White Adipose Tissue

Subjects: Cell & Tissue Engineering

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The immune and endocrine dysfunctions of white adipose tissue are a hallmark of metabolic disorders such as obesity and type 2 diabetes. In humans, white adipose tissue comprises distinct depots broadly distributed under the skin (hypodermis) and as internal depots (visceral). Depot-specific ASCs could account for visceral and subcutaneous adipose tissue properties, by regulating adipogenesis and immunomodulation. More importantly, visceral and subcutaneous depots account for distinct contributions to obesity and its metabolic comorbidities. Distinct ASCs subpopulations were also described in subcutaneous adipose tissue. Interestingly, the superficial layer closer to the dermis shows hyperplastic and angiogenic capacities, whereas the deep layer is considered as having inflammatory properties similar to visceral.

Keywords: obesity ; adipose tissue ; adipose stem/stromal cells ; spheroids ; organoids

1. Subcutaneous and Visceral Depots and the Intrinsic Differences of Adipose Tissue-Derived Stem/Stromal Cells

White adipose tissue (WAT) acts primarily as a regulatory center for the homeostasis of the body's energy metabolism. This is accomplished through the regulation of adipocyte lipid storage or release in response to the body's energy demands, regulation of blood glucose levels due to the high insulin sensitivity of adipocytes [1], and through its secretory function [1][2]. The array of adipose secretory products, called adipokines, mediates inter-organ communication. This influences the metabolism and function of central and peripheral organs, including the immune system function [2][3].

WAT is one of the body's pathways that, through remodeling of adipose tissue, allows adaptation to metabolic challenges posed by different external environmental changes, including food deficit, energy excess, stress, infection, or cold [4]. Crosstalk between different cell types composing the WAT stromal-vascular fraction (SVF) and adipocytes orchestrates the mechanisms of WAT remodeling [5]. Physiological remodeling that occurs during WAT expansion is characterized by angiogenesis, extracellular matrix remodeling, minimal inflammation, and adipocyte hyperplasia, through the recruitment of adipocyte precursors that provide capacity to store extra lipids during its adipogenic differentiation into small adipocytes (i.e., adipogenesis). The ability of WAT depots to expand through hyperplasia may be crucial for adequate lipid storage during WAT expansion and to avoid harmful ectopic lipid deposition in non-adipose tissues [6]. However, the chronic demand for excess energy storage that occurs during the development of obesity can trigger mechanisms of an unhealthy —or pathologic—WAT remodeling during its expansion, characterized by dysfunctional hypertrophic adipocytes, insufficient vascularization and hypoxia, fibrosis [4][5], infiltration of immune cells, and pro-inflammatory responses that contribute to tissue inflammation and subsequently insulin resistance [7][8][9].

WAT depots with distinct expansion and remodeling patterns are widely distributed in the human body, comprising mainly subcutaneous and visceral depots. They have intrinsic biological differences [10] and differentially impact on obesity-induced metabolic complications: in obesity, visceral adipose tissue (VAT) is more associated with the risk to develop insulin resistance and type 2 diabetes than subcutaneous adipose tissue (SAT) [11]. These functional differences among WAT depots and their distinct contributions to obesity and its metabolic comorbidities are being attributed not only to differences in their SVF composition (i.e., frequency of mesenchymal stem cells, pre-adipocytes, endothelial progenitor cells, mature endothelium, and immune cells subtypes), but also to the functional diversity of adipocyte stem cells and progenitors cells that derive specialized adipocyte subtypes [12][13][14][15].

The SVF of obese abdominal SAT in humans has the highest content of preadipocytes compared to the VAT. More importantly, adipocyte stem and progenitor cells' in vitro counterparts, here referred to as the adipose-derived stromal/stem cells (ASC), are depot-specific, with an inverse relationship between the adipogenic and immunogenic status: ASC from VAT are more pro-inflammatory in terms of cytokine secretion, with lower adipogenic potential [13]. Indeed, proliferation of adipose precursors induced by a high-fat diet is regulated in a depot-dependent manner in mice [16][17][18]. Moreover, a subpopulation of murine adipose perivascular progenitor cells (platelet-derived growth factor receptor-beta positive, PDGFRβ$^+$), termed "fibro-inflammatory progenitors" (FIPs; LY6C$^+$PDGFRβ$^+$ cells), regulates

visceral WAT macrophage accumulation in mice fed a high-fat diet in a Toll-like receptor 4-dependent manner resulting in WAT dysfunction [19]. PDGFRβ+ perivascular cells are precursors of adipocytes in mice, both in inguinal and visceral WAT pads, with varying levels of the zinc finger protein 423 preadipocyte commitment factor (Zfp423) that distinguish subpopulations of adipogenic from inflammatory cells [20]. These authors also described that the frequency of adipogenic precursors and adipogenesis is depot-specific.

Recent efforts on single-cell level profiling of adipocyte progenitors and on clonal analysis of their in vitro counterparts (ASC) derived from different WAT depots of donors with a variety of metabolic phenotypes have clarified the understanding of ASC heterogeneity, their depot-dependent characteristics, and their contribution to the obesity scenario [15]. Vijay and coworkers [21] characterized different cell types that are WAT depot-specific or correlate with metabolic status (with or without type 2 diabetes) using single-cell RNA sequencing (scRNA-Seq) of WAT depots from obese donors. The authors identified progenitor cells with expression signatures that were dependent on their respective WAT depot. More importantly, a higher abundance of a subtype of preadipocytes was identified in individuals with hyperglycemia levels compared to those with normal glycemia. In addition, lymphatic-derived endothelial cells were more frequent in the VAT samples.

The intrinsic differences in ASC derived from VAT and SAT are also present in a non-obese state and are retained during obesity. The immunogenic factor bone marrow stromal cell antigen 2 (*BST2*) was identified as a marker of visceral ASC in a non-obese state in humans. However, the difference in *BST2* expression between subcutaneous and visceral ASC is more pronounced in obesity and with insulin resistance. In addition, in vitro-derived adipocytes from non-obese ASCs of VAT have lower gene expression of adipogenic markers and higher gene expression of immunogenic markers than those derived from SAT [22]. Raajendiran and collaborators [14] identified three adipocyte progenitor cell subtypes with distinct molecular patterns, but with similar adipogenic capacity. The characterization of the adipocyte progenitor cell subtypes was based on the expression of CD34 among $CD31^{-}CD45^{-}CD29^{+}$ SVF cells. Interestingly, adipocytes derived from each progenitor subtype display distinct metabolic and endocrine phenotypes. Furthermore, adipocyte progenitors were more frequent in gluteo-femoral SAT than in abdominal SAT and VAT, and the frequency of adipocyte progenitor subtypes varies among donors with type 2 diabetes. ASCs showing depot-specific genetic [23], adipogenic, immunogenic, endocrine, and even extracellular vesicles [24] profiles are an interesting source of cells to decipher the cellular and molecular mechanisms that govern WAT physiology and dysfunction in a depot-specific manner.

The preferable expansion of gluteo-femoral SAT depots is typical of women and is associated with a lower risk of cardiometabolic dysfunction. On the other hand, VAT depot is expanded preferentially in men and is a predictor of cardiometabolic disease [25][26][27][28]. A recent study supported these clinical findings showing a depot- and sex-dependent adipose progenitor cell heterogeneity in mice [29].

Adipocyte stem and progenitor cell heterogeneity and depot-dependent characteristics from adipose SVF have been widely explored at the single-cell level in murine-derived samples [30]. However, the extent to which the tissue architecture and composition of mouse adipose tissue resembles that of humans is still unknown and limits the translation of obesity-related findings from murine to human. Vijay and coworkers [21] have recently explored SVF heterogeneity in adipose samples derived from obese donors using single-cell transcriptomics. In addition, ASCs' depot-dependent profile has been described for human WAT during the last decade [12][13][22]. Therefore, there is a need to specifically study human adipose tissue-related physiology and diseases in experimental systems engineered with human-derived cells with depot-specific profiles to mimic key morphofunctional properties.

2. Subcutaneous Adipose Tissue Layers as Adipose Tissue-Derived Stem/Stromal Cells Microenvironments

Human SAT is separated into two layers by a dense conjunctive tissue named fascia [31]. The superficial layer is located close to the dermis, having well-defined and compacted lobules, while the deep layer shows more loose and disorganized larger lobules [32]. The superficial layer of SAT supports properties already described for the entire tissue such as anti-inflammatory and regenerative, acting as a protective depot in metabolic syndromes [33][34]. This layer contains a higher number of small adipocytes, as well as larger lipid droplets compared with deep SAT, showing a high potential for adipogenesis [35][36][37][38][39][40].

The SVF of the superficial layer showed the highest percentage of preadipocytes and a predominant presence of arterioles [35]. The adventitia layer of arterioles was previously described as a preadipocyte niche in SAT [41]. Furthermore, ASCs derived from the superficial layer have a significantly greater surface of lipidic droplets together with the highest

number of unilocular cells and up-regulation of CEBPα and FABP4 genes [35], supporting a high adipogenic potential previously described for this layer.

On the other hand, the deep layer of SAT is correlated with high levels of inflammatory cytokines and adipokines, besides its disproportionate expansion observed in obese Caucasian males [42][43]. ASCs derived from the deep layer revealed the lowest levels of adipogenic and secretory capacities [35]. Monzon and collaborators [44] compared the lipolysis in adipocytes isolated from the deep and superficial layers and found an increase in lipolysis in the deep layer.

3. The Stem/Progenitor Cells Derived from the Fascial System of Subcutaneous Adipose Tissue

SAT is inserted into the fascia to form a structural and functional continuity over the body [45]. Each fascia system at distinct tissues and organs operates independently, but at the same time is interdependent with the whole system [46].

Young rats have a primitive SAT, making them an ideal animal model for investigating adipose tissue origin. In this context, Su and collaborators [47] described the generation of adipocytes from the adventitia of blood vessels leading to the formation of primitive adipocytes lobules in the fascia. Zhang and collaborators [48][49] demonstrated that the superficial fascia of rats has a population of adipocyte progenitor cells capable of forming adipose tissue organoids with functional unilocular adipocyte-like cells.

In human SAT, the conjunctive extensions derived from the fascia are more prominent at the superficial layer, being named retinacula cutis [50]. The retinacula cutis of the superficial layer showed double positive staining for CD34 and CD31, revealing the presence of endothelial progenitor cells. Interestingly, Pref-1 staining was found exclusively in retinacula cutis and in adventitia of blood vessels while it was absent in adipose tissue itself [35]. Furthermore, ASCs derived from retinacula cutis showed the highest secretion in vitro for vascular endothelial growth factor (VEGF) compared with ASCs from both superficial and deep layers [35]. Recently, Ziegler and collaborators [51] showed an angiogenic genetic profile in the human fascia matrix, supporting the results with ASCs derived from retinacula cutis.

Interestingly, retinacula cutis revealed a continuity with the adventitia of blood vessels, being the adventitia niche more frequent in the superficial layer of SAT [35]. A previous study by a research group found that SAT samples from ex-obese subjects had a higher number and size of blood vessels and revealed a preferential location of these blood vessels close to the dermis [52]. These results were later associated with an increase in preadipocytes in SVF of ex-obese SAT samples [53].

4. The Dermis Can Be Stratified According to Its Fibroblasts Subpopulations

The dermis is a connective tissue located between the epidermis and the hypodermis. The dermis is composed of two different layers, a papillary dermis just below the dermo–epidermal junction and deeper at the reticular dermis. Fibroblasts are the most abundant cells in the dermis; they secrete and remodel the extracellular matrix (ECM) which allows the dermis to be a support tissue for the skin. There is an ECM signature of the different fibroblasts subpopulation [54]. The papillary dermis presents an important cellular density with more proliferative fibroblasts than the reticular dermis [55][56]. ECM of the papillary dermis present collagen fibrils loosely organized, thin unstriated fibrillar material, and proteoglycan aggregates [57]. The papillary fibroblast regulates hair growth and plays an indispensable role in re-epithelialization during wound healing [58]. The papillary dermis role is to interact physically and chemically through growth factors with the epidermis. The reticular dermis is thicker and presents an ECM with aligned collagen fibrils and a dense network of elastin [57]. Its role is to confer tensile strength to the dermis. The reticular fibroblast initiates healing by matrix production [59].

In addition to their role in ECM regulation, fibroblasts also interact with other cell types such as ASCs, located in SAT just beneath the reticular dermis. Haydont and collaborators [60] recently described a high adipogenic potential for a subpopulation of fibroblasts located at the dermo–hypodermal junction of the skin. The fascia has extensions originating from the dermis passing through the superficial layer and becoming looser and scarcer until reaching the deep SAT [50]. If dermo–hypodermal fibroblasts dwell at these extensions, one hypothesis is that these cells can infiltrate the SAT, and due to their plasticity, these fibroblasts can assume different behaviors according to the niche.

If fibroblasts can interact with ASCs modulating their behavior, ASCs can also do the same with fibroblasts, as suggested by in vitro assays.

ASC's conditioned culture medium increased proliferation of dermal fibroblasts [61][62]. This proliferative effect of ASC's conditioned culture medium in fibroblasts can be decreased by TGF-beta1 [63]; however, it does not interfere with the secretion of ECM; more specifically, the secretion of type I collagen and fibronectin are increased [61][63]. Interestingly, ASC's conditioned culture medium can decrease the secretion of metalloproteinase, which can explain, in part, the increase of ECM secretion by fibroblasts [64]. Auxenfans et al. [65] also demonstrated that a 3D-reconstructed skin model co-cultured with ASCs increased thickness of the dermis, specifically the papillary dermis. In direct juxtacrine co-culture and in indirect paracrine co-culture, ASCs improved collagen maturation and metalloproteinase secretion compared to monoculture [66].

The beneficial effect of ASCs in dermis is even observed in altered conditions, such as keloid scar, since TGF-beta1-induced myofibroblast differentiation and human dermal fibroblasts' function were inhibited by ASC's conditioned culture medium [67]. In addition, Borrelli et al. [68] identified a subpopulation of ASCs positive for CD74 with enhanced antifibrotic effects. Dermal fibroblasts incubated with ASC's conditioned culture medium from CD74 positive cells produced less collagen under TGF-beta1 stimulus compared to those incubated with ASC's conditioned culture medium from CD74 negative cells. ASCs positive for CD74 may attenuate production of pro-fibrotic ECM components by fibroblasts and could promote improvement of detrimental histologic and biomechanical changes to skin following skin radiation injury.

To conclude, the scientific literature supports the crosstalk between dermal fibroblasts and ASCs that impact skin quality. Soluble factors secreted by ASCs seem to regulate the skin microenvironment according to the needs. Surprisingly, a recent study showed that diabetic microenvironment-preconditioned ASCs effectively strengthen the capacity against inflammation and modulate the progress of long-term T2D complications [69]. In addition, patients receiving fat grafting in subcutaneous areas exposed to radiation injury show improved cosmetic and functional skin outcomes, such as skin softness and pliability increase, volume restoration, hair growth improvement in areas of alopecia, and pain decrease [70]. It is suggested that endogenous stem and progenitor cells that reside within the SVF of adipose tissue could drive regenerative mechanisms by which fat grafting can slow or reverse skin radiation-induced fibrosis [71]. Indeed, fat grafts enriched with human CD34+CD146+ adipose-derived stromal cells enhance fat graft retention and vascularization and promote recovery of soft tissue after radiotherapy in mice [72]. However, there is a lack of research studies focusing on the specific interaction of ASCs with fibroblasts' subpopulations, and more importantly, to what extent the ontogeny of fibroblasts and ASCs is correlated, since dermal fibroblasts can infiltrate the SAT.

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