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Adipose tissue remodeling and obesity

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To fulfill its role as the major energy-storing tissue, adipose has several unique properties that cannot be seen in any other organ, including an almost unlimited capacity to expand in a non-transformed state. As such, the tissue requires potent mechanisms to remodel, acutely and chronically. Adipocytes can rapidly reach the diffusional limit of oxygen during growth; hypoxia is therefore an early determinant that limits healthy expansion. Proper expansion requires a highly coordinated response among many different cell types, including endothelial precursor cells, immune cells, and preadipocytes. There are therefore remarkable similarities between adipose expansion and growth of solid tumors, a phenomenon that presents both an opportunity and a challenge, since pharmacological interventions supporting healthy adipose tissue adaptation can also facilitate tumor growth.

Introduction

Adipose tissue (AT) can respond rapidly and dynamically to alterations in nutrient deprivation and excess through adipocyte hypertrophy and hyperplasia, thereby fulfilling its major role in whole-body energy homeostasis. AT remodeling is an ongoing process that is pathologically accelerated in the obese state, and thus, features such as reduced angiogenic remodeling, ECM overproduction, a heightened state of immune cell infiltration and subsequent proinflammatory responses prevail in many obese fat-pads (1). However, not all AT expansion is necessarily associated with pathological changes. The concept of the “metabolically healthy obese” state (2) suggests that some individuals can preserve systemic insulin sensitivity on the basis of “healthy” AT expansion, bypassing all of the aforementioned pathological consequences associated with obesity (3), thereby also avoiding the obesity-associated lipotoxic side effects. Many physiologically relevant processes important for human AT remodeling can be studied in rodent models, with the added advantage that processes related to AT expansion and reduction can occur at an extremely rapid rate. A 24-hour fast in a mouse is associated with a dramatic loss of AT mass and an acute remodeling process that involves rapid infiltration of macrophages; moreover, merely 24 to 48 hours of exposure to a high-fat diet (HFD) can cause a prompt increase in adipocyte size (4). AT is therefore an ideal model system to study rapid alterations in tissue expansion and reduction, as it adapts to a differential nutrient supply. Here, we will focus on key aspects of the intricate dynamics of AT remodeling and subsequent inflammatory consequences that arise from obesity.

Macrophages, major constituents of AT and mediators of remodeling

In 2003, two independent articles in the *JCI* highlighted the infiltration of macrophages into expanding AT as an important physiological phenomenon (5, 6). While not only opening up a newfound fundamental role for the macrophage in metabolism, these reports also fueled the publication of numerous additional papers, thus firmly establishing the phenomenon of a macrophage-orchestrated inflammatory response co-existing with obesity-induced insulin resistance.

Supporting this hypothesis, large clusters of macrophage-related inflammatory genes were identified as significantly altered in obese AT (5). Rodent studies revealed increased levels of macrophage markers present in AT of leptin-deficient, leptin receptor-deficient and diet-induced obese (DIO) mouse models (5). In parallel, obese subjects were found to harbor increased AT macrophage (ATMΦ) content, particularly in more insulin-resistant visceral depots (7). Conversely, weight reduction regimens resulted in a lowering of inflammatory markers, which suggested that this infiltration is reversible (7).

The “initiation” of macrophage infiltration into adipose: four different mechanisms

Adipocyte hyperplasia and hypertrophy can both contribute to AT expansion. This expansion can lead to a myriad of effects, including hypoxia, adipocyte cell death, enhanced chemokine secretion, and dysregulation in fatty acid fluxes (8). Such adipocyte-related consequences pose major logistical challenges and require macrophages to create a permissive environment for the remodeling process. Adipocytes are therefore at the very core of initiation of macrophage recruitment.

Adipocyte death. As “professional” phagocytes, macrophages are extremely proficient in the removal of numerous molecules, ranging from small lipids to colonies of pathogens to dead cells (9). Cinti and colleagues hypothesized that necrosis of adipocytes, driven by hypertrophy and accelerated by obesity, is a prominent phagocytic stimulus that regulates ATMΦ infiltration (10). Indeed, macrophages have been shown to aggregate, forming crown-like structures (CLSs) surrounding necrotic adipocytes in advanced obesity (10–13). In this state, they fuse to phagocytose the residual lipid droplet, forming large lipid-laden multinucleated syncytia in the process, a commonly accepted hallmark of chronic inflammation (10, 14). Using a transgenic model of inducible lipoatrophy, we have previously demonstrated that massive adipocyte death can indeed drive rapid accumulation of ATMΦs as an integral element in the remodeling of fat pads (15). While we used a model of inducible apoptosis in this particular case, the apoptosis in our model recapitulates the necrosis observed by Cinti and colleagues (10) in many aspects. It is almost impossible to determine whether adipocytes *in vivo* preferentially undergo a simple necrotic death or apoptosis, due to the technical difficulties of reproducibly demonstrating apoptosis in adipocytes. However, the death of an adipocyte, necrotic or apoptotic, leaves an inert

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lipid droplet behind that is devoid of the protein coat usually associated with an intracellular lipid droplet, as well as devoid of any lipases that could mediate hydrolysis of triglycerides. The removal of these lipid droplets is an integral aspect of AT remodeling. If the situation in the inducible lipodystrophy model in the mouse is an indication of the rate of lipid droplet removal under normal physiological conditions, then this is likely a relatively slow process, occurring over a period of days to weeks (15).

Chemotactic regulation. Chemokines are small proinflammatory molecules that promote macrophage mobilization from bone marrow into tissues. There is considerable evidence for the pathophysiological role of macrophage- and/or hypertrophic adipocyte-derived chemotactic MCP-1/CCR2 pathways in the regulation of monocyte accumulation in obese AT (16, 17). In particular, increased expression levels of MCP-1, CXCL14, MIP-1 α , MCP-2, MCP-3, and RANTES can be observed in AT of mice with genetic or DIO (5, 16, 18).

Hypoxia. Adipocyte hypertrophy creates areas of local AT microhypoxia at the earliest stages of expansion (19). This has been verified by recent clinical observations in humans, which suggest that AT is poorly oxygenated in the obese state (20, 21). The effects of the local AT hypoxia have been investigated both in isolated murine adipocytes and in animal models (4, 22–25). These papers suggest that many adipokines that are related to inflammation, such as macrophage migration inhibitory factor (MIF), the matrix metalloproteinases MMP2 and MMP9, IL-6, Angptl4, PAI-1, VEGF, and leptin are all upregulated by hypoxia (22–25).

A master regulator of hypoxia and oxygen homeostasis is HIF-1 (26, 27). Several important hypoxia-associated genes, such as leptin and VEGF, are directly regulated by HIF-1 (25). As a transcription factor, HIF-1 functions as a heterodimer, consisting of an oxygen-sensitive HIF-1 α subunit and a constitutively expressed HIF-1 β subunit. While HIF-1 α synthesis is O₂ independent, its degradation is enhanced under normoxic conditions (28). As such, O₂ can mediate the prolyl hydroxylation of HIF-1 α , which subsequently facilitates ubiquitination by E3 ligase. This ligase contains a von Hippel-Lindau (VHL) tumor-suppressor protein, which specifically recognizes the hydroxylated form of HIF-1 α and targets it for ubiquitination and degradation.

To investigate the effects of HIF-1 α in white AT, we analyzed a transgenic mouse model in which we overexpressed a dominant active (degradation-resistant) deletion mutant of HIF-1 α (HIF-1 α - Δ ODD) specifically in adipocytes (4). Unexpectedly, our model failed to induce any classical HIF-1 α targets, such as VEGF-A. Moreover, we did not detect any increases in key components of the angiogenic or anaerobic glycolytic programs (4). The most striking alteration we observed in this model was an enhanced fibrotic response, which included an upregulation lysyl oxidase (LOX), elastin, collagens I and III, tissue inhibitor of metalloproteinase I, and connective tissue growth factor. As the result of this aberrant HIF-mediated transcriptional program, we proposed that the ECM in white AT accumulates fibrillar collagens; the upregulation of ECM thus causes local fibrosis. Collectively, our results suggest that hypoxia-induced fibrosis in AT may be a key factor that ultimately stimulates the local inflammatory responses.

Fatty acid flux. FFAs, stored in the form of triglycerides in AT, are released from hypertrophic adipocytes through lipolysis during fasting. Some of these FFAs are shunted to the liver and stored in lipid droplets, while some of them are oxidized in other organs. However, many of them are locally re-esterified in adipocytes

(29–31). Those FFAs that escape reesterification play a critical role in several organs as a primary energy source during prolonged fasting (32). However, FFAs can also serve as ligands for the TLR4 complex (33), thereby activating the classical inflammatory response in the context of increased local extracellular lipid concentrations, which ultimately drives ATM Φ accumulation (34, 35). Even though we do not consider AT as a conventional tissue suffering from lipotoxic side effects, either high rates of lipolysis or an influx of saturated FFAs into adipocytes do cause temporary inflammation within the tissue (33).

While these four mechanisms of macrophage recruitment and infiltration into AT may act independently, the metabolic and inflammatory pathways are tightly interconnected. Future studies should reveal the precise temporal steps required for macrophage recruitment to AT in expanding fat pads.

AT remodeling: the crosstalk between adipocytes and macrophages

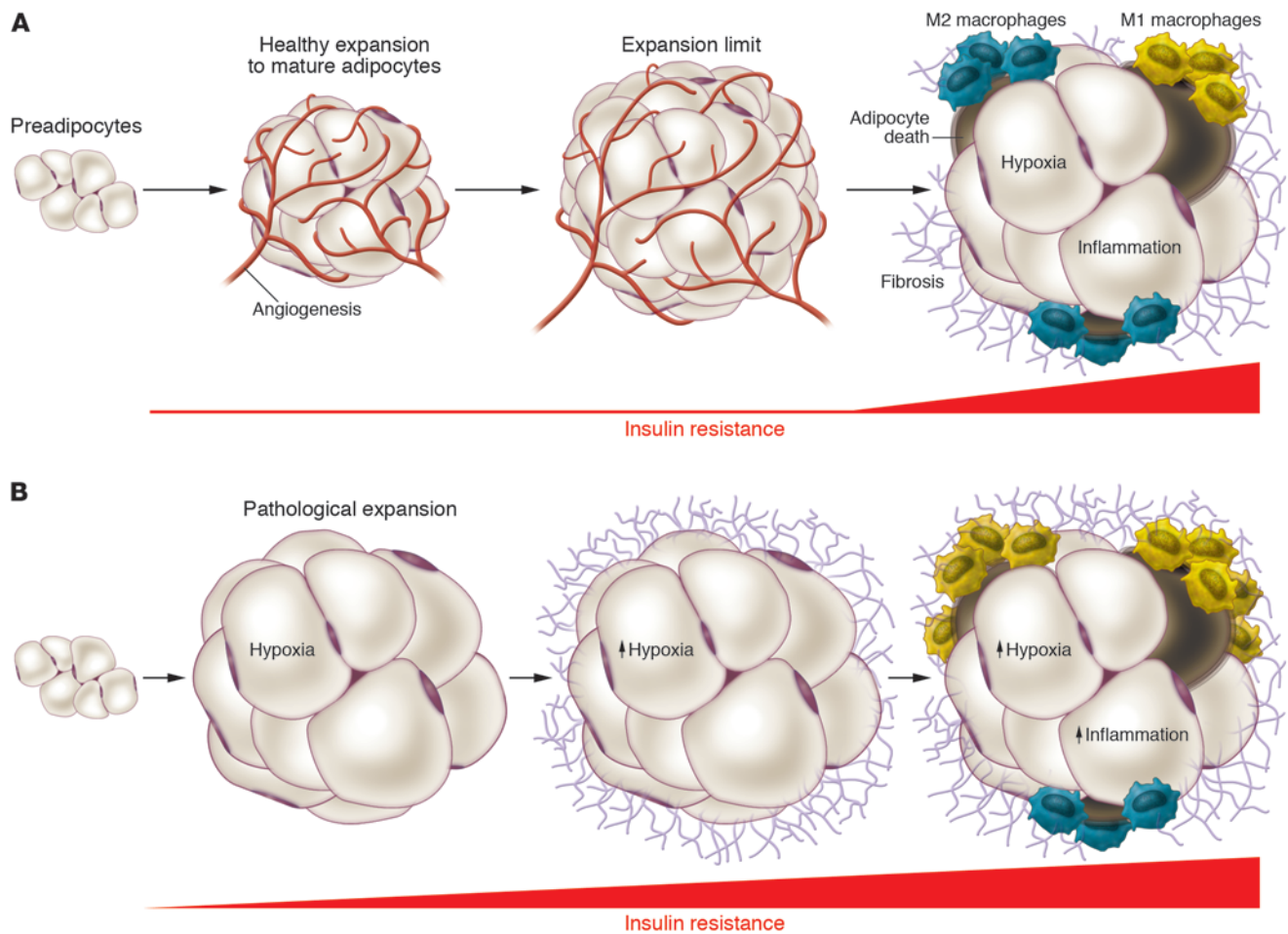
Although the adipocyte is the key player orchestrating local changes in the microenvironment, much evidence also points toward pivotal role for macrophages in such remodeling events. Resident ATM Φ s display remarkable heterogeneity in their activities and functions (36), largely reflecting the complex events occurring in AT during metabolic and immune perturbations.

The obesity-driven phenotypic switch in ATM Φ s. ATM Φ s can be characterized in two broad classes based on the expression of particular antigens (37, 38). In 2007, Saltiel and colleagues proposed a model of “phenotypic switching” that captured the very essence by which enhanced ATM Φ infiltration exacerbates the milieu of obesity-related inflammation (38). Their model emphasized that obesity is accompanied by a transformation in the polarized states of macrophages, from an antiinflammatory “alternatively activated” M2 form that primarily accumulates during negative energy balance (39), to a more proinflammatory “classically activated” M1 form (38). The M1 population demonstrates a positive correlation with insulin resistance and dominates in states of overnutrition by targeting FFA-mediated increases in proinflammatory responses (14, 40).

In the lean state, resident macrophages are polarized toward an M2 status. A hallmark feature of the M2 population is that they express F4/80, CD301, IL-10, and arginase 1 (37, 38), the latter of which has been shown to inhibit NOS (iNOS) activity (41). Such a combination of factors may help to preserve normal adipocyte function by promoting tissue repair and angiogenesis in the increasing AT mass (42). Conversely, M1 macrophages, induced by LPS and the Th1 cytokine IFN- γ , express a repertoire of proinflammatory factors, which include F4/80, CD11c, TNF- α , IL-6, iNOS, and CCR2 (14, 38).

In light of this, Patsouris and colleagues utilized a conditional cell-ablation system based on the transgenic expression of the diphtheria toxin receptor, under the control of the CD11c promoter, to specifically ablate CD11c⁺ macrophages in the AT of HFD-fed obese mice (43). Such conditional ablation resulted in a profound reduction of proinflammatory ATM accumulation, accompanied by rapid whole-body normalization of insulin sensitivity (43). This further highlights the direct contribution of these cells toward local and systemic insulin sensitivity.

Taken together, these studies suggest that a delicate balance of polarized populations of macrophages is necessary to maintain adequate adipocyte function. Identifying factors that quench

**Figure 1**

Healthy and unhealthy AT expansion. **(A)** Healthy AT expansion consists of an enlargement of AT through effective recruitment of adipogenic precursor cells to the adipogenic program, along with an adequate angiogenic response and appropriate remodeling of the ECM. There are strong individual differences with respect to the potential for AT expansion. **(B)** In contrast, pathological AT expansion consists of massive enlargement of existing adipocytes, limited angiogenesis, and ensuing hypoxia. As a result, HIF-1 α is induced, which in turn can cause the induction of a fibrotic program. Ultimately, M1-stage macrophages prevail, leading to an inflammatory phenotype that is strongly associated with systemic insulin resistance.

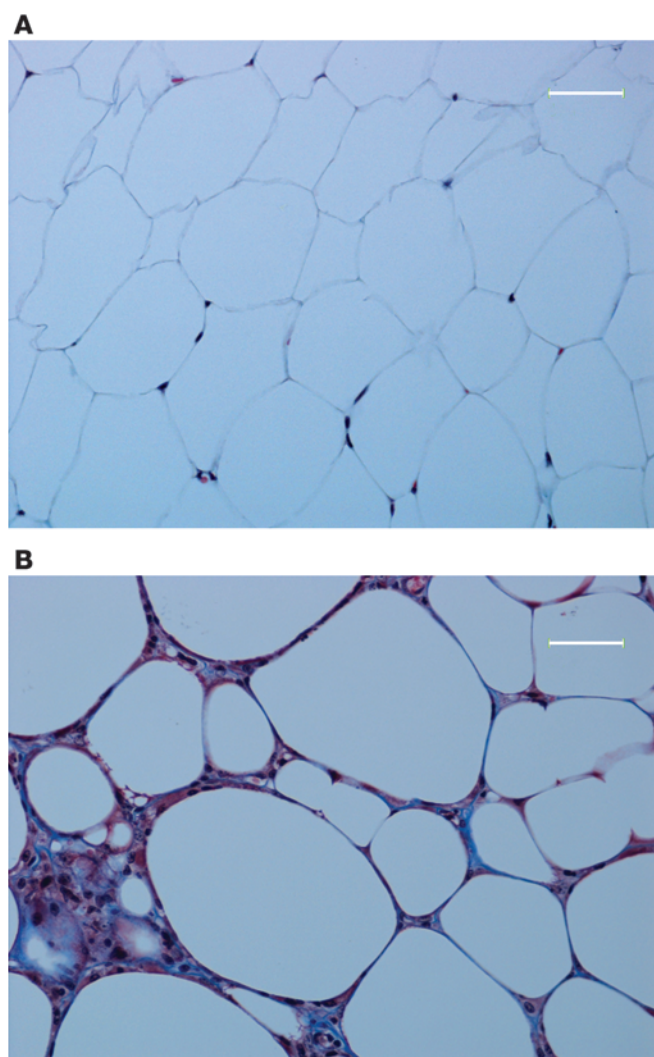
inflammatory signals in AT through modification of ATMs to retain an M2 polarization, or by triggering the phenotypic switch from M1 to M2, may be beneficial to preserve adequate adipocyte function and insulin action in an obese setting (Figure 1).

An important distinction needs to be established between healthy fat pad expansion and pathological fat pad expansion. We define healthy expansion as an enlargement of the fat pad mass through enhanced recruitment of adipocyte precursor cells that are differentiated into small adipocytes, along with the recruitment of other stromal cell types with appropriate ratios, and subsequent vascularization, minimal induction of ECM and minimal inflammation. In contrast, pathological expansion of AT can be described by rapid growth of the fat pad through enlargement of existing fat cells, a high degree of macrophage infiltration, limited vessel development, and massive fibrosis (ref. 8 and Figure 2). Such pathological expansion is associated with chronic inflammation, which ultimately results in the development of systemic insulin resistance.

The intricate dynamics of AT remodeling

Acute remodeling in context of weight loss. Recently, Kosteli and colleagues demonstrated that acute weight loss is unexpectedly associated with a rapid – albeit transient – lipolytic-driven recruitment of macrophages into AT (39), thereby confirming similar reports by Granneman and colleagues (44).

In contrast to the obese state, in which the occurrence of increased fat mass, adipocyte hypertrophy, and elevated basal lipolytic rates exacerbate the invasion of classically activated CD11c⁺ macrophage populations that permanently reside in the inflamed AT, we anticipate that remodeling due to weight reduction would lower ATM Φ content and subsequent inflammation. This was indeed observed by Kosteli et al. during chronic weight loss, during which they report that a progressive decrease in lipolysis and ATM Φ content followed 21 days of caloric restriction (39); F4/80⁺ and CD11b⁺ macrophages were further shown to be prominent during times of negative energy balance (39). Ironically, acute weight loss (days 0–7), a 24-hour fast, or pharmacological induction of

**Figure 2**

Healthy and unhealthy AT. (A) Trichrome stain of a healthy murine epididymal fat pad with densely packed, hexagonal adipocytes and limited immune cell infiltration. (B) Trichrome stain of an unhealthy *ob/ob* epididymal fat pad, containing a high level of immune cell infiltration and enhanced ECM (blue areas). Scale bars: 50 μ m.

adipocyte lipolysis using a β 3-adrenergic agonist all resulted in a transient accumulation of lipid-laden ATM Φ s (an increase of up to 65% of macrophage infiltration was observed). This was reflected by an increase in the levels of macrophage-specific genes, F4/80, CD38 and CSF-R1 (39). Collectively, these studies suggest that a transient influx of ATM Φ s is a physiologically healthy response and further implicates a maladaptive inflammatory reaction in the chronic state. In addition, a chronic negative energy balance, which results in the mobilization of triglyceride stores through enhanced lipolytic rates, acts as a signal to circulating monocytes, providing an excellent paradigm for the adaptations and the beneficial interplay between adipocytes and macrophages that arise as a consequence of a reduction in AT mass. While we appreciate that during this process the egression of ATM Φ s from the adipose back into circulation following a period of fasting is fully effective, it is

not known how we can prevent the lipid-laden proinflammatory ATM Φ s from permanently residing in AT in the obese state. When and how do we transition from a physiologically healthy adaptive immune response to a pathological maladaptive state?

Understanding how AT remodels and acclimatizes to an acutely altered nutrient environment may provide key mechanistic insights into solving the puzzle of chronic ATM Φ recruitment into AT.

Chronic remodeling of AT: the adipocyte in the spotlight. With an exceptionally high level of adipokine production, it is legitimate to view the adipocyte as a “professional” secretory cell. In times of sustained excess of caloric intake, adipocytes undergo hypertrophy and hyperplasia, thus setting into motion the dynamic events of AT expansion. Hypertrophy results in an altered complement of secretory products, which over time adversely affects the remodeling of AT (45). In parallel, the hypertrophy provides valuable protection against the detrimental systemic lipotoxic effects of excess lipid exposure. These events entail mechanical stress through increased ECM overproduction, limited angiogenesis, and the induction of local inflammation, all of which continuously compromise the functional integrity of AT (46, 47).

Hypertrophy versus hyperplasia? Two distinct mechanisms can lead to increased AT size: hypertrophy (an increase in adipocyte volume) or hyperplasia (an increase in adipocyte cell number). Although we recognize that adipocyte hypertrophy prevails in obesity, there remains some debate as to whether the adipocyte number remains constant in an adult individual, or whether the ability to undergo hyperplasia is age dependent. Key questions to take into consideration are: what is the actual half-life of an adipocyte? Can certain processes alter the rate of adipocyte turnover? And if so, is the adipocyte indeed programmed to survive for a certain period of time, or can its fate be derailed under extreme metabolic oscillations?

Using incorporation of environmental 14 C as a tracer, Spalding and colleagues documented that “new adipocytes form constantly to replace lost adipocytes” and estimated the half-life of the average adipocyte to be in the order of 8.3 years (48). This group postulated that adipocyte cell number is relatively fixed by early adulthood, and that any alterations in fat mass during adulthood are merely credited to alterations in adipocyte hypertrophy (48). The rate of appearance of newly emerging adipocytes is balanced by adipocyte death, with the total number of adipocytes being tightly controlled, while the whole system is in a state of constant flux. This hypothesis suggests that adipocyte progenitor cells are either recruited into the stromal vascular fraction of adult AT (49) or propagated as precursor cells into mature AT, thus allowing them to differentiate into lipid-laden mature adipocytes at the same rate that existing adipocytes undergo cell death.

These studies, however, raise further questions: given that obesity is associated with a higher adipocyte turnover due to an elevated rate of cell death (10), are there conditions in which a higher rate of preadipocyte recruitment, rather than loss of mature adipocytes through cell death, can occur? Can we ever tip the balance to have an uneven “apoptotic-to-adipogenic ratio” (48), and what would be the metabolic consequences? Moreover, what are the signals that trigger an influx of preadipocytes and enhanced recruitment of preadipocytes towards the adipogenic axis? One plausible mechanism may be that once adipocytes reach a critical volume, they secrete factors that recruit new adipocytes (50); however, the details of these processes are far from understood.

The effect of age on adipocytes and AT remodeling has also recently been the focus of several studies. Adipogenesis and the



intrinsic functional dynamics of preadipocytes en route to develop into fully functional mature adipocytes have been documented to decline markedly with age (51, 52). In particular, the capacity of preadipocytes to proliferate, differentiate, and confer resistance to cell death is severely blunted with age (51, 53). As such, the inherent characteristics of these cells are shown to contribute to age- and depot-dependent variations in AT function, collectively resulting in metabolically unfavorable ectopic fat accumulation (51, 54, 55).

Is the macrophage involved in adipocyte turnover? Strissel and colleagues provided key answers to this question by assessing the progression of adipocytes and macrophages in AT of HFD-fed mice. As expected from previous studies, the group initially observed that following 16 weeks of HFD feeding, a high degree (approximately 80%) of adipocyte cell death was evident in epididymal AT (13). However, a fascinating twist to these findings was that at the 20-week stage, the percentage of necrotic cells was reduced to approximately 16%; this was accompanied by an increased emergence of smaller adipocytes ($<5,000 \mu\text{m}^2$), thus restoring the adipocyte number (13). These key findings lend support to a model in which the progression of obesity is associated with AT remodeling, expanding largely through hypertrophy, subsequently requiring adipocytes to undergo an initial “necrotic wave,” with later stages relying on hyperplastic expansion.

So, where do ATMΦs fit into this equation of adipocyte turnover and AT expansion? And does a macrophage polarization shift occur in response to fluctuations in adipocyte turnover? The initial surge of cell death was shown to be associated with the presence of M1-like ATMΦs. In contrast, progressing on to the hyperplastic stage of AT transition, a reduction in M1-type markers was evident with a concomitant appearance of M2 macrophages (13).

Other immune cells: the emerging physiological role of T cells in AT physiology. In addition to macrophages, recent studies have revealed a growing list of other immune cells that may orchestrate AT remodeling. These include subsets of the T lymphocyte lineage, including CD4^+ and CD8^+ T cells, Tregs, and mast cells (56, 57). In particular, levels of a specific subpopulation of CD8^+ T cells are enriched in the early stages of obesity, in fact preceding the accumulation of ATMΦs (56). This certainly implicates a potential role for CD8^+ T cells in the initiation of the subsequent inflammatory cascade. Mast cells have recently been highlighted as another AT-associated cell type; abundant in obese AT, these inflammatory cells may elicit a pathogenic role in AT functionality (58). As a whole, these studies underscore that, in order to design therapeutic strategies, it is important to understand the imbalance of Tregs in AT during the progression of inflammation in expanding AT.

Angiogenesis, a rate-limiting step for AT expansion and remodeling

Angiogenesis is the process of formation of new blood vessels. Tumor expansion critically depends on an appropriate blood supply, which can be provided by new blood vessels from surrounding tissues. Angiogenesis plays a critical role in tumor growth and metastasis formation, and blocking angiogenesis prevents tumor growth and forces regression of existing lesions (59–61). However, in healthy adults, the majority of tissues do not expand in size, and the embedded blood vessels are thus quiescent (62). One notable exception is AT, which displays by nature a higher level of plasticity and retains the potential to grow throughout the entire lifetime. AT can rapidly expand or regress under different nutritional conditions; therefore newly emerging adipocytes

are highly angiogenic. The angiogenic activity of white AT has been demonstrated in a number of in vitro systems, and has been further utilized clinically to promote wound healing (63). The newly formed vascular network is crucial for adipogenesis and AT expansion (64, 65). These vessels provide O_2 , nutritional components, growth factors, hormones, inflammatory cells, and bone marrow-derived stem cells to maintain adequate homeostasis of AT, all of which are crucial for further expansion (66). Effective development of the vascular supply through angiogenesis is therefore a rate-limiting step in AT expansion (67).

Different fat pads vary with respect to their degree of angiogenic potential. The vascular density and abundance of endothelial cells is higher in visceral AT in comparison to subcutaneous fat pads (68). Moreover, endothelial cells in visceral AT exhibit more potent angiogenic and inflammatory properties (68).

During AT remodeling in metabolically challenging conditions, adipokines are subjected to differential regulation, thereby orchestrating the growth and expansion of various fat pads. Leptin can stimulate the critical steps required to evoke an angiogenic program (69). In addition, bFGF can promote vascular endothelial cell growth (70) and hence the process of angiogenesis. Of note, bFGF is secreted by preadipocytes, and its levels are increased during caloric excess. Moreover, upon proper nutritional stimulation, AT also synthesizes HGF and VEGF (71), both of which play key pro-angiogenic roles (71). The situation seems more complex for adiponectin, however. Some studies have suggested anti-angiogenic properties for adiponectin (72), but these studies rely on recombinant preparations of the protein, and the quality of these preparations varies widely. Other in vivo studies demonstrating adiponectin-mediated inhibition of angiogenesis in mouse cornea assays suffer from the same shortcomings (73). In contrast, data from several other more reliable in vivo studies suggest potent pro-angiogenic effects (74). Using a genetic approach, our laboratory identified that adiponectin has potent angiogenic properties in tumor vascularization (75).

VEGF-A is the only bona fide endothelial cell growth factor; moreover, its presence is essential for initiation of the angiogenic program (76, 77). VEGF-A exhibits predominantly pro-angiogenic activity in AT (78). Furthermore, VEGF levels are known to be regulated by hypoxia, insulin stimulation, certain growth factors, and specific cytokines (79) and vary during adipogenesis (80). In brief, VEGF functions by binding two tyrosine kinase receptors, VEGF-R1 and VEGF-R2; the latter is expressed in vascular endothelial cells, and signaling through this receptor is critical for both physiological and pathological blood vessel formation. Blockade of VEGF-R2, but not VEGF-R1, restricts AT expansion and thus prevents the progression of DIO (81). Remarkably, blockade through use of a VEGF-R2 antibody can further inhibit preadipocyte differentiation (82). Due to their dramatic effects in angiogenesis and hence AT remodeling, VEGF and its receptors should be considered as key targets worth further exploration in the context of development of pharmacological anti-obesity approaches.

The angiopoietin proteins are the most important functional partners of VEGF. Both Ang-1 and Ang-2 can bind to the common endothelial cell tyrosine receptor, Tie-2 (83, 84). More specifically, Ang-1 is an agonist of the Tie-2 receptor, which is constitutively expressed in several tissues, including AT (83). The activity of Ang-1 can enhance VEGF receptor function for vascular development, remodeling, stabilization, and final maturation (85–87). Ang-2, on the other hand, is an antagonist for the Tie-2 receptor and is exclu-



sively expressed in sites of vascular remodeling (83, 84, 88). In these regions, Ang-2 can block the action of Ang-1 through competitive inhibition, thereby destabilizing the vessel structure (84). However, delineating an unequivocal definition of the precise function of Ang-2 is proving somewhat challenging, since it very much depends on the context; for instance, in the presence of VEGF, Ang-2 can prime vessels to mount a robust pro-angiogenic response (89).

Several reports suggest a role of angiopoietins and Tie-2 in AT, but to date, the functions of this family of proteins have not been directly examined. Xue and colleagues recently reported that AT FOXC2 can mediate certain aspects of angiogenesis and vascular patterning (90). Given that Ang-2 is an established transcriptional target of FOXC2 (90), it is likely that Ang-2 plays an important role in this pathway. When inhibiting the function of Ang-2 with a neutralizing antibody, the effects caused by FOXC2 are equally blocked (90). Further studies are therefore required to better define the precise function of angiopoietins in AT remodeling.

Promoting an angiogenic cascade in healthy fat pads can effectively counteract hypoxic conditions and could thus be associated with metabolically beneficial effects. Adiponectin, for example, has been shown to induce angiogenesis in hypoxic fat pads and tumors (75, 91). Moreover, we have previously demonstrated that overexpression of adiponectin in an *ob/ob* background improves vascularization and expansion of the subcutaneous fat pad in particular (91).

O₂ tension in the center of solid tumors may be so low that cells essentially become anoxic; this is recognized to have profound effects on the tumor metabolism (92–94). We have discussed hypoxia as a key player in expanding AT that serves as a driving force for macrophage infiltration. Compared to brown AT, white AT is not particularly well vascularized (4, 20). The O₂ tension in obese white AT can reach levels as low as 15 mmHg, much lower than that in normal lean AT, in which values would typically reach 45–50 mmHg (23). Moreover, the postprandial increase in blood flow that is so frequently observed in lean individuals is dramatically reduced in obese individuals (95, 96). Here we should take into consideration the large diameter that an individual adipocyte can acquire (150–200 μ m); this is markedly larger than the average diffusion distance of O₂ in tissues (97). Several mouse models of hypoxia have been described in the literature (4, 22, 23, 98, 99). We have also shown that exposure to a HFD for merely a few days results in a significant increase in adipocyte cell size; this consequently leads to an acute hypoxic response within the adipocytes themselves (4).

The role of collagens and matrix metalloproteinases in the remodeling process

Adipocytes are enmeshed in a dense network of ECM (100, 101). The ECM not only functions to provide mechanical support for a fat pad, but also regulates the physiological and pathological events of AT remodeling through a variety of signaling pathways (102). During AT expansion, the ECM actively remodels to accommodate the growth. We and others have demonstrated that several ECM components are upregulated during fat mass expansion in states of obesity (102, 103). AT fibrosis, with its associated reduced plasticity, is therefore a

key hallmark of the metabolically dysfunctional AT. Collagen VI, for example, is a collagen complex that is highly enriched in the ECM of AT (102). We have demonstrated that the weakening of the ECM that surrounds AT by elimination of collagen VI leads to improved survival rates of adipocytes and improvements in metabolism (102). Others have further reported correlations between elevated collagen VI levels, hyperglycemia, and insulin resistance (104, 105).

The ECM components in AT are known to be regulated by matrix metalloproteinases, a family of neutral endopeptidases that cleave ECM components, thereby enabling remodeling of ECM (106). Chun and colleagues highlighted the essential role for one of these proteases by demonstrating that MT1-MMP (MMP14) has a direct impact on adipose differentiation *in vitro* and *in vivo* (101). The absence of MT1-MMP impairs white AT development and hence causes lipodystrophy (101). The function of several other MMPs highly expressed in AT remains to be further identified. Maquoi and colleagues further demonstrated that in DIO mice, expression levels of MMP3, -11, -12, -13, and -14 are all upregulated, whereas levels of MMP7, -9, -16, and -24 are downregulated (106). In parallel, specific tissue inhibitors of MMPs (TIMPs) can also be dysregulated. More specifically, TIMP1 is upregulated with obesity, and conversely, TIMP4 is downregulated in states of obesity (106). Such striking regulatory patterns of MMPs and their corresponding inhibitors suggest important roles of these factors in the pathophysiology of AT.

Concluding remarks

We underestimate the complex events that must occur in order for an organ such as AT to rapidly remodel and either release or accept a large number of calorically dense lipids that have the potential to be potentially cytotoxic. A well-orchestrated set of interactions between a number of critical cell types has to take place in a defined chronological order, with many pathological changes that can occur during that process. While research in the past decade has focused on the macrophage as an important player in AT remodeling, future efforts will unquestionably address the unique properties of other cells, including AT-associated endothelial cells as key players for a healthy adipocyte microenvironment.

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