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STATE-OF-THE-ART REVIEW

Krüppel-Like Factors

Crippling and Uncrippling Metabolic Pathways

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SUMMARY

Krüppel-like factors (KLFs) are deoxyribonucleic acid–binding transcriptional factors that regulate various pathways that control metabolism and other cellular mechanisms. Various KLF isoforms have been associated with cellular, organ, or systemic metabolism. Altered expression or activation of KLFs has been linked to metabolic abnormalities, such as obesity and diabetes, as well as with heart failure. This review article summarizes the metabolic functions of KLFs, as well as the networks of different KLF isoforms that jointly regulate metabolism in health and disease. (J Am Coll Cardiol Basic Trans Science 2018;3:132–56) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

KRÜPPEL-LIKE FACTOR BIOLOGY

Krüppel-like factors (KLFs) are zinc finger proteins with the ability to bind CACCC or GT box deoxyribonucleic acid (DNA) elements and act as either transcriptional activators or repressors. The regions of KLFs that do not participate in DNA binding are highly divergent and participate in protein-protein interactions. The name "Krüppel-like factor" was derived from the homologous *Drosophila* protein Krüppel, which means "cripple" and is associated with development. The founding member of the mammalian KLF family was discovered in red blood cell lineage and plays a major role in β-globin expression and erythrocyte development. The KLF family has 18 members thus far, with a broad range of expression profile among several tissues. KLF isoforms have been associated with regulation of metabolic pathways and energetic homeostasis in various organs, such as the liver, adipose tissue, heart, skeletal muscle, lungs, and myeloid cells. The present review article summarizes studies in cell systems and animal models (**Table 1**) that implicate KLFs in the regulation of organ and systemic metabolism, as well as their role in the pathophysiology of metabolic diseases. Thus far, no metabolic functions have been attributed to KLF12, KLF17, or KLF18.

KLFs have 3 conserved zinc finger motifs at the C-terminal domain of 23-25 amino acids, which bind on GC-rich sequences with a preference for the CACCC sequence (1). The zinc finger domains also contain nuclear localization signals (2–6). Although the C-terminal domain of the KLFs contains primarily nuclear localization signals and the DNA-binding region, the N-terminal domain bears regions that interact with other proteins.

Post-translational modifications of KLFs, as well as the proteins that KLFs interact with, alter their

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transcriptional activity or subcellular localization. KLFs with structural homology, particularly in the N-terminal domains, share functional similarity. Depending on their structural features and transcriptional roles, KLFs have been divided into 3 major groups (**Figure 1**) (1). Group 1 includes KLF3, KLF8, and KLF12, which interact with transcriptional repressors. Group 2 comprises KLF1, KLF2, KLF4, KLF5, KLF6, and KLF7, which function primarily as transcriptional activators although interaction with transcriptional repressors has also been reported. Finally, group 3 includes KLF9, KLF10, KLF11, KLF13, KLF14, and KLF16, which have mostly been described as transcriptional repressors. KLF15 and KLF17 have not been categorized in any of these groups because little is known about their protein interaction motifs. Various KLF members of group 2, such as KLF1, KLF2, KLF4, KLF5, and KLF6, as well as KLF13 from group 3, bind to acetyl-transferases, such as cAMP response element-binding protein (CREB) binding protein (CBP), p300, and p300/CBP-associated factor (7–11). This interaction leads to acetylation of KLFs that stimulates their transcriptional activity (12,13) or acetylation of histones followed by chromatin remodeling that ignites transcription in regions that are targeted by KLFs (14–17). Accordingly, binding of KLFs, such as KLF1, KLF4, KLF5, and KLF11, with histone deacetylases (HDACs) suppresses transcriptional activity due to deacetylation of either KLFs (18,19) or histones (20,21). Another mechanism of transcriptional repression by KLFs involves interaction with the transcriptional repressors Sin3A (22), C-terminal binding protein (CtBP)1, and CtBP2 (23–25), which in turn recruit HDACs (26), methyltransferases (27), and other silencing complexes such as polycomb proteins (28) and Ikaros (29).

Post-translational modifications of KLFs alter their subcellular localization, interactions with other proteins, and transcriptional activity. Histone acetyltransferases mediate acetylation of KLFs, which generally promotes binding of KLFs on DNA and transcriptional activation (7–12). Phosphorylation also modulates transcriptional activity by regulating protein-protein interaction of KLFs with other transcriptional regulators, including either activators (14,30–36) or repressors (37). The stability of KLFs is regulated by ubiquitination that is mediated by ubiquitin ligases, such as WWP1, Itch, and FBXO22, which eventually activate the proteasome pathway (38–46). KLFs undergo modification by the small ubiquitin-like modifier (SUMO) peptide. SUMOylation may either promote (47–49) or suppress (50,51) KLF transcriptional activity by promoting nuclear translocation (47), degradation (50), or interaction with transcriptional corepressors (51). Thus, KLFs may either activate or suppress gene transcription depending on post-translational modifications that affect stability of the protein or interaction with transcriptional coregulators.

KLF BIOLOGY IN ORGANS OF METABOLIC INTEREST

HEART. The heart is a major regulator of systemic metabolism; it consumes a large amount of lipids and glucose to produce the 5 to 6 kg of adenosine triphosphate (ATP) that is needed for pumping approximately 7,000 l of blood per day. To meet this energetic demand, the heart relies primarily on fatty acid (FA) β-oxidation, which supplies approximately 70% of the heart's ATP (52). Therefore, a robust regulatory network is in place to coordinate cardiac and systemic metabolism. Disruption of this network results in altered uptake, transport, and use of FAs in the heart and systemic metabolic changes. To illustrate the consequences of disrupting the network, deletion of the triglyceride (TG) hydrolytic enzyme lipoprotein lipase in cardiomyocytes reduces uptake of circulating TG, leading to hypertriglyceridemia (53). Similarly, expression of lipoprotein lipase only in the heart normalizes TG levels in mice with lipoprotein lipase deletion in skeletal muscle and adipose (54). As such, changes in the energy demands of the heart affect cardiac function and may also influence the systemic metabolic balance.

Various members of the KLF family interact with critical regulators of cardiac mitochondrial function and FA oxidation and are therefore necessary for maintaining the metabolic function of the heart (**Figure 2**). Mitochondrial biogenesis is regulated in large part by the coactivator peroxisome proliferator-activated receptor (PPAR)γ coactivator 1 (PGC-1), which interacts with nuclear receptors, including estrogen-related receptor, PPARα, and nuclear respiratory factor, to regulate genes critical to mitochondrial function (55). KLF4 forms a transcriptional complex with estrogen-related receptor and PGC-1, in which KLF4 is required for optimal activity of the estrogen-related receptor-PGC-1 complex (56). Concordantly, cardiac-specific *Klf4*$^{-/-}$ mice develop heart dysfunction with aging or pressure overload associated with

ABBREVIATIONS AND ACRONYMS

APOE = apolipoprotein E
ATP = adenosine triphosphate
BAT = brown adipose tissue
BCAA = branched chain amino acid
C/EBP = CCAAT/enhancer-binding protein
CBP = cAMP response element binding protein binding protein
CREB = cAMP response element binding protein
CtBP = C-terminal binding protein
DIO = diet-induced obesity
Dlk1 = delta-like noncanonical notch ligand 1
DNA = deoxyribonucleic acid
FA = fatty acid
FGF = fibroblast growth factor
GLUT4 = glucose transporter type 4
HDAC = histone deacetylases
KLF = Krüppel-like factor
MODY = maturity-onset diabetes of the young
PEPCK = phosphoenolpyruvate carboxykinase
PGC = PPARγ coactivator 1
PPAR = peroxisome proliferator-activated receptor
RNA = ribonucleic acid
SCD1 = stearoyl-coenzyme A desaturase 1
SK1 = sphingosine kinase-1
SREBP-1c = sterol regulatory element-binding protein-1c
SUMO = small ubiquitin-like modifier
T2DM = type 2 diabetes mellitus
TG = triglyceride
TGF = transforming growth factor
WAT = white adipose tissue