Chapter 12
Role of Krüppel-like Factor 15 in Adipocytes

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Abstract Krüppel-like factor 15 (KLF15) has been implicated in energy metabolism in various tissues including muscle, heart, liver, and adipose tissue. The expression of KLF15 is induced by the synergistic action of CCAAT/enhancer-binding protein β (C/EBPβ) and C/EBPδ during the differentiation of preadipocytes into adipocytes. The time course of KLF15 expression during this process is similar to that for C/EBPα, and these two proteins appear to promote the differentiation program in a cooperative manner through induction of the peroxisome proliferator-activated receptor γ (PPARγ) gene and other adipocyte-specific genes. A combination of microarray-based chromatin immunoprecipitation and gene expression analyses identified six genes whose promoters bound KLF15 and whose expression was either increased or decreased by forced expression of KLF15 in 3T3-L1 adipocytes. The gene for adrenomedullin, a vasodilatory hormone implicated in the pathogenesis of obesity-induced hypertension and insulin resistance, was one of these genes whose expression appears to be regulated by KLF15. KLF15 may thus also control the function of mature adipocytes through regulation of such genes.

Introduction

Krüppel-like factor 15 (KLF15) was first identified as a protein that binds to the promoter of the gene for CLC-K1, a kidney-specific CLC chloride channel (Uchida et al. 2000); KLF15 was thus formerly designated kidney KLF (KKLF).

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KLF15 was subsequently shown to regulate the expression of various genes whose products contribute to energy metabolism. For example, KLF15 regulates transcription of the gene for GLUT4 (Gray et al. 2002), an insulin-sensitive glucose transporter expressed in muscle and adipocytes. It is also implicated in the fasting-induced expression in skeletal muscle of the gene for acetyl-coenzyme A (CoA) synthetase 2 (Yamamoto et al. 2004), an enzyme of the mitochondrial matrix that provides acetyl-CoA for the citric acid cycle. Moreover, KLF15 has been shown to control glucose production by the liver through regulation of genes for enzymes involved in either gluconeogenesis (Teshigawara et al. 2005) or amino acid catabolism (Gray et al. 2007). It also appears to influence energy metabolism in the whole body through regulation of the differentiation of adipocytes as well as through control of the expression of various adipocyte-specific genes in mature adipocytes. In this chapter, we first summarize the functions of KLF15 and other members of the KLF family in differentiation of preadipocytes into adipocytes and then address the possible role of KLF15 in mature adipocytes.

Roles of KLF15 and Other KLF Family Proteins in Adipocyte Differentiation

The amount of adipose tissue in the body is an important determinant of energy homeostasis in living animals and is altered in various physiological or pathological conditions (Rosen and Spiegelman 2006). An increase in adipose tissue mass can result from an increase in cell size, cell number, or both (Rosen and Spiegelman 2000). The number of adipocytes is thought to increase as a result of the proliferation of preadipocytes and their subsequent differentiation into mature adipocytes. Such differentiation of preadipocytes is characterized by marked changes in the pattern of gene expression that are achieved by the sequential induction of various transcription factors. Preadipocytes exposed to hormonal inducers of differentiation thus manifest an early, transient increase in expression of the transcription factors CCAAT/enhancer–binding protein β (C/EBPβ) and C/EBPδ, which in turn promote a subsequent increase in the expression of C/EBPα and peroxisome proliferator-activated receptor γ (PPARγ) (Rangwala and Lazar 2000; Rosen and Spiegelman 2006) (Fig. 1). C/EBPα and PPARγ are thought to act synergistically in the transcriptional activation of a variety of adipocyte-specific genes, with each also reciprocally activating the expression of the other (Rangwala and Lazar 2000; Rosen and Spiegelman 2006).

Certain members of the KLF family of proteins have been implicated in the differentiation of preadipocytes. The differentiation of mouse 3T3-L1 preadipocytes into mature adipocytes in response to various hormonal inducers is a widely studied model of adipogenesis. Whereas 3T3-L1 preadipocytes express KLF2 and KLF3 at high levels, the amounts of these two proteins decrease rapidly after exposure of the cells to hormonal inducers of differentiation (Banerjee et al. 2003; Sue et al. 2008) (Fig. 2). Overexpression of KLF2 or KLF3 in 3T3-L1 preadipocytes has been shown

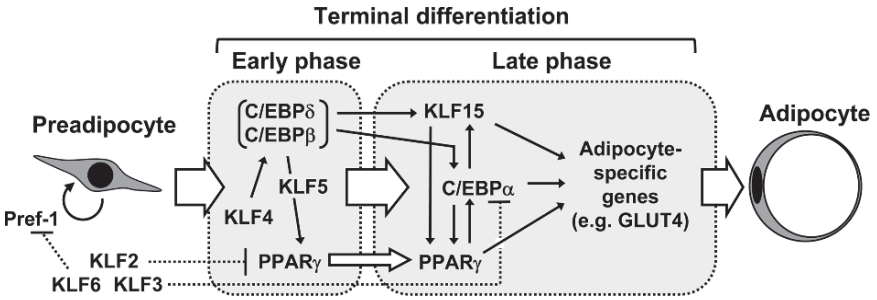

Fig. 1 Model for transcriptional control of adipocyte differentiation. Various members of the Krüppel-like factor (KLF) family of transcription factors contribute to transcriptional control of the differentiation of preadipocytes into adipocytes

to inhibit the differentiation of these cells by preventing the expression of PPARγ and C/EBPα, respectively (Banerjee et al. 2003; Sue et al. 2008). These observations suggest that the downregulation of these KLF proteins is important for differentiation of preadipocytes into adipocytes. Indeed, differentiation of embryonic fibroblasts derived from mice lacking KLF3 into adipocytes is enhanced compared with that for the corresponding wild-type cells (Sue et al. 2008). Preadipocyte factor-1 (Pref-1), also known as delta-like 1 (Dlk1), is a transmembrane protein that inhibits differentiation of preadipocytes. KLF6, which is transiently induced at an early phase of differentiation, inhibits the expression of Pref-1 and thereby promotes the differentiation of preadipocytes (Li et al. 2005) (Figs. 1, 2). The expression of KLF4 is also increased at an early phase of preadipocyte differentiation (within 1 hour after exposure to hormonal inducers), and this protein appears to contribute to differentiation by stimulating the expression of C/EBPβ and C/EBPδ (Birsoy et al. 2008).

KLF15 is also implicated in the differentiation of preadipocytes. Expression of KLF15 is markedly increased at a relatively late phase of the differentiation process (Mori et al. 2005) (Fig. 3A). Given that forced expression of C/EBPβ or C/EBPδ in NIH 3T3 fibroblasts results in a synergistic increase in the amount of KLF15 mRNA (Mori et al. 2005), the former two proteins likely trigger expression of KLF15 during the differentiation of preadipocytes (Fig. 2). Forced expression of KLF15 in NIH 3T3 fibroblasts or C2C12 myoblasts directs these nonadipocyte cell lines into the adipocyte lineage (Mori et al. 2005). Inhibition of the function of KLF15 in 3T3-L1 preadipocytes, by expression of a dominant negative mutant or by RNA interference, was found to attenuate expression of PPARγ as well as adipocytic differentiation (Mori et al. 2005), indicating that KLF15 is essential for the differentiation process in these cells. The time course of KLF15 expression during differentiation is similar to that for C/EBPα. KLF15 and C/EBPα stimulate, in an additive manner, both adipocytic differentiation of nonadipocyte cell lines as well as the activity of the PPARγ gene promoter (Mori et al. 2005), suggesting that these two proteins coordinately regulate gene transcription associated with the terminal differentiation of adipocytes.

Although a dominant negative mutant of KLF15 was shown to inhibit expression of PPARγ during the late phase of adipocytic differentiation in 3T3-L1 cells, it did not

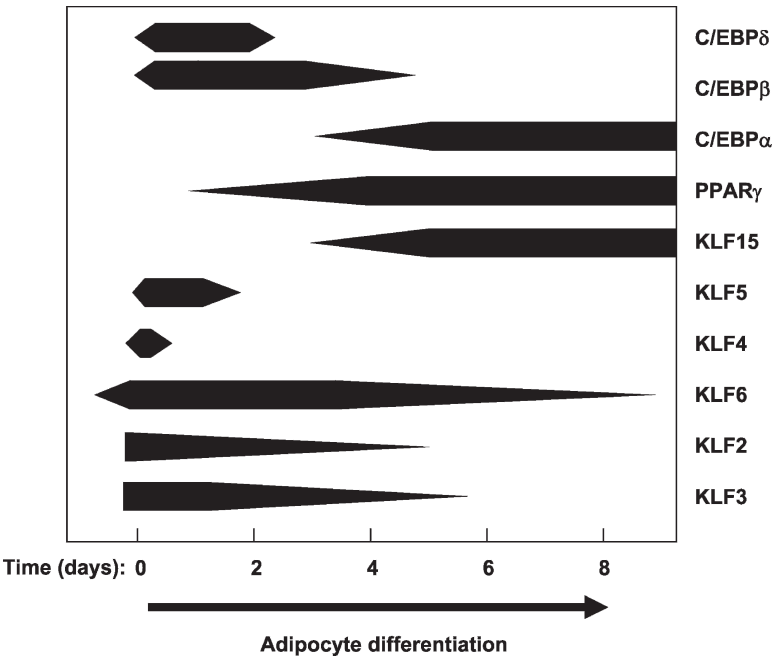

Fig. 2 Time course and extent of the expression of various transcription factors involved in regulation of the differentiation of 3T3-L1 preadipocytes into adipocytes in response to hormonal inducers. *IgG* = immunoglobulin G; *ChIP-chip* = chromatin immunoprecipitation with a promoter oligonucleotide microarray

affect early adipogenesis (on day 2) before the onset of KLF15 expression (Mori et al. 2005). These observations suggest that whereas KLF15 contributes to maintenance of the expression of PPARγ at a high level during the late phase of differentiation the early induction of PPARγ is achieved by a mechanism independent of KLF15. Both C/EBPβ and C/EBPδ bind directly to the promoter of the PPARγ gene and stimulate expression of PPARγ (Rangwala and Lazar 2000). Moreover, KLF5, whose expression is induced at an early phase of differentiation, upregulates the expression of PPARγ, and loss of function of KLF5 was shown to inhibit adipocyte differentiation (Oishi et al. 2005). It is thus likely that KLF5 contributes to the induction of PPARγ in coordination with C/EBPβ and C/EBPδ at an early phase of differentiation (Fig. 2).

Function of KLF15 in Mature Adipocytes

Given that KLF15 is expressed at a high level in mature adipocytes, it likely contributes not only to the differentiation of preadipocytes but also to maintenance of the function of mature adipocytes. Forced expression of KLF15 in mature adipocytes increases the expression of GLUT4 (Gray et al. 2002), a marker protein for mature adipocytes. This effect of KLF15 is likely attributable to its direct activation of the promoter of the GLUT4 gene (Gray et al. 2002), suggesting that KLF15

contributes to the function of mature adipocytes by directly regulating the expression of adipocyte-specific genes.

To characterize further the function of KLF15 in mature adipocytes, we attempted to identify novel target genes of this transcription factor in adipocytes by a combination of chromatin immunoprecipitation with a promoter oligonucleotide microarray (ChIP-chip) and analysis of gene expression with an oligonucleotide expression microarray (Nagare et al. 2009). We performed ChIP-chip analysis with immunoprecipitates prepared from fully differentiated 3T3-L1 adipocytes with antibodies to KLF15 or control immunoglobulin. We found that among ~6000 genes on the microarray the promoter regions of 132 genes showed a reproducibly significant difference in hybridization signal between the two samples (Fig. 3) (Nagare et al. 2009). We next profiled genes whose level of expression changed in association with adenovirus-mediated overexpression of KLF15 in 3T3-L1 adipocytes with the use of an oligonucleotide expression microarray. This analysis revealed that forced expression of KLF15 in the mature adipocytes was accompanied by an increase in expression of 337 genes and a decrease in that of 274 genes (Fig. 3) (Nagare et al. 2009). Comparison of the ChIP-chip data with the expression microarray data resulted in identification of six genes whose promoters bound KLF15 and whose expression was either increased (*Slc16a9*, *Cdk9*, *P4ha2*, *Klf3*) or decreased (*Aprt, Adm*) by forced expression of KLF15 (Nagare et al. 2009).

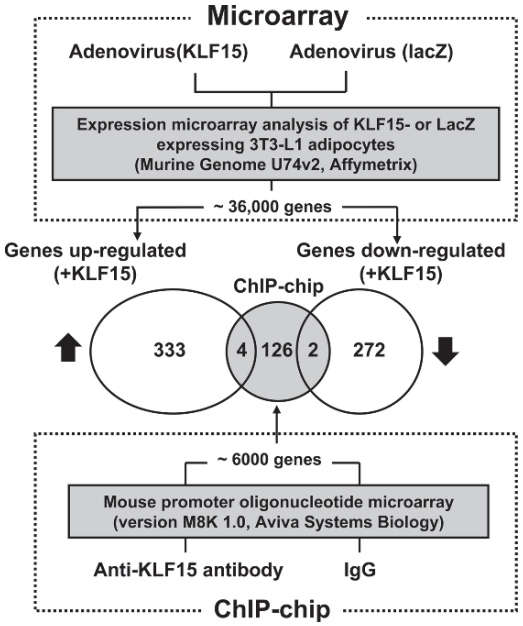

Fig. 3 Identification of target genes of KLF15. Strategy for the identification of target genes of KLF15 and a Venn diagram of the numbers of genes whose promoters were found to bind KLF15 by ChIP-chip analysis and whose expression was found to be increased or decreased in response to KLF15 overexpression by expression microarray analysis in 3T3-L1 adipocytes (Nagare et al. 2009)

Slc16a9 encodes a transporter for monocarboxylic acids, which are important metabolites of carbohydrates and fatty acids (Halestrap and Price 1999). The protein encoded by *Cdk9* is a member of the cyclin-dependent kinase (CDK) family and has been shown to participate in adipocyte differentiation through direct interaction with and phosphorylation of PPARγ (Iankova et al. 2006). *P4ha2* encodes prolyl 4-hydroxylase, which contributes to the synthesis of collagen and to oxygen homeostasis (Myllyharju 2008), the latter of which has been shown to influence the function of adipocytes (Trayhurn et al. 2008). The protein encoded by *Klf3* (KLF3), as mentioned above, appears to regulate adipogenesis by inhibiting the expression of C/EBPα (Sue et al. 2008). *Aprt* encodes adenine phosphoribosyltransferase, an enzyme involved in purine nucleotide metabolism (Delbarre et al. 1974).

Adrenomedullin, the protein encoded by *Adm*, is a potent vasodilatory hormone that was originally identified in pheochromocytoma cells (Kitamura et al. 1993). Mature adipocytes were subsequently shown to be a major source of adrenomedullin in the body (Harmancey et al. 2007; Nambu et al. 2005). We found that the expression of adrenomedullin in 3T3-L1 adipocytes was increased as a result of KLF15 depletion by RNA interference and that KLF15 inhibits the activity of the adrenomedullin gene promoter by directly binding to the most proximal CACCC element (Nagare et al. 2009), confirming the notion that KLF15 is a negative regulator of the adrenomedullin gene. Adipocyte-derived adrenomedullin is thought to protect against the development of hypertension, insulin resistance, and the complications of these conditions in obese subjects (Paulmyer-Lacroix et al. 2006). Expression of KLF15 is decreased in adipose tissue of mice with diet-induced or genetic (*ob/ob*) obesity (H.S. and M.K., unpublished observations), animals in which the expression of adrenomedullin in adipose tissue is increased (Harmancey et al. 2007). It is thus possible that obesity-induced downregulation of KLF15 in adipose tissue is related to the pathogenesis of obesity-induced health disorders induced by a decrease in adrenomedullin production.

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