Pref-1, a Preadipocyte Secreted Factor That Inhibits Adipogenesis

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Abstract

Preadipocyte factor 1 (Pref-1) belongs to the Notch/Delta/Serrate family of epidermal growth factor-like repeat-containing proteins. Pref-1 is highly expressed in 3T3-L1 cells but is extinguished during adipocyte differentiation. Pref-1 serves as an excellent marker for preadipocytes. Furthermore, Pref-1 is an inhibitor of adipogenesis. Constitutive expression of Pref-1 inhibits, whereas antisense Pref-1 enhances, 3T3-L1 adipocyte differentiation. We found that Pref-1 is synthesized as a transmembrane protein but processed to generate soluble forms, including a large 50-kDa soluble form and the small soluble forms. Furthermore, only the large soluble form, but not the small soluble or the transmembrane forms of Pref-1, is biologically active to inhibit adipogenesis. We recently elucidated that the 50-kDa soluble form of Pref-1 is released by an ADAM family member, tumor necrosis factor-α-converting enzyme (ADMA 17). In vivo, mice lacking Pref-1 show accelerated fat deposition; conversely, mice overexpressing soluble Pref-1 in adipose tissue show a decrease in fat mass, reduced expression of adipocyte markers, and lower adipocyte-secreted factors. These findings clearly demonstrate the inhibitory effect of Pref-1 on adipogenesis in vivo.

Introduction

Adipose tissue develops late during the embryonic stage and rapidly expands postnatally. Although deposition of adipose tissue in adults is largely the result of increased fat cell size with increased triacylglycerol storage, the number of fat cells may also increase from the differentiation of preadipocytes into mature adipocytes. PPARγ and the CCAAT/enhancer-binding protein α (C/EBPs)² have been found to play critical roles in adipocyte differentiation. These transcription factors activate many of the genes involved in fatty acid and lipid metabolism, including adipocyte fatty acid binding protein (aP2), stearoyl CoA desaturase 1, and fatty acid synthase (FAS). Hormones, growth factors, and extracellular matrix proteins play regulatory roles (1,2) in adipocyte proliferation and/or differentiation.

Our laboratory has been largely interested in the mechanisms that regulate adipose tissue development. We identified several genes encoding regulatory molecules, such as Ectoderm-Neural Cortex 1 (3), preadipocyte factor 1 (Pref-1), and adipocyte-specific secretory factor (ADSF)/resistin (4,5). In this review, we summarize our current knowledge of the role of Pref-1, a preadipocyte secreted factor in adipose tissue development. We will emphasize the latest studies carried out in our laboratory on the inhibitory effects of Pref-1 on adipogenesis.

Pref-1 protein structure and processing to generate the soluble forms. Pref-1 is a protein of 385 amino acids that contains an extracellular domain with 6 epidermal growth factor (EGF)-like repeats, a juxtamembrane region, a single transmembrane domain, and a short cytoplasmic tail. The EGF-repeats of Pref-1 maintain both the conserved spacing of 6 cysteines for the formation of 3 disulfide bonds and other amino acids characteristic of the EGF-repeat motif that appear to be for protein-protein interaction and are found in proteins that affect growth and differentiation. This motif was initially described for EGF and is present in TGFα and heparin-binding EGF, where it signals for proliferation, growth inhibition, or differentiation by binding to the cell surface EGF receptor (6). However, Pref-1 does not contain conserved amino acid residues that are required for EGF receptor binding. Rather, Pref-1 shares higher structural homology with another class of EGF-like repeat-containing signaling proteins, the Notch/Delta/Serrate family, which are involved in cell signaling and cell fate determination. Pref-1, however, also lacks the so called DSL domain that mediates receptor-ligand interaction (7) and that is conserved in all Notch ligands. Therefore, it is unlikely that Pref-1 acts as a Notch ligand.

In preadipocytes, multiple transmembrane forms of Pref-1, ranging from 50 to 60 kDa, are found in the cell membrane, due in part to post-translational modification by N-linked glycosylation (8). Moreover, Pref-1 has multiple alternate splicing forms. In addition to the largest full-length form (Pref-1A), alternate splicing also generates 3 major shorter forms of Pref-1 (Pref-1B–D), and each contains in-frame deletions in the extracellular juxtamembrane region or EGF-like repeat domain (9). Membrane forms of Pref-1A and Pref-1B contain 2 proteolytic cleavage sites at the extracellular domain; one is located near the fourth EGF repeat and the other in the juxtamembrane domain. By undergoing cleavage at those sites, Pref-1A and Pref-1B generate 2 soluble forms, a 50-kDa large soluble form and a 24–25-kDa small soluble form. Conversely, Pref-1C and Pref-1D only produce the small soluble form by cleavage at the processing site distal to the membrane region (Fig. 1) (10). The relative abundance of the different spliced forms varies depending on the tissue or developmental stage investigated (11).
Pref-1 inhibits adipocyte differentiation. We originally cloned Pref-1 by differential hybridization screening of a 3T3-L1 cDNA library, using the criteria of preadipocyte specific expression and absence in other tissues. Pref-1 mRNA and protein are highly expressed in 3T3-L1 preadipocytes, but their expression is decreased during differentiation following addition of the adipogenic agents, DEX/MIX, and is absent in mature adipocytes (8,12). Pref-1 expression is under negative control of glucocorticoids, because DEX can suppress Pref-1 transcription (12,13). DEX, a component of the routinely used adipogenic agents, may induce adipocyte differentiation partly via suppressing Pref-1 expression. Using various in vitro approaches, we demonstrated the inhibitory role of Pref-1 in adipocyte differentiation. In 3T3-L1 cells, constitutive expression of Pref-1 by stable transfection of Pref-1A markedly lowers the degree of adipocyte differentiation. Conversely, decreasing Pref-1 levels by transfection of antisense sequence greatly enhances adipogenesis. Because 3T3-L1 preadipocytes express high endogenous levels of Pref-1 mRNA, we recently used the Pref-1 null mouse embryonic fibroblasts (MEFs) to examine adipocyte differentiation. Compared with the 50% differentiation of wild-type MEFs into adipocytes, 90% of Pref-1 null MEFs differentiated into adipocytes. Furthermore, infection of lentivirus-containing Pref-1A decreased the degree of differentiation of Pref-1 null MEFs by 60%, demonstrating the inhibitory effort of Pref-1 (14).

To test, specifically, the effect of soluble Pref-1 on adipocyte differentiation, 3T3-L1 preadipocytes were treated with the purified Pref-1 protein encoded by extracellular domain. Pref-1 protein markedly inhibited differentiation, with only 10% of cells converting into adipocytes with similarly decreased levels of the terminal adipocyte marker mRNAs FAS, stearoyl CoA desaturase 1, and adipocyte fatty acid binding protein. The mRNAs for C/EBPα and PPARγ were also suppressed, indicating the inability of cells to express these transcription factors in the presence of soluble Pref-1. To further elucidate the structural requirement for Pref-1 in inhibiting adipocyte differentiation, artificial forms of Pref-1 were constructed in addition to Pref-1A–D. These included Pref-1EC, containing the full extracellular domain of the 50-kDa fragment, and Pref-1Δ21, an artificial form constructed by deleting the 21-amino acid juxtamembrane sequence (EQHLKVSMLNKKSTPLL), containing the cleavage site proximal to the membrane. With this change, the membrane form of Pref-1 is made but cannot be processed to generate the large soluble form. All of these constructs, however, can produce small soluble forms by cleavage at the processing site distal to the membrane region (Fig. 1). By transfection of these constructs, we further demonstrated that only the Pref-1 variants (Pref-1A and B) that generate the 50-kDa larger soluble fragment or the large soluble form of Pref-1 (Pref-1EC) inhibit adipocyte differentiation, whereas Pref-1C, Pref-1D, and the membrane form (Pref-1Δ21) do not affect the differentiation process. These studies clearly show that only the 50-kDa soluble Pref-1 is effective in inhibiting adipocyte differentiation. These studies also suggest that alternate splicing determines generation of biologically active soluble Pref-1.

Figure 1  S (signal sequence), 6 EGF-repeats (1–6), Jm (juxtamembrane domain), Tm (transmembrane domain), Cy (cytoplasmic region), D (distal), and P (proximal) cleavage sites.

Figure 2  Inhibition of adipocyte differentiation by TACE-mediated Pref-1 processing (from Fig. 4A and 7B,C from Wang and Sul [14]). (A) COS cells stably expressing Pref-1A (HA-tagged in extracellular domain) were infected with lentivirus-containing control vector or TACE-expressing vector (Myc-tagged in C terminus). Both basal and phorbol 12-myristate 13-acetate-stimulated Pref-1A cleavages were increased by TACE lentivirus infection. The 50-kDa Pref-1 soluble form and 65-kDa Pref-1 membrane form were detected in medium and lysates by anti-HA antibody. (B) Transfection of TACE siRNA markedly decreased the releasing of Pref-1A from cells. (C) Compared with the control 3T3-L1 cells, infection of TACE lentivirus inhibited adipocyte differentiation; in contrast, transfection of TACE siRNA enhanced adipocyte differentiation. Oil red O staining for lipid accumulation is shown (upper panel). Northern-blot analysis for PPARγ and C/EBPα (lower left panel) and RT-PCR analysis for adipocyte markers (lower right panel) are also shown.
Pref-1 knockout and Pref-1 overexpressing transgenic mouse models. Pref-1 is expressed in multiple mouse embryonic tissues, such as liver, lung, tongue, pituitary, developing vertebrae (8), and placenta, and detectable amounts of circulating Pref-1 are found in maternal serum in concentrations that correlate with the number of fetuses (15). Delta-like 1 (Dlk1), the human homolog of Pref-1, also identified as Fetal Antigen 1 (16), has been shown to be expressed in a wide array of human embryonic tissues that include glandular cells of pancreas (17), cells of the ovaries, and skeletal myotubes (18). However, after birth, expression of Pref-1 is rapidly abolished in most tissues and becomes restricted to certain cellular types, such as preadipocytes (8), pancreatic islets cells (19), thymic stromal cells (17), and adrenal gland cells (20). Pref-1 is coded by the gene (dlk1) located in an imprinted region of mouse chromosome 12, and paternal monoallelic expression of Pref-1 due to differential methylation has been demonstrated (21–23). Given the role of imprinted genes in fetal growth and development, in general, and the expression of Pref-1 in embryonic tissues, Pref-1 may have functions beyond the regulation of adipogenesis (21–25). To define the in vivo role of Pref-1, we generated Pref-1 knockout mice (26) as well as transgenic mice overexpressing Pref-1 in adipose tissue (27). In the Pref-1 knockout mice in which Pref-1 expression is totally abolished, at the weaning age, both Pref-1 null male and female mice weighed significantly less than wild-type mice at weaning. Nevertheless, the weight of major fat depots (inguinal, retroperitoneal, and gonadal) was significantly higher in null mice than in wild type, indicating that accelerated body wt gain in Pref-1 knockout mice was due to an increase in adipose tissue mass. Histological analysis of fat depots revealed that adipocytes from Pref-1 null mice were bigger than those from wild-type littermates. Moreover, mRNA levels of various markers of adipocyte differentiation were significantly higher in adipose tissue of Pref-1 null mice. Interestingly, those mice also showed enlarged fatty livers as well as an increase in circulating levels of triglycerides, cholesterol, and free fatty acids, characteristics usually associated with obesity. These data demonstrated that ablation of Pref-1 expression enhances adipogenesis in vivo and support the proposed role of Pref-1 as a negative regulator of the adipogenic process (26).

We next generated transgenic mice overexpressing the soluble form of Pref-1 as an Fc-fusion protein in adipose tissue using the aP2 promoter. aP2-Pref-1/hFc transgenic mice showed a marked decrease in adipose tissue mass and reduced expression of adipocyte markers, including SCD, C/EBPα, FAS, and ADSF/resistin, as well as lower adipocyte-secreted factors. As adipose tissue development decreased, lipodystrophy mice showed hypertriglyceridemia, decreased glucose tolerance, and lower insulin sensitivity (27). Mice expressing the Pref-1/hFc transgene exclusively in liver under the control of the albumin promoter also showed a decrease in adipose mass and adipocyte marker expression, suggesting an endocrine mode of action of Pref-1 (27). Data from these in vivo studies of Pref-1 knockout and transgenic mice are consistent with in vitro studies strongly demonstrating the inhibitory effect of Pref-1 in adipogenesis. Our studies of Pref-1 knockout and Pref-1 overexpressing transgenic mice also suggest that proper development of adipose tissue is critical for maintenance of glucose/insulin homeostasis. In addition to its role in adipose tissue development, Pref-1 in knockout mice displayed >50% perinatal lethality, and surviving animals show growth retardation, skeletal malformations, and eyelid defects (26). Pref-1 transgenic mice also showed skeletal defects in addition to the lean phenotype (27). Overall, Pref-1 knockout and transgenic mice showed distinct defects similar to murine mUPD12 and pUPD12, respectively, and syntenic UPD14 syndromes in humans. These data demonstrate that Pref-1 is also involved in embryonic development in addition to adipogenesis. Pref-1 may function as a soluble factor, maintaining proliferating cells in an undifferentiated state, highlighting the importance of Pref-1 during mouse development.

In conclusion, obesity, lipodystrophy, or dysfunctions in the secretory function of adipose tissue are commonly associated with diverse pathologies, including diabetes, cardiovascular diseases, and immunodepression. In this regard, it is important to elucidate and characterize the mechanisms involved in adipogenesis as well as the factors that regulate adipocyte differentiation and function. Here we have presented the current knowledge on the function and mechanism of Pref-1 on adipogenesis. We have unequivocally demonstrated in vivo the inhibitory role of Pref-1 on adipogenesis by generating Pref-1 knockout mice and transgenic mice. By a variety of in vitro experimental approaches using 3T3-L1 cells and Pref-1 null MEFs, we have demonstrated that Pref-1 prevents cell differentiation into adipocytes. We also found that the biologically active Pref-1 form is generated by TACE cleavage of the juxtamembrane domain, releasing full ectodomain. It remains unknown how Pref-1 activates downstream signaling. Identification of the receptor through which Pref-1 signaling occurs is critical in understanding the molecular mechanisms underlying the function of this secreted factor in adipogenesis.
Literature Cited