fasting hyperglycemia while female mice showed increased plasma triglycerides.[33] Furthermore, it was found that high glucose and fructose can stimulate *Klf10* expression in hepatocytes.[32] *Klf10* deficiency led to increased expression of glycolysis genes *Slc2a4, Pklr* (Fig. 3), gluconeogenic gene *Pck1* (Fig. 3), and lipogenesis genes *Fasn* and *Elovl6* (Fig. 3) in the livers from high sugar-fed mice and high glucose and fructose (HGF)-treated primary hepatocytes (PH) compared to the control groups. These results suggest that KLF10 can alleviate liver damage under high sugar stress. Using ChIP-seq analyses, researchers found that genes that exhibited differential expression between hepKO and wild type (WT) PH under HGF treatment were involved in extensive metabolic pathways, including lipid and glucose metabolism, protein phosphorylation as well as oxidation-reduction.[32] These results reveal the important role of KLF10 in hepatic glucose and lipid metabolism, and indicate that KLF10 can protect mice against liver injury under high glucose and fructose diet feeding condition. It is also demonstrated that KLF10 can regulate an extensive metabolic network in the liver which needs further studies.

In another study, mice injected with adenovirus overexpressing *Klf10* through the tail veins exhibited higher blood glucose level, impaired glucose tolerance and increased expression of gluconeogenic genes, including *Pgc-1α*, *Pck1* and *G6pc* compared with control mice.[33] Conversely, in db/db and diet-induced obese (DIO) mice, adenovirus-mediated knockdown of *Klf10* in the livers led to lower blood glucose level and improved insulin sensitivity and decreased expression of *Pgc-1α*, *Pck1* and *G6pc*.[33] The results above indicated that KLF10 could enhance gluconeogenesis in the liver, which were opposite to the results mentioned earlier that KLF10 deficiency induced increased expression of *Pck1*.[32] The difference in the genetic background of the mice or experimental procedures between the two studies might lead to these contradictory phenotypes, which awaits further investigation.

Carbohydrate response element binding protein (ChREBP) plays an important role in facilitating carbohydrate and lipid metabolism in hepatocytes.[34] Iizuka et al found that *KLF10* was a target gene of ChREBP.[35] And overexpression of *Klf10* inhibited glucose-stimulated expression of ChREBP target genes, such as *Pklr*, *Fasn*, and *Acc 1* (Fig. 3). Thus, ChREBP induced the expression of *Klf10*, and in turn, KLF10 inhibited glucose-induced expression of ChREBP target genes, which would form a negative feed-back loop.[35] Therefore, the ChREBP/KLF10 axis may play an important role in regulating the homeostasis of carbohydrate and lipid metabolism in hepatocytes, which merits further exploration.

As mentioned earlier, after being phosphorylated by AMPK, KLF10 can inhibit the transcriptional expression of *Srebf1*, thereby inhibiting the expression of SREBP-1C target genes, including *Facn*, *Acly* and *Accl* in the liver (Fig. 3).[28] By comparing the transcriptome of the KLF10 KO mice livers and that of control WT mice livers, it was shown that genes showing altered expression were most enriched in lipid metabolism pathways.[28] Moreover, KLF10 deficiency led to upregulation of the genes involved in lipogenesis.[28] These results further indicate a functional role of KLF10 in hepatic lipid metabolism.

Collectively, *Klf10*, a clock-controlled gene, is critical for the maintenance of hepatic circadian rhythm, an event that is highly associated with metabolism, and it also plays an important role in regulating glucose and lipid homeostasis in the liver.

KLF10 plays important roles in adipogenesis and adipocytes metabolism

Adipocytes are important type of cells for the energy balance of organism. Adipogenesis is the process during which mesenchymal stem cells (MSCs) commit to preadipocytes, then preadipocytes differentiate into mature adipocytes.[36] Adipogenesis is critical for the generation of adipocytes and maintaining the metabolic homeostasis in adipose tissue. Besides, white adipose tissue (WAT), brown adipose tissue (BAT) and beige adipose tissue are three types of adipose tissue in mammals. The transformation of WAT to BAT-like tissue or beige adipose tissue, namely the browning of WAT, can enhance energy consumption and thermogenesis.[37–39]

3T3-L1 cell line is a well-established model for the study of adipogenesis. CCAAT/enhancer-binding protein (C/EBPβ), an important transcription factor for early 3T3-L1 preadipocyte differentiation, can facilitate mitotic clonal expansion (MCE) (Fig. 4A).[40,41] During 3T3-L1 adipocyte differentiation, there are cell division and differentiation coupling phenomena. Pre-adipocytes will first grow to a contact inhibitory state, and then reenter the cell cycle upon the treatment of adipogenic hormones. After about two rounds of cell division (namely MCE), cells will enter the terminal differentiation phase, forming mature adipocytes.[40] MCE is a critical step for 3T3-L1 adipocyte differentiation and contributes to adipocyte hyperplasia.[42] After 36–48 h of adipogenic induction, MCE was completed, and the expression of C/EBPα and PPARγ were increased.[40,43,44] C/EBPα and PPARγ are anti-proliferation genes and are transactivated by C/EBPβ, functioning as the two key adipogenic factors. But they showed delayed expression during 3T3-L1 adipocyte differentiation, which may guarantee the MCE (Fig. 4A).[40,43] *Klf10* gene could be transactivated by C/EBPβ (Fig. 4A) and then KLF10 would inhibit the transcription of *C/EBPα* and *PPARγ* by interacting with HDAC1. KLF10 could inhibit the transcription of *C/EBPα* through the

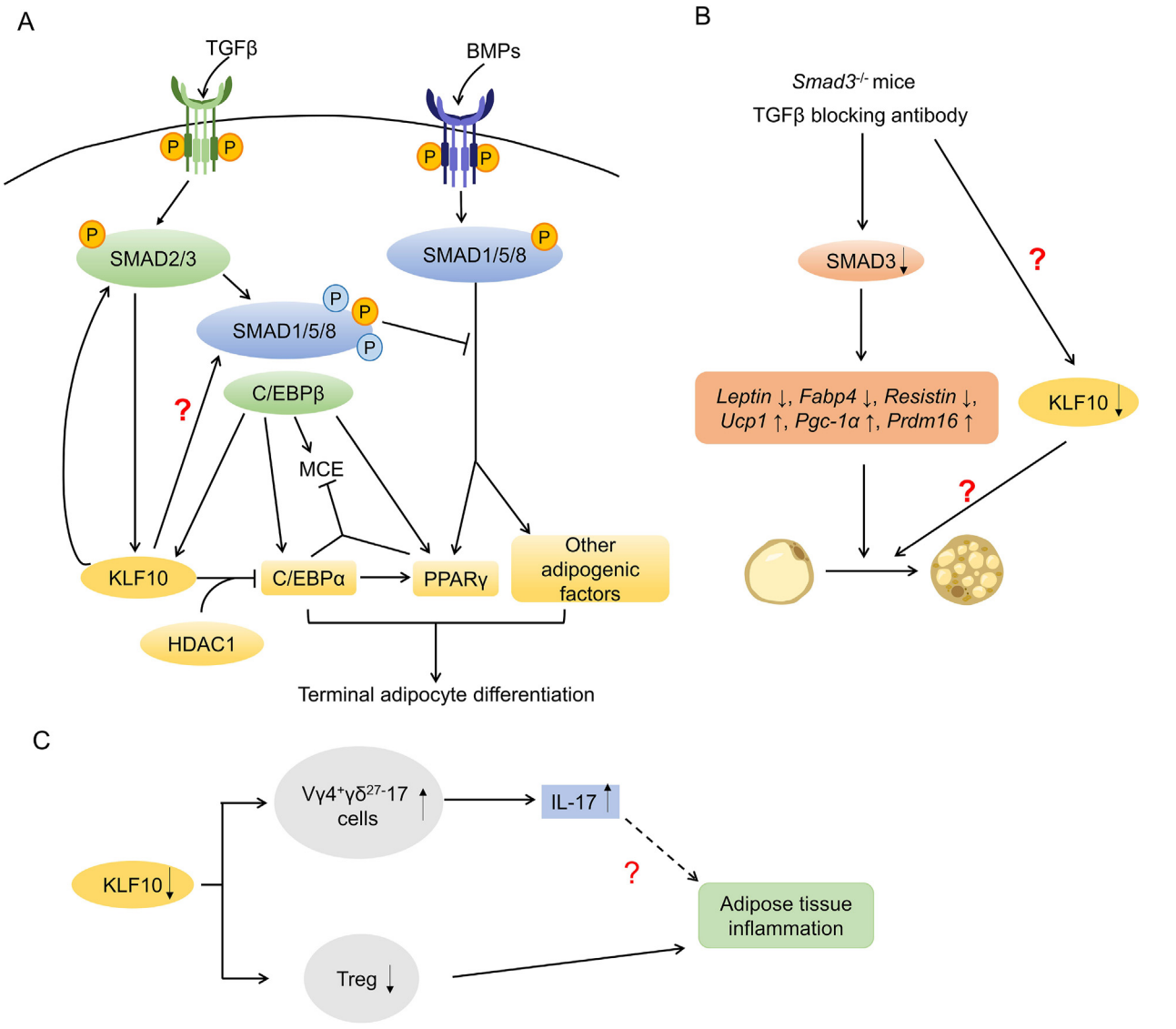

Figure 4 The role of KLF10 in adipogenesis, adipocytes metabolism and T cell immunity **(A)** SMAD 1/5/8 can be phosphorylated by BMPs, which will then increase the expression of *PPARγ* and other adipogenic factors. TGFβ can activate SMAD2/3, which will then induce the hyper-phosphorylation of SMAD 1/5/8 to inhibit the expression of PPARγ and other adipogenic factors. As a downstream factor of TGFβ/SMAD signaling pathway, KLF10 may be also involved in regulating adipogenesis through this pathway, which is worthy of further investigation. During the early stage of 3T3-L1 adipocyte differentiation (0—48 h post-adipogenic induction), C/EBPβ can facilitate mitotic clonal expansion (MCE). After 48 h of MCE, C/EBPα and PPARγ which inhibit MCE and enhance terminal adipocyte differentiation, are transactivated by C/EBPβ. During the early phase of 3T3-L1 adipocyte differentiation, KLF10 can be transactivated by C/EBPβ and then it would inhibit the transcription of *C/EBPα* and *PPARγ* by interacting with HDAC1 to facilitate MCE, thereby ensuring adipogenesis at an appropriate level **(B)** Blockade of TGFβ/SMAD3 signaling contributes to the decreased expression of WAT-fat genes *Leptin, Fabp4, Resistin* and increased expression of BAT-fat genes *Ucp1, Pgc-1α, Prdm16*, which promotes the browning of WAT. Because KLF10 is a downstream factor of TGFβ/SMAD signaling pathway, the potential role of KLF10 in regulating the browning of WAT merits further investigation **(C)** KLF10 deficiency in $CD4^+$ T Cells can cause decreased Treg differentiation and mobilization to the adipose tissue pool and liver, which leads to more severe inflammation in adipose tissue. And KLF10 deficiency also causes higher IL-17 production in $V\gamma4^+\gamma\delta^{27-}$-17 cells, whose role in regulating adipose tissue inflammation merits further study.

KLF10-mediated recruitment HDAC1 to *C/EBPα* promoter (Fig. 4A).[40] Thus, during 3T3-L1 adipocyte differentiation, KLF10 plays an important role in facilitating MCE and ensuring adipogenesis at an appropriate level. In future studies, preadipocyte specific KO or overexpression of KLF10 in mice can be done to examine the role of KLF10 in adipogenesis and adipose tissue metabolic homeostasis *in vivo*.

On the cell membrane, TGFβ superfamily receptors can bind with their ligands, such as TGFβ, Activin, BMPs, and so on, which will activate the intracellular SMAD family proteins.[45] SMAD1/5/8 are downstream factors of BMPs. It is

found that in 3T3-L1 preadipocytes, BMPs such as BMP2, BMP4, BMP6 and BMP7, lead to the phosphorylation of SMAD1/5/8, which will then translocate into the nucleus, inducing the expression of adipocyte marker genes to promote adipogenesis (Fig. 4A).[45] While TGFβ can activate SMAD2/3, and then induce the hyper-phosphorylation of SMAD 1/5/8 to inhibit its nucleus translocation (Fig. 4A).[45] Thus SMAD1/5/8 signaling drives adipogenesis, but the TGFβ-mediated SMAD2/3 signaling blunts the role of SMAD1/5/8, thereby inhibiting adipogenesis.[45] Because KLF10 is a downstream effector of TGFβ signaling pathway, it may also be involved in regulating adipogenesis through this pathway, which is worthy of further studies.

One study showed that systematic blockade of TGFβ/SMAD signaling rendered white adipose tissue (WAT) increased mitochondrial biogenesis and brown adipose tissue (BAT)-like gene expression profile, thus protecting mice from obesity and diabetes (Fig. 4B).[46] And the level of TGFβ was positively associated with obesity as the circulating TGFβ level was higher in individuals with higher BMI.[46] Therefore, as an important downstream effector of TGFβ/SMAD signaling pathway, the potential role of KLF10 in regulating the browning of WAT merits further investigation.

Taken together, the results above suggest an important role of KLF10 in regulating preadipocytes differentiation and maintaining the metabolic homeostasis of adipose tissue. Further studies are needed to confirm the functional role and mechanism of KLF10 in adipogenesis and adipose tissue metabolism *in vivo* by using mice with preadipocyte or mature adipocyte specific KO or overexpression of *Klf10*.

KLF10 regulates T cell differentiation to inhibit inflammation

Obesity related metabolic disorders are usually triggered and aggravated by chronic inflammation.[47,48] Regulatory T cells (Treg) is a subtype of $CD4^+$ T cell, differentiated from resting $CD4^+$ T cell, and can ameliorate inflammation.[49] It was found that due to defects in $CD4^+$ Treg differentiation and mobilization to the adipose tissue pool and liver, mice lacking *Klf10* in $CD4^+$ T cells developed obesity, insulin resistance, and fatty liver (Fig. 4C), which could be completely alleviated by adoptive transfer of WT $CD4^+$ Treg to the mice.[49] Tregs lacking *Klf10* showed obvious intrinsic defects in mitochondrial respiration, oxidative phosphorylation, glycolysis, and PI3K-Akt-mTOR signaling, which might impair the mobilization and anti-inflammation ability of Tregs.[49]

KLF10 can constrain the development of $V\gamma4^+$ IL-17-committed $CD27^-$ γδ T cells ($V\gamma4^+$ $\gamma\delta^{27-}$-17 cells) to regulate thymic and peripheral immune homeostasis.[50] The γδ T cells are major sources for IL17 production. $V\gamma4^+\gamma\delta^{27-}$-17 cell is a subtype of $\gamma\delta^{27-}$ T cells. IL17 plays an important role in initiating the inflammatory response, coordinating the functions of neutrophils, and recruiting monocytes. KLF10 is shown to be important for controlling the immune responses initiated by the above cells.[50] In *Klf10*-deficient mice, $V\gamma4^+\gamma\delta^{27-}$-17 cells were selectively increased with higher IL-17 production (Fig. 4C).[50] In this work, the effects of *Klf10* deletion on T cells function was studied. Further studies could be done to investigate whether overexpression of *Klf10* would increase the population of Tregs and decrease the amount of $V\gamma4^+\gamma\delta^{27-}$-17 cells to inhibit inflammation and attenuate obesity related metabolic disorders.[50]

In conclusion, the studies above demonstrate the important role of KLF10 in ameliorating inflammation by regulating the function of T cells. KLF10 also has other roles in immune system which has been reviewed by previous articles.[2,3,51]

KLF10 is required for the functional and structural homeostasis of skeletal muscle

KLF10 is highly expressed in skeletal muscle and also plays essential roles in regulating the function of skeletal muscle.[4] It was reported that the skeletal muscles in KLF10 deficient mice exhibited hypertrophy, hyperplasia, and changes in muscle area distribution in female mice (Fig. 5).[52] Muscle fibers are generally classified into oxidative fibers (I, and IIA) and glycolytic fibers (IIX, and IIB). Soleus (Sol) is the oxidative muscle and extensor digitorum longus (EDL) is the glycolytic muscle. The *Klf10* KO Sol showed stronger static and dynamic stress compared to WT Sol, while weaker static and dynamic stress was shown in *KLF10* KO EDL (Fig. 5).[53] It was found that the viscosity of *KLF10* KO Sol was greater than WT Sol, while the viscosity of *KLF10* KO EDL was less than WT EDL (Fig. 5).[53] And it was shown that in both Sol and EDL, the percentage of glycolytic fibers (IIX, and IIB) were increased and oxidative fibers (I, and IIA) were decreased when *Klf10* was knocked out (Fig. 5).[53] This study demonstrates that in Sol, lack of *Klf10* leads to increased static and dynamic stress and enhances elastic properties, but *Klf10* deficiency weakens the above parameters in EDL.[53] The mechanism for the different roles of KLF10 in different types of muscle fibers needs further investigation.

KLF10 may regulate the contractile properties of the skeletal muscle by altering the binding between the crossbridge and actin.[54] In *Klf10* KO female mice muscle, the maximum stress, stiffness and maximum force of Sol were lower, while those of EDL were higher compared with WT mice muscle (Fig. 5).[54] Transmission electron microscope showed that compared with WT mice muscle, the average number of myosin filaments in *Klf10* KO mice Sol and EDL was decreased.[54] The decrease in the maximum stress for *Klf10* KO Sol may due to a smaller proportion of attached cross bridges or a weaker adhesion between the myosin head and the actin molecule. And further studies are needed to determine whether differences in extracellular matrix composition and excitation contraction coupling exist in these two types of *Klf10* KO muscles and whether such differences contribute to the weakened contractile properties in *Klf10* KO Sol and enhanced contractile properties in *Klf10* KO EDL.

In one study, the effect of KLF10 on muscle mitochondrial oxidative capacity and muscle metabolism was explored in female mice.[55] The number of mitochondria was significantly decreased in muscle and many genes encoding mitochondrial components were down-regulated in the muscle of *Klf10* KO mice. The mitochondria showed ultrastructural disorganization in the Sol and EDL of *Klf10*

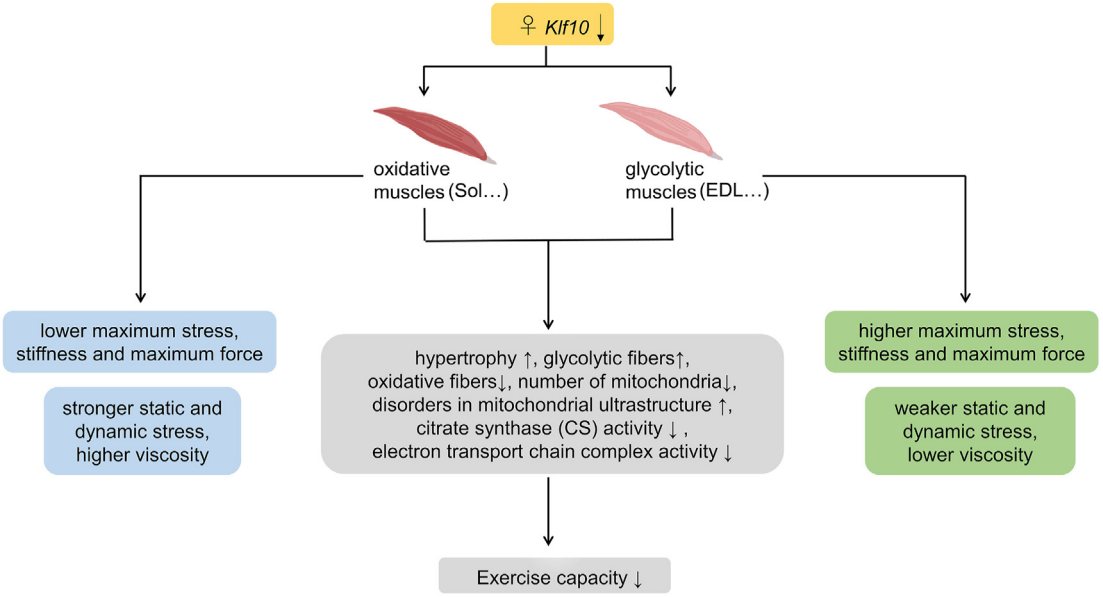

Figure 5 The role of KLF10 in skeletal muscle. *Klf10* deficiency leads to hypertrophy, increased glycolytic fiber property, decreased oxidative fiber property, fewer number of mitochondria, disorganized mitochondrial ultrastructure, less citrate synthase (CS) activity and electron transport chain complex activity in both oxidative muscles and glycolytic muscles, which impairs the exercise capacity of the mice. *Klf10* deficiency also affect contractile properties in oxidative muscles and glycolytic muscles. *Klf10* deficient oxidative muscle shows lower maximum stress, stiffness and maximum force, stronger static and dynamic stress, and higher viscosity, while *Klf10* deficient glycolytic muscle shows higher maximum stress, stiffness and maximum force, weaker static and dynamic stress, and lower viscosity. The above effects of *Klf10* deficiency on skeletal muscle function are observed only in female mice. Sol, Soleus; EDL, extensor digitorum longus.

deficient mice (Fig. 5).[55] Citrate synthase (CS) activity and electron transport chain complex activity were significantly decreased in *Klf10* KO muscles (Fig. 5).[55] And the levels of phosphocreatine and ATP were also significantly lower in the muscle of *Klf10* KO mice compared with WT mice.[55] In addition, *Klf10* KO mice showed markedly decreased exercise capacity.[55] Therefore, deficiency of *Klf10* in mice can cause altered muscle gene expression, disorders in mitochondrial ultrastructure, decreased mitochondrial activity, disrupted mitochondrial metabolism in muscle and impaired exercise ability.

Notably, muscle hypertrophy, alterations in muscle metabolism, disturbance of mitochondrial ultrastructure, and altered skeletal muscle function induced by *Klf10* deficiency was observed only in female mice. This sex difference may be attributable to the interaction between KLF10 and estrogen signaling, which needs further investigation.

KLF10 also displays other biological functions

KLF10 is recognized as a positive regulator of bone growth. KLF10 can enhance the sub-cellular localization of β-catenin and Wnt signaling.[6] The increased expression of β-catenin and its localization in nuclei both lead to a significant increase in bone content. As a result, KLF10 can promote the increase of bone mass.[6]

The inhibitory effect of KLF10 on cell proliferation has been demonstrated by several studies.[2,51] In addition, although liver had a marked capacity of regeneration in partially hepatectomized mouse model, deletion of *Klf10* suppressed the proliferation of hepatocytes as the liver/body weight ratio was significantly lower and the proliferation-associated genes were downregulated in the livers of *Klf10* KO mice compared with WT mice, suggesting critical role of KLF10 in liver regeneration.[56] KLF10 is also involved in many other biological processes, which have been reviewed previously.[3]

Roles of KLF10 in disease states

KLF10 plays critical roles in regulating the initiation and progression of nonalcoholic steatohepatitis (NASH)

Non-alcoholic fatty liver disease (NAFLD) covers a spectrum of lesions ranging from non-alcoholic fatty liver (NAFL) to NASH.[57–60] NASH is the progressive form of NAFLD and is characterized by steatosis, ballooning hepatocytes and fibrosis.[61–63] Among these characteristics, fibrosis is a major feature. Regardless of the presence or severity of other histological features, the level of fibrosis influences mortality independently.[64] TGFβ signaling is shown to be involved in liver fibrosis. When TGFβ signaling is activated, SMAD4 binds to SMAD3. And SMAD3 can translocate into the nucleus and upregulate a number of fibrogenic genes in hepatic stellate cells (HSCs) (Fig. 6).[13] In contrast, SMAD2 can translocate into the nucleus to protect the hepatocytes from fibrosis as it was reported that disruption of SMAD2 upregulated type I collagen expression (Fig. 6).[13,65] Activated liver HSCs causes excessive liver mesenchymal

extracellular matrix (ECM) deposition, thereby exacerbating liver fibrosis (Fig. 6). TGFβ can inhibit the apoptosis of HSCs, suppress the production of matrix-degrading proteases, and increase the expression of protease inhibitors.[13] As a downstream effector of TGFβ/SMAD pathway, KLF10 may also play a certain role in the activation of HSCs and NASH.

It was found that the hepatic expression of KLF10 was significantly increased in mice with high fat, sucrose diet (HFSD)-induced NASH, which was accompanied with upregulated TGFβ gene expression and suppressed expression of *Mlxipl* (the gene encoding ChREBP).[66] Moreover, the expression of *Col1a1*, *Klf10*, *TGFβ*, *SMAD2*, *SMAD3* and *SMAD7* were all elevated in the livers of NASH mice.[66] These results suggest that increased KLF10 expression coincides with progression of liver fibrosis and is associated with the activation of HSCs.[66] This study is the first one that takes KLF10 into consideration in NASH progression and HSCs activation.[66] Although it does not elucidate whether increased KLF10 acts as a cause or the result of NASH, this study has provided new insights into the potential role of KLF10 in NASH.

In *Klf10* deficient mice fed with NASH-inducing diet, the level of serum ALT was significantly higher compared with WT mice.[31] And *Klf10* deficient mice displayed significantly higher hepatic expression of fibrosis markers including *TGFβ*, *Timp1* and *Col1α1*, indicating that *Klf10* deficiency could worsen liver injury and exacerbate liver fibrosis in mice upon NASH-inducing diet feeding.[31] In another study, it was observed that *Klf10* KO mice showed more severe hepatic steatosis, inflammation, and liver injury after the mice were fed with high sucrose diet (HSD).[67] Triglycerides (TG) and cholesterol levels in liver, plasma ALT and AST levels were all significantly increased in *Klf10* KO mice, whereas WT mice showed mild hepatic steatosis without obvious liver injury.[67] HSD-fed *Klf10* KO mice showed significant increase in endoplasmic reticulum stress, oxidative stress, and expression levels of pro-inflammatory cytokines in the livers.[67] Furthermore, decreased very low density lipoprotein (VLDL) packaging and secretion might promote lipid accumulation in the livers of *Klf10* KO mice. Most importantly, deficiency of *Klf10* significantly increased fibrogenesis and the deposition of collagen in mice livers.[67] These two studies above both indicate that deficiency of *Klf10* can lead to liver injury and fibrosis in mice liver under NASH-inducing diet feeding.

As described earlier, KLF10 can be phosphorylated by AMPK on Thr189.[28] This will facilitate the binding of KLF10 to the promoter of *Srebf1* to inhibit its transcription, thereby suppressing lipogenic genes expression (Fig. 6).[28]

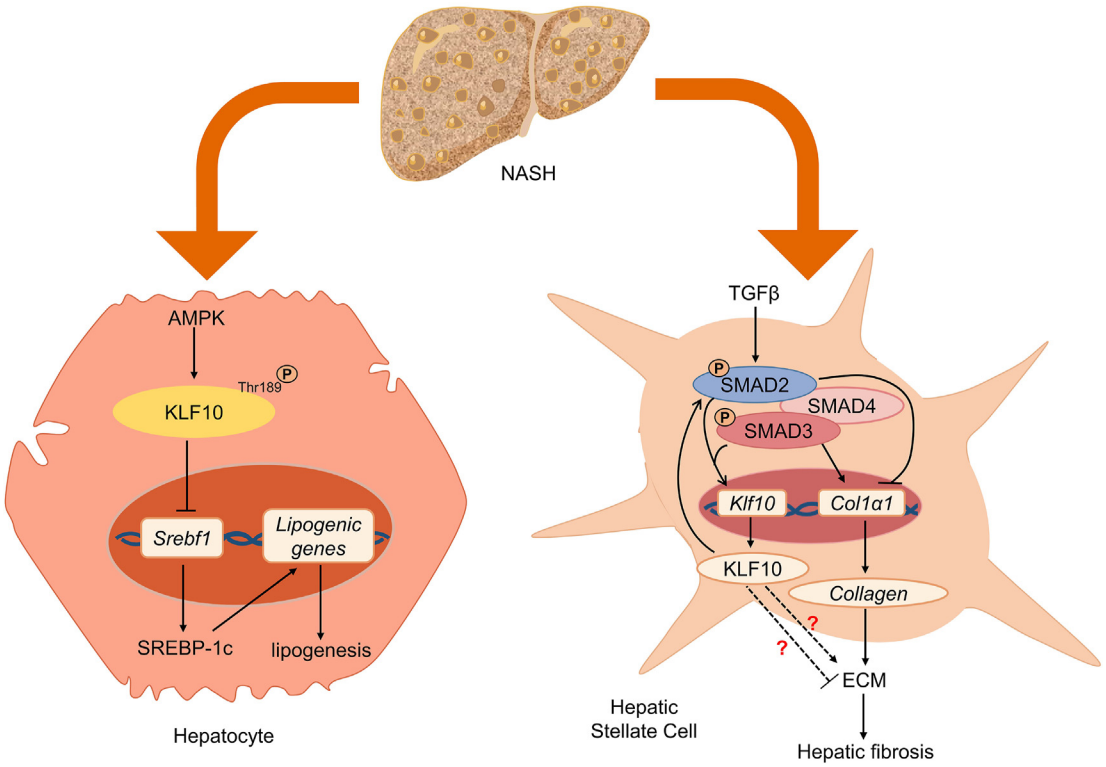

Figure 6 The effect of KLF10 on hepatocytes and hepatic stellate cells (HSCs) during the initiation and progression of nonalcoholic steatohepatitis (NASH). In hepatocytes, KLF10 can be phosphorylated by AMPK on Thr189 and then it will inhibit the transcription of *Srebf1* by binding to its promoter, thereby suppressing lipogenic genes expression. TGFβ is produced and released by the injured hepatocytes and it will bind to the TGFβ receptor on the plasma membrane of HSCs to activate SMAD signaling. SMAD4 binds to phosphorylated SMAD3, which will translocate into the nucleus and upregulate the expression of *Col1α1* gene, thereby leading to excessive liver mesenchymal extracellular matrix (ECM) deposition and exacerbating liver fibrosis. However, activated SMAD2 will translocate into the nucleus to suppress the expression of *Col1α1* gene. Because KLF10 is a downstream effector of TGFβ/SMAD signaling, the effect of KLF10 on SMAD2 or SMAD3-mediated signaling and fibrosis genes expression in HSCs merits further study.

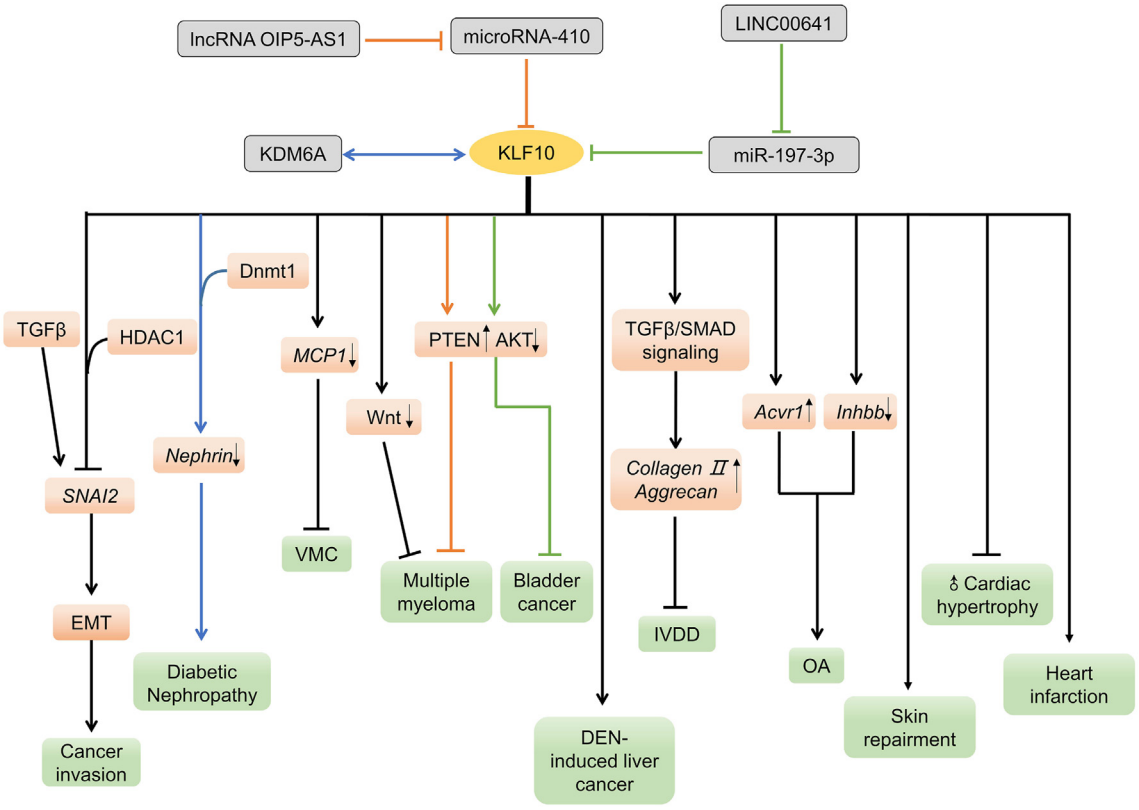

Figure 7 The roles of KLF10 in tumors and many other diseases. KLF10 can increase the expression of PTEN to blunt AKT activity, thereby inhibiting multiple myeloma (MM) and bladder cancer. In MM, microRNA-410 inhibits the expression of KLF10, which can be blocked by lncRNA OIP5-AS1 (see the pathway indicated by orange lines). KLF10 can also inhibit the activity of Wnt signaling to suppress MM. In bladder cancer, the inhibitory effect of miR-197—3p on KLF10 expression can be suppressed by LINC00641 (see the pathway indicated by green lines). KLF10 may promote DEN-induced liver cancer. KDM6A can transactivate the expression of *Klf10*, and in turn KLF10 can bind on *KDM6A* gene promoter to increase its expression, forming a positive feedback loop. Furthermore, KLF10 can recruit Dnmt1 to inhibit the expression of *Nephrin* and thus exacerbates diabetic nephropathy (see the pathway indicated by blue lines). Through recruiting HDAC1 to suppress the expression of *SNAI2*, KLF10 can inhibit TGFβ-induced epithelial-to-mesenchymal transition (EMT) to suppress cancer invasion. By decreasing the expression of *MCP1*, KLF10 can attenuate acute viral myocarditis (VMC). KLF10 can activate TGFβ/SMAD signaling pathway, and then stimulate the expression of *collagen II*, *Aggrecan*, thereby alleviating intervertebral disc degeneration (IVDD). In osteoarthritis (OA), KLF10 can increase the expression of *Axvr1* and decrease the expression of *Inhbb* and thus exacerbates OA. KLF10 can protect against cardiac hypertrophy in male mice. However, KLF10 may be not good for ameliorating heart infarction. KLF10 can be beneficial in promoting skin repairment.

In *Klf10* deficient hepatocytes, the expression levels of NAFLD markers *Pnpla3*, which can lead to increased lipid accumulation, was increased, and *Mat1a*, whose deficiency can lead to NASH and HCC, was decreased.[28] These results indicate that KLF10 plays important roles in alleviating liver steatosis and NASH by downregulating the expression of SREBP-1c and its target genes involved in lipogenesis.[28]

These studies above all suggest that KLF10 may protect liver from steatosis and fibrosis. However, TGFβ is known as a factor capable of activating HSCs to induce fibrosis.[13] It is possible that the function of KLF10 independent of regulating TGFβ signaling may play important roles in controlling NASH progression, which merits further study. In particular, mice models with liver cell type (hepatocyte, HSCs and so on)-specific KO or overexpression of *KLF10* are needed to further dissect the liver cell type-specific role of KLF10 in the initiation and progression of NASH.

KLF10 regulates tumorigenesis acting mainly as a tumor suppressor

Cancer is a major cause of death around the world.[68] In 2018, the roles of KLF10 in tumors has been reviewed clearly by Azra Memon and Woon Kyu Lee.[2] KLF10 is recognized mainly as a tumor suppressor in many types of cancers. After the above review was published, growing number of new studies on the role of KLF10 in different tumors have also been reported.

MicroRNA-410 can facilitate the development of multiple myeloma (MM). MicroRNA-410 promotes proliferation and inhibits apoptosis of MM tumor cells.[69] Acting as the downstream target of microRNA-410, KLF10 expression can be inhibited by microRNA-410 (Fig. 7), which is associated with decreased PTEN expression and increased phosphorylation of AKT.[69] Both overexpression of KLF10 and inhibition of AKT can abolish the effect of microRNA-410 on promoting

MM.[69] In addition, the long non-coding RNA (lncRNA) OIP5-AS1 is a molecular sponge whose overexpression can inhibit miR-410 expression, increase KLF10 expression and inhibit the stimulation of phosphorylated AKT, thereby suppressing the development of MM (Fig. 7).[69] Mimi Zhou et al reported that *Kfl10* overexpressing in MM cells can inhibit Wnt signaling pathway by reducing β-catenin nuclear accumulation and GSK3β phosphorylation, which can inhibit proliferation, migration and drug resistance of MM cells (Fig. 7).[70] It was also shown that regulator of sister chromatid separation, securin (*PTTG1*), the downstream target of KLF10, was downregulated by KLF10 in MM.[70] And knockdown of PTTG1 in MM cells could mimic the role of KLF10 in suppressing MM cells.[70] Collectively, these two studies above both showed the inhibitory effect of KLF10 on MM and may provide new therapeutic strategies for treating MM.

In 2018, Zhijia Li et al reported that in bladder cancer, miR-197−3p could bind to the 3′-UTR of *Klf10* mRNA and inhibit its translation, which would then activate PTEN/PI3K/AKT pathway to facilitate tumor cell proliferation (Fig. 7).[71] LINC00641, a novel LncRNA, could bind with miR-197−3p and abolish its translational inhibition of KLF10, thus increasing the protein expression of KLF10 and inactivating PTEN/PI3K/AKT pathway (Fig. 7).[71] While in bladder cancer, the LINC00641 level was down-regulated both in bladder cancer tissues and bladder cancer cell lines compared with the normal controls.[71] Overexpressing LINC00641 in bladder cancer cell line could inhibit bladder cancer cell proliferation, but knockdown KLF10 blunted the above effect.[71] These results indicate that KLF10 is important in the LINC00641-mediated inhibition of bladder cancer.

It is interesting that although KLF10 acts as a downstream factor of TGFβ and mostly has inhibitory effects on the development of tumor, the role of TGFβ in tumor growth is complicated and not straightforward. TGFβ acts as a tumor suppressor in the early stage of tumor growth, but in the late stage, it can become a tumor activator and enhance the invasion and migration of tumor cells.[72] In the late stage of tumor development, TGFβ signaling can enhance epithelial-to-mesenchymal transition (EMT) which is a pathological property of cancer invasion.[72,73] KLF10, however, can inhibit TGFβ-induced EMT. Vivek Kumar Mishra et al found that KLF10 protected mice from DMBA feeding-induced lung adenocarcinoma. *SNAI2*, a downstream target gene of TGFβ and an EMT-promoting transcription factor, was significantly upregulated in *Klf10*-depleted lung adenocarcinoma cells and pancreatic ductal adenocarcinoma cells that were treated with TGFβ (Fig. 7).[74] KLF10 can bind to the proximal promoter region of *SNAI2* gene and histone deasetylases-1 (HDAC1) can be recruited by KLF10 to remove histone acetylation markers H3K9ac and H3K27ac on *SNAI2* gene promoter, thereby reducing the transcription of *SNAI2* (Fig. 7).[74] Thus, TGFβ can act as a tumor activator by inducing EMT, which can be inhibited by KLF10.

In most cases, KLF10 serves as a tumor inhibitor. But in some tumors, KLF10 can act as a tumor activator. In one study, mice were injected with diethylnitrosamine (DEN) to induce liver cancer.[75] The *Klf10* KO mice exhibited lower tumor incidence and decreased hepatocytes proliferation compared with WT mice.[75] It also showed increased expression levels of SMAD3, TGF-β1, TGF-βRI, and p15 in the tumor tissues of *KLF10* KO mice compared with WT mice. The level of phosphorylated SMAD3 was also significantly increased in the tumor tissue of *Klf10* KO mice.[75] These results suggest that *Klf10* deficiency can enhance the TGFβ-SMAD signaling pathway and protect against chemically-induced liver cancer (Fig. 7).[75] Therefore, the role of KLF10 in tumorigenesis may vary depending on the type of tumor cells and the microenvironment of the tumors.

As described above, KLF10 mostly serves as a tumor suppressor. But as mentioned earlier, KLF10 has the ability to enhance the proliferation of hepatocytes and is required for liver regeneration in partially hepatectomized mouse model.[56] It is possible that the different tissue microenvironment and cellular context may influence the effect of KLF10 on the capacity of cell proliferation, which needs further exploration. The important roles of KLF10 in the control of tumor development are updated and discussed here. A previous review provides detailed information about the roles of KLF10 in a variety of other tumors.[2]

KLF10 also exerts impact on other diseases

The function of KLF10 in promoting bone growth has been elucidated in previous studies.[6] Besides promoting bone growth, KLF10 also plays an important role in the bony union. KLF10 can prevent intervertebral disc from degeneration (Fig. 7). In intervertebral disc degeneration (IVDD) patients, inflammatory cytokine IL-1β was accumulated in nucleus pulposus (NP) cells, and the accumulation of IL-1β inhibited the expression of KLF10.[7] The more severe IVDD was associated with lower expression of KLF10, as well as lower expression of collagen II and Aggrecan in NP cells, the two important components of the extracellular matrix forming NP (Fig. 7).[7] In NP cells treated with IL-1β, cell migration and cell proliferation were inhibited. KLF10 can suppress IL-1β-mediated cell apoptosis and enhance cell migration and proliferation in NP cells.[7] Moreover, KLF10 can alleviate IVDD through activating TGFβ/SMAD signaling pathway and reducing the negative effects of IL-1β.[7] This study above provides a potential way for the treatment of IVDD.

Chondrocyte dysfunction is one of the main causes of osteoarthritis (OA). KLF10 expression was significantly higher in the cartilaginous tissue of OA patients and OA mice than in the normal tissue of control groups.[8] And *Klf10* overexpression in chondrocytes led to increased expression of Acvr1 which facilitated endochondral ossification, and decreased expression of Inhbb which might enhance chondrocyte migration, thereby inhibiting the proliferation and migration of chondrocytes to cause the pathology changes in OA (Fig. 7).[8]

In cardiovascular diseases, deficiency of KLF10 has been shown to promote cardiac hypertrophy (Fig. 7), increase heart weight, and exacerbate heart fibrosis.[9] The expression level of PTTG1, which plays an important role in enhancing cardiac hypertrophy, was elevated in *KLF10* KO mice. And this KLF10 deficiency-mediated cardiac hypertrophy is only detected in male mice, but not in female mice.[9]

Although KLF10 deficiency can cause cardiac hypertrophy, *Klf10* deficiency can protect against heart infarction (Fig. 7).[10] Cardiomyocytes isolated from *Klf10* deficient mice exhibited reduced apoptosis rate, increased proliferation and angiogenesis. And *Klf10* deficient mice with heart infarction exhibited improved cardiac function compared with WT mice. *Klf10* deficiency also led to reduced infarcted size of the heart.[10] Finally, decreased expression ratio of PTEN/AKT and increased expression ratio of Bcl 2/Bax were both observed in both myocytes and endothelial cells of *Klf10* deficient mice.[10] Therefore, down-regulation of KLF10 may be beneficial in alleviating heart infarction by the regulation of PTEN/AKT signaling pathway and Bcl2/Bax signaling pathway.

KLF10 also has a protective effect on acute viral myocarditis (VMC) (Fig. 7). In VMC induced by coxsackievirus B3 (CVB3), the decreased expression of KLF10 enhanced the development of VMC.[11] In cardiomyocytes, KLF10 can recruit histone deacetylase 1 (HDAC1) and bind to the CACCC site on the promoter of monocyte chemoattractant protein 1 (*MCP1*) gene which encodes MCP1, a chemokine that stimulates the migration of mononuclear cells (Fig. 7). This would inhibit the transcription of *MCP1*, reduce monocyte infiltration and consequently protect against VMC.[11] This study provides a potential KLF10-based therapeutic strategy for treating VMC.

KLF10 also promotes skin repairment (Fig. 7). Overexpression of KLF10 can protect normal human fibroblasts (NHF) from ultraviolet radiation-B (UV—B)-induced damages and strengthen the healing ability of fibroblasts.[76] And KLF10 also facilitated actin cytoskeleton rearrangement as the study showed that overexpression of KLF10 increased stress fibers and upregulated the expression of myofibroblast marker genes such as α-*SMA*, *RhoB* and *Cofilin*. These genes are involved in regulating actin cytoskeleton rearrangement.[76]

The injury of podocytes is known to promote the progression of diabetic nephropathy. In diabetic podocytes, the expression of a histone demethylase lysine demethylase 6 A (KDM6A) is increased, and as a downstream target gene of KDM6A, *Klf10* can be upregulated by KDM6A.[12] Then KLF10 protein in turn bind on *KDM6A* gene promoter to increase its expression, forming a positive feedback loop (Fig. 7).[12] Besides, KLF10 can bind on the promoter element from −1,893 to −1,931 of the *Nephrin* gene, repress its transcriptional expression by recruiting DNA methyltransferase 1 (Dnmt1) (Fig. 7).[12] *Nephrin* is a podocyte-specific marker gene and its downregulation would lead to kidney dysfunction.[12] Thus, the above work suggests a potential therapeutic strategy to attenuate diabetes-induced kidney injury through targeting KDM6A/KLF10 axis.

The newly reported roles of KLF10 in disease processes have been updated and discussed in this paper. In addition, the functional roles of KLF10 in many other pathophysiological conditions have been reviewed previously.[2,3,51,77]

Conclusion

KLF10 plays important roles in multiple tissues, exhibits tissue-specific roles and has positive or negative effects on various diseases. TGFβ/SMAD signaling pathway is a canonical pathway that KLF10 is involved in. Besides TGFβ/SMAD signaling pathway, KLF10 is also involved in many other pathways. Through inhibiting Wnt signaling pathway, KLF10 protects against multiple myeloma. Through repressing PTEN/PI3K/AKT pathway, KLF10 acts as an inhibitor of bladder cancer and multiple myeloma. But the knowledge about how KLF10 regulates these pathways and what roles KLF10 plays in these pathways was still limited. NASH is a disease with worldwide attention because of its high incidence and potential to develop into end-stage liver diseases. KLF10 is believed to play essential roles in regulating lipogenesis, cell death, inflammation and fibrosis, suggesting its important role in NASH. However, the exact role and mechanism of KLF10 in NASH awaits to be clarified by using animal models with liver cell type-specific knockout or over-expression of *Klf10*. The functional roles of KLF10 can be sex-dependent. The *Klf10* deficiency-mediated changes in muscle morphology, muscle function and disturbance of mitochondrial ultrastructure were observed in female mice, but not in male mice. And KLF10 deficiency-caused cardiac hypertrophy was only detected in male mice, but not in female mice. To avoid interference by estrogen signaling, most zoopery was performed in male mice. However, the roles of KLF10 in female mice may need further study, which could provide more insights into the interplay between KLF10 and sex hormones. Further high throughput studies, such as metabolomics, ChIP-seq, epigenomics, protein—protein interaction screening, that are performed in various cellular or tissue context and under different physiological or pathophysiological conditions, may reveal new functions and new targets of KLF10, which could help us better understand how KLF10 regulate the biological and disease processes.

Author contributions

The search and collection of literatures was performed by H.-Y.L., J.-Y.Z., M.C., W.-J.M., and L.G. The first draft of the manuscript was written by H.-Y.L. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interests.

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