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Cellular Heterogeneity in Adipose Tissues

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Abstract

Adipose tissue depots in distinct anatomical locations mediate key aspects of metabolism, including energy storage, nutrient release, and thermogenesis. Although adipocytes make up more than 90% of adipose tissue volume, they represent less than 50% of its cellular content. Here, I review recent advances in genetic lineage tracing and transcriptomics that reveal the identities of the heterogeneous cell populations constituting mouse and human adipose tissues. In addition to mature adipocytes and their progenitors, these include endothelial and various immune cell types that together orchestrate adipose tissue development and functions. One salient finding is the identification of progenitor subtypes that can modulate adipogenic capacity through paracrine mechanisms. Another is the description of fate trajectories of monocyte/macrophages, which can respond maladaptively to nutritional and thermogenic stimuli, leading to metabolic disease. These studies have generated an extraordinary source of publicly available data that can be leveraged to explore commonalities and differences among experimental models, providing new insights into adipose tissues and their role in metabolic disease.

INTRODUCTION

The ability to safely sequester and release energy stores when needed provides a great evolutionary advantage to organisms. Cells of many lower organisms perform this feat in the form of lipid droplets within their cytoplasm (1). The adipocyte, a cell type specialized for the storage and release of energy, appears in bony fish (2) and has evolved to control key aspects of systemic energy metabolism, including fuel storage sensing, thermogenesis, mechanical support, and immune protection. These multiple functions are mediated through specialized types of adipocytes with distinct morphologies and anatomical distributions. White adipocytes, which are optimized for energy storage and release, are typically uniocular and can be found in most adipose depots. Thermogenic adipocytes are smaller, multilocular, and mitochondria rich and can be found in dedicated depots, such as the interscapular depot in rodents and newborn humans, or interspersed with white adipocytes in the supraclavicular, paravertebral, omental, periaortic, and inguinal depots. In recent years, it has become clear that neither white nor thermogenic adipocytes are homogeneous, and that specialized functions of adipocytes are mediated by specific adipocyte subtypes. This finding is important, as it raises the possibility that adaptive or maladaptive changes in adipose tissue can occur through changes in the proportion of different adipocyte subtypes, rather than through changes within a homogeneous set of cells.

Adipose tissues are composed of many cell types in addition to differentiated adipocytes. Interest in these cells was significantly accelerated by the discoveries that adipose tissue is a source of multipotent mesenchymal progenitor cells that can differentiate into various lineages, including adipogenic, osteogenic, and chondrogenic (3–5), and that immune cell composition of adipose depots is highly sensitive to metabolic and nutritional states (6, 7). These findings have led to the recognition that adipocytes represent less than 50% of adipose tissue cells (8), despite contributing to more than 90% of its volume. As an example, the simple experiment of staining human or mouse adipose tissue whole mounts to detect adipocytes and nuclei indicates that less than 15% of nuclei correspond to adipocytes (**Figure 1**). New technologies, including single-cell (sc) and single-nuclei (sn) RNA sequencing (RNA-Seq), genetic models for lineage tracing, multicolor fluorescence-activated cell sorting (FACS), and tissue clearing are providing a deeper understanding of nonadipocyte cells and their localization within depots. These technologies are evolving rapidly and have been reviewed recently (9–12). All articles covered in this review have made their primary raw data available, allowing for reanalysis. In addition, new resources, such as Tabula Muris, comprising single-cell transcriptomic data from more than 100,000 cells from 20 organs and tissues, including adipose tissue, can be useful for providing context for new data (13).

MORPHOLOGICAL AND FUNCTIONAL EVIDENCE FOR ADIPOCYTE HETEROGENEITY

The first studies to probe the structure and cellular composition of adipose tissue took advantage of the emerging high resolution of electron microscopy to discern the fine structural details of adipocytes within diverse adipose depots of numerous species under diverse physiological conditions (14–24). Much insight into the mechanisms that control adipose tissue cellularity and morphology was gleaned from these anatomical studies. For example, early histological studies demonstrated how the volume of adipocytes in newborn mice was unaffected by litter size, but the subsequent growth of each adipocyte could increase markedly with decreasing litter size. These studies revealed developmentally programmed mechanisms that dictate adipocyte number and the remarkable ability of white adipose tissue to store energy through adipocyte hypertrophy (21). Similarly, the features of thermogenic interscapular adipose tissue and its responsiveness to

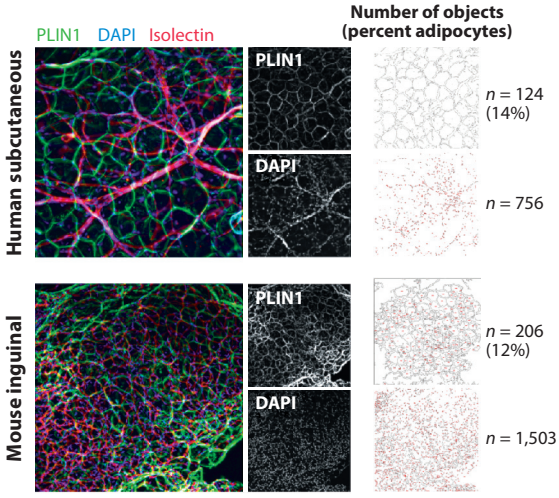

Figure 1

Relative proportion of adipocytes in human subcutaneous and mouse inguinal adipose tissue depots as estimated by perilipin-1 (PLIN1) and DAPI (4′,6-diamidino-2-phenylindole) staining of whole-mount specimens. Analysis of human and mouse adipose tissue whole mounts stained with antibodies to PLIN1, with human or mouse isolectin to label the vasculature, and with DAPI to identify nuclei. Images represent maximal-intensity projections of 8 (human) or 6 (mouse) optical sections taken at 25-μm intervals. Grayscale images of PLIN1 and DAPI are shown, and the drawing was produced by the "analyze particles" algorithm in Fiji/ImageJ after thresholding. The numbers of objects representing either adipocytes or nuclei are shown. The percentages shown in parentheses represent the number of nuclei that correspond to adipocytes, whereas the remainder correspond to other cell types in the tissue.

thermogenic stress was precisely described through electron micrographs depicting immediate hyperemia, decrease in droplet size (lipolysis), new droplet generation (lipogenesis), and remodeling of mitochondrial cristae in response to cold (25). Many important insights into the cellular mechanisms governing adipose tissue development, including the perivascular localization of adipocyte progenitors, were obtained from observations of the close relationship between vascular growth and the emergence of differentiated adipocytes and the close anatomical apposition of preadipocytes, adipocytes, and capillaries (21).

Development of methods to isolate and study both the morphological features and functional properties of adipocytes provided evidence of heterogeneity among white adipocytes, even within the same depot. For example, analyses of large and small adipocytes from rat epididymal fat pads revealed decreased insulin-mediated glucose uptake (26) but not maximal lipogenesis rates (27) in larger adipocytes. More recently, using a single-cell imaging assay that resolves fatty acid uptake in single adipocytes in subcutaneous adipose tissue explants, Varlamov et al. (28) reported that subcutaneous adipocytes are heterogeneous in size and intrinsic insulin sensitivity. Using optimized FACS conditions for sorting live adipocytes from the interscapular and inguinal depots, distinct size differences and content of UCP1 were revealed (29). Importantly, these studies also revealed populations of adipocytes from obese insulin-resistant subjects that lack beta-adrenergic receptors. These results explain previous findings of a decrease in beta-adrenergic receptor levels in insulin resistance and further reveal that these changes are attributable to dichotomous receptor expression in different cells, rather than a homogeneous decrease in the abundance of receptors per cell.

While the observations of size distributions and functional differences between adipocytes could potentially be attributable to an expected degree of biological variation within normally distributed traits, more recent studies support the notion that true developmentally distinct adipocyte subtypes coexist in the same depot. For example, Sanchez-Gurmaches & Guertin (30) reported multiple developmental origins for white and thermogenic adipocytes within the same depots of the mouse. These investigators leveraged a Cre-recombinase-sensitive, dual-color labeling system that utilizes a membrane-targeted fluorescent reporter (R26R-mTmG) to identify adipocytes from distinct lineages at single-cell resolution in whole-mount preparations. More recent studies on the transcriptional profiles of adipocyte progenitor cells in adipose depots, reviewed in detail below, are consistent with the existence of distinct progenitor populations that give rise to functionally distinct adipocyte subtypes. Imaging techniques that allow preservation of the three-dimensional (3D) structure of tissues are consistent with this heterogeneity and are further revealing the differential distribution of thermogenic adipocytes to highly innervated regions (31).

STUDIES OF DIFFERENTIATED WHITE ADIPOCYTES AND ADIPOCYTES DIFFERENTIATED FROM PROGENITOR CELLS

The development of technologies that allow an unbiased comprehensive analysis of thousands of single cells has ushered in the possibility of more precisely defining the transcriptomes of adipocytes. However, obtaining transcriptomes from differentiated adipocytes is complicated by the large size, buoyancy, and fragility of cells owing to their large lipid content, largely precluding droplet-based approaches. This has led to efforts to use snRNA-Seq to obtain transcriptomes of differentiated adipocytes isolated by flotation. Nonetheless, this analysis is also complicated by the fact that many nonadipocyte cells are in close contact with adipocytes, leading to contamination during preparation. For example, a 3D image analysis of the isolated adipocyte-containing floating layer revealed lipid-engorged macrophages, macrophages in contact with lipid droplets, and sheath-like assemblies of macrophages surrounding adipocytes (32). Moreover, very tight contacts between developing adipocytes and endothelial cells have been observed (21, 33). Taking these caveats into consideration, Rajbhandari et al. (34) performed snRNA-Seq on 10,000 nuclei obtained from adipocytes isolated by trypsin digestion and flotation from the inguinal depot of mice (34). As pointed out by the authors and based on prior reports (35), these nuclei could include those from cells tightly bound to floating adipocytes. Transcriptomes of these nuclei could be clustered into 14 groups, one of which (Cluster 9) expressed high levels of the adipocyte marker *Plin1*, and of thermogenesis-associated genes including *Acsl1* and the β3-adrenergic receptor *Adrb3*. A subcluster expressing thermogenic genes *Dio2*, *Ucp1*, and *Cidea* emerged within Cluster 9 following cold exposure or β3-adrenergic agonist CL316,243 treatment. Most relevant to this study was the finding that this subpopulation was expanded in mice lacking expression of the *Il10* receptor in adipocytes, demonstrating that Il-10 signaling impairs thermogenic pathways (34).

Alternative experimental approaches also support the existence of heterogeneity of white adipocytes within the same depots (**Figure 2**, *right*). To identify differentiated adipocyte subtypes in humans, our laboratory leveraged our previous finding that progenitor cells from human adipose tissue proliferate with minimal loss of multipotency in organoid-like cultures consisting of Matrigel and proangiogenic media, and they robustly differentiate into functional mature adipocytes (36, 37). Single progenitor cells were expanded through approximately seven doublings and split into three replicate wells. One well remained undifferentiated, while the other two were differentiated, and one of the differentiated wells was exposed to forskolin to simulate thermogenic conditions. This strategy allowed a direct comparison of transcriptomes between

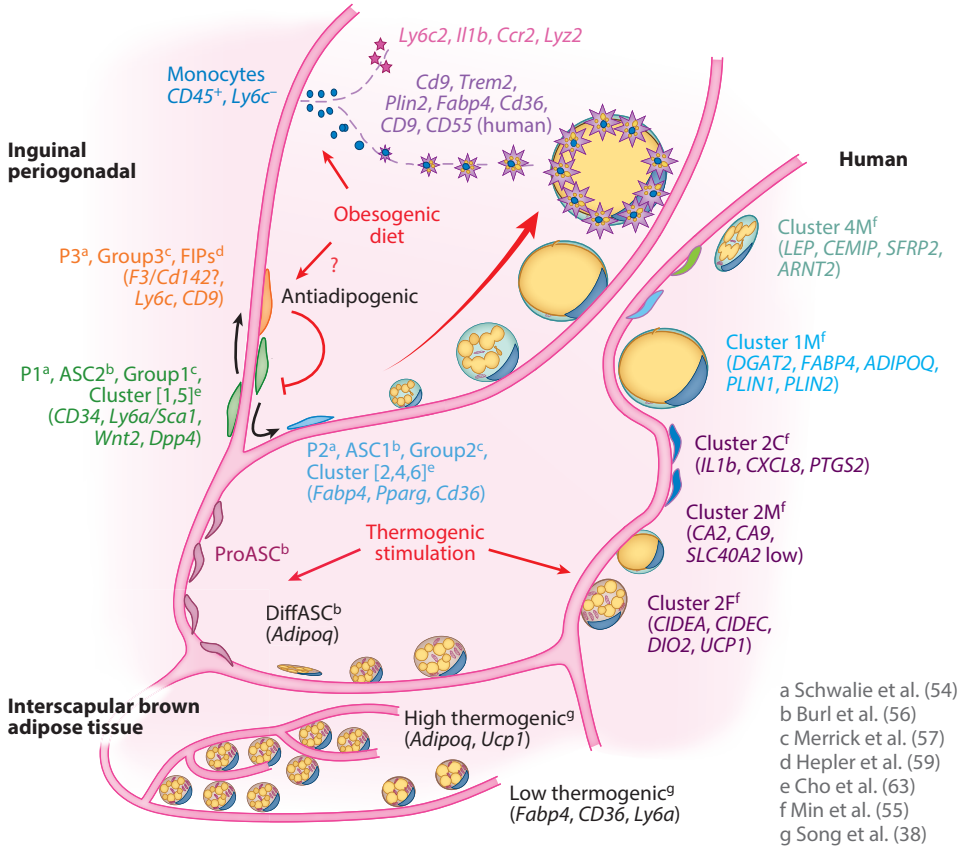

Figure 2

Summary of findings and interpretations of transcriptomic studies of adipocytes, adipocyte progenitors, and immune cells in adipose tissue. Heterogeneous populations of cells identified in inguinal and perigonadal mouse adipose tissue or derived from human adipose tissue progenitors are illustrated. The names assigned to each subtype and the corresponding reference (superscripts) are shown, as are the genes associated with each population common between studies. The monocyte/macrophage trajectory reflects data in References 56 and 83–86.

the undifferentiated, differentiated, and thermogenically stimulated states for each original single cell analyzed. Transcriptomes of adipocytes differentiated from 52 single-cell-derived clones could be clustered into four groups, one of which displayed a significantly higher induction of genes associated with thermogenesis (e.g., *DIO2*, *CIDEA*, *CIDEC*) in response to forskolin. This adipocyte subtype was enriched in genes that enable iron accumulation and protect from oxidative stress, suggesting that thermogenic adipocytes are primed for iron-intensive metabolic pathways, such as mitochondrial biogenesis, which are rapidly elicited by thermogenic stimuli. Additional differences observed between adipocyte subtypes included markedly different levels of *ADIPOQ* and *LEP* gene and protein expression, correlating with differences in expression of lipogenesis and lipolysis-associated pathways. This finding is consistent with that seen by Song et al. (38) in brown adipocytes from mouse interscapular depots (see below). Importantly, the genetic signature of these adipocyte subtypes was enriched in human thermogenic depots, supporting the concordance between phenotypes of adipocytes from diverse depots differentiated in vitro and in vivo.

Also using a clone-based approach, Lee et al. (39) characterized adipocytes generated from conditionally immortalized cell lines derived from the stromovascular fraction of the scapular white, inguinal, perigonadal, perirenal, and mesenteric fat pads of 6-week-old male mice. Single-cell-derived clones from both the subcutaneous and visceral depots displayed varying degrees of adipocyte differentiation but also differences in acidification rates that were not explained by degree of differentiation. Transcriptomes of single-cell clones clustered into three groups. Type 1 progenitors were enriched in *Wt1*, *Lrrn4*, and *Upk3b*, the latter being a known mesothelial cell marker, consistent with a mesothelial origin of visceral adipose tissue (40). Type 2 progenitors were enriched in *Tagln*, *Ctgf*, and *Krt19*, while Type 3 progenitors were enriched in *Mx1*, *Casp1*, and *Cxcl12*. None of the adipocytes differentiated from these immortalized progenitors induced thermogenic genes in response to stimulation and thus did not correspond to beige/brite adipocyte subtypes. Instead, these subtypes varied in triglyceride content and responded differentially to insulin, TNF-α, and growth hormone. Importantly, lineage tracing experiments indicated that the proportion of these adipocyte subtypes varied and could not account for the totality of adipocytes in most depots, pointing to additional adipocyte subtypes that remain to be identified (39).

HETEROGENEITY OF INTERSCAPULAR THERMOGENIC ADIPOCYTES

Adipocytes in interscapular adipose tissue in rodents are characterized by small size, small lipid droplets, and abundant, cristae-rich mitochondria; they respond to adrenergic stimulation with enhanced fuel oxidation, uncoupled respiration, and heat production. These thermogenic adipocytes also display distinct mechanisms of insulin signaling to stimulate glucose transport compared to unilocular white adipocytes (41). Despite morphological homogeneity, single-cell transcriptomes of plated, aspirated, primary brown adipocytes (42) revealed significant heterogeneity in *Ucp1* expression, as well as heterogeneity in numerous additional genes associated with the brown thermogenic phenotype. Song et al. (38) were able to obtain 50,000–100,000 reads from 3,600 single cells from a total of 10,000 using the Chromium Single Cell v2 (10x Genomics, Pleasanton, California) platform. Analysis of this data set identified two main clusters of cells, classified as corresponding to high- or low-thermogenic capacity, at an approximate 2:1 ratio. High-thermogenic-capacity cells expressed high levels of *Adipoq*, *Ucp1*, and other canonical thermogenic genes associated with mitochondrial oxidative metabolism. These high-thermogenic-capacity cells could be further divided into three subclusters, BAH1–3, differing in expression of genes related to insulin responsiveness (*Irs2*, *Scd4*, *Lpin1*), mitochondrial biogenesis (*Pdk4*, *Ppargc1*, *Ppargc2*), and oxidative phosphorylation (*Cox6b1*, *Cox8b*, *Cox7a1*). Low-thermogenic-capacity cells were defined by low content of Adipoq and Ucp1, but were enriched in genes associated with lipid uptake pathways (*Fabp4*, *Cd36*), as well as creatine metabolism-related pathways. These cells may be specialized to conduct the recently described creatine futile-cycle-associated thermogenesis (43, 44). It is worth noting that low-thermogenic-capacity cells also expressed higher levels of *Ly6a* (*Sca1*), *Ly6c1*, and *Id1*, suggesting a less differentiated state (**Figure 2**, *bottom*).

A complementary study (45) sequenced 8,827 nuclei from interscapular brown adipose tissue of adult mice, which were identified as adipocyte nuclei by virtue of expression of red fluorescent protein driven by the adiponectin promoter. This population is likely to correspond to the high-thermogenic-capacity cells in the study by Song et al. (38) on the basis of their high expression of *Adipoq*. These nuclear transcriptomes could be clustered into four groups (P-RT-1 through 4), respectively, defined by enrichment in the genes *Pde3a*, *Cish*, *Atp5e*, and *Cyp2e1*. Interestingly, *Cyp2e1* was also found to be differentially expressed in the study by Song et al. but in cells resembling white adipocytes and slightly expressed in one of the high-thermogenic-capacity clusters

(BA-H2). Interestingly, in their report, Sun et al. (45) find that *Cyp2e1* appears to be enriched in nuclei from human adipose tissue obtained from supraclavicular depots, suggesting that human thermogenic adipocytes are most similar to the BA-H2 and P-RT-4 in the studies by Song et al. and Sun et al., respectively. This adipocyte subpopulation was further characterized by Sun et al. and appears to exert paracrine interactions with other thermogenic adipocytes potentially through acetate metabolism.

Other marker genes found by Sun et al. (45) to distinguish brown adipocyte populations were not detected as highly differentially expressed within the populations defined by Song et al. (38). This divergence is likely due to substantial differences in the protocols employed; Song et al.'s scRNA-Seq results could have been subject to selection bias based on levels of difficult-to-dissociate cells, dissociation-induced cellular stress, cellular lipid content, uneven cell breakage, and other factors, whereas nuclear sequencing performed by Sun et al. could have been affected by bias toward larger genes and against genes involved in mitochondrial function, as observed in other snRNA-Seq studies (46, 47). Notwithstanding these differences, both studies point to substantial heterogeneity within brown adipocytes from the mouse interscapular depot. This heterogeneity may be functionally important, as it may reflect cellular specialization for recently described UCP1-dependent and -independent mechanisms of thermogenesis (48, 49).

Together, all studies of mature adipocytes, from both mice and humans, support the existence of diverse subtypes with distinct functional properties. A better understanding of mechanisms that lead to the development of each subtype and the relationships between these mechanisms and metabolic disease susceptibility will be the goal of future studies.

STUDIES OF ADIPOCYTE PROGENITORS

Though it was known for many years that multipotent mesenchymal progenitors were present in the stromovascular fraction of mouse adipose tissue (50), whether and how they gave rise to adipocytes in vivo were not known. To understand the in vivo origin of adipocytes, Tang et al. (51) leveraged the use of genetic tracing to follow cells expressing *Pparg*, an absolute prerequisite for adipocyte differentiation. Using an inducible Cre-lox system to conditionally mark cells expressing or having expressed *Pparg*, they found that virtually all adipocytes developed within the first month of life arise from a population of cells that expressed *Pparg* prenatally. These cells were capable of proliferation and could repopulate the stromovascular compartment while giving rise to adipocytes. These progenitors were found to express Sca1/Ly6a and *Cd34* and to reside in the adipose vasculature, where they colabel with the mural cell markers Pdgfrβ, Ng2, and Sma. Using only Pdgfrβ to FACS sort cells from the stromovascular fraction, it was found that Pdgfrβ$^+$ cells displayed significant spontaneous and insulin-stimulated adipogenic differentiation and could develop adipose tissue when introduced into nude mice. This work thereby identified a Pdgfrβ$^+$ subpopulation of mural cells as bona fide adipocyte progenitors.

Contemporaneously, Rodeheffer et al. (52) used a comprehensive FACS strategy to define the immunophenotype of mouse adipose tissue stromovascular cells depleted of endothelial and immune cell lineages. To identify a signature for adipose progenitors, they quantified the capacity of immune-isolated cells to generate adipocytes in vitro and adipose tissue in lipodystrophic mice. These investigators found a Cd29(Itgb1)$^+$:Cd34$^+$:Sca1(Ly6a)$^+$ population, comprising approximately half of the Lin$^-$ cells, that was capable of multilineage differentiation in vitro, but it was not able to give rise to adipocytes in vivo. However, a small subpopulation characterized by Cd24 positivity was both highly adipogenic in vitro and able to give rise to adipocytes in vivo (52). Whether the Cd24$^+$ subpopulation represents a subpopulation within progenitor cells with retained multilineage differentiation potential or whether it represents a more

committed step toward adipose differentiation (53) was not ascertained in this study. It is likely that the Cd29(Itgb1)$^+$:Cd34$^+$:Sca1(Ly6a)$^+$ cells identified by Rodeheffer et al. correspond to the Pdgfrβ$^+$ population identified by Tang et al. (51), given the common expression of Sca1/Ly6a and Cd34 in both populations.

In the past 24 months, several studies have used single-cell transcriptomics to further characterize the nature of adipocyte progenitors, and a consensus is emerging around several important points (**Figure 2**, *left*). Schwalie et al. (54) used 10x Genomics to interrogate 1,804 Lin$^-$ single cells separated by FACS from the mouse inguinal adipose depot stromovascular fraction. Interestingly, most of the cells express progenitor markers *Cd34*, *Cd29/Itgb1*, *Sca1/Ly6a*, and *Pdgfrb*, consistent with findings from FACS analysis (52) and lineage tracing (51). These results suggest that progenitor cells constitute the majority of nonendothelial and nonimmune cells in mouse adipose tissue. Transcriptomes of these cells clustered into three main groups, where two (P1 and P2) comprise more than 90% of the cells. The P1 population was distinguished by higher enrichment in stem cell–associated genes such as *Cd34* and *Sca1/Ly6a*, while the P2 population was enriched in genes associated with adipose differentiation such as *Fabp4*, *Pparg*, and *Cd36*. The third, smaller subpopulation (P3) was distinguished by its higher content of *F3/Cd142*. Using available FACS-grade antibodies, the authors removed each population selectively and measured their differentiation capacity, as well as that of remaining cells. They observed that the presence of the P3 subpopulation exerted an inhibitory effect on adipose differentiation that could not be explained by the lack of differentiation of the P3 subpopulation, per se. In addition, the P2 population displayed a similar capacity for lipid accumulation, but markedly lower *Adipoq* expression compared to others, pointing to large variation in the capacity of adipocyte subtypes to express this adipokine (54, extended data figure 3*e*,*f*). This is consistent with that seen in mouse thermogenic and human adipocytes (38, 55).

A comparable study performed by Burl et al. (56) was conducted with the purpose of determining whether thermogenic adipocytes are derived from a specific subgroup of progenitor cells. This study analyzed both Lin$^+$ and Lin$^-$ cells from both the inguinal and perigonadal depots from mice treated without or with CL316,243 to stimulate the β3-adrenergic receptor and activate thermogenic cell development. Approximately 6,000 Lin$^-$ cells from gonadal adipose tissue from untreated mice were clustered into two major groups (ASC1 and ASC2), both expressing *Pdgfra* and *Ly6a/Sca1* and representing 69% of Lin$^-$ cells, with the remainder consisting of vascular endothelial cells, fibroblasts, and cells escaping Lin$^+$ selection. The ASC1 cluster contained more cells expressing genes associated with adipose differentiation, including *Pparg*, suggesting correspondence to the P2 cluster identified by Schwalie et al. (54). The relative proportions of ASC1 and ASC2 varied with depot of origin; gonadal tissue contained more ASC1-type cells, whereas inguinal tissue contained more ASC2-type cells. In response to CL316,243 treatment, cells in the ASC1 and ASC2 clusters of gonadal fat shifted within the cluster, reflecting increased expression of cell migration and extracellular matrix–remodeling genes, and two new populations (ProASC and DiffASC) appeared. The ProASC cluster was enriched in cell cycle genes, indicating active proliferation, and the DiffASC cluster was enriched in specific adipogenesis genes, including *Adipoq*. These data indicate that thermogenic stimulation elicits proliferation and differentiation of gonadal adipose tissue progenitors, probably existing within ASC1 and ASC2. In contrast, these changes were much less prominent in cells from inguinal adipose tissue, in which CL316,243 treatment did not elicit appearance of the ProASC and DiffASC populations. These results are consistent with those of Rajbhandari et al. (34), who performed scRNA-Seq on 10,000 cells isolated from the stromovascular fraction of mouse inguinal adipose tissue with no prior FACS selection. Transcriptomes from the stomovascular fraction could be grouped into 15 clusters, including 4 clusters representing adipocyte precursor cells, 4 clusters of B cells, 4 clusters of

T cells, and 3 clusters of macrophages. Treatment with CL316,243 resulted in a transcriptomic shift in the adipocyte progenitor cell clusters, similar to that seen in Burl et al.'s analysis of inguinal adipose tissue (56).

A third comparable study was performed by Merrick et al. (57), who analyzed the developing and adult inguinal fat pads of mice. Single-cell transcriptomes of 11,423 $Cd45^-$ cells could be categorized into three subgroups, all expressing *Pdgfra*, *Ly6a/Sca1*, and *Cd34*, albeit to varying extents. Group 1 cells were most highly enriched in *Ly6a* and *Cd34* and were selectively enriched in *Dpp4*, *Wnt2*, *Bmp7*, and *Pi16*, suggesting a more stem-like state, similar to the P1 and ASC2 clusters identified by Schwalie et al. (54) and Burl et al. (56). Group 2 cells expressed genes associated with adipocyte differentiation, including *Pparg* and *Fabp4*, suggesting a more differentiated state corresponding to the P2 and ASC1 clusters identified by Schwalie et al. and Burl et al. Group 3 expressed *F3/Cd142*, indicating general correspondence to the P3 group identified by Schwalie's team. Similar groupings were seen in Lin^- cells obtained from inguinal depots from adult mice maintained at thermoneutrality, indicating maintenance of the progenitor cell phenotype of adipose tissue with development. Further examination of Group 1 cells, isolated on the basis of Dpp4 positivity, revealed that these cells proliferated at a higher rate and expressed a higher induction of osteocyte-specific genes when cultured under osteogenic conditions, consistent with a more multipotent phenotype. Consistent with these findings, prospective isolation of cells from the human stromovascular fraction using DPP4 and CD55 (58) had previously identified a population expressing increased levels of general stem cell markers (such as *CD34* and *CD73*) and genes associated with cancer and embryonic stem cells (*CD99*, *ITGB3*, *GGT1*), suggesting increased regenerative potential.

Although Schwalie et al. (54) and Merrick et al. (57) identified a similar overall composition of the mouse adipose tissue progenitor cell population and similarities in their precursor-product relationships, the function of cells enriched in *F3/Cd142* was discrepant. Schwalie et al. found limited adipose differentiation potential of cells positively selected for F3/Cd142 and antiadipogenic effects of this population, but Merrick et al. found that cells selected for F3/Cd142 displayed substantial differentiation potential. While many possible experimental differences might account for these discrepant findings, one could be the different FACS gating strategies employed. Specifically, in Schwalie et al.'s study, $Cd142^+$ cells included only ~4% of Lin^-, $Cd34^+$, and $Sca1^+$ cells (54, extended data figure 3), whereas Merrick et al.'s strategy included ~15% (57, figure S7); Thus, it is possible that cells from Group 2 could have been included, a result consistent with the finding by RNA-Seq that cells positively selected with Cd142 express *Icam1* at similar levels compared to Group 2 cells (57, figure S7). Indeed, when introduced into developing fat pads, a subset of $Cd142^+$ cells downregulated *Cd142* and retained *Icam1*, indicating at least two phenotypes within this $Cd142^+$ subpopulation. An additional explanation can reside in the strategies used to measure adipogenic potential. Adipogenic induction in Merrick et al.'s study was carried out in the presence of 20 nM insulin, 1 nM T3, 1 μM dexamethasone, 0.5 μM 3-isobutyl-1-methylxanthine, and 125 nM indomethacin, or in a minimal adipogenic cocktail containing 20 nM insulin only. In contrast, differentiation in the study by Schwalie et al. was carried out in the presence of 170 nM insulin, 1 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine. Differentiation conditions were critical in revealing differences between groups in Merrick et al.'s study, as $Dpp4^+$ cells only displayed comparatively lower differentiation capacity in the minimal adipogenic cocktail. Therefore, differences between $Cd142^+$ cells in the studies by Schwalie et al. and Merrick et al. could potentially also be attributable to differences in adipogenic conditions.

In a more targeted study to characterize cells specifically associated with the microvasculature, Hepler et al. (59) leveraged previous findings that *Pdgfr*-β^+ cells residing directly adjacent to the endothelium in white adipose tissue blood vessels can differentiate into adipocytes in both inguinal

and perigonadal fat (60). Cells in which inducible green fluorescent protein expression was driven by the *Pdgfrb* promoter were isolated by FACS and subjected to scRNA-Seq (59). Two major clusters of cells were identified, which were defined by the abundance of the cell surface marker *Ly6c*. *Ly6c*-positive cells were also positive for *Cd9*, which was previously identified as a marker of fibrogenic cells induced in response to obesity in adipose tissue in mice (61). This marker had been previously identified as an abundant protein in human mesenchymal progenitor cells, with proliferative and proangiogenic activities (62). This population, termed FIPs for fibro-inflammatory progenitors, displayed poor adipose differentiation capacity and exerted an antiadipogenic effect through factors secreted into the media. This finding is analogous to that made by Schwalie et al. (54) of a cell population that exerts antiadipogenic effects (P3) through secreted factors; however, the marker chosen by Schwalie et al. to identify the antiadipogenic population (*F3/Cd142*) was not enriched in the Ly6c$^+$ population identified by Hepler et al. However, analysis of the genes expressed in FIPs and P3 populations reveals common enrichment for *Cd9* (59, figure 2b; 54, extended data figure 4k). Thus, while some discrepancy exists in the identification of these cells through cell surface markers, the existence of a population of antiadipogenic cells is consistent between studies. It bears mentioning that cell surface markers are usually selected on the basis of availability of FACS-validated antibodies and are not directly mechanistically associated with the specific cell function of interest. Because of plasticity in progenitor cell transcriptomes, association of any particular cell surface marker with a particular function may be stochastic.

Further support for the existence of antiadipogenic populations is found in other studies. For example, Rodeheffer et al. (52) noted that the total stromovascular fraction of mice is poorly adipogenic in comparison to the Cd29(Itgb1)$^+$:Cd34$^+$:Sca1(Ly6a)$^+$ population, which represents 53.5% of the stomovascular fraction, suggesting the presence of inhibitory factors in the unfractionated pool. Another example is the finding by Lee et al. (39) of robust differentiation of 37% of the clones derived from the perigonadal stromovascular fraction but a much lower degree of differentiation in the unfractionated pool of cells from which the clones were derived, suggesting the presence of an antiadipogenic factor in the mixed cell culture. Further understanding of the mechanisms by which antiadipogenic effects are exerted by specific populations of cells will identify the specific genes involved and likely further explain apparent discrepancies.

Cho et al. (63) conducted a comparable study using mouse visceral adipose tissue to identify alterations caused by acute diet-induced obesity. They found that Lin$^-$ cells in the gonadal fat pad of mice comprised 32.9% and 20.3% of total viable cells, indicating a decrease in adipocyte progenitors in response to obesogenic diet. A total of 2,636 and 2,143 adipocyte progenitor cells were profiled by 10x Genomics, and >280,000 reads per cell were acquired. Reanalysis of existing data sets, including those of Burl et al. (56), Schwalie et al. (54), and Merrick et al. (57), revealed concordance between subpopulations, with their identities refined by increased read depth. The majority of transcriptomes could be assigned to two clusters, comprising three [2,4,6] and two [1,5] subclusters, respectively. Subclusters 1 and 5 were enriched in *Cd34* and *Dpp4*, indicating a less-differentiated phenotype, whereas subclusters 2, 4, and 6 were enriched in cells expressing markers associated with adipose differentiation, including *Fabp4*. Subclusters 1 and 5 were distinguished by the presence of genes associated with negative regulation of adipocyte differentiation. Differential expression analysis between subclusters 2, 4 and 6, as well as assessment of their proliferative and adipogenic differentiation capacity in vitro, is consistent with these subclusters representing different stages along the adipose differentiation trajectory. Moreover, the findings of Cho et al. are consistent with those acquired by Merrick et al. in that high-fat feeding and/or obesity depletes the *Dpp4*$^+$ mesenchymal progenitor pool and impairs the adipogenic differentiation competency of preadipocytes in visceral white adipose tissue.

HUMAN ADIPOSE TISSUE PROGENITORS

Although much conservation undoubtedly exists in the biological role and mechanisms of adipose tissue function between species, differences in longevity, metabolic rates, and anatomical features between mice and humans are likely to be reflected in important functional differences between their adipose tissues. Studies of human adipose tissue progenitors have been conducted largely ex vivo, but recently the possibility of studying genetically engineered human tissue in humanized mouse models to understand mechanisms underlying its development of function has come closer to being feasible (36, 37).

Human adipose tissue progenitors were identified almost 20 years ago as a population of multipotent mesenchymal cells that can differentiate along multiple lineages (3, 5, 64). Interest in their empirical use in the field of regenerative medicine is very high, as exemplified by the 5,716 publications containing the words "adipose" and "stem" in the title over the past 10 years. Adipose mesenchymal progenitor cells have been defined as plastic adherent cells positive for CD73, CD90, and CD105, and negative for CD45, CD14, CD11b, CD79α, CD19, and HLA-DR (65). Whether these cells can be subdivided into functional classes has been explored. For example, Osathanon et al. (66) isolated 14 individual clones using expressed embryonic and mesenchymal stem cell marker genes. All of these clones could differentiate into both osteogenic and adipogenic lineages but exhibited varying differentiation potential suggestive of heterogeneity within the progenitor class.

Acosta et al. (67) obtained the transcriptomes of 574 single cells from the stromovascular fraction of four healthy women. These transcriptomes clustered into four distinct populations, three of which corresponded to adipose tissue–resident macrophage subtypes. The remaining population corresponded to adipose progenitor cells, which could not be subclustered into distinct subtypes. An increased resolution was achieved by Merrick et al. (57), who obtained transcriptomes of 11,338 CD45-depleted cells from the stromovascular fraction of human abdominal subcutaneous adipose tissue. The majority of cells expressed mesenchymal markers *PDGFRA*, *PDGFRB*, and *SCA1* and were associated in a large cluster that could be subdivided into two groups; Group 1 cells selectively expressed *DPP4*, *CD55*, and *WNT2*, reflecting a more stem-like phenotype as seen previously by Rennert et al. (58), and the larger cluster of Group 2 cells expressed *ICAM1*, *PPARG*, and *GGT5*, reflecting a more differentiated stage.

More recently, Vijay et al. (68) obtained the transcriptomes of 26,350 cells from the stromovascular fraction from 25 abdominal subcutaneous and visceral adipose tissue samples from individuals undergoing bariatric surgery. Transcriptomes from subcutaneous adipose tissue cells were clustered into 5 groups. SP1 and SP3 were enriched in more stem-like markers, including *MGP*, *APOD*, *CXCL14*, and *WISP2*; SP2 contained genes associated with adipocyte differentiation, including *FABP4* and *CD36*, representing a more differentiated state; SP4 was enriched in fibrosis and extracellular matrix genes, including *CD55*; and SP5 was enriched in immune cell markers including *CD45*. Of note, top genes highly expressed in subcutaneous adipose tissue progenitor clusters (68, supplementary table 15) did not include *PDGFRA*, *PDGFRB*, *CD34*, *CD9*, *DPP4*, *F3/CD142*, *LY6A*, *PPARG*, *CD73/NT5E*, *CD90/THY1*, and *CD105/ENG*, which other studies described throughout this review have identified as markers of mouse or human adipose tissue progenitor cells. The absence of these markers in progenitor cells described by Vijay et al (68) is potentially attributable to the obesity status of the cohort studied; alternatively, as mentioned previously, these cell surface markers have not been functionally associated with specific cell behaviors, and thus might vary stochastically.

In general, scRNA-Seq of human adipose tissue stromovascular fraction reveals a more homogeneous set of progenitor cells compared to mouse tissue, despite the fact that progenitor cells cultured from different human depots can be readily distinguished from each other by bulk

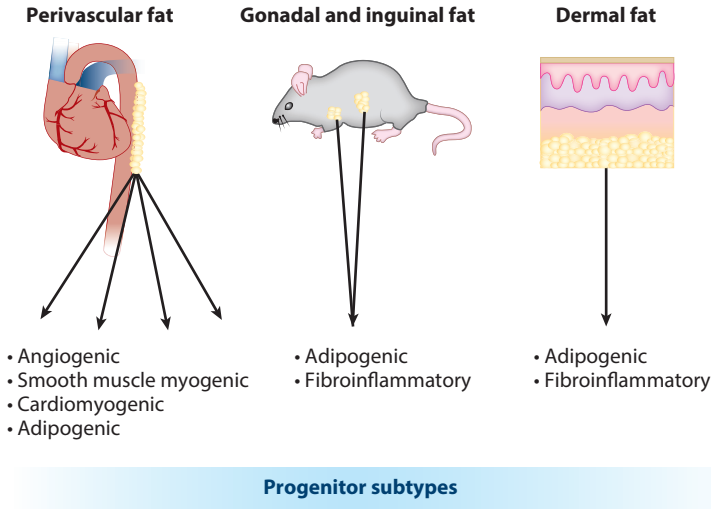

Figure 3

Fates of mesenchymal progenitors isolated from diverse tissues reflect the cellular composition of vicinal structures.

transcriptomics (69). These findings may reflect differences in stage along the adipogenic trajectory of progenitors within different depots. Our laboratory analyzed progenitor cells from human subcutaneous adipose tissue and found a very homogeneous distribution of transcriptomes upon principal component analysis (55). Interestingly, differences among the progenitors were revealed upon differentiation, as the transcriptomes of adipocytes derived from the same exact clones could be clustered into at least four groups. These results suggest that much of the heterogeneity within adipocyte progenitors may be latent and only manifests upon activation of the adipose differentiation program. Further studies of the epigenome of human progenitor cells will be required to further test this possibility. Nevertheless, progenitors that gave rise to thermogenic adipocytes were found to express a unique set of cytokines and transcriptional regulators, including *IL11*, *CXCL8*, and *IL1B*, which could have a potential role in organizing distinct adipose tissue niches (**Figure 2**, *right*).

ADIPOCYTES AND PROGENITORS FROM DERMAL, BONE MARROW, AND PERIVASCULAR ADIPOSE DEPOTS

Recent studies have also explored the cellular composition of other important adipose depots (**Figure 3**). Zhang et al. (70) developed protocols to isolate dermal adipocytes from mice and found specific features that differentiate these cells from adipocytes from other depots. In particular, the expression of the cathelicidin antimicrobial peptide Camp serves as a specific identifier of dermal adipocytes. Transcriptomes from ~7,000 *Pdgfra*$^+$ progenitors isolated from dermal fat were categorized into two clusters: Cluster 1 was enriched for genes related to adipogenesis and mature adipocyte markers, such as *Pparg*, *Fabp4*, *Lpl*, and *Plin2*, while Cluster 2 was more highly enriched in fibro-inflammatory genes including *Cxcl2*, *Il6*, *Ccl2*, and *Cscl1*. In general, this classification is similar to that defined in gonadal and inguinal fat depots: one group with more stem-like qualities, and another potentially further along an adipocyte differentiation trajectory. Importantly, lineage tracing studies of dermal adipocytes strongly suggest that these cells have the capacity to dedifferentiate and redifferentiate during skin development and in response to environmental stimuli,

consistent with prior reports on the behavior of human adipocytes (71). This suggests that this process could further contribute to progenitor cell heterogeneity.

Similarly, transcriptomes of 2,847 nonhematopoietic and nonepithelial cells from the bone marrow of normal 8- to 16-week-old C57BL/6 mice could be assigned to two clusters along a continuum of cell states, which diverged into proadipogenic and pro-osteo/chondrogenic lineages (72). More recent work has provided a comprehensive analysis of the bone marrow niche of mice (73, 74). Baccin et al. (74) performed scRNA-Seq on 7,497 cells, which could be arranged into 32 clusters corresponding to distinct cell types or stages of differentiation, nine of which corresponded to different Pdgfra-positive mesenchymal populations. One of these, termed the Adipo-CAR (CAR, Cxcl12-abundant reticular) cluster is characterized by higher levels of *Lepr* and *Adipoq* compared to Osteo-CAR and other mesenchymal populations. Adipo-CAR cells display a predominantly sinusoidal localization, suggesting a role in establishing a specialized niche within the bone marrow to regulate hematopoietic stem cell maintenance. Zhong et al. (73) conducted scRNA-Seq on mesenchymal lineage cells defined through Td-tomato expression driven by Col2-Cre (Col2:Td mouse). They identified nine clusters of mesenchymal lineage cells, completely consistent with the findings of Baccin et al. Similarly, all mesenchymal clusters expressed PDGFRα, but adipogenic progenitors were distinct owing to high levels of *Adipoq* expression and a perivascular localization. Pseudo-temporal analysis placed these clusters at different points along differentiation trajectories toward osteogenic and adipogenic lineages. Notably, the adipogenic lineage displayed a longer differentiation pseudotime compared to the osteogenic lineage, with a greater number of uniquely associated transcription factors, potentially reflecting differentiation into adipocyte subtypes. Additionally, chemokine signaling pathways were overrepresented in adipocyte progenitors, consistent with an important regulatory role of adipocyte progenitors in the bone marrow environment that was also suggested by the data of Baccin et al.

Adipose tissue surrounding major blood vessels provides critical functions through thermogenesis and secretion of specific adipokines that can directly affect the vessel wall (75, 76). Two recent studies have investigated the cellular composition of perivascular adipose tissue and its relationship with vascular function. Gu et al. (77) performed scRNA-Seq of freshly isolated $CD45^-/Cdh5^-/Sca1^+/CD29^+$ cells from the adipose tissue surrounding the mouse thoracic aorta. Transcriptomes of 85 cells could be segregated into two clusters. Cluster 1 expressed angiogenesis markers *Pecam1* and *Cdh5*, together with adipogenesis markers *Fabp4* and *Pparg*, while Cluster 2 featured the expression of *Tgfbr2* and multiple genes associated with smooth muscle cell differentiation. Greater resolution was sought by scRNA-Seq analysis of these cells after expansion in culture. However, culture conditions resulted in selective expansion of cells constituting Cluster 2 (77), and their transcriptomes could be ordered into a trajectory of spontaneous smooth muscle cell differentiation (**Figure 3**). In a comparable study, Pan et al. (78) cultured $Cd45^-/Cd90^+/Cd34^+/Sca1^+$ cells from thoracic aortas from young and old mice for three passages after isolation. Cultured cells were capable of differentiating into endothelial cells, smooth muscle cells, osteoblasts, and adipocytes when incubated with specific differentiation cocktails. Transcriptomes of 3,000 cells could be categorized into 10 subgroups and differential expression representing 10 putative cell types in cells from young mice and 7 in cells from older mice. These cell types included adipogenic, cardiomyocyte, epithelial, neural, and endothelial lineages. Interestingly, adipocytes differentiated from these expressed *Ucp1* even without thermogenic activation, consistent with prior findings of distinct regional phenotypes of progenitor cells in mice (79).

These studies demonstrate remarkable diversity of progenitors from different adipose depots, where progenitors seem specialized to maintain the functions of tissues and organs with which the depot is associated. Progenitors from the perivascular depot seem particularly diverse, with the potential to have a critical role in vascular remodeling. Whether the commitment of progenitors

from different depots to specific lineages is fixed and whether all progenitors can undertake differentiation into any lineage in a way dictated by regional stimuli are important questions for future studies. Another important question involves the mechanisms that control progenitor cell replenishment. Although some caveats are associated with this approach (80), studies on gene expression in specific progenitor subsets have already revealed important aspects of mechanisms that control progenitor cell replenishment. For example, Jiang et al. (81) pointed to a central role of *Pparg* expression in progenitor cells to induce expression of *Vegf*, which acts on endothelial cells to expand the progenitor niche (81); Vegf production by mature adipocytes also stimulates endothelial cell proliferation and angiogenesis, while inducing differentiation of progenitors toward the thermogenic lineage (82). Further studies will continue to elucidate the mechanisms that replenish diverse progenitor populations in different adipose depots and help us understand their role in development, aging, and adaptation of environmental conditions, for example, in response to obesogenic stress.

IMMUNE CELLS

The finding that immune cell composition of adipose depots is highly sensitive to metabolic and nutritional states (6, 7) led to an explosion of research on the composition and functional relevance of these cells. In one of the first single-cell approaches to understand the characteristics of immune cells in adipose tissue, Hill et al. (83) analyzed the transcriptomes of adipose tissue macrophages from mice subjected to high- or low-calorie diets for 12 weeks. Transcriptomes from $Cd11b^{+}$ $Ly6c^{-}$ cells from high-calorie diet-fed animals clustered into two groups distinguished by expression of *Cd9* as well as genes associated with lipid metabolism, including *Lpl* and *Plin2*. This subgroup of macrophages was associated with crown-like structures that surround hypertrophied adipocytes (**Figure 2**, *top*). Importantly, lipid-laden $CD9^{+}$ adipose tissue macrophages that increase with body mass were also seen in human adipose tissue (83).

Sharma et al. (84) obtained transcriptomes from 2,740 and 1,697 $Cd45^{+}$ cells from gonadal adipose tissue from mice fed normal or obesogenic diets, respectively, which could be categorized into 17 clusters, of which 7 corresponded to macrophage/monocyte cells. The majority of the macrophage clusters were contiguous, suggesting a spectrum of related activation states. Monocyte/macrophage transcriptomes decomposed into a two-phase pseudotime trajectory, with monocytes branching into two states, one of which was characterized by genes associated with protein synthesis, metabolism, and inflammation. The second branch diverged into a smaller proportion (7.5%) characterized by high expression of migration and immune response genes, including *Ccl6*, *Cfp*, *Ctsc*, *Ccl2*, and *Arg1*, and an additional proportion (12.6%) of cells branched into a state characterized by high expression of genes associated with lipid handling and transport, including *Plin2*, *Fabp4*, *Cd36*, *Cd9*, and *Trem2* (84, figure 6). In this study, it was also found that the absence of the neuroimmune guidance cue netrin-1 resulted in an altered trajectory with activation of gene programs for lipid uptake, lipolysis, and lipid droplet formation, and suppression of inflammatory pathways (84).

A high-resolution data set was obtained by Jaitin et al. (85), who characterized the transcriptomes of 21,210 $Cd45^{+}$ cells from the gonadal depot of mice at 6, 12, and 18 weeks of obesogenic diet and age-matched controls. Transcriptomes were categorized into 15 clusters, including B cells, naïve cells, $Cd4^{+}$ and $Cd8^{+}$ T dendritic cells, innate lymphoid cells, macrophages, monocytes, mast cells, neutrophils, natural killer (NK) cells, plasma cells, and regulatory T cells (Tregs). The most prominent changes observed with obesogenic diet were an expansion of adipose tissue macrophages and a reduction in Tregs and type 2 innate lymphoid cells. Two out of three subpopulations of macrophages emerged only under obesogenic conditions and

expressed *Cd9* consistent with the findings of Hill et al. (83). Pseudotime trajectories suggest that these macrophages are recruited from monocytes through a fate-determination trajectory characterized by the loss of expression of *Ly6c2*, *Il1b*, *Ccr2*, *Lyz2*, and *S100a10* and acquisition of *C1qa*, *Cd9*, *Cd63*, and *Cd68*, dependent on the function of Trem2.

Weinstock et al. (86) merged the data set from Burl et al. (56) with transcriptomes from Lin^+ cells obtained from mice fed an obesogenic diet for 24 weeks and from mice subjected to caloric restriction following an obesogenic diet. Transcriptomes of the combined data sets, comprising 2,268, 5,232, and 2,458 cells from lean, obese, and calorie-restricted obese mice, respectively, could be categorized into 15 populations: The major subpopulation containing 51% of cells was composed of macrophages, and these could be subdivided into 7 subtypes, which they categorized as major, phagocytic, activated, resident, stem like, heme like, and B cell like. For cells other than macrophages, 14% corresponded to dendritic cells, which could be subdivided into 3 classes: 11% corresponded to T cells, which could be subdivided into 2 classes, and 9%, 8%, and 6% of cells were NK cells, monocytes, and B cells, respectively. In response to an obesogenic diet, they observed a marked increase in major macrophage class and appearance of a new "phagocytic" macrophage subclass, likely corresponding to the $Cd9^+$- and $Trem2^+$-expressing macrophages. These results are completely consistent with those of Burl et al. (56), Sharma et al. (84), Hill et al. (83), and others (68, 87). They indicate that heterogeneity in macrophage composition of adipose tissue reflects stages along a continuum of fate determination steps, where an obesogenic diet leads to the development of a unique macrophage type characterized by a high capacity for lipid handling and expression of a specific gene signature, including *Trem2* and *Cd9*. Perturbations in this dynamic, elicited by diet or thermogenic stimuli, can profoundly influence adipose tissue and systemic physiology.

A physiological role for the *Cd9*-positive population of macrophages seems to be conserved in humans. Analyzing the entire complement of stromal cells in omental and subcutaneous adipose tissue of obese humans, Vijay et al. (68) found that progenitor and endothelial cells comprised 55% and 6% of transcriptomes, respectively, and immune cells represented ~30%. Immune cells could be segregated into 14 clusters: Five represented macrophage subtypes, one of which was enriched for lipid metabolism genes *LIPA*, *LPL*, *CD36*, and *FABP4*. Importantly, this subpopulation was also enriched for *CD9*, which was positively correlated with obesity traits. In addition, four clusters comprised NK cells and T cells, two clusters represented monocytes, and one cluster each represented dendritic cells, B cells, and an unidentified cell type.

To define the role of immune cells in the thermogenic response, Burl et al. (56) performed scRNA-Seq on 7,725 Lin^+ cells from the gonadal adipose tissue of mice under control conditions and in response to CL316,243. Transcriptomes were segregated into clusters, representing reticulocytes, vascular smooth muscle cells, vascular endothelial cells, NK T cells, dendritic cells, and two clusters of macrophages (MAC1 and MAC2). CL316,243 treatment resulted in an increase in the number of macrophages and a bias toward a larger MAC2 population. Importantly, they found that markers previously associated with CL316,243 action and M2 macrophages such as *Chil3*, *Clec10a*, and *Arg1* (88) are distributed equally in MAC1 and MAC2, suggesting a spectrum of activation states rather than a dichotomous classification into M2 or M1 phenotypes. More recently, Rajbhandari et al. (34) obtained transcriptomes of 10,000 non-FACS-selected cells from the stromovascular fraction of inguinal adipose tissue of mice exposed to CL316,243 treatment (34). In addition to progenitor and endothelial cells, they could identify four clusters of B cells, three clusters of macrophages and four clusters of T cells, a cluster of NK cells, and a few mast cells. They found that CL316,243 caused a major increase in B cell populations and a transcriptomic shift in the macrophage population, consistent with the observations of Burl et al. (56). The comparison between results of Burl et al. and Rajbhandari et al. reveals a very different complement

of immune cells in gonadal versus inguinal depots in mice; nevertheless, in both cases, CL316,243 treatment results in an increase in total macrophages and a transcriptomic shift into a population that is enriched in genes associated with lipid uptake and metabolism (*Cd36*, *Fabp5*, *Lpl*, and *Lipa*). Further analysis will be necessary to determine whether this macrophage population is similar to the *Cd9*-enriched population that arises in response to the obesogenic diet; in both cases, excess fatty acids can accumulate in the tissue (due to overfeeding or lipolysis, respectively) and may drive development of this macrophage class.

In addition to the critical role of macrophages, other immune cells contribute to adipose tissue cell heterogeneity. $Cd4^+/Cd25^+/Foxp3^+$ Tregs play a role in tissue homeostasis during inflammation, and they are also found in adipose tissue (89), where their phenotype is strongly sex dimorphic (90). Kalin et al. (91) found that Tregs comprised approximately 10% of $Cd4^+/Cd25^+$ T cells in male mouse gonadal fat and that their numbers doubled in response to cold exposure, β3-adrenergic stimulation, or short-term obesogenic diet. Depletion of Tregs using a monoclonal antibody to Cd25, which induces macrophage engulfment (92), resulted in a small but significant decrease in genes associated with thermogenesis in response to cold or CL316,243 treatment, demonstrating the functional role of this immune cell population (91). Other less well-characterized subpopulations of immune cells may also play functional roles. For example, Vijay et al. (68) found that a subpopulation of $CD8^+$ T cells in human adipose tissue express metallothionein genes and that expression of these genes was associated with obesity-related traits. The data sets resulting from the several scRNA-Seq studies described above will allow further high-resolution unbiased analysis of immune cells in adipose depots and their relative responses to developmental and environmental perturbations.

ENDOTHELIAL CELLS

Although subgroups of endothelial cells were not reported in studies of the stromovascular fraction of mice, Vijay et al. (68) identified three types of endothelial cells (EC1–3) in omental and subcutaneous adipose tissues from obese humans. EC1 cells were enriched in genes associated with lipid handling, including *FABP4*, *CD36*, *LGALS1*, *RBP7*, and *GPX3*, while EC2 cells express classic endothelial markers, including *PECAM1*, *VWF*, *VCAM1*, and *ICAM1*. EC3 cells were enriched in LYVE1, a marker of lymphatic endothelial cells, indicating the presence of lymphatic vasculature in these depots. An intriguing feature of endothelial cells is that they contribute to cellular heterogeneity of adipose tissue in a mechanistically unique way, through the transfer of proteins and lipids from endothelial cells to adipocytes. Crewe et al. (93) found that levels of caveolin 1 in adipocytes in which the *Cav1* gene was excised reached 50% of wild-type levels as a result of transfer from endothelial cells and was incorporated into caveolae. Thus, protein and lipid transfer from endothelial cells to adipocytes is significant enough to affect functional features and contribute to heterogeneity.

SUMMARY POINTS

1. Multiple studies reviewed here converge on the finding of multiple differentiated adipocyte subtypes within the same depot of both mouse and humans, with distinct transcriptomic profiles indicating specialized biological functions. This finding implies that adaptive or maladaptive changes in adipose tissue, leading to systemic metabolic phenotypes, may occur through mechanisms that define the proportion of different adipocyte

subtypes within depots, rather than to changes in the functions of a homogeneous set of cells. There is substantial heterogeneity within brown adipocytes from the mouse interscapular depot. This heterogeneity may reflect cellular specialization for recently described UCP1-dependent and -independent mechanisms of thermogenesis.

2. The existence of a population of antiadipogenic progenitors within adipose tissue is consistently seen in various studies, although markers associated with these cells are inconsistent. Identifying molecular markers mechanistically associated with this antiadipogenic effect will help clarify the reasons for these differences.
3. Studies also converge in identifying a more proliferative subpopulation of progenitors in mouse gonadal and inguinal adipose depots and in human adipose tissue. This population is enriched with *Dpp4*/*DPP4* and may give rise to a less proliferative progenitor population that diverges toward the adipocyte lineage. It will be interesting to determine whether Dpp4 activity is mechanistically associated with the phenotype of these progenitors, and whether it is associated with antidiabetic and antiatherogenic effects of Dpp4 inhibition (94, 95).
4. Comparison of progenitors from different adipose depots reveals substantial differences, with proliferative progenitors diverging toward lineages of cells that compose vicinal structures, e.g., bone and large blood vessels. It will be interesting to determine whether adipose tissue depots are the reservoir for mesenchymal progenitors that repair and maintain homeostasis of vicinal organs and tissues.
5. Robust changes in abundance and transcriptomic composition of adipose tissue macrophages are seen in response to environmental perturbations. Heterogeneity in macrophage composition of adipose tissue reflects stages along a continuum of fate determination steps, where an obesogenic diet leads to development of a unique macrophage type characterized by a high capacity for lipid handling and expression of a specific gene signature, including *Trem2* and *Cd9*. Perturbations in this dynamic, elicited by diet or thermogenic stimuli, can profoundly influence adipose tissue and systemic physiology.
6. Although scRNA-Seq and snRNA-Seq are just beginning to reveal fascinating features of adipose tissue biology, additional approaches to understanding the cellular composition of adipose tissue and its relationship to disease are being developed. For example, the identification of cell-specific-expressed quantitative trait loci has great potential to allow in silico deconvolution of bulk tissue transcriptomes to define cell type composition of adipose tissue at a population level (96).

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