Cell Metabolism
Minireview

Before They Were Fat: Adipocyte Progenitors

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Adipose tissue mass can expand throughout adult life. Therefore, proliferative adipocyte precursor cells must stand at the ready to respond to increased demand for energy storage. Recent provocative studies have identified discrete immature cell populations from which brown and white adipocytes are derived. This work not only brings fundamental insight into adipose tissue formation but also provides new tools to study how adipogenesis is regulated in pathological conditions such as obesity and diabetes.

Adipocytes are central to the control of energy balance and lipid homeostasis. The ability to store excess energy in adipose tissue is an important evolutionary adaptation. However, obesity, the excess accumulation of adipose tissue, is a risk factor for type 2 diabetes and cardiovascular disease (Kopelman, 2000). As the global rates of obesity are expected to rise for the foreseeable future, this once beneficial adaptation now poses a major health threat. Conversely, lipodystrophy also results in insulin resistance, diabetes, and aberrant lipid metabolism. Thus, understanding the origins of adipose tissue and its links to metabolic diseases is of enormous public interest.

In mammals, adipocytes have been classified into two distinct types: white adipose tissue (WAT), the primary site of energy storage, and brown adipose tissue (BAT), specialized for energy expenditure (Spiegelman and Flier, 2001). White adipocytes express cell type-selective machinery required for triglyceride synthesis from lipoprotein-derived fatty acids as well as hormone-stimulated glucose uptake and lipolysis. In addition, adipocytes have an endocrine role, producing adipokines such as TNFα, leptin, resistin, RBP4, and adiponectin that modulate systemic metabolism (Waki and Tontonoz, 2007). This endocrine function is not selective for white fat cells, as many of these factors are also made by brown adipocytes. WAT is also an important target for the action of human therapeutics—the thiazolidinedione (TZD) antidiabetic drugs being the most notable example (Nolan et al., 1994). The inability to properly store triglycerides in adipose tissue results in adverse effects on glucose metabolism in the liver and skeletal muscle (Saltiel and Kahn, 2001). TZDs may act in large measure by promoting appropriate lipid storage in adipocytes and diverting excess lipid away from skeletal muscle.

In contrast with WAT, the physiological role of BAT is to metabolize fatty acids and generate heat (Spiegelman and Flier, 2001). This specialized function of brown fat cells derives from high mitochondrial content and the ability to uncouple cellular respiration through the action of uncoupling protein-1 (UCP-1). Due to these functional differences, the balance between WAT and BAT affects systemic energy balance and may contribute to the development of obesity. Although the role of BAT in adult humans for many years had been considered to be insignificant, this is now the subject of significant debate in the adipocyte biology community. Recent studies have shown that brown adipocytes are dispersed throughout the human adipose tissues and are metabolically active (Nedergaard et al., 2007). Modulation of brown adipocyte activity has been proposed as a potential strategy to combat obesity and its associated diseases. Dissection of the regulatory pathways that control BAT and WAT development may therefore uncover new opportunities for intervention in metabolic diseases.

The transcriptional cascade required for adipocyte differentiation has been the subject of intense investigation and has been reviewed extensively elsewhere (Gesta et al., 2007; Tontonoz and Spiegelman, 2008). Briefly, the adipogenic program centers on the expression and activation of PPARγ, a lipid-activated nuclear hormone receptor that serves as the master transcriptional regulator of adipogenesis. PPARγ heterodimerizes with RXR and regulates downstream target gene expression leading to the formation of differentiated adipocytes. Three members of the C/EBP family (α, β, δ) also play important roles in differentiation and act in a feedback loop to regulate PPARγ expression. In addition to these central players, Krox20, KLFs, and EBFs have been reported to promote differentiation, while GATA2/3, KLF2, HES-1, and TCF/LEF are inhibitory (Gesta et al., 2007; Tontonoz and Spiegelman, 2008).

Despite substantial progress in defining adipocyte transcriptional control mechanisms, we still have only limited information regarding the identity and localization of adipocyte precursors in vivo. Although most WAT development occurs during late prenatal and early postnatal life, it retains the ability to expand during adult life when energy intake exceeds energy expenditure. This is accomplished through both increased adipocyte numbers (hyperplasia) as well as increased adipocyte size (hypertrophy) (Hirsch and Batchelor, 1976). Furthermore, recent studies suggest that approximately 10% of the body's fat cells are regenerated each year (Spalding et al., 2008). However, mature adipocytes are postmitotic. In order to support the expansion of adipose tissue mass if needed and to maintain adipose dynamics in adult, proliferative adipocyte precursor cells must exist and be poised to respond to metabolic demands. A number of laboratories have embarked upon the search for such precursors, and these efforts have recently begun to yield hefty fruit.

In a recent publication in *Science*, Graff and colleagues generated PPARγ-reporter strains based on the reasoning that PPARγ expression is the defining feature of the adipocyte (Tang et al., 2008). To perform lineage analysis, the authors generated PPARγ-R26R (PPARγ-tTA [tet TransActvator]; TRE-CRE

[tTA-responsive Cre]; Rosa-flox-stop-flox-LacZ [express LacZ in response to Cre]) mice. Surprisingly, the expression of lacZ from the PPARγ-R26R allele within adipose depots remained relatively constant at postnatal day 30 (P30) despite the administration of the tTA inhibitor doxycycline from P1. This result suggested that even at P1 there were PPARγ-cells already present and appeared to contradict the classical idea that adipocyte formation occurs later during development. However, it should be noted that it has been clear to adipocyte researchers for some time that undifferentiated preadipocytes express low levels of PPARγ and can be driven to differentiate simply by providing PPARγ ligand (Tontonoz and Spiegelman, 2008). Tang et al. verified their result using a second reporter, PPARγ-GFP (PPARγ-tTA; TRE-H2B-GFP), that is stably expressed in postmitotic cells but whose signal becomes attenuated in proliferating cells upon tTA inhibition. This approach also confirmed the proliferative capacity of these GFP^+ PPARγ-expressing cells. Further analysis of these GFP^+ cells found in the adipose stroma-vascular fraction by FACS revealed high expression of the preadipocyte markers Pref1, GATA3, Wisp2, Smo, and Gli3 but lower levels of mature adipocyte markers. These GFP^+ putative precursor cells were capable of differentiating into mature white adipocytes in vitro and formed ectopic GFP^+ fat when transplanted into nude mice.

Previous work had postulated that precursors may exist in the adipose vasculature (Sengenes et al., 2005). In support of this idea, Tang et al. localized their GFP^+ PPARγ-expressing precursor cells to the vasculature. Furthermore, these GFP^+ cells colocalized with so-called mural cells as shown by SMA, PDGFRβ, and NG2 staining. The authors also found that $PDGFR\beta^+$ cells from adipose tissue exhibited adipogenic potential both in vitro and in vivo. On the other hand, $PDGFR\beta^+$ cells from other tissues and $SM22^+$ (a vascular smooth muscle cell marker) cells did not have adipogenic potential, suggesting that the adipocyte precursor cells are a discrete subset of mural cells.

Working independently, Friedman and colleagues have also reported in a recent publication in *Cell* the isolation of white adipocyte precursor cells (Rodeheffer et al., 2008). Using FACS to analyze the expression of cell-surface and stem cell markers, these investigators isolated two cell populations, one $CD24^+$ (lin^-:$CD29^+$:$CD34^+$:$Sca\text{-}1^+$:$CD24^+$) and one $CD24^-$, that were capable of developing into adipocytes in vitro. Remarkably, the precursor cells isolated by the Graff group were also found to display similar markers, suggesting that the two laboratories have identified similar (if not identical) precursor populations.

To determine whether their FACS-sorted subpopulations could develop into fat cells in vivo, Rodeheffer et al. transplanted 50,000 GFP-labeled donor cells into the fat depots of A-Zip lipodystrophic mice (Rodeheffer et al., 2008). These fatless mice express a dominant-negative bHLH protein specifically in adipocytes and exhibit a lipoatrophic diabetic phenotype (Moitra et al., 1998). The authors found that injection of the $CD24^+GFP^+$ precursor cells led to the development of fat depots showing normal adipocyte morphology that were almost entirely GFP^+. However, the $CD24^-$ and $CD34^-$ populations failed to reconstitute WAT after transplantation into A-ZIP mice, suggesting that $CD24^+$ populations are the true source of white adipocyte precursors in vivo. The question of what is special about the lipoatrophic background that allows these cells to develop into adipocytes remains to be addressed. It would also be interesting to know whether cells with similar surface marker expression derived from other tissues could also give rise to adipocytes.

Rodeheffer et al. also used noninvasive imaging methods, as previously developed by this group, to follow the maturation of adipocyte precursors in a living animal (Birsoy et al., 2008). $CD24^+$ cells derived from mice expressing luciferase from the adipocyte-specific leptin promoter were injected into the fat depots of A-Zip mice. In vivo, the $CD24^+$ cells displayed a steady increase in luciferase expression beginning 2 weeks after injection. Interestingly, luciferase activity was detected only in adipose tissue, demonstrating the unique developmental potential of this cell population. Moreover, consistent with the expected increase in adipocyte number in response to dietary challenge, a robust luciferase signal from $CD24^+$ cells was observed following injection into high-fat-fed mice but not in chow-fed mice.

In prior studies of A-Zip mice, Reitman and colleagues were able to correct the insulin resistance and hyperglycemia by transplanting a large mass of normal WAT (~1 g) (Gavrilova et al., 2000). Remarkably, in their current work Friedman and colleagues showed that injection of only 50,000 $CD24^+$ cells enabled the formation of normal-sized WAT depots and rescued the lipodystrophic phenotype. Plasma glucose and insulin levels in A-Zip mice injected with $CD24^+$ cells were also normalized and the plasma level of adiponectin, an adipokine secreted from mature fat, was increased. These data clearly established that the fat cells reconstituted from $CD24^+$ cells are functional. The identification of adipocyte precursor cells by both of these research teams may lead to a better understanding of adipose tissue formation and provide the tools to study factors that modulate formation of adipose tissue in pathological contexts.

Adipose tissues, muscle, and bone originate from the mesoderm. The prevailing model has held that a common mesoderm/mesenchymal stem cell (MSC) gives rise to bone, muscle, WAT, and BAT in response to appropriate developmental cues (Figure 1). An intermediate step in this developmental program has been assumed to be the differentiation of a common adipose precursor cell capable of giving rise to both WAT and BAT. One observation pointing to the possible existence of such a common precursor is the ability of WAT to transdifferentiate into BAT under certain experimental conditions. However, recent work by Timmons and colleagues strongly argued that brown and white preadipocytes are distinct (Timmons et al., 2007). This observation was verified and extended by Spiegelman and colleagues, who showed that WAT and BAT in fact are derived from distinct precursor populations (Seale et al., 2008). These authors showed that skeletal muscle progenitor cells can give rise to either muscle cells or brown fat cells, but not white fat cells.

Seale et al. further showed that the decision of this precursor to become muscle or BAT is controlled by the transcription factor PRDM16. Expression of PRDM16 causes Myf5-expressing muscle precursors to commit to becoming brown fat cells, whereas reduction of PRDM16 expression by shRNA targeting induces the myogenic differentiation program. Interestingly, other groups had previously observed that myogenin-deficient neonatal mice not only have less muscle but also exhibit an accumulation of brown fat cells in areas where muscle would normally develop (Hasty et al., 1993). Also consistent with the idea of a common muscle and BAT precursor is the fact that muscle performs

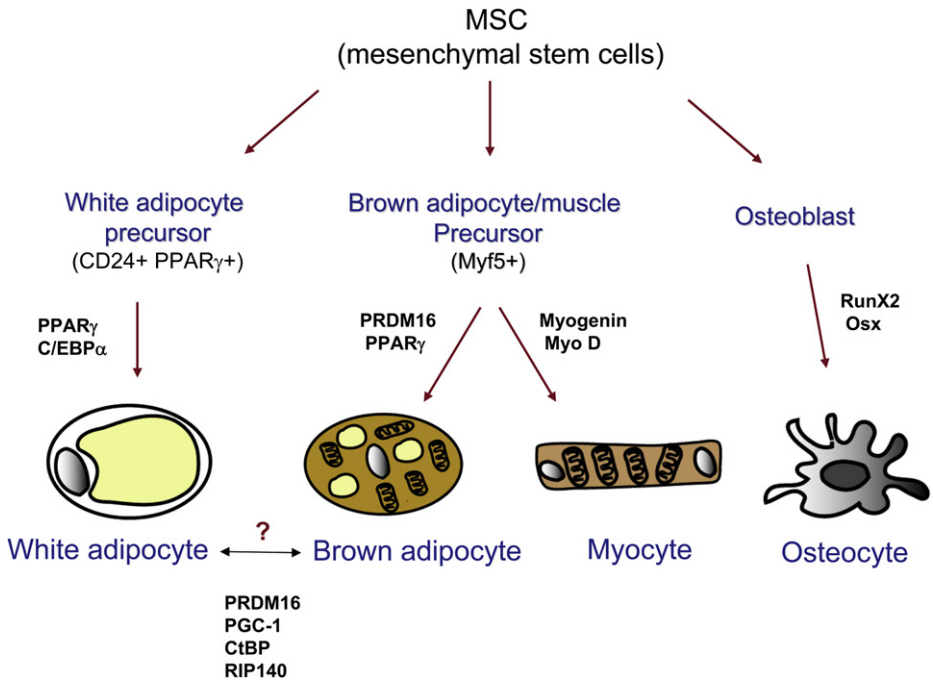

Figure 1. A Model for the Development of White and Brown Fat Cells

Mesenchymal stem cells give rise to precursor cells of bone, muscle, and fat cells under appropriate conditions. PPARγ$^+$ CD24$^+$ white adipocyte precursor cells reside in mural cell compartments of the adipose vasculature. White adipocyte differentiation is driven by the transcription factors PPARγ and C/EBPs, giving rise to triglyceride-storing WAT. This proliferating, WAT precursor cell population continually reconstitutes WAT depots throughout adult life and can respond to increased demand for energy storage with increased differentiation. Brown fat cells share precursors (Myf5$^+$) with muscle cells but not with white adipocytes. Induction of PRDM16 expression in Myf5$^+$ cells directs them to develop into brown fat cells. In the absence of PRDM16, these precursor cells will develop into muscle cells under the influence of the transcription factors myogenin and MyoD. PRDM16 is able to interact with PGC-1 and CtBPs to activate brown fat genes and suppress white fat genes, respectively. The cofactor RIP140 and CtBPs can also suppress mitochondrial gene expression in white adipocytes. An outstanding issue is whether "transdifferentiation" between WAT and BAT occurs in physiological contexts.

BAT-like function in some species. For example, birds are homeotherms dependent on the expression of UCP in muscle for thermoregulation (Mozo et al., 2005). The discovery that muscle and BAT share common progenitor cells may also help to explain the functional dimorphism of adipose tissues, with WAT acting primarily to storage lipid and BAT serving to metabolize lipids to produce heat. The work of Spiegelman and colleagues provides a vivid illustration of the axiom that looks can be deceiving. Although the WAT and BAT are superficially similar in that they both contain lipid droplets, they appear to stem from divergent developmental programs.

Investigation of their developmental origins will undoubtedly lead to better understanding of WAT and BAT biology. Looking further into the future, it is intriguing to contemplate potential translational applications of these discoveries for the treatment of metabolic diseases. However, the journey toward this goal must begin with the investigation of a number of outstanding questions. For example, are there two routes toward BAT formation (i.e., one from muscle and one from transdifferentiated WAT)? When do white and brown precursor cells arise during embryonic development? Are the WAT adipocyte precursor cells identified by Friedman and Graff and the muscle/BAT precursor identified by Spiegelman derived from a common mesenchymal cell? What are the molecular determinants that control PRDM16 expression in BAT precursors and PPARγ expression in WAT precursors? Can we identify small molecules that modulate the development of BAT and WAT from precursor cells? Addressing these and other issues may provide the foundation for the development of new approaches for the therapeutic manipulation of energy balance and the prevention or treatment of metabolic diseases.

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