MINIREVIEW

Adipocyte Differentiation: From Fibroblast to Endocrine Cell

FRANCINE M. GREGOIRE[1]
Metabolex, Hayward, California 94549

Recent advances regarding the biology of adipose tissue have demonstrated that white adipose tissue (WAT) plays a central role in the regulation of energy balance and acts as a secretory/endocrine organ that mediates numerous physiological and pathological processes. Dysregulation of WAT mass causes obesity or lipoatrophy, two disorders associated with life-threatening pathologies, including cardiovascular diseases and diabetes. Alterations in WAT mass result from changes in adipocyte size and/or number. Change in adipocyte number is achieved through a complex interplay between proliferation and differentiation of preadipocytes. Adipocyte differentiation or adipogenesis is a highly controlled process that has been extensively studied for the last 25 years. *In vitro* preadipocyte culture systems that recapitulate most of the critical aspects of fat cell formation *in vivo* have allowed a meticulous dissection of the cellular and molecular events involved in the adipogenesis process. The adipogenic transcription factors peroxisome proliferator-activated receptor-γ and CCAAT/enhancer binding protein-α play a key role in the complex transcriptional cascade that occurs during adipogenesis. Hormonal and nutritional signaling affects adipocyte differentiation in a positive or negative manner, and components involved in cell-cell or cell-matrix interactions are also pivotal in regulating the differentiation process. This knowledge provides a basis for understanding the physiological and pathophysiological mechanisms that underlie adipose tissue formation and for the development of novel and sound therapeutic approaches to treat obesity and its related diseases. [Exp Biol Med Vol. 226(11):997–1002, 2001]

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Obesity is a disorder that results from excess white adipose tissue (WAT) and is a major risk factor for type 2 diabetes (NIDDM) and cardiovascular disease (1). A dramatic increase in the incidence of both obesity and NIDDM is currently observed in Western countries, but so far strategies to combat excess body weight and/or WAT mass have not been effective in most individuals. Lipoatrophy, less frequent in humans, is characterized by a paucity of adipose tissue and is associated with insulin-resistant diabetes (2), stressing that both increased and decreased WAT mass can have profound effects at extra-adipose sites. Therefore, understanding the cellular and molecular basis of adipose tissue growth in physiological and pathophysiological states is an important area of research that may lead to the development of innovative therapies to treat WAT-related disorders. WAT was originally viewed as an inert tissue containing a fixed number of adipocytes, its main cellular component. It was believed to act as a passive energy depot, storing or releasing lipid under the influence of various hormones. However, it is now undisputed that WAT is a very dynamic endocrine organ with pleiotropic functions.

Adipocytes secrete factors that play a central role in the regulation of energy balance, insulin sensitivity, immunological responses, and vascular diseases (3, 4). Moreover, several studies have suggested that WAT expansion during adulthood not only results from increased adipocyte size, but also from increased adipocyte number. Adipocyte hyperplasia is observed in various rodent models, including high fat-fed rats, obese Zucker rats treated with troglitazone, transgenic mice overexpressing GLUT4 selectively in adipose tissue, and estrogen-receptor-α knockout mice (5–9). Although the occurrence of adipocyte hyperplasia in humans remains controversial, fat cell precursors that can be differentiated *in vitro* into mature adipocytes have been isolated from human adult WAT (10–12), demonstrating that adults retain the potential to acquire new fat cells. Moreover, human WAT-derived stromal vascular cells were recently reported to differentiate *in vitro* into adipogenic, chondrogenic, myogenic, and osteogenic cells in the pres-

[1] To whom requests for reprints should be addressed at Metabolex, Inc., 3876 Bay Center Place, Hayward, CA 94549. E-mail: fmgregoire@metabolex.com

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ence of lineage-specific induction factors (13), emphasizing WAT plasticity. This suggests that in addition to fibroblast-like cells already determined for adipocyte lineage (preadipocytes), human adult WAT also contains multipotent precursor cells. However, because differentiation into these lineages was only assessed morphologically, the findings of this study need to be confirmed at the molecular level. Numerous gaps still exist in our knowledge, mostly regarding the early molecular events that lead to the determination of primitive precursor cells to the adipocyte lineage. Therefore, if real, the availability of multipotent precursor cells may provide a new model system for identification of novel adipogenic regulatory genes.

In contrast, the cellular and molecular events that take place during the transition from undifferentiated fibroblast-like preadipocytes into mature adipocytes have been extensively characterized. Adipogenesis results from the transcriptional activation and repression of adipocyte genes, a process that has been recently reviewed comprehensively (14, 15). This review summarizes this knowledge as well as other aspects of adipocyte biology, with a particular emphasis on recently identified adipocyte-specific secretory factors.

Adipogenesis: from Fibroblast-Like to Mature Adipocyte

Various cell culture models, including preadipocyte cell lines and primary culture of adipose-derived stromal vascular precursor cells, have been used to study the molecular and cellular events that take place during the adipocyte differentiation process. The characteristics of most commonly used cell culture models have been reviewed in detail (5) and are not addressed here.

Committed preadipocytes have to withdraw from the cell cycle before undergoing adipose conversion. For preadipose cell lines as well as for primary preadipocytes, growth arrest is required for adipocyte differentiation and is normally achieved through contact inhibition. However, contact inhibition *per se* is not a prerequisite for adipogenesis since cells plated at low density in serum-free medium or kept in a methylcellulose suspension still undergo differentiation (5). Following growth arrest, preadipocytes must receive an appropriate combination of mitogenic and adipogenic signals to continue through the subsequent differentiation steps, leading to the progressive acquisition of the morphological and biochemical characteristics of the mature adipocytes. The nature of the induction depends on the specific cell culture model used because the responsiveness to inducing agents may vary considerably between preadipose cell lines and primary preadipocytes (5). Overall, in serum-containing medium, the standard adipogenic cocktail, contains supraphysiological concentrations of insulin, dexamethasone (DEX), and isobuthylmethylxanthine (MIX), highlighting the involvement of the insulin/IGF-1, glucocorticoid, and cAMP signaling pathways in the adipocyte differentiation process (5). Post-confluent preadipocytes undergo at least one round of DNA replication and cell doubling, leading to the clonal amplification of committed cells (16). Although the clonal amplification step has been viewed as required for the subsequent differentiation events, this view has recently been questioned. That is, primary preadipocytes derived from human adipose tissue do not require cell division to enter the differentiation process (17). In addition, data collected on 3T3-L1 preadipocytes corroborate that DNA synthesis and mitotic expansion are not required steps for preadipocyte differentiation into adipocytes (18). When induced by MIX and DEX, a significant proportion of preadipocytes differentiates in the absence of mitotic clonal expansion. Moreover, treatment of normally induced 3T3-L1 preadipocytes with a mitogen-activated protein kinase-1 (MEK-1) inhibitor that blocks mitotic clonal expansion by inhibiting mitogen-activated protein kinase and activation of extracellular signal-regulated kinases 1 and 2 does not affect differentiation (18).

The first hallmark of the adipogenesis process is the dramatic alteration in cell shape as the cells convert from fibroblastic to spherical shape. These morphological modifications are paralleled by changes in the level and type of extracellular matrix (ECM) components and the level of cytoskeletal components (5). Recent findings indicate that these events are key for regulating adipogenesis as they may promote expression of critical adipogenic transcription factors, including CCAAT/enhancer binding protein-α (C/EBPα) and/or peroxisome proliferator-activated receptor-γ (PPARγ). Mediation of the proteolytic degradation of the stromal ECM of preadipocytes by the plasminogen cascade is required for cell-shape change, adipocyte-specific gene expression, and lipid accumulation (19). Plasma kallikreine or plasminogen deficiencies lead to inhibition of adipocyte differentiation *in vitro* and *in vivo*, probably through suppression of fibronectin degradation. Addition of exogenous fibronectin during adipocyte differentiation also inhibits adipogenesis, confirming the negative regulatory role of this ECM molecule (5, 19). ENC-1, a Drosophila kelch-related actin-binding protein may also play a regulatory role early in adipocyte differentiation by affecting cytoskeletal reorganization and cell-shape change. In preadipocytes, ENC-1 colocalizes with actin filaments, and its mRNA levels are transiently increased 8- to 12-fold early in adipocyte differentiation. ENC-1 induction precedes expression of PPARγ and C/EBPα, and decreasing endogenous ENC-1 levels effectively inhibit adipocyte differentiation (20).

Many of the changes that occur during preadipocyte differentiation take place at the gene expression level. Several reports have attempted to schematize the stages of adipocyte differentiation into a simple hierarchy of molecular events. Genes differentially regulated during adipogenesis have been categorized into early, intermediate, and late mRNA/protein markers (4, 5, 14, 15, 21, 22). However, obtaining an accurate chronology of the molecular events that take place during adipocyte differentiation is a daunting task. Growth arrest and clonal expansion, when present, are

accompanied by complex changes in the pattern of gene expression that can differ with cell culture models and the specific differentiation protocols employed. Moreover, progressive acquisition of the adipocyte phenotype is associated with changes in the expression of over 2000 genes, as highlighted in a recent study using microarray technology to monitor global changes in gene expression profiles during 3T3-L1 differentiation (23). Over 100 expressed sequence tags representing uncharacterized genes were expressed only in preadipocytes or in adipocytes, clearly indicating that further research is needed to fully understand the adipogenesis process.

Several transcription factor families that exhibit diverse modes of activation and function are key regulators of the adipogenesis process. The roles played by PPARγ2 and the C/EBPs have been intensively investigated and recently reviewed (14, 15). Members of the C/EBP family and PPARγ2 are involved in terminal differentiation by their subsequent transactivation of adipocyte-specific genes. Exposure of confluent preadipocytes to the adipogenic cocktail induces expression of C/EBPβ and C/EBPδ, which in turn activate PPARγ2 and C/EBPα. Production of the appropriate ligand of PPARγ2 by the differentiating preadipocyte is likely a limiting step in this transcriptional cascade. The nature of PPARγ2 endogenous ligand and the molecular mechanisms that regulate its production are still unknown. ADD1/SREBP-1c is another key transcription factor known to modulate transcription of numerous liver genes involved in lipid metabolism as well as adipocyte differentiation (15, 24). ADD1/SREBP-1c and more recently C/EBPβ and C/EBPδ were proposed to play roles in regulation of PPARγ2 ligand production (15, 25), but additional studies will be necessary to confirm their direct involvement in this process. The regulatory role of PPARδ, another member of the PPAR family, is still a matter of intense debate. In contrast to PPARγ2, PPARδ expression is not restricted to WAT. Ectopic expression of PPARδ in fibroblastic cell lines was originally reported to have either no effect on adipogenesis or to promote it (26, 27). Recent reevaluation of the role of PPARδ in adipocyte differentiation has confirmed that its overexpression in both fibroblastic and adipocytic cell lines supports adipose conversion in the presence of non-specific (2-bromopalmitate) or specific (L165041) PPARδ ligands (28–30). However, activation of endogenous PPARδ by L165041 is not sufficient to significantly increase 3T3-L1 differentiation (30, 31); and expression of a dominant negative mutant PPARδ in differentiating ob17 preadipocytes does not completely abolish differentiation induced by 2-bromopalmitate (29). Therefore, the impact of PPARδ on the processes that lead to terminal differentiation is unclear, and its precise biological function remains to be determined.

In addition to C/EBPs, PPARγ2, and ADD1/SREBP-1c, several other transcription factors, including GATA-binding transcription factors GATA-2 and GATA-3, and cAMP response element binding protein (CREB), play a critical role in the molecular control of the preadipocyte-adipocyte transition (32, 33). GATA-2 and GATA-3 are specifically expressed in white preadipocytes, and their mRNAs are downregulated during adipocyte differentiation. Constitutive expression of GATA-2 and GATA-3 suppresses adipocyte differentiation and traps cells at the preadipocyte stage. This effect is mediated, at least in part, through direct suppression of the activity of the PPARγ2 promoter. Moreover, GATA-3-deficient embryonic stem cells exhibit an enhanced capacity to differentiate into adipocytes, and GATA-2 and GATA-3 expression is severely downregulated in WAT derived from genetically obese mice (33). The transcription factor CREB is constitutively expressed prior to and during adipogenesis, and is upregulated by conventional differentiation-inducing agents such as insulin, DEX, and dibutyryl cAMP. Overexpression of a constitutively active CREB in 3T3-L1 preadipocytes is necessary and sufficient to initiate adipogenesis, whereas overexpression of a dominant-negative CREB alone blocks adipogenesis in cells treated with conventional differentiation-inducing agents (32).

In addition to transcription factors, other signaling molecules such as pref-1 and Wnts also regulate adipocyte differentiation (34, 35). Pref-1 is an inhibitor of adipocyte differentiation and is synthesized as a plasma membrane protein containing six EGF repeats in the extracellular domain. Pref-1 is highly expressed in 3T3-L1 preadipocytes, but is not detectable in mature fat cells. DEX, a differentiation agent, inhibits pref-1 transcription and thereby promotes adipogenesis. Downregulation of pref-1 is required for adipose conversion, and constitutive expression of pref-1 inhibits adipogenesis. Conversely, decreasing pref-1 levels by antisense pref-1 transfection greatly enhances adipogenesis (35). Wnt signaling also appears to be a molecular switch that governs adipogenesis. Preadipocytes that constitutively express Wnt-1 failed to differentiate when treated with the adipogenic cocktail. Moreover, activation of Wnt signaling downstream of the receptor also inhibits differentiation, indicating that Wnt signaling maintains preadipocytes in an undifferentiated state. This effect seems to be mediated through inhibition of C/EBPα and PPARγ. Furthermore, disruption of Wnt signaling by expression of a dominant-negative TCF-4 that cannot be activated by β-catenin induces adipogenesis in 3T3-L1 preadipocytes, C3H10T1/2 cells, and NIH-3T3 fibroblasts. It also causes transdifferentiation of myoblasts into adipocytes *in vitro*, suggesting that the Wnt pathway plays a role in mesodermal cell fate determination (34).

During the terminal phase of differentiation, activation of the transcriptional cascade leads to increased activity, protein, and mRNA levels for enzymes involved in triacylglycerol synthesis and degradation. Glucose transporters, insulin receptor number, and insulin sensitivity also increase. Synthesis of adipocyte-secreted products including leptin, adipsin, resistin, and adipocyte-complement-related protein (Acrp30) begins, producing a highly specialized en-

docrine cell that will play key roles in various physiological processes. These complex series of events require the cell to process a variety of combinatorial inputs during the decision to undergo differentiation. The identification of agents and molecules that modulate the adipogenesis process has provided insights into the signal transduction pathways involved. So far, a plethora of hormones, cytokines, and growth factors able to act as positive or negative adipogenic regulators have been identified. Their respective role on preadipocyte differentiation was recently reviewed in detail (5). Briefly, insulin, IGF-1, glucocorticoids, and agents that increase intracellular cAMP levels are generally recognized as positive effectors. Cytokines, growth factors belonging to the TGF-β family, and protein kinase C (PKC) inhibitors are viewed as negative regulators.

The Mature Adipocyte: a Highly Specialized Endocrine Cell

Mature adipocytes, the main cellular component of WAT, are uniquely equipped to function in energy storage and balance under tight hormonal control. However, with the realization that adipocytes secrete factors known to play a role in immunological responses, vascular diseases, and appetite regulation, a much more complex and dynamic role of WAT has emerged. In addition to proteins involved in lipid and lipoprotein metabolism, cytokines, and growth factors, adipocytes also synthesize factors involved in the regulation of food intake and energy homeostasis. Adipocyte-derived factors include leptin, adipsin, acylation stimulation protein, agouti, angiotensin II, prostaglandins, Acrp30, resistin, TNF-α, macrophage migration inhibitory factor, secreted protein acidic and rich in cysteine (SPARC), and PPARγ angiopoietin related (PGAR)/ fasting-induced adipose factor (FIAF).

Leptin, the obese (*ob*) gene product, is a hormone that is primarily made and secreted by mature adipocytes and that plays a crucial role in the regulation of energy balance. Current knowledge on leptin production, regulation, and action has been recently reviewed (3, 36, 37). The functions of agouti, acylation stimulation protein, angiotensin II, and prostaglandins in the regulation of energy balance and whole-body homeostasis have also been recently described (3, 38). Here, we concentrate on four newly described secreted factors that may play a role in energy homeostasis and/or affect key functions in adipose tissue physiology and pathology: resistin, Acrp30, SPARC, and PGAR/ FIAF.

Resistin is an adipocyte-derived secreted product that potentially links obesity to diabetes (39). It belongs to a family of tissue-specific secreted proteins that include resistin-like molecules α and β (RELM-α and RELM-β). Resistin is exclusively expressed in adipose tissue; RELM-α has a restricted tissue distribution with highest levels in adipose tissue and RELM-β is expressed only in the gastrointestinal tract (39–41). Resistin is a thiazolidinedione-regulated protein as evidenced by the finding that thiazolidinedione treatment decreases resistin mRNA in 3T3-L1 adipocytes. Resistin mRNA is markedly induced during adipocyte differentiation as well as in diet-induced and genetic forms of obesity. Its expression is under nutritional and hormonal regulation, with resistin message being very low during fasting and in WAT derived from streptozotocin-treated (i.e., insulin-deficient) rats, and increasing upon refeeding and insulin administration, respectively (39, 40). Immunoneutralization of circulating resistin improves blood glucose levels and insulin action in high fat-fed mice, suggesting that elevated resistin levels may result in decreased insulin sensitivity in obese rodents (39). Interestingly, resistin also exerts an inhibitory effect on adipocyte differentiation; i.e., treatment of 3T3-L1 preadipocytes with conditioned medium from COS cells transfected with murine resistin markedly decreases expression of adipocyte markers. This indicates that resistin may function as a feedback signal to restrict adipocyte formation (40). Such a finding implies that adipocytes express resistin receptors and that resistin is likely to have autocrine, paracrine, and endocrine functions. However, further investigations are required to determine the precise role played by resistin *in vivo*.

Acrp30 (synonyms: AdipoQ, ApM-1, and adiponectin) is an adipocyte-specific secreted protein that displays sequence homology with C1q, the first component of the classical complement activation pathway. Acrp30 mRNA is dramatically induced during adipocyte differentiation (42, 43), and its human ortholog apM1 (renamed adiponectin) is the most abundant gene transcript in human adipose tissue (44). Acrp30 is abundant in normal serum and, *in vitro*, its secretion is enhanced by insulin (43). Its message level is dysregulated in obesity, being significantly reduced in WAT derived from obese mice and humans (42). Moreover, in obese subjects, plasma concentrations of adiponectin are significantly lower than in lean controls (44), suggesting that this adipocyte-secreted factor plays a role in the regulation of energy balance. Supporting this hypothesis is the recent report that the proteolytic cleavage product of Acrp30 (gAcrp30) is able to regulate body weight and lipid metabolism in mice (45). Acrp30 effects on energy homeostasis are mediated, at least in part, by increased free fatty acid oxidation in muscle. Although the molecular mechanisms through which Acrp30 achieves this effect are currently unknown, the possibility of pharmacologically increasing free fatty acid oxidation in muscle may provide new ways to control body weight without interfering with food intake (45).

SPARC (also known as osteonectin or BM-40) is a newly identified factor secreted by the adipocyte (46). SPARC belongs to the matricellular group of proteins known to modulate cell adhesion, differentiation, and angiogenesis. SPARC is not an adipocyte-specific product. In adult animals, its expression is limited largely to remodeling tissues, and it is prominent in tumors and in disorders associated with fibrosis (47). Interestingly, SPARC mRNA is markedly upregulated in several models of experimental obesity, including genetic and chemically and diet-induced

models, suggesting that elevated SPARC gene expression may be a general feature of this pathology (46). SPARC is hormonally and nutritionally regulated, with insulin and high fat feeding each increasing its message levels. Moreover, addition of purified SPARC to cultured rat adipose tissue increases plasminogen activator inhibitor I (PAI-1) mRNA expression, indicating that SPARC is an autocrine/paracrine factor that might contribute to the elevation in plasma PAI-1 observed in obesity (46). Increased PAI-1 levels are associated with increased risk for a coronary event (48), but it is not yet known if SPARC expression is also altered in human obesity or if it plays a significant role in the pathophysiology of this disease. In addition to modulating PAI-1 levels, SPARC may influence several other cellular processes in WAT, including adipocyte hyperplasia and WAT neovascularization, a prerequisite for WAT development (46).

PGAR/FIAF is a peroxisome proliferator-activated receptor target gene that was independently discovered by Kersten *et al.* (2000) and Yoon *et al.* (2000) (49, 50). Murine PGAR was identified by a subtractive cloning strategy designed to obtain gene products expressed in NIH 3T3 cells in a PPAR-γ ligand-dependent way (50). Murine FIAF is identical to PGAR at the amino acid level and was identified by subtractive hybridization comparing liver mRNA from wild-type and PPARα null mice. FIAF mRNA is undetectable in livers of PPARα null mice, indicating it behaves as a PPARα target gene in mouse liver (49). PGAR/FIAF is a novel member of the fibrinogen/angiopoietin-like secreted proteins and is predominantly expressed in adipose tissue and placenta. It is detected in various tissues and in blood plasma, suggesting that it has an endocrine function. Induction of PGAR/FIAF transcript coincides with adipocyte differentiation and, *in vivo*, its expression is modulated by alterations in nutrition and by genetic obesity. PGAR/FIAF mRNA is strongly upregulated by fasting in WAT and liver and is markedly elevated in WAT derived from *ob/ob* and *db/db* obese mice. Moreover, PGAR/FIAF plasma levels are increased by fasting and are decreased by chronic high fat feeding, suggesting that this novel signaling molecule may play a role in the regulation of metabolism, especially under fasting conditions (49, 50). However, further studies are needed to identify the tissues and cell types targeted by PGAR/FIAF and to establish more precisely its role in the regulation of energy homeostasis.

Summary and Perspectives

The exponential increase in knowledge regarding processes involved in fat cell formation has radically modified our thinking regarding WAT function. Identification of PPARγ as a key transcriptional regulator of adipogenesis has led to major advancements in obesity and diabetes research and has provided a molecular link between lipid metabolites and lipid and glucose homeostasis. WAT plays a central role in the regulation of energy balance in physiological and pathological situations. The list of factors produced by the mature adipocytes is growing rapidly, further emphasizing its pleiotropic function. Adipocytes modulate metabolic processes, immune response, reproduction, and hematopoiesis. WAT is a critical endocrine player in obesity and its associated disorders, stressing the need for further identification of novel factors produced by adipocytes as well as for further characterization of the transcription factors that regulate adipocyte determination, differentiation, and function. This will lead to development of innovative therapeutic approaches to human obesity that will most likely involve modulation of adipocyte function rather than manipulation of the adipogenesis process *in vivo*. The latter approach is indeed questionable in the light of the consequences of forced leanness in mice and humans.

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