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Review

White and brown adipose stem cells: From signaling to clinical implications ☆

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ABSTRACT

Epidemiological studies estimate that by the year 2030, 2.16 billion people worldwide will be overweight and 1.12 billion will be obese [1]. Besides its now established function as an endocrine organ, adipose tissue plays a fundamental role as an energy storage compartment. As such, adipose tissue is capable of extensive expansion or retraction depending on the energy balance or disease state of the host, a plasticity that is unparalleled in other organs and – under conditions of excessive energy intake – significantly contributes to the afore mentioned obesity pandemic. Expansion of adipose tissue is driven by both hypertrophy and hyperplasia of adipocytes, which can renew frequently to compensate for cell death. This underlines the importance of adipocyte progenitor cells within the distinct adipose tissue depots to control both energy storage and endocrine functions of adipose tissue. Here we summarize recent findings on the identity and plasticity of adipose stem cells, the involved signaling cascades, and potential clinical implications of these cells for the treatment of metabolic dysfunction in obesity. This article is part of a Special Issue entitled Brown and White Fat: From Signaling to Disease.

1. Introduction

Once considered an inert mass of stored energy, the past two decades have seen a surge in interest in the complexity of adipose tissue and its role in disease. In addition to serving as a site for energy storage, adipocytes secrete proteins involved in inflammation, appetite regulation, blood pressure control and energy balance [2–5]. Adipocytes are unique in their ability to store large quantities of lipids that can be rapidly released and used for energy by other organs, when necessary; however, excessive adipose tissue, particularly in the visceral adipose tissue depot, is associated with increased risk of insulin resistance, cardiovascular disease and cancer [6–9]. There exists two distinct kinds of adipose tissue: white adipose tissue (WAT), specifically designed for energy storage, and brown adipose tissue (BAT), which derives from a muscle-like precursor and, when activated, burns energy under conditions of non-shivering thermogenesis. Through the expression of uncoupling protein 1 (UCP1) BAT uncouples oxidative phosphorylation from the synthesis of ATP, resulting in the generation of heat and serves to protect an organism from hypothermia. The role of BAT in human physiology has been overlooked in the past; however, since the discovery of metabolically active BAT in adult humans [10], it resumed a place in the spotlight. Given the advent of the obesity pandemic in "westernized" countries, much attention has been paid to understanding the signals involved in the differentiation of such "energy burning" brown adipocytes. Adipose tissue is capable of extensive expansion or retraction depending on the energy balance or disease state of the host, a plasticity that is unparalleled in other organs. Expansion of adipose tissue is driven by both hypertrophy and hyperplasia of adipocytes, which can renew frequently to compensate for cell death, suggesting the necessity of adipocyte progenitor cells within the adipose tissue depot, that are capable of differentiating into mature and functional adipocytes.

2. Developmental origin of adipose tissue

Formation of adipose tissue commences shortly after birth in most rodents and during mid-gestation in higher mammals, including humans with both types of adipose (white adipose tissue and brown adipose tissue) originating from the mesoderm. While the formation of white adipose tissue commences shortly after birth, brown adipose tissue develops before birth, as its function is to protect a newborn against cold after transition from intrauterine environment [11].

The generation of mature adipocytes is comprised of two phases: determination and differentiation. In this process, multipotent stem cells become adipoblasts that can further differentiate into preadipocytes, cells already committed to become fat cells. Under appropriate stimulation, in the ultimate phase of differentiation, preadipocytes convert to mature, lipid-laden adipocytes.

The first phase, determination, involves the commitment of multipotent stem cells to the adipocyte lineage, at which point the

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cell is referred to as a pre-adipocyte. During the second phase, terminal differentiation, the pre-adipocyte undergoes multiple rounds of mitosis before exiting the cell cycle and differentiating into a mature adipocyte. In the progression from pre-adipocyte to adipocyte, the cell takes on the characteristics and metabolic capabilities of a mature adipocyte while under the control of a series of tightly regulated transcriptional and morphological changes. WAT is readily available for study from human patient samples and experimental animal models; however, it is difficult to maintain *in vitro* and cannot be expanded. Terminal differentiation is more extensively characterized in immortalized cell lines, such as the mouse line 3T3-L1 [12,13], which can undergo one or two rounds of cell division prior to differentiation and pre-adipocyte cell lines that differentiate without post-confluence mitosis (e.g. C3H10T1/2). Pre-adipocytes maintain the ability to divide and have been reported to have a turnover rate of up to 4.5% and 5% per day for humans and mice, respectively. Mature adipocytes are widely believed to have lost the ability to divide following the completion of terminal differentiation, and adipocyte turnover has been reported to be up to 10% per year [14].

2.1. White adipogenesis

2.1.1. Adipose tissue resident progenitors

Adipose tissue is characterized by striking plasticity, namely the capability of expansion and retraction, depending on changes in energy supply and demand, respectively. In adulthood, two means of adipose tissue expansion exist, hypertrophy and hyperplasia, which are complementary to each other. Hypertrophy is characterized by the enlargement of adipocytes, while hyperplasia leads to an increased number of adipocytes. As mature adipocytes have lost the ability to divide, hyperplasia of adipose tissue must be achieved by differentiation of precursor cells. Adipocyte progenitors derive from pluripotent mesenchymal stem cells (MSC) (Fig. 1), which are capable of differentiating into cells of both mesodermal (myocytes, adipocytes, chrondrocytes and osteoblasts) [15,16] and non-mesodermal [17–19] origin.

Adipocyte progenitors arise from the mesoderm and are localized to areas along blood capillaries [11], indicating that adipose tissue develops in coordination with vasculature. MSC can be isolated from the stromal vascular fraction (SVF) of adipose tissue; however, the proliferative and adipogenic capacity of these cells is significantly affected by the location of the depot from which they were isolated and diverse conditions such as aging and obesity [20].

In contrast to hematopoietic stem cells, a clear definition of cells considered to be adipose tissue stem cells is lacking; however, Rodeheffer et al. [21] largely contributed to the characterization of adipocyte precursors by applying fluorescence-activated cell sorting (FACS) on freshly isolated SVF to separate distinct cell subpopulations based on well-defined stem cell markers. Thus, hematopoietic and endothelial cells were sorted out from the SVF, leading to the creation of a lineage-negative (Lin−) population. From the Lin− population, cells positive for antigens CD34 and CD29 were selected as they demonstrated high adipogenic potential. Finally, based on staining for stem cell antigen 1 (Sca-1) and CD24, two surface markers known to be expressed on stem cells in different tissues, the progenitor population was defined as: Lin−/CD29+/CD34+/Sca-1+/CD24+, and its adipogenic potential was confirmed *in vivo* by injecting Lin−/CD29+/CD34+/Sca-1+/CD24+ cells into lipodystrophic or high fat diet fed mice, where this cell population was capable of forming functional adipose tissue.

In order to identify adipose tissue progenitors and their localization within fat tissue, Tang et al. [22] used lineage tracing based on the labeling of cells that express peroxisome proliferator-activated receptor gamma (PPARγ), the master regulator of adipogenesis. Labeled cells were found in the SVF and exhibited considerable proliferative capacity and adipogenic potential, both in culture and after transplantation to nude mice. Immunohistochemical examination revealed that PPARγ expressing cells were localized to the adipose vasculature and express the above mentioned Sca-1 and CD34, in addition to known mural cell markers (smooth-muscle actin (SMA), platelet-derived growth factor receptor beta (PDGFRβ) and chondroitin sulfate proteoglycan 4 known as NG2), indicating that adipocyte progenitors reside in the mural cell compartment of adipose depot. A close relationship between newly arising adipocytes and vasculature was also shown [23] using reporter genes expressed under the VE-cadherin promoter. VE-cadherin is indispensable for vasculature formation; however, it was also found to be expressed in pre-adipocytes that are characterized by the pre-adipocyte determination marker Zfp423, a multi zinc finger transcriptional regulator [24]. Together with morphological, immunohistochemical and gene expression analysis these results support the notion that pericytes (endothelial cells that surround capillaries) could serve as a source of adipocyte progenitors.

Despite the widely accepted model that WAT arises exclusively from Myf5 (myogenic regulatory factor) negative progenitors, it has recently been shown that a deletion of PTEN (phosphatase and tensin homolog) in Myf5 positive cells unexpectedly affected adipogenesis of WAT. Lineage tracing experiments in mice expressing yellow fluorescent protein in Myf5 positive cells revealed interscapular and retroperitoneal WAT derived from Myf5 positive progenitors [25]. These white depots did not display the BAT gene expression signature, which indicates that Myf5 positive progenitors give rise to a subset of white adipocytes. Studies of progenitor cells in humans are primarily based on FACS analysis and immunohistochemistry. Similar to mice, human adipose tissue-derived stem cells were shown to express stem cell markers Sca-1 and CD34 and were localized in close proximity to the vascular network [26]. Furthermore, pericyte marker CD146 has been detected on adipose-derived progenitors and the adipogenic potential of those cells was confirmed after cell sorting [27]. Under angiogenic conditions, human adipose tissue explants have angiogenic capabilities and can form new blood vessels, *ex vivo*, lined with cells containing lipid droplets [23]. Overall, recent reports strongly support the endothelial origin of human adipocytes, and propose a close relationship between adipogenic and angiogenic processes.

2.1.2. Adipose tissue non-resident progenitors

Previously, the proposed source of progenitors for formation of new adipocytes has been restricted to adipose tissue resident cells; however, a reason for re-evaluation of this notion has emerged. It was shown that progenitors from other tissues could also contribute to the generation of new adipocytes [20,28,29]. Bone marrow containing a marked number of hematopoietic and mesenchymal progenitors appears to be a promising candidate (Fig. 1). After transplantation of a green fluorescent protein (GFP)-labeled bone marrow-derived hematopoietic cell subpopulation into irradiated wild-type mice, GFP-expressing adipocytes appeared in the adipose tissue of recipient animals [28]. Selection of cells positive for a murine myeloid marker, CD11b, confirmed the myeloid origin of GFP-labeled adipocytes. Furthermore, adipocytes originating from the myeloid lineage were characterized by increased expression of inflammatory and chemotactic genes. These cells accumulated preferentially in visceral adipose tissue, which is consistent with the increased inflammatory state of this depot, compared to subcutaneous adipose tissue [20].

2.2. Brown adipogenesis

Although conventional brown and white adipocytes differ considerably in function and morphology, they have long been considered to share a common progenitor. Both adipose tissues originate from MSC; however, brown adipocytes, unlike white adipocytes, are derived from the myogenic lineage, characterized by Myf5 expression (Fig. 1). After deletion of PR domain containing 16 (PRDM16), the transcriptional regulator of brown adipocyte determination, in brown adipocyte precursors, the resulting cells exhibited a skeletal