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Review

Angiogenesis and obesity

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Development of obesity is associated with substantial modulation of adipose tissue structure, involving adipogenesis, angiogenesis, and extracellular matrix remodelling. These processes require proteolytic activity, provided mainly by the fibrinolytic (plasminogen/plasmin), matrix metalloproteinase, and ADAM/ADAMTS systems. In early-stage development of adipose tissue, adipogenesis is tightly associated with angiogenesis. Thus, adipose tissue explants trigger blood vessel formation, and in turn adipose tissue endothelial cells promote pre-adipocyte differentiation. Modulation of angiogenesis and of proteolytic systems may have the potential to impair adipose tissue development.

1. Introduction

Over the last decades, obesity and its consequences worldwide have become a major health problem. Between 1976 and 2002, the prevalence of overweight [body mass index (BMI) $\geq$ 25 kg/m^2] in the USA has increased from 46 to 66% of the population and that of obesity (BMI $\geq$ 30 kg/m^2) from 15 to 31%.[1] Excess weight increases the risk of multiple conditions, including hypertension, cardiovascular, and cerebrovascular disease, type 2 diabetes, certain types of cancer, gallstones, and osteoarthritis. In addition, obesity negatively affects physical functioning, vitality, and general quality of life.[2,3]

To supply growing adipose tissue with nutrients and oxygen, the vasculature responds by increasing the number and/or size of blood vessels. In early stage development of adipose tissue, adipogenesis is tightly associated with angiogenesis. We will review the pro- and anti-angiogenic components that have been demonstrated in adipose tissues, and discuss how modulation of proteolysis and/or angiogenesis may affect obesity.

2. Adipose tissue vasculature

Adipose tissues exhibit extensive vascularity with every adipocyte surrounded by one or more capillaries. Fat cell development is characterized by the appearance of a number of fat cell clusters, or 'primitive organs,' which are vascular structures in the adipose tissue with few or no fat cells. In addition to pre-adipocytes, mature adipocytes, and ECs fat pads consist of many different cell types, including pericytes, fibroblasts, macrophages, and mesenchymal stem cells.

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The growth of white adipose tissue (WAT) requires continuous remodelling of the vascular network, primarily of primitive capillary networks. Expansion of adipose tissue can be supported by both neovascularization (for adipocyte hyperplasia) and dilation and remodelling of existing capillaries (for adipocyte hypertrophy). Brown adipose tissue (BAT) is mainly responsible for energy metabolism, and its function requires efficient blood perfusion to supply nutrients and oxygen and to export heat. BAT hyperplasia is critically dependent on angiogenesis, as it requires rapid activation of mitosis in fat precursor cells and ECs to develop capillaries.[4]

In vitro studies revealed that adipose tissue explants in fibrin or collagen gels trigger blood vessel formation,[5] and that in turn adipose tissue ECs promote pre-adipocyte differentiation.[6] Mature adipocytes in culture can de-differentiate followed by differentiation into adipocytes or ECs, suggesting that these are derived from a common lineage.[7]

Blood vessel density in adipose tissue may not truly reflect angiogenic activity. Indeed, in tumours it was suggested that the number of cells that can be supported by a blood vessel varies, influencing in turn the vascular density.[8] Similarly, the number and/or size of adipocytes in adipose tissue may affect blood vessel density. This is supported by a study on capillary fenestrations in adipose tissue, showing that microvessel density was lower in genetically obese *ob*/*ob* mice than in wild-type (WT) controls, possibly as a result of increased adipocyte size in *ob*/*ob* mice.[9] To take this into account, blood vessel density in adipose tissues can be normalized to the adipocyte density.

3. Regulation of adipose tissue-related angiogenesis

It is generally accepted that the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/

VEGFR) system accounts for most of the angiogenic activity in adipose tissue, making it an attractive target to reduce obesity.[10–12] In addition, many components affecting angiogenesis have been identified in adipose tissues, including PlGF, Np-1, FGF-2, Ang-2, leptin, TSP-1, osteonectin, adiponectin, resistin, TF, TNF-α, FGF-β, IGF, HGF, etc. The angiogenic potential of the main pro- or anti-angiogenic components is sometimes context dependent and different in different fat pads (see below).

3.1 Vascular endothelial growth factors and placental growth factor

VEGF-A (17–23 kDa) is a major angiogenic factor that stimulates proliferation and migration of ECs.[13] Three forms of VEGF-A are produced in the mouse as a result of alternative splicing (VEGF-A121, VEGF-A165, and VEGF-A189). Several studies indicate that VEGF-A stimulates both physiological and pathological angiogenesis by signalling through VEGFR-2 in a strict dose-dependent manner. Loss of a single allele causes embryonic vascular defects, while reduction of VEGF-A levels by only 25% impairs spinal cord perfusion, resulting in motor neuron degeneration reminiscent of amyotrophic lateral sclerosis.[13–15] VEGF-B (21 kDa) is 43% identical to VEGF-A165; it also promotes angiogenesis and is implicated in extracellular matrix (ECM) degradation via regulation of plasminogen activation.[16] VEGF-C (23 kDa) displays 30% homology with VEGF-A165 and plays an important role both in angiogenesis and lymphangiogenesis.[17,18] VEGF-D (22 kDa) is 48% identical to VEGF-C and also promotes the growth of lymhatic vessels.[19]

Placental growth factor (PlGF), a 25 kDa homologue of VEGF-A (53% sequence identity with VEGF-A165), enhances angiogenesis, but only in pathological conditions. Loss of PlGF impairs angiogenesis in the ischaemic retina, limb, and heart, in wounded skin and in tumours, without affecting physiological angiogenesis.[20]

3.2 Vascular endothelial growth factor receptor and placental growth factor receptors

The members of the VEGF family bind to transmembrane tyrosine kinase receptors [VEGFR-1 (206 kDa), VEGFR-2 (218 kDa), and VEGFR-3 (150 kDa)]. VEGF-A interacts with both VEGFR-1 and VEGFR-2, whereas VEGF-B and PlGF bind to VEGFR-1. VEGF-C and VEGF-D activate VEGFR-3, but VEGF-C can also bind to VEGFR-2.

VEGFR-1 and VEGFR-2 mediate angiogenesis, whereas VEGFR-3 is involved mainly in lymphangiogenesis.[21] VEGF-A165, PlGF, and VEGF-B also bind to another transmembrane receptor, neuropilin-1 (Np-1). Inactivation of the *Np-1* gene in mice causes disturbances in development of the vascular and nervous system.[22]

3.3 Fibroblast growth factor-2 and osteonectin

Fibroblast growth factor (FGF)-2 (25 kDa) is a potent stimulator of differentiation, migration, and proliferation of ECs, and enhances adipocyte differentiation *in vivo*.[23] During angiogenesis, FGF-2 stimulates the synthesis of proteinases such as collagenase and urokinase-type plasminogen activator (u-PA), and of integrins to form new capillary cord structures.[24,25]

Osteonectin binds to VEGF-A, impairs VEGFR-1 activation, and inhibits FGF-2, resulting in inhibition of EC proliferation. Osteonectin is produced by the adipose tissue and its expression is upregulated in obesity.[26] Osteonectin deficient mice on high fat diet (HFD) develop larger fat pads as compared to WT mice.[27]

3.4 The angiopoietins, TIE-1, and TIE-2

Another signalling system contributing to maintenance, growth, and stabilization of blood vessels involves the tyrosine kinase (T) with Ig (I) and epidermal (E) growth factor (EGF) homology domains (TIE)-1 and -2 receptors (140–145 kDa). TIE-2 binds the angiopoietins (Ang-1 and Ang-2), whereas the ligands of TIE-1 are not known yet. Unlike Ang-2, which activates TIE-2 on some cells but blocks it on others,[28] Ang-1 consistently activates TIE-2.[29] The role of Ang-1 in vascularization is pleiotropic and context-dependent. Ang-1 tightens vessels by affecting junctional molecules,[30] by promoting the interaction between ECs and mural cells, and by recruiting pericytes.[31] Ang-2 may stimulate vessel growth by loosening endothelial–periendothelial cell interactions and degrading the ECM.[32] TIE-1 and TIE-2 are expressed in adipose tissues, but their role in adipose tissue associated angiogenesis has not been clearly established.

3.5 Leptin

Leptin (16 kDa), a satiety hormone produced by the adipose tissue, promotes migration of ECs. Interaction of leptin with its receptor on ECs leads to activation of the Stat3 pathway and enhancement of its DNA-binding activity.[33] Besides a direct pro-angiogenic activity, leptin upregulates VEGF expression via activation of the Jak/Stat3 signalling pathway.[34] Similar to VEGF-A, leptin induces the formation of fenestrated capillaries, as confirmed by the absence of fenestrations in leptin deficient *ob/ob* mice.[9] Leptin has a synergistic effect on stimulation of angiogenesis by VEGF or FGF-2.[9]

3.6 Thrombospondins

Thrombospondins [TSP-1 (145 kDa) and TSP-2 (145 kDa)] are components of the ECM in remodelling tissues and, like other matricellular proteins, binds to matrix proteins and cell-surface receptors, including proteoglycans, non-integrin, and integrin receptors. Despite its pleiotropic biological role, mice deficient in TSP-1 are viable and exhibit only subtle abnormalities in development, although they develop pneumonia and show delayed organization and neovascularization of skin wounds.[35]

4. Proteolysis in growing adipose tissue

4.1 General aspects

The excessive growth of body fat requires substantial modulation of adipose tissue structure, involving adipogenesis, angiogenesis, and ECM remodelling.[36] There is growing evidence that proteolysis is required for several aspects of adipose tissue expansion. Hypertrophy of adipocytes requires remodelling of their basement membrane and the surrounding ECM. Proteolysis is also required for cell migration during the development of blood vessels and peripheral nerves, and for migration of macrophages in the growing adipose tissue. Thus, both adipogenesis and angiogenesis require proteolytic activity, which is mainly

mediated via the plasminogen/plasmin (fibrinolytic) and matrix metalloproteinase (MMP) systems. Recently, some evidence has emerged that proteins of the ADAM (A Disintegrin And Metalloproteinase) and ADAMTS (ADAM with TSP motif) families may also be implicated. Structural and functional aspects of these proteins have recently been reviewed.[37]

These proteinases are collectively able to cleave a wide variety of substrates, including ECM components, other proteinases and their inhibitors, and matrix receptors, whereby adipose tissue remodelling may be facilitated. MMPs and plasmin can also release, activate, or degrade several growth factors and cytokines implicated in obesity and play major roles in angiogenesis.

4.2 The fibrinolytic system and adipose tissue development

The fibrinolytic system comprises an inactive proenzyme, plasminogen, that can be converted to the active enzyme, plasmin, that degrades fibrin into soluble fibrin-degradation products. Two immmunologically distinct plasminogen activators have been identified: tissue-type plasminogen activator (t-PA) and u-PA. u-PA binds to a specific cellular receptor (u-PAR or CD87) resulting in enhanced activation of cell-bound plasminogen. Inhibition of the fibrinolytic system may occur either at the level of plasmin by α_2-antiplasmin, or at the level of the plasminogen activators, mainly by plasminogen activator inhibitor-1 (PAI-1).[38] While different studies support a relationship between fibrinolytic activity and obesity, the interactions between individual fibrinolytic parameters and other systems, such as angiogenesis, appear more complex than anticipated.

Several nutritionally induced obesity models in transgenic mice have been used to study the role of the fibrinolytic system in the development of obesity (*Table 1*). t-PA-deficient mice, kept on HFD, had higher body weight and adipose tissue mass than WT controls.[39] There was an increase in the number of ECs and stroma cells in the fat tissues, suggesting that targeted inactivation of t-PA increases angiogenesis in the adipose tissue, which may promote adipose tissue formation. Deficiency in u-PA, in contrast, had no effect on nutritionally induced obesity,[39] although it was previously shown that overexpression of u-PA in the brain resulted in reduced body weight and size.[40] Mice deficient in plasminogen, the substrate for both plasminogen activators, showed reduced fat accumulation associated with reduced differentiation of stromal cells.[41] Deficiency of α2-antiplasmin had no significant effect on adipose tissue development in mice.[42]

PAI-1 is strongly expressed in murine and in human adipose tissue.[43–45] In human adipose tissue, its expression is positively correlated with BMI.[46] Increased levels of PAI-1 are also observed in patients with the insulin resistance syndrome.[47] In obese women, plasma PAI-1 levels during body weight reduction and body weight regain are positively correlated with the amount of body fat.[48] The role of PAI-1 in adipose tissue development at present still remains controversial. Some studies with transgenic mice reported that PAI-1 deficiency has little or no effect on adipose tissue development,[49,50] whereas others also using models of nutritionally induced obesity found impaired development.[51] Disruption of the PAI-1 gene in genetically obese and diabetic *ob*/*ob* mice also resulted in reduced adiposity and improvement of the metabolic profile.[52] To further elucidate the role of PAI-1 in the development of obesity, transgenic mice were generated with overexpression of murine PAI-1 under control of the adipocyte-specific promoter aP2. High circulating PAI-1 levels and reduced fibrinolytic activity in adipose tissue resulted in a reduction of nutritionally induced obesity whereas analysis of blood vessels did not reveal significant differences.[53] This finding is in agreement with a study of Eren *et al*.[54] who showed that transgenic mice overexpressing a stable human PAI-1 variant had virtually no intraperitoneal fat. Overexpression of PAI-1 thus seems to modify the cellularity of adipose tissue, however without significantly affecting angiogenesis. It may enhance expression of anti-differentiation factors such as Pref-1, which may contribute to reduced tissue mass.[53]

The discrepancies between these studies may be explained in part by the fact that nutritionally induced models depend on the composition and timing of the diet, and on the age and genetic background of the mice used. It can also not be excluded that modifier genes in a specific genetic background affect the outcome of such obesity studies. There is also a difference between genetically determined obesity models (mainly *ob*/*ob* or *db*/*db* mice lacking leptin or its receptor) and nutritionally induced models in WT mice with intact genome. Leptin provides a local angiogenic signal and improves the efficiency of lipid release from fat stores to maintain energy homeostasis.[33]

Table 1 Role of proteolytic systems in adipose tissue development, as studied in transgenic mouse models

Deficiency	Effect on adipose tissue	Reference
Fibrinolysis		
t-PA	Enhanced	Morange *et al*.[39]
u-PA	No effect	Morange *et al*.[39]
Plasminogen	Impaired	Hoover-Plow *et al*.[41]
α2-antiplasmin	No effect	Lijnen[42]
PAI-1	No effect	Morange *et al*.[49] and Lijnen[50]
	Impaired	Ma *et al*.[51] and Schäfer *et al*.[52]
MMP systems		
MMP-3	Enhanced	Maquoi *et al*.[62]
MMP-11	Enhanced	Lijnen *et al*.[64]
MMP-2	Impaired	Unpublished
MMP-9	No effect	Unpublished
TIMP-1	Impaired	Lijnen *et al*.[65]
ADAMTS-1	Impaired	Luque *et al*.[69]

4.3 The matrix metalloproteinase system and adipose tissue development

The MMPs belong to a family of over 25 neutral endopeptidases that are collectively able to cleave all of the ECM components as well as several non-ECM proteins, such as adhesion molecules, cytokines, protease inhibitors, and other (pro) MMPs. Generally, MMPs are expressed at low levels but are rapidly induced at times of active tissue remodelling. Most MMPs are secreted as inactive proenzymes and require proteolytic processing to become active. MMP

activity is modulated through interactions with tissue inhibitors of MMPs (TIMPs). Four TIMPs have been characterized that are able to inhibit the activities of all known MMPs. Consequently, the net MMP activity in tissues is locally determined by the balance between the levels of activated MMPs and TIMPs.[55]

Several lines of evidence suggest a potential role of MMPs in the development of adipose tissue. Conditioned medium of rat adipocytes contains a MMP-2 (gelatinase A), like gelatinolytic activity, that may play a role in their organization into large multicellular clusters.[56] High expression of MMP-2 was reported in adipose tissue of mice with nutritionally induced obesity as well as in genetically obese mice.[57,58] MMP-2 and MMP-9 expression and secretion have also been demonstrated in human adipose tissue.[59] Furthermore, MMP-2 levels increase and TIMP-1 levels decrease during adipocyte differentiation.[60]

To gain further insight into the involvement of the MMPs in the development of adipose tissue, the expression of MMPs and TIMPs was monitored in lean and obese mice.[61] This revealed upregulation with obesity of mRNA levels of some MMPs (MMP-3, -11, -12, -13, -14) and downregulation of others (MMP-7, -9, -16, -24). Most of these modulations were specific to the gonadal (GON) fat, supporting the concept that the different fat depots [subcutaneous (SC) and GON) are not identical].

TIMP-1, which is synthesized by most types of connective tissue cells as well as macrophages, acts against all members of the collagenase, stromelysin, and gelatinase classes. Analysis of mRNA expression in adipose tissue of lean and obese mice revealed significant upregulation of TIMP-1 with obesity. In contrast, TIMP-4 was downregulated with obesity, whereas TIMP-2 and TIMP-3 expression levels were not significantly modulated, at least in gonadal adipose tissue.[61]

Several nutritionally induced obesity models were used to study the role of MMPs and TIMP-1 in the development of adipose tissue (*Table 1*). Inactivation of the stromelysin-1 (MMP-3) gene in mice leads to enhanced development of adipose tissue when fed a HFD.[62] The higher body weight of MMP-$3^{-/-}$ mice resulted essentially from a specific increase of their adiposity, characterized by hypertrophic adipocytes in the SC and GON fat pads. A higher blood vessel density was observed in the adipose tissue of MMP-$3^{-/-}$ mice, suggesting that MMP-3 affects adipose tissue-related angiogenesis. A regulatory role of MMP-3 has also been suggested in adipogenesis during mammary gland involution in mice; MMP-$3^{-/-}$ mice showed accelerated differentiation and hypertrophy of adipocytes.[63] These data thus suggest an inhibitory effect of MMP-3 on adipocyte metabolism and differentiation. Similar results were seen with inactivation of the stromelysin-3 (MMP-11) gene. Indeed, MMP-11 deficiency promoted adipose tissue development and resulted in adipocyte hypertrophy.[64] We have recently shown that MMP-2-deficient mice when kept on a HFD, but not MMP-9-deficient mice, show significantly reduced obesity associated with adipocyte hypotrophy, without effect on angiogenesis (unpublished results).

TIMP-$1^{-/-}$ mice on HFD gained less weight than their WT counterparts and developed less adipose tissue.[65] Plasma leptin levels were significantly elevated in WT as compared to TIMP-$1^{-/-}$ mice on HFD. Leptin acts as a satiety factor and increases energy expenditure, while its secretion is strongly correlated with body fat mass and adipocyte size.[66] This suggests an effect of TIMP-1 deficiency on leptin secretion, or may merely be due to the lower body fat mass in the TIMP-$1^{-/-}$ mice. To further substantiate a role of TIMP-1 in nutritionally induced obesity, the effect of TIMP-1 overexpression by adenoviral gene transfer in mice was studied on adipogenesis and adipose tissue development.[67] Long-term expression of highly elevated levels of human TIMP-1 was associated with reduced MMP activity in plasma, as well as in adipose tissue. There was no significant effect on body weight or fat mass when the mice were kept on HFD. This is somewhat surprising since TIMP-1 deficiency resulted in impaired adipose tissue development. However, it is possible that physiologic TIMP-1 concentrations in mice are sufficient to promote adipogenesis and adipose tissue development, whereas overexpression has no further effect, and deficiency results in impairment.

4.4 ADAM and ADAMTS families and adipose tissue development

ADAMTS-1 and ADAMTS-8 can inhibit VEGF-induced angiogenesis and suppress FGF-2-induced vascularization.[68] They both mediate a greater anti-angiogenic response than either TSP-1 or endostatin, with ADAMTS-1 showing a greater inhibitory capacity than ADAMTS-8. The anti-angiogenic activity of ADAMTS-1 and -8 is mediated through their TS motifs. Interestingly, ADAMTS-1 deficient mice are exceptionally lean, their volume of epididymal fat is significantly smaller than in WT mice and the formation of capillaries in the adrenal gland is drastically impaired.[69]

The expression of ADAM and ADAMTS family members was investigated in adipose tissue of lean and obese mice. mRNA levels of ADAM-17 (TACE or TNF-α converting enzyme), ADAMTS-1, and ADAMTS-8 were quantified in isolated adipose tissues and cell fractions, and during differentiation of murine pre-adipocytes. ADAM-17, ADAMTS-1, and ADAMTS-8 mRNA were detected in both SC and GON adipose tissue of lean mice. In SC adipose tissue of obese mice (HFD), the expression of ADAM-17 was enhanced and that of ADAMTS-1 reduced, whereas in GON adipose tissue expression of ADAMTS-8 was reduced. In lean and obese mice, expression of ADAM-17, ADAMTS-1, and ADAMTS-8 was higher in the stromal-vascular cell fraction than in mature adipocytes. During differentiation of murine 3T3-F442A pre-adipocytes, expression of ADAM-17 and ADAMTS-1 remained virtually unaltered, whereas that of ADAMTS-8 decreased as adipocytes matured.[70] Aggrecan, as well as the two aggrecanases ADAMTS-4 and ADAMTS-5 mRNAs, are expressed in SC and GON adipose tissues of mice.[71] In mice with nutritionally induced obesity (HFD) as well as in lean controls, aggrecan mRNA expression was downregulated whereas ADAMTS-4 and ADAMTS-5 were upregulated with time. In mice with genetically determined obesity (*ob*/*ob*), ADAMTS-5 mRNA was upregulated in both SC and GON adipose tissues, as compared to WT mice. Thus, aggrecan levels were high at the early stages of adipose tissue development in mice, whereas its production decreased and its degradation increased during development of obesity. A functional role of aggrecan in promoting early stages of adipogenesis is supported by the findings that

it stimulated the *in vitro* differentiation of 3T3-F442A pre-adipocytes and the *de novo in vivo* accumulation of fat in Matrigel plaques injected into WT mice.[71]

Proteoglycans in the ECM of adipose tissue, such as aggrecan, may thus contribute to the regulation of lipid uptake and obesity in mice.

5. Modulation of angiogenesis in adipose tissue

During differentiation of 3T3-F442A pre-adipocytes into mature adipocytes mRNAs for Ang-1, the VEGF-A isoforms and PlGF are upregulated; FGF-2 and Np-1 expression are transiently upregulated, whereas the expression of Ang-2 is high in confluent cultures but decreases during maturation. TSP-1 and TSP-2 expression are downregulated in the early phase of differentiation but increase again in later phases. VEGF-B, VEGF-C, and VEGFR-1 mRNA levels are less modulated during differentiation. TIE-1, TIE-2, VEGFR-2, and VEGFR-3 are not expressed at detectable levels.[12]

Development of vasculature and mRNA expression of pro- or anti-angiogenic factors were studied during adipose tissue development in nutritionally induced (HFD) or genetically determined (*ob*/*ob*) murine obesity models.[12] *ob*/*ob* mice and C57Bl/6 mice on HFD had significantly larger SC and GON fat pads, accompanied by significantly higher blood content, increased total blood vessel volume, and higher number of proliferating cells, as compared to lean controls. Ang-1 expression was downregulated, whereas TSP-1 was upregulated in developing adipose tissue in both obesity models. Despite this upregulation, TSP-1 deficiency in mice did not significantly affect adipose tissue development;[72] its role in adipose tissue associated angiogenesis thus remains unclear. Ang-1 mRNA levels correlated negatively, and TSP-1 mRNA levels positively with adipose tissue weight. PlGF and Ang-2 expression were increased in SC adipose tissue of *ob*/*ob* mice and TSP-2 was increased in both their SC and GON fat pads. mRNA levels of VEGF-A isoforms, VEGF-B, VEGF-C, VEGFR-1, -2, and −3, and neuropilin-1 were not markedly modulated by obesity.[12]

To establish a functional role in obesity, PlGF deficient ($PlGF^{-/-}$) and WT mice with the same genetic background were kept on HFD for 15 weeks. $PlGF^{-/-}$ mice had a significantly lower body weight and less total SC plus GON adipose tissue. Blood vessel size was lower in GON adipose tissue of $PlGF^{-/-}$ mice. Blood vessel density, normalized to adipocyte number, was significantly lower in SC adipose tissue of $PlGF^{-/-}$ mice. These differences were not observed for $PlGF^{-/-}$ and WT mice kept on normal chow. These observations are in line with the emerging concept that PlGF deficiency has little or no effect on angiogenesis under normal (feeding) conditions, but is associated with impaired angiogenesis under stress conditions (HFD) during the early stages of adipose tissue formation.[73]

6. Pharmacologic inhibition of proteolysis/angiogenesis impairs adipose tissue development

6.1 Inhibition of angiogenesis

3T3-F442A pre-adipocytes injected into severe combined immunodeficient (SCID) mice induce angiogenesis and differentiate into adipocytes.[74] It was reported that neovasculature in the new adipose tissue originates by sprouting from larger host-derived blood vessels that run parallel to peripheral nerves and that endothelial progenitor cells do not play an important role in this process.[75] Adipogenesis and *de novo* fat pad formation in this model can be impaired by inhibition of PPAR-γ or VEGFR-2[10] and by administration of a PlGF neutralizing monoclonal antibody.[73] Furthermore, adipose tissue growth in mice can be impaired with angiogenesis inhibitors such as TNP-470 (a synthetic analog of fumagillin, that selectively inhibits EC growth by suppression of methionine aminopeptidase), angiostatin (a plasminogen fragment containing kringles 1–4), and endostatin (a COOH-terminal fragment of collagen XVIII).[76] These agents are also able to cause weight reduction in aged, relatively weight-stable *ob*/*ob* mice, suggesting that adipose tissue blood vessels are relatively immature and susceptible to inhibitors even when no longer growing. A recent study, using living adipose tissue-imaging techniques, demonstrated that angiogenesis in obesity requires a close interplay between differentiating adipocytes, stromal cells, and blood cells.[77] The use of an anti-VEGF antibody indeed inhibited not only angiogenesis, but also the formation of adipo/angiogenic cell clusters indicating that coupling of adipogenesis and angiogenesis is essential for differentiation of adipocytes in obesity, confirming that VEGF is a key mediator.[77]

Another strategy to reduce fat mass via its vasculature may be to target prohibitin, a multifunctional membrane protein selectively expressed in adipose tissue ECs.[78] Ablation of fat tissue can be achieved with a synthetic peptide that binds to prohibitin and induces apoptosis in adipose tissue blood vessels.

6.2 Inhibition of proteolysis

Tiplaxtinin, designed as a synthetic inhibitor of PAI-1, reduced body weight in WT mice kept on HFD.[79] The weights of the isolated SC and GON fat deposits were also significantly reduced, associated with adipocyte hypotrophy. Inhibitor-treated adipose tissues displayed similar blood vessel size, but a higher blood vessel density.

Other studies showed that Tiplaxtinin, in a model of diet-induced obesity, exhibited a dose-dependent reduction in body weight, epididymal adipose tissue weight, adipocyte volume, and circulating plasma active PAI-1.[80]

Administration of galardin, a hydroxamate-based broad-spectrum MMP inhibitor, to WT mice kept on HFD resulted in significant reduction of adipose tissue weight.[81] Ro 28-2653, a synthetic MMP inhibitor with enhanced selectivity for MMP-2, MMP-9, and MMP-14, did not affect adipose tissue development significantly.[82] In contrast, genetically obese *ob*/*ob* mice treated with the MMP inhibitor Bay 12-9566 gained somewhat less weight than controls.[76] The more specific gelatinase inhibitor Tolylsam also significantly reduced body weight and adipose tissue mass in the nutritionally induced obesity model in mice. This was associated with significant adipocyte hypotrophy (unpublished results).

Proteinase inhibitor treatments revealed a specific role of MMP-9 in the differentiation of human adipocytes.[83] However, MMP-9 deficiency in mice did not affect adipose tissue development on HFD.

7. Future lines of investigation

Pharmacological inhibition of PAI-1 could be beneficial in diseases associated with expansion of adipose tissue mass. Therefore, PAI-1 inhibitors will be needed that target active circulating PAI-1 as well as PAI-1 bound to vitronectin.

Furthermore, available data suggest the potential to impair adipose tissue development by using protease inhibitors. Identification of the MMPs that play key roles in adipose tissue development and generation of more specific inhibitors will be required to further explore their potential to affect obesity.

Modulation of angiogenesis appears to have the potential to impair the development of obesity. In particular, the VEGF/VEGFR signalling system may be an attractive target. Recent studies have suggested that the expression pattern of pro- and anti-angiogenic components in adipose tissue is fat depot dependent. A better understanding of the regulation of their expression will thus be instrumental in the development of specific targeting approaches. Little is known either on the interactions between these angiogenic components in adipose tissues and on the interplay with adipokines, such as leptin, resistin, and adiponectin. Also the interactions between adipocytes and ECs remain to be elucidated in more detail. The contribution of inflammatory cell derived cytokines, such as TNF-α and several interleukins (e.g. IL-6 and IL-8), to adipogenesis and angiogenesis in adipose tissues also needs further study. Since more and more antiangiogenic agents are characterized in the cancer field, there is a real option to evaluate such components in obesity models *in vivo*. However, since the development of adipose tissue is a complex and multifactorial process, it is unlikely that a single protease or angiogenesis inhibitor will allow reduction of obesity without associated side effects. It should indeed be kept in mind that the proteolytic systems and angiogenic components involved in adipogenesis and adipose tissue development also are critical to many other biological processes.

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