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## Commentary

Adipose extracellular matrix remodelling in obesity and insulin resistance<sup>☆</sup>De Lin<sup>a</sup>, Tae-Hwa Chun<sup>b,c</sup>, Li Kang<sup>d,\*</sup><sup>a</sup> Division of Cancer Research, School of Medicine, University of Dundee, Dundee, UK<sup>b</sup> Division of Metabolism, Endocrinology & Diabetes (MEND), Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA<sup>c</sup> Biointerfacing Institute, University of Michigan, Ann Arbor, MI, USA<sup>d</sup> Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee, Dundee, UK

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## ABSTRACT

The extracellular matrix (ECM) of adipose tissues undergoes constant remodelling to allow adipocytes and their precursor cells to change cell shape and function in adaptation to nutritional cues. Abnormal accumulation of ECM components and their modifiers in adipose tissues has been recently demonstrated to cause obesity-associated insulin resistance, a hallmark of type 2 diabetes. Integrins and other ECM receptors (e.g. CD44) that are expressed in adipose tissues have been shown to regulate insulin sensitivity. It is well understood that a hypoxic response is observed in adipose tissue expansion during obesity progression and that hypoxic response accelerates fibrosis and inflammation in white adipose tissues. The expansion of adipose tissues should require angiogenesis; however, the excess deposition of ECM limits the angiogenic response of white adipose tissues in obesity. While recent studies have focused on the metabolic consequences and the mechanisms of adipose tissue expansion and remodelling, little attention has been paid to the role played by the interaction between peri-adipocyte ECM and their cognate cell surface receptors. This review will address what is currently known about the roles played by adipose ECM, their modifiers, and ECM receptors in obesity and insulin resistance. Understanding how excess ECM deposition in the adipose tissue deteriorates insulin sensitivity would provide us hints to develop a new therapeutic strategy for the treatment of insulin resistance and type 2 diabetes.

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## 1. Introduction

The global epidemic of overweight and obesity is escalating and has become a major health challenge. Obesity is implicated as a cause of many devastating diseases, including diabetes, cardiovas-

cular disorders, and cancers [1]. Insulin resistance is a pathological condition closely associated with obesity, which may underlie the links between obesity and chronic metabolic diseases [2]. Adipocytes undergo dramatic expansion during the development of obesity. At the same time, the adipose tissue of obese individuals becomes fibrotic in both subcutaneous and omental fat depots [3,4]. Of note, obese insulin-resistant subjects with a similar body mass index display increased fibrosis in adipose tissues than obese insulin-sensitive subjects [5]. These studies suggest that fibrosis in the adipose tissue is closely associated with obesity and insulin resistance. However, how adipose tissue fibrosis occurs and exerts its metabolic impacts on the pathophysiology of obesity and insulin resistance is unknown. It is suggested that the excess deposition of extracellular matrix (ECM) components, such as collagens and osteopontin (OPN), in adipose tissues triggers the necrosis of adipocytes, which attracts classically activated pro-inflammatory macrophages and causes tissue inflammation and metabolic dysfunction (Fig. 1). In addition to imposing physical restriction on adipose tissue expansion, excess ECM deposition may cause adipocyte death and adipose inflammation through the signalling via

**Abbreviations:** ECM, extracellular matrix; HFD, high fat diet; OPN, osteopontin; HA, hyaluronan; THBS, thrombospondin; MMPs, matrix metalloproteinases; MT-MMPs, membrane-type matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases; HOMA-IR, homeostasis model assessment index of insulin resistance; ADAM, a disintegrin and metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; RGD, Arg-Gly-Asp; FFA, free fatty acid; JNKs, c-Jun N-terminal kinases; NF- $\kappa$ B, nuclear factor- $\kappa$ B; VEGF, vascular endothelial growth factor; HIF1, hypoxia inducible factor 1; FAK, focal adhesion kinase; ERM, Ezrin-radixin-moesin; MAPKs, mitogen-activated protein kinases; ATM, adipose tissue macrophage.

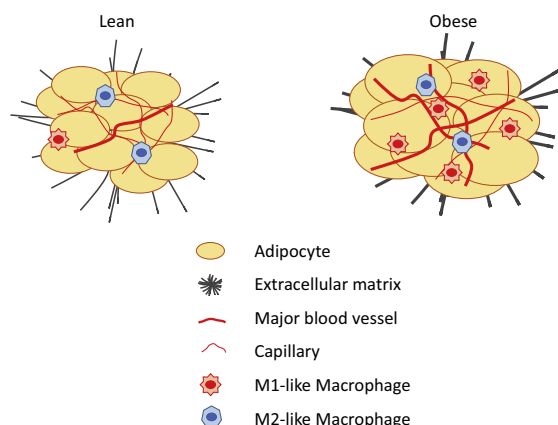
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**Fig. 1.** Adipose tissue remodelling during the development of obesity. Adipose tissues undergo dramatic remodelling during the development of obesity. These include enlargement of adipocytes, accumulation of extracellular matrix components, increased formation of new blood vessels and increased perfusion of capillaries, and increased infiltration of pro-inflammatory macrophages (M1-like).

integrins and CD44. In this review, we summarize the recent findings on adipose tissue ECM remodelling and the roles played by ECM receptors, e.g. integrins, CD44, and CD36. We propose a new concept that the interaction of adipose ECM molecules with their cognate receptors expressed not only by adipocytes but also by a diverse array of cells, i.e. pre-adipocytes, macrophages, and vascular endothelial cells, should contribute to adipose tissue inflammation, apoptosis, angiogenesis, and subsequent metabolic deteriorations in obesity. A similar concept has been proposed in the biology of the skeletal muscle and liver, which was recently reviewed elsewhere [6]. Despite a novel perception in the context of obesity and insulin resistance, ECM–ECM receptor pathways have been long implicated in the biology of pulmonary fibrosis, wound healing, and tumour growth [7–9].

## 2. ECM components in the adipose tissue

### 2.1. Collagens

Collagens, as the most abundant structural components of the ECM, not only support tissue architecture but also cell functions, including cell adhesion, migration, differentiation, morphogenesis, and wound healing [10]. In adipose tissues, it is known that the ECM undergoes constant remodelling to allow adipocytes to rapidly expand and shrink in parallel with weight gain and loss [11]. Abnormal expression of ECM components, modifiers, and

receptors in adipose tissues is a hallmark of obesogenic adipose tissue remodelling (Table 1). Excessive collagen deposition in adipose tissues has been seen in various animal models of metabolic diseases. In genetically obese and diabetic *db/db* mice, the mRNA levels of a group of collagens (mainly types I, III, V, and VI) are increased in white adipose tissues, and high-fat diet (HFD) further increases those collagen expressions [12]. Type VI collagen is highly enriched in adipose tissues, and its gene-targeted deletion (*Col6a1*) results in less restricted expansion of adipose tissues coupled with a substantial improvement in whole-body energy homeostasis [3]. The overexpression of a cleaved fragment of the  $\alpha$ -3 chain of collagen VI (*Col6a3*), named endotrophin, in mice stimulates fibrotic collagen deposition in adipose tissues and triggers adipose inflammation and insulin resistance [13]. In obese humans, the expression of collagen V is increased in adipose tissues that demonstrate a decreased number of capillaries [14]. Increased collagen V is colocalized with blood vessels, and the addition of collagen V to an angiogenesis assay inhibits endothelial budding, suggesting an inhibitory role of collagen V in angiogenesis [14]. These data suggest that excessive collagen deposition in the adipose tissue poses physical barriers against adipocyte hypertrophy during obesity progression and may also inhibit angiogenesis within adipose tissues.

### 2.2. Osteopontin

Osteopontin (OPN), also known as secreted phosphoprotein 1, is an ECM glycoprotein expressed in various cell types and tissues including the adipose tissue [15]. OPN expression is drastically increased in adipose tissues of HFD-induced and genetically obese mice as well as obese humans [16]. OPN is highly expressed in adipose tissue macrophages [17]. The genetic deletion of OPN in mice prevents HFD-induced obesity [18,19] and attenuates macrophage infiltration in adipose tissues, improving insulin sensitivity [17]. Similarly, neutralization of OPN using a monoclonal OPN antibody [20] or OPN gene silencing selective to adipose tissue macrophages [21] in mice suppresses adipose tissue inflammation and insulin resistance. It is hypothesized that action of OPN is mediated through engagement of a number of receptors, but particularly through CD44 and integrin  $\alpha_v\beta_3$  [15].

### 2.3. Hyaluronan

Hyaluronan (HA) is a linear glycosaminoglycan consisting of chemically unmodified repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine [22]. HA binds to cell-surface receptors (CD44 and HA-mediated motility receptor)

**Table 1**  
The ECM, ECM modifiers and ECM receptor remodelling in the adipose tissue of obesity and insulin resistance.

		Mice	Human	References
ECM	Collagen I, III, V, and VI	↑ ( <i>db/db</i> ; <i>ob/ob</i> ; HFD)	↑	[3,12,14]
	Osteopontin	↑ ( <i>db/db</i> ; HFD)	↑	[16,17]
	Hyaluronan	↑ ( <i>ob/ob</i> ; HFD)		[24]
	Thrombospondin 1	↑ (HFD)	↑	[27,29]
ECM modifier	MMP2, 3, 11, 12, 13, 14, 19	↑ (HFD, <i>ob/ob</i> , <i>db/db</i> )		[41,42]
	MMP7, 16, 24	↓ (HFD, <i>ob/ob</i> , <i>db/db</i> )		[41,42]
	MMP9	↓ (HFD)	↑	[37,41]
	MMP15		↓	[37]
	TIMP-1	↑ (HFD)		[41]
	TIMP-2	↓ (HFD males)		[64]
	TIMP-3	↓ ( <i>ob/ob</i> , <i>db/db</i> )		[42]
	TIMP-4	↓ (HFD)		[41]
ECM receptor	$\beta$ 2 integrin ( $\alpha$ L $\beta$ 2, $\alpha$ M $\beta$ 2, $\alpha$ X $\beta$ 2, and $\alpha$ D $\beta$ 2)	↑ (HFD)	↑	[64]
	CD44	↑ (HFD)	↑	[86–88]
	CD36		↑	[96]

and influences cellular responses such as proliferation and migration [23]. HA content is increased in hypertrophic 3T3-L1 cells and in the adipose tissues of diabetogenic LDL receptor-deficient and *ob/ob* mice, possibly due to an increased expression of HA synthase 2 [24]. Increased HA content has been demonstrated to facilitate monocyte adhesion and chemotaxis [24]. In contrast, the reduction of HA by exogenous hyaluronidase inhibits adipogenesis of 3T3-L1 cells [25]. Moreover, chronic treatment of HFD-fed obese mice with a PEGylated human recombinant hyaluronidase PH-20 decreases adiposity and adipose inflammation to prevent insulin resistance [26].

#### 2.4. Thrombospondins

Thrombospondin 1 (THBS1) is a large adhesive ECM glycoprotein expressed predominantly in visceral adipose tissues and its expression is elevated in insulin-resistant, obese humans [27,28]. In mice, HFD acutely induces *Thbs1* expression in visceral adipose tissues and increases the circulating THBS1 level [29]. The genetic deletion of *Thbs1* renders mice protected from adipose tissue inflammation and insulin resistance [29,30]. Most importantly, a recent study suggests that circulating THBS1 may induce fibrotic damage to skeletal muscle and insulin resistance as *Thbs1*-null skeletal muscles are protected from HFD-induced collagen deposition [29]. This is the first study that suggests a potential role of circulating ECM protein in the crosstalk between the adipose tissue and the skeletal muscle in obesity and insulin resistance. Despite the important role played by THBS1 in adipose tissue inflammation and insulin resistance, THBS2 does not seem to play a substantial role in adipose tissue development and HFD-induced obesity, at least in mice [31].

### 3. ECM modifiers in the adipose tissue

#### 3.1. MMPs

Matrix metalloproteinases (MMPs), a family of calcium-dependent and zinc-containing endopeptidase, are responsible for the degradation of virtually all ECM proteins [32,33]. MMPs play an essential role in regulating ECM remodelling in both normal physiology and diseases [33,34]. MMP family members are categorized into soluble collagenase (MMP1, -8, -13), gelatinase (MMP2, -9), stromelysin (MMP3, -10, -11), matrilysin (MMP7, -26), membrane-type MMPs (MT-MMPs) (MMP14, -15, -16, -17, -24, -25), and elastase (MMP12) [34]. Dysregulation of MMPs are implicated in the pathophysiology of obesity and diabetes in humans [35–37]. Plasma concentrations of gelatinases (MMP2 and -9), two major circulating MMPs, are increased in obese [38] and diabetic humans [39,40]. The adipose expression of MMP9 positively correlates with the homeostasis model assessment index of insulin resistance (HOMA-IR) in obese humans [37].

The specific role played by each MMP in the pathogenesis of obesity and insulin resistance has not been fully defined. MMP expression in the adipose tissue is differentially regulated in HFD-fed obese mice [41,42]. A series of MMP gene targeting were tested in mice to determine the role of each MMP in obesity and diabetes, and the results have been variable. The genetic deletion of MMP3 (stromelysin-1) causes hyperphagia and obesity in HFD-fed mice [43]. The responsible substrate or the site of action of MMP3 in metabolism is unknown. MMP3 cleaves OPN [44]; therefore, the loss of MMP3 may exacerbate OPN-dependent adipose inflammation. Similarly, MMP11 (stromelysin-3)-null mice are more prone to HFD-induced obesity [45]. The gene targeting of MMP10 (stromelysin-2) did not cause any significant changes in adipose tissue size and function after 15-week HFD [46].

Mice lacking a gelatinase, MMP2 (gelatinase A), are resistant to obesity induced by HFD feeding, displaying smaller fat pads and smaller adipocytes [47]. The genetic deletion of another gelatinase, MMP9 (gelatinase B), however, did not demonstrate a significant change in weight, fat mass, fasting blood glucose and insulin levels after 15 weeks of HFD [48]. As MMP9 is highly expressed by adipose tissue macrophages [49], a further study should be needed to fully define the impact of genetic deletion of MMP9 on adipose inflammation and metabolism. Interestingly, a pharmacological inhibition of MMPs with a relative specificity to MMP2 and MMP9 reduces weight gain and fat pad weights in *ob/ob* mice [50].

Among MT-MMPs, MMP14 (MT1-MMP) and MMP15 (MT2-MMP) act as major pericellular collagenases [51]. The loss of MMP14 causes severe lipodystrophic phenotype, underscoring its dominant role in adipose tissue development in mice [52]. MMP14 haploinsufficiency confers mice a protection from diet-induced obesity and a genetic variance in human MMP14 gene is associated with obesity and diabetes [36]. While MMP14 is the major regulator of MMP2 activation [53], the gene deletion of both MMP2 and MMP14 causes a synthetic lethality, underscoring the critical biological pathways regulated through the interplay between MMP2 and MMP14 [54]. In humans, MMP15 (MT2-MMP) is down-regulated in white adipose tissues of obese humans [37]. The exact role of MMP15 in regulating adipose tissue size and function is unknown. Unlike MMP14, the gene deletion of MMP15 alone does not cause a significant developmental defect; however, the loss of both MMP14 and -15 causes embryonic lethality due to the defective development of the placenta [55]. As such, the functional interplay of MMP14 with MMP2 and/or MMP15 may play a synergistic role in regulating adipose tissue function as well. The roles played by other MT-MMPs (MMP16, -17, -24, -25) in the regulation of obesity and diabetes are unknown.

Elastin is another major component of adipose ECM [56]. The expression of elastin in adipose tissues was found to be less abundant in obesity [14]. MMP12 (macrophage elastase) is the major MMP that degrades elastin in mice [57]. In HFD (60% fat)-induced obesity, adipose macrophages, particularly CD11c<sup>+</sup> residential macrophages (M2-like) express a high level of MMP12 [58,59]. In their study, the loss of MMP12 exacerbated HFD-induced adipose hypertrophy but improved insulin sensitivity [58]. The loss of MMP12 alone, however, did not change elastin content in adipose tissues under either normal or HFD condition [59]. Another group reported that the loss of MMP12 did not exert any significant effects on HFD (42% fat)-induced obesity [60]. It is unclear whether a difference in dietary fat content or genetic background may account for the difference in the reported obesity phenotypes.

Together, these data suggest that MMPs play important but diverse roles in regulating adipose tissue homeostasis in obesity; however, the exact substrates of each MMP responsible for the regulation of obesity and diabetes phenotypes have not been fully defined. The functional interplays between MMPs, e.g. MMP2 and -14, MMP14 and -15, in the regulation of adipose tissue homeostasis and metabolism should require further investigation.

#### 3.2. TIMPs

The MMPs are inhibited by specific endogenous tissue inhibitors of metalloproteinases (TIMPs), which comprise a family of four protease inhibitors: TIMP-1, -2, -3 and -4 [61]. Circulating levels of TIMP-1 and -2 are increased in patients with metabolic syndrome and diabetes [40]. Hypothalamic TIMP-1 expression is regulated by an adipose-derived hormone, leptin, and the gene deletion of TIMP-1 causes increased food intake and obesity in female mice [62]. The overexpression of TIMP-1 in pancreatic  $\beta$ -cells protects

mice from streptozotocin-induced  $\beta$ -cell death and diabetes [63]. While TIMP-1 mostly inhibits soluble MMPs alone, TIMP-2 can inhibit both soluble and MT-MMPs [51]. The genetic deletion of TIMP-2 in mice exacerbates HFD-induced obesity and diabetes [64]. TIMP-2 gene deletion impairs MMP14 (MT1-MMP)-dependent MMP2 activation [65]; therefore, the phenotype of TIMP-2-null mice might be partly modified by the impaired MMP2 activation. TIMP-3 expression is reduced in the adipose tissue of mouse obesity models [42]. The genetic deletion of TIMP-3 in mice causes hepatic steatosis and adipose tissue inflammation [66], whereas TIMP-3 overexpression in macrophages protects mice from insulin resistance, adipose inflammation, and hepatic steatosis [67].

These data may suggest that increased activities of TIMPs in tissues are protective in metabolic regulation, but in a tissue- and context-dependent manner. While TIMPs are endogenous inhibitors of MMPs that are responsible for degrading excess ECM, it is unclear whether the beneficial effects of increased TIMP activities is solely due to the suppressed activity of MMPs and increased ECM stability or through different target molecules, including ADAM (a disintegrin and metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) [68].

#### 4. ECM receptors in the adipose tissue

##### 4.1. Integrins

Integrins are heterodimeric transmembrane receptors ensuring the communication between ECM and the intracellular environment. In mammals, there are eighteen  $\alpha$  and eight  $\beta$  subunits that can be non-covalently assembled into 24 heterodimeric combinations [69]. The specific integrin expression patterns determine which ECM substrate can bind to the cell and further regulate the downstream signalling events. In brief, integrins are classified into several subfamilies including collagen receptors, laminin receptors, Arg-Gly-Asp (RGD) receptors and leucocytes-specific receptors [69]. Collagen and laminin receptor integrins share common  $\beta 1$  subunit and leucocyte-specific receptor integrins share common  $\beta 2$  subunit. It has been shown that integrin  $\beta 1$  is critical in regulating HFD-induced insulin resistance in skeletal muscles [70,71]; however, its role in adipose tissues has not been studied. On the other hand, leucocyte-derived  $\beta 2$  integrin has been associated with HFD-induced obesity and insulin resistance in the adipose tissue. Under a HFD condition, mutated  $\beta 2$ -integrin knockin mice display increased neutrophil numbers in white adipose tissues and show significantly increased peripheral insulin resistance [72]. The  $\beta 2$  integrin subfamily is comprised of 4 members,  $\alpha L\beta 2$  (CD11a/CD18),  $\alpha M\beta 2$  (CD11b/CD18),  $\alpha X\beta 2$  (CD11c/CD18), and  $\alpha D\beta 2$  (CD11d/CD18). CD11b, CD11c and CD11d expression is increased in the adipose tissue and circulating monocytes of obese humans and rodents [73–75]. The majority of macrophages infiltrated in white adipose tissue in obesity co-express CD11b and CD11c [76]. Moreover, CD11b deficient mice are protected from development of HFD-induced insulin resistance through reduction of alternative activation and proliferation of adipose tissue macrophages [77]. CD11c-positive adipose tissue macrophages are identified as markers of insulin resistance in human obesity [78]. These studies are consistent and may suggest a contributing role of  $\beta 2$  integrin expressed by neutrophils and macrophages in diet-induced insulin resistance. Integrin  $\alpha 4$  associates with either  $\beta 1$  or  $\beta 7$  subunit to form an integrin that may play a role in cell motility and migration [79]. Although inhibiting  $\alpha 4$  integrin function and signalling has been shown to block inflammatory responses associated with mononuclear cell-mediated diseases such as multiple sclerosis and Crohn's disease [80,81], their role in low-grade

chronic inflammatory conditions, such as obesity-induced insulin resistance is not well studied. However, it is shown that mice bearing an  $\alpha 4$  (Y991A) mutation are protected from development of HFD-induced insulin resistance through mediating the trafficking of monocytes into adipose tissues [82].

##### 4.2. CD44

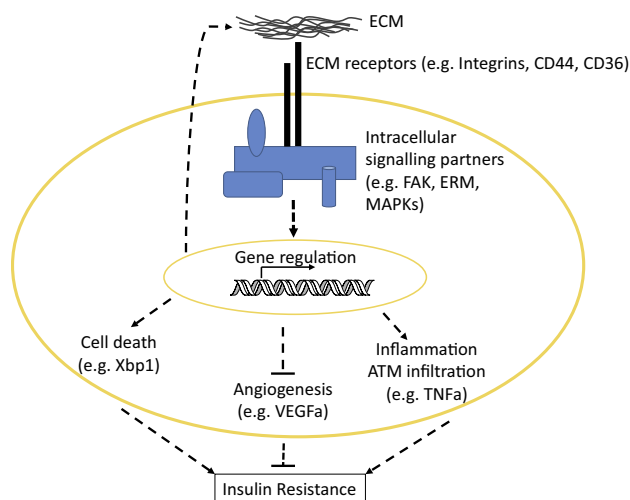
CD44 is a multifunctional cell membrane receptor for ECM components, mainly HA and OPN [83]. CD44 transcripts are subject to alternative splicing, resulting in the expression of CD44 standard isoform (CD44s) and multiple CD44 variants (CD44v) [84]. CD44s is ubiquitously expressed in most tissues, whereas the larger variant isoforms are expressed only in a few epithelial tissues and several cancers [85]. The expression of CD44v in adipose tissues has not been identified and studied. Current studies of CD44 in the adipose tissue in the context of obesity and diabetes have focused on the standard form of CD44. CD44s is associated with type 2 diabetes from expression-based genome-wide association studies [86]. CD44s expression level in the adipose tissue is positively correlated with adipose inflammation and an index of insulin resistance, HOMA-IR in obese individuals and HFD-fed obese mice [86–88]. Serum CD44s levels are positively correlated with insulin resistance and glycemic control in human subjects [86]. HFD-fed CD44 knockout mice remain considerably more insulin sensitive and glucose tolerant than HFD-fed wildtype control mice and exhibit lower blood insulin levels [89]. Treatment of CD44 monoclonal antibody suppresses visceral adipose tissue inflammation and reduces fasting blood glucose levels, weight gain, liver steatosis, and insulin resistance in a HFD-fed mouse model [88]. These of course cannot rule out the potential expression and importance of CD44v in the adipose tissue of obesity and insulin resistance.

##### 4.3. CD36

CD36 also known as fatty acid translocase is an integral membrane protein, which binds many ligands including collagen, THBS, lipoproteins and fatty acids [90]. CD36 facilitates FFA transport into the adipose tissue in humans [91]. HFD-fed mice harbouring CD36 deletion display improved insulin signalling and reduced macrophage infiltration in the adipose tissue compared with wild-type mice, with variable effects on HFD-induced whole-body insulin resistance [92–94]. Genetic variation within the CD36 locus is suggested to contribute to metabolic disease via its effect on body adiposity [95]. Gene expression studies indicate that CD36 is significantly upregulated in the mesenteric adipose tissue of diabetic patients [96]. AP5258, a CD36 specific inhibitor significantly increases cell survival of oleic acid-treated mouse and human adipocytes, and partially restores the transcriptional response to oleic acid in the presence of insulin through JNKs (c-Jun N-terminal kinases) pathway [97]. Although most of these studies of CD36 in adipose tissue in obesity and insulin resistance are attributed to its role as a FFA transporter, the role of ECM binding in the process of FFA uptake is potentially significant. This is evidenced by the fact that an ECM ligand, such as THBS induces the dimerization of membrane-bound CD36, which is proposed to play an important role in signal transduction [98].

#### 5. Proposed model for how ECM-receptor interaction is linked to obesity-associated insulin resistance

Numerous studies have demonstrated that the increased deposition of ECM components and the presence and activation of ECM receptor pathways in the adipose tissue are associated with obesity-associated inflammation and insulin resistance. The under-



**Fig. 2.** Proposed model for how the activation of ECM receptor pathway in the adipose tissue is linked to obesity-associated insulin resistance. It is proposed that activation of ECM-receptor pathway would induce the expression of genes that mediate the metabolically unfavourable processes, including adipocyte death, inhibition of angiogenesis and attraction of pro-inflammatory macrophage infiltration which culminate in insulin resistance. Potential downstream intracellular signalling partners of each ECM receptor include FAK for integrin receptors [119], ERM for CD44 receptor [85] and MAPKs for CD36 receptor [122]. ECM: extracellular matrix; FAK: focal adhesion kinase; ERM: ezrin–radixin–moesin; MAPKs: mitogen-activated protein kinases; VEGF: vascular endothelial growth factor; ATM: adipose tissue macrophage.

lying mechanisms however, are not fully understood. We propose the following potential downstream pathways of ECM-receptor signalling that may mediate the process. These include induction of adipocyte death, inhibition of angiogenesis in adipose tissues and the promotion of inflammatory cytokine production and macrophage infiltration (Fig. 2). It is worth noting that these pathways share analogies to those leading to pulmonary fibrosis, wound healing and tumour growth [7–9]. Similarities and differences of adipose ECM remodelling in comparison to cancer ECM dynamics are highlighted in the following section.

### 5.1. Induction of adipocyte death

The ECM in the adipose tissue surrounding adipocytes not only provides structural support but regulates cell proliferation and death. Adipocyte death is increased progressively during the development of obesity with a frequency of 80% death rate in mice after 16 weeks of HFD feeding, coincident with widespread deposition of collagen [99]. It is hypothesized that excessive deposition of adipose ECM components physically constraints the expansion of adipocytes and causes adipocyte death [3]. We hypothesize that ECM receptor pathways (e.g. integrins) would trigger downstream gene regulation that mediates processes that regulate adipocyte necrosis or apoptosis. This hypothesis is supported by the fact that *ob/ob* mice that lack collagen VI (*Col6a1*) display a reduced necrotic cell death accompanied by enlarged adipocytes and improved systemic insulin resistance [3]. Reduced adipocyte death in these mice is associated with a significant reduction of spliced form of Xbp1, a marker for endoplasmic reticulum stress which causes cells to undergo apoptosis through activation of CHOP and JNK [3]. Adipocyte death may cause adipose inflammation and insulin resistance because necrotic adipocytes become a phagocytic stimulus that attracts macrophages [99].

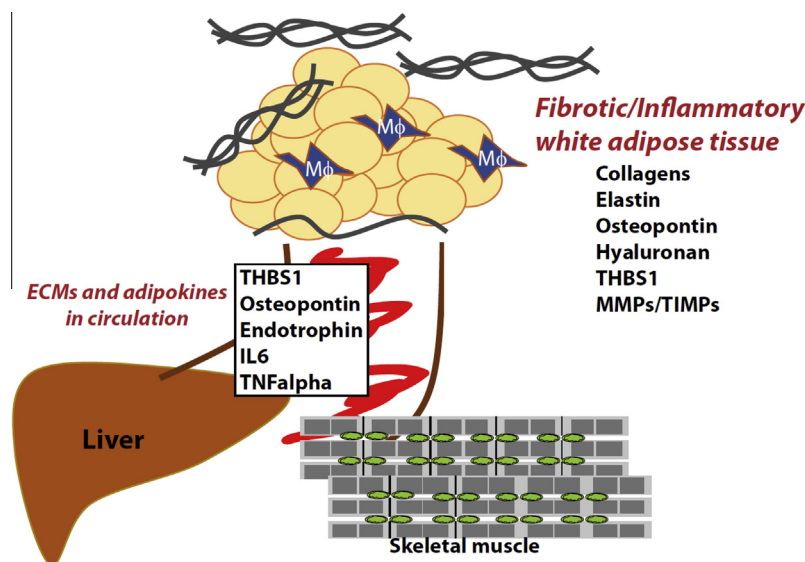
The concept that augmented ECM receptor signalling in adipose tissue induces adipocyte death is at odds with its proposed role in tumour biology. Many of the changes in the ECM, ECM modifiers

and ECM receptors in expanding adipocytes occur during tumour cell growth including increased deposition of various collagens (e.g. I, II, III, V and IX) [9], increased levels of MMPs (e.g. MMP1, 2, 3, 7, 9, 12, 14, 21, 24, 25) and TIMPs (e.g. TIMP 1, 2, 3) [100], and increased ECM receptor signalling (e.g. hyaluronan and CD44 signalling) [101]. However, it is shown that this ECM remodelling in cancer facilitates tumour cell growth, invasion, and metastasis [9,101]. In cancer, activated integrin signalling upon ECM binding initiates pro-survival signals through increased nuclear factor- $\kappa$ B (NF- $\kappa$ B) or PI3K-AKT activity, decreased p53 activation and increased expression of the pro-survival molecules BCL-2 and FLIP [102]. Although disparate from our proposal that activated ECM signalling in the adipose tissue would cause adipocyte death and associated inflammatory response (Fig. 2), research in cancers would provide insight to our understanding of adipose tissue biology in obesity and insulin resistance.

### 5.2. Inhibition of angiogenesis in the adipose tissue

White adipose tissues are highly vascularised and expansion of the adipose tissue is necessarily accompanied by angiogenesis. It is hypothesized that excessive deposition of ECM limits the angiogenic capacity of white adipose tissue in obesity. It is shown that the hypoxic response in the adipose tissue of *ob/ob* mice is paradoxically associated with decreased gene expression of vascular endothelial growth factor A (VEGFa), vascular endothelial cell markers, and decreased vessel density [103]. Overexpression of dominant active hypoxia inducible factor 1 (HIF1) fails to increase VEGFa expression but induces the gene expression causal for tissue fibrosis [103]. Likewise, overexpression of VEGFa leads to increased adipose vascularity and reduced tissue hypoxia [104]. These findings are in contrast to what is found in cancers wherein hypoxia stimulates angiogenesis via HIF1a/VEGFa pathway [105], and suggest the presence of an obesity-specific relationship between hypoxia, fibrosis, and angiogenesis. Moreover, increased collagen V inhibits endothelial budding, suggesting its inhibitory role in angiogenesis [14]. As adipose tissue fibrosis inhibits the angiogenic capacity of the tissue, it is reasonable to propose that the suppressed expression of genes necessary for adipose angiogenesis (e.g. VEGFa) should be mediated by the activation of ECM receptor pathways by excess ECM deposition. We have previously showed that genetic deletion of integrin  $\alpha$ 2 $\beta$ 1, one of the collagen binding receptors is associated with increased vascularization in muscle of HFD-induced obese mice [70]. The angiogenic capacity of white adipose tissues is positively associated with glucose homeostasis. Mice with adipose-specific deletion of VEGFa display exacerbated insulin and glucose tolerance on a HFD; in contrast, induction of VEGFa expression in the adipose tissue reverses glucose intolerance in HFD-induced obese mice [104]. It is hypothesized that reduced angiogenesis in white adipose tissues leads to reduced exchange of insulin and other hormones, cytokines and adipokines from blood to fat, leading to insulin resistance. Although not specifically shown in the adipose tissue, we have successfully demonstrated such a relationship in an insulin-sensitive metabolic tissue, i.e., the skeletal muscle. Our previous studies have shown that defects in recruitment of muscle capillaries contribute to the development of muscle insulin resistance [106,107]; whereas improved muscle insulin resistance is associated with increased muscle capillary density [26,70]. Further studies are needed to investigate the metabolic impacts of integrin-dependent regulation of angiogenesis in adipose tissues.

Transcriptional co-activators PGC-1 $\alpha$  and PGC-1 $\beta$  have been shown to induce VEGF expression and angiogenesis in muscles [108–111]. As these two PGC-1 isoforms are operative in white adipose tissues, it is possible that inhibition of angiogenesis in obese, expanding adipocytes is due to decreased expression and function



**Fig. 3.** Fibrotic and inflammatory white adipose tissue remodelling in crosstalk with the liver and skeletal muscles. Fibrotic and inflammatory adipose tissue remodelling is associated with the increased circulating levels of THBS1, OPN, endotrophin in parallel with IL6 and TNF $\alpha$ . These circulating factors derived from expanding adipose tissues induce insulin resistance of the liver and skeletal muscles.

of PGC-1 $\alpha$  and PGC-1 $\beta$ . This hypothesis is highly supported by the fact that the expression of both PGC-1 $\alpha$  and PGC-1 $\beta$  is decreased in obesity and mice lacking PGC-1 $\alpha$  specifically in the adipose tissue develops exacerbated insulin resistance on a HFD [112,113]. However, the role of ECM receptor pathways in the regulation of PGC-1 isoform expression is still unknown and may require further investigations.

Angiogenesis is another shared pathway which is proven to be important in both obesity and cancer. Anti-angiogenesis therapy for cancers has been proposed for more than 40 years; however, in both preclinical and clinical settings, the arise of resistance mechanisms limits the long-term benefit of anti-angiogenesis therapy [114]. In obesity, the therapeutic angiogenesis for treatment of obesity and metabolic diseases remains a paradoxically disputed issue [115]. Controversial results exist. For example, early studies using genetic and HFD-induced obese mice show that treatment of generic angiogenesis inhibitors including TNP-470 and angiostatin, suppresses adipose angiogenesis and prevents obesity in mice [116,117]. In contrast, systemic anti-VEGF-A treatment to HFD-fed mice induced weight gain and caused exacerbated systemic insulin resistance [118]. Targeting angiogenesis in white adipose tissues for treating obesity and insulin resistance remains controversial and has been well reviewed previously [115].

### 5.3. Induction of adipose tissue macrophage infiltration and inflammation

We propose that activation of ECM binding to ECM receptor mediates intracellular signalling to regulate expression of genes that mediate inflammation and adipose tissue macrophage infiltration. Focal adhesion kinase (FAK), a ubiquitously expressed tyrosine kinase, which is essential for development and cellular proliferation, transmits extracellular signals via integrin signalling. Adipocyte-specific deletion of FAK increases adipose tissue inflammation shown by increased macrophage infiltration and adipocyte apoptosis [119]. These results suggest that FAK may be essential for gene expression for adipose tissue remodelling and inflammation. Chronic treatment of human recombinant pegylated hyaluronidase decreases adipose tissue ECM HA and decreases adipocyte size and the gene expression of pro-inflammatory markers (e.g.

TNF $\alpha$ ) in adipose tissue of HFD-fed mice [26]. Genetic deletion of the main HA receptor CD44 consistently decreases adipose tissue inflammation in mice following a HFD [89]. These results suggest that the activation of HA-CD44 pathway regulates macrophage infiltration and inflammation in the adipose tissue of HFD-fed mice. It has been previously shown that the genetic deletion of  $\beta$ 2 integrin CD11b protects mice from development of HFD-induced insulin resistance by suppressing the alternative activation and the proliferation of adipose tissue macrophages [77].

## 6. Concluding remarks

It is recently ascertained that fibrosis, excess deposition of ECM components, in metabolically active, insulin-sensitive tissues, including the skeletal muscle, adipose tissue and liver has damaging impact on glucose homeostasis [6,120,121]. Obesogenic ECM remodelling of white adipose tissues is closely linked with the increased levels of circulating ECM proteins and ECM-derived peptides in parallel with increased levels of adipose-derived cytokines. These white adipose tissue-derived ECM or ECM-related molecules may exert metabolically deleterious effects on metabolic crosstalk between the adipose tissue, liver, and skeletal muscles (Fig. 3). Despite a recent implication of ECM-receptor pathway in determining glucose homeostasis in the skeletal muscle and liver [6], its role in the adipose tissue has not been fully defined. We postulate that the ECM receptor pathway of adipocytes as well as other cell types found in adipose tissues, i.e. inflammatory monocytes and macrophages and vascular endothelial cells are important in transducing intracellular signalling of adipocyte death, angiogenesis, and the infiltration of inflammatory cells, which culminate in insulin resistance. Tissue-specific mouse models that lack a key ECM, ECM modifier, ECM receptor, or intracellular mediator, will help us decipher the importance of the ECM receptor pathway and its regulators in determining metabolic tissue remodelling, function and glucose homeostasis.

We propose the potential of developing therapeutic strategies that target ECM matrix of metabolically active tissues, including the liver, skeletal muscle and the adipose tissue. Current anti-fibrotic drugs being tested in clinical settings have been focused on cancers (e.g. PEGPH20), heart failure (e.g. FT011) and glaucoma

surgery (e.g. CLT-28643). The effectiveness of their use in obesity, insulin resistance and type 2 diabetes is unknown and may worth further investigation.

## References

- [1] D.W. Haslam, W.P. James, Obesity, *Lancet* 366 (9492) (2005) 1197–1209.
- [2] F.S. Facchini, N. Hua, F. Abbasi, G.M. Reaven, Insulin resistance as a predictor of age-related diseases, *J. Clin. Endocrinol. Metab.* 86 (8) (2001) 3574–3578.
- [3] T. Khan, E.S. Muise, P. Iyengar, Z.V. Wang, M. Chandalia, N. Abate, B.B. Zhang, P. Bonaldo, S. Chua, P.E. Scherer, Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI, *Mol. Cell. Biol.* 29 (6) (2009) 1575–1591.
- [4] A. Divoux, J. Tordjman, D. Lacasa, N. Veyrie, D. Hugol, A. Aissat, A. Basdevant, M. Guerre-Millo, C. Poitou, J.D. Zucker, P. Bedossa, K. Clement, Fibrosis in human adipose tissue: composition, distribution, and link with lipid metabolism and fat mass loss, *Diabetes* 59 (11) (2010) 2817–2825.
- [5] H.M. Lawler, C.M. Underkofler, P.A. Kern, C. Erickson, B. Bredbeck, N. Rasouli, Adipose tissue hypoxia, inflammation, and fibrosis in obese insulin-sensitive and obese insulin-resistant subjects, *J. Clin. Endocrinol. Metab.* 101 (4) (2016) 1422–1428.
- [6] A.S. Williams, L. Kang, D.H. Wasserman, The extracellular matrix and insulin resistance, *Trends Endocrinol. Metab.* 26 (7) (2015) 357–366.
- [7] T. Kulkarni, P. O'Reilly, V.B. Antony, A. Gaggar, V.J. Thannickal, Matrix remodeling in pulmonary fibrosis and emphysema, *Am. J. Respir. Cell Mol. Biol.* (2016).
- [8] L.E. Tracy, R.A. Minasian, E.J. Caterson, Extracellular matrix and dermal fibroblast function in the healing wound, *Adv. Wound Care (New Rochelle)* 5 (3) (2016) 119–136.
- [9] P. Lu, V.M. Weaver, Z. Werb, The extracellular matrix: a dynamic niche in cancer progression, *J. Cell Biol.* 196 (4) (2012) 395–406.
- [10] M. Aumailley, B. Gayraud, Structure and biological activity of the extracellular matrix, *J. Mol. Med. (Berl.)* 76 (3–4) (1998) 253–265.
- [11] E.C. Mariman, P. Wang, Adipocyte extracellular matrix composition, dynamics and role in obesity, *Cell. Mol. Life Sci.* 67 (8) (2010) 1277–1292.
- [12] J. Huber, M. Löffler, M. Bilban, M. Reimers, A. Kadl, J. Todoric, M. Zeyda, R. Geyeregger, M. Schreiner, T. Weichhart, N. Leitinger, W. Waldhausl, T.M. Stulnig, Prevention of high-fat diet-induced adipose tissue remodeling in obese diabetic mice by n-3 polyunsaturated fatty acids, *Int. J. Obes. (Lond.)* 31 (6) (2007) 1004–1013.
- [13] K. Sun, J. Park, O.T. Gupta, W.L. Holland, P. Auerbach, N. Zhang, R. Goncalves Marangoni, S.M. Nicoloso, M.P. Czech, J. Varga, T. Ploug, Z. An, P.E. Scherer, Endothelin triggers adipose tissue fibrosis and metabolic dysfunction, *Nat. Commun.* 5 (2014) 3485.
- [14] M. Spencer, R. Unal, B. Zhu, N. Rasouli, R.E. McGehee Jr., C.A. Peterson, P.A. Kern, Adipose tissue extracellular matrix and vascular abnormalities in obesity and insulin resistance, *J. Clin. Endocrinol. Metab.* 96 (12) (2011) E1990–E1998.
- [15] F. Kahles, H.M. Findeisen, D. Bruemmer, Osteopontin: a novel regulator at the cross roads of inflammation, obesity and diabetes, *Mol. Metab.* 3 (4) (2014) 384–393.
- [16] F.W. Kiefer, M. Zeyda, J. Todoric, J. Huber, R. Geyeregger, T. Weichhart, O. Aszmann, B. Ludvik, G.R. Silberhumer, G. Prager, T.M. Stulnig, Osteopontin expression in human and murine obesity: extensive local up-regulation in adipose tissue but minimal systemic alterations, *Endocrinology* 149 (3) (2008) 1350–1357.
- [17] T. Nomiya, D. Perez-Tilve, D. Ogawa, F. Gizard, Y. Zhao, E.B. Heywood, K.L. Jones, R. Kawamori, L.A. Cassis, M.H. Tschop, D. Bruemmer, Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice, *J. Clin. Invest.* 117 (10) (2007) 2877–2888.
- [18] J. Chapman, P.D. Miles, J.M. Ofrecio, J.G. Neels, J.G. Yu, J.L. Resnik, J. Wilkes, S. Talukdar, D. Thapar, K. Johnson, D.D. Sears, Osteopontin is required for the early onset of high fat diet-induced insulin resistance in mice, *PLoS ONE* 5 (11) (2010) e13959.
- [19] A. Lancha, A. Rodriguez, V. Catalan, S. Becerril, N. Sainz, B. Ramirez, M. A. Burrell, J. Salvador, G. Fruhbeck, J. Gomez-Ambrosi, Osteopontin deletion prevents the development of obesity and hepatic steatosis via impaired adipose tissue matrix remodeling and reduced inflammation and fibrosis in adipose tissue and liver in mice, *PLoS ONE* 9 (5) (2014) e98398.
- [20] F.W. Kiefer, M. Zeyda, K. Gollinger, B. Pfau, A. Neuhofer, T. Weichhart, M.D. Saemann, R. Geyeregger, M. Schleder, L. Kenner, T.M. Stulnig, Neutralization of osteopontin inhibits obesity-induced inflammation and insulin resistance, *Diabetes* 59 (4) (2010) 935–946.
- [21] M. Aouadi, M. Tencerova, P. Vangala, J.C. Yaw, S.M. Nicoloso, S.U. Amano, J.L. Cohen, M.P. Czech, Gene silencing in adipose tissue macrophages regulates whole-body metabolism in obese mice, *Proc. Natl. Acad. Sci. U.S.A.* 110 (20) (2013) 8278–8283.
- [22] J.R. Fraser, T.C. Laurent, U.B. Laurent, Hyaluronan: its nature, distribution, functions and turnover, *J. Intern. Med.* 242 (1) (1997) 27–33.
- [23] R. Stern, Devising a pathway for hyaluronan catabolism: are we there yet? *Glycobiology* 13 (12) (2003) 105R–115R.
- [24] C.Y. Han, S. Subramanian, C.K. Chan, M. Omer, T. Chiba, T.N. Wight, A. Chait, Adipocyte-derived serum amyloid A3 and hyaluronan play a role in monocyte recruitment and adhesion, *Diabetes* 56 (9) (2007) 2260–2273.
- [25] E. Ji, M.Y. Jung, J.H. Park, S. Kim, C.R. Seo, K.W. Park, E.K. Lee, C.H. Yeom, S. Lee, Inhibition of adipogenesis in 3T3-L1 cells and suppression of abdominal fat accumulation in high-fat diet-feeding C57BL/6J mice after downregulation of hyaluronic acid, *Int. J. Obes. (Lond.)* 38 (8) (2014) 1035–1043.
- [26] L. Kang, L. Lantier, A. Kennedy, J.S. Bonner, W.H. Mayes, D.P. Bracy, L.H. Bookbinder, A.H. Hasty, C.B. Thompson, D.H. Wasserman, Hyaluronan accumulates with high-fat feeding and contributes to insulin resistance, *Diabetes* 62 (6) (2013) 1888–1896.
- [27] V. Varma, A. Yao-Borengasser, A.M. Bodles, N. Rasouli, B. Phanavanh, G.T. Nolen, E.M. Kern, R. Nagarajan, H.J. Spencer 3rd, M.J. Lee, S.K. Fried, R.E. McGehee Jr., C.A. Peterson, P.A. Kern, Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance, *Diabetes* 57 (2) (2008) 432–439.
- [28] Y. Matsuo, M. Tanaka, H. Yamakage, Y. Sasaki, K. Muranaka, H. Hata, I. Ikai, A. Shimatsu, M. Inoue, T.H. Chun, N. Satoh-Asahara, Thrombospondin 1 as a novel biological marker of obesity and metabolic syndrome, *Metabolism* 64 (11) (2015) 1490–1499.
- [29] M. Inoue, Y. Jiang, R.H. Barnes 2nd, M. Tokunaga, G. Martinez-Santibanez, L. Geletka, C.N. Lumeng, D.A. Buchner, T.H. Chun, Thrombospondin 1 mediates high-fat diet-induced muscle fibrosis and insulin resistance in male mice, *Endocrinology* 154 (12) (2013) 4548–4559.
- [30] Y. Li, X. Tong, C. Rumala, K. Clemons, S. Wang, Thrombospondin1 deficiency reduces obesity-associated inflammation and improves insulin sensitivity in a diet-induced obese mouse model, *PLoS ONE* 6 (10) (2011) e26656.
- [31] M. Van Hul, L. Frederix, H.R. Lijnen, Role of thrombospondin-2 in murine adipose tissue angiogenesis and development, *Obesity (Silver Spring)* 20 (9) (2012) 1757–1762.
- [32] K.M. Thraikill, R. Clay Bunn, J.L. Fowlkes, Matrix metalloproteinases: their potential role in the pathogenesis of diabetic nephropathy, *Endocrine* 35 (1) (2009) 1–10.
- [33] C. Bonnans, J. Chou, Z. Werb, Remodelling the extracellular matrix in development and disease, *Nat. Rev. Mol. Cell Biol.* 15 (12) (2014) 786–801.
- [34] H. Nagase, R. Visse, G. Murphy, Structure and function of matrix metalloproteinases and TIMPs, *Cardiovasc. Res.* 69 (3) (2006) 562–573.
- [35] M.T. Traurig, P.A. Permana, S. Nair, S. Kobes, C. Bogardus, L.J. Baier, Differential expression of matrix metalloproteinase 3 (MMP3) in preadipocytes/stromal vascular cells from nonobese nondiabetic versus obese nondiabetic Pima Indians, *Diabetes* 55 (11) (2006) 3160–3165.
- [36] T.H. Chun, M. Inoue, H. Morisaki, I. Yamanaka, Y. Miyamoto, T. Okamura, K. Sato-Kusubata, S.J. Weiss, Genetic link between obesity and MMP14-dependent adipogenic collagen turnover, *Diabetes* 59 (10) (2010) 2484–2494.
- [37] F.J. Tinahones, L. Coin-Araguez, M.D. Mayas, E. Garcia-Fuentes, C. Hurtado-Del-Pozo, J. Vendrell, F. Cardona, R.M. Calvo, M.J. Obregon, R. El Bekay, Obesity-associated insulin resistance is correlated to adipose tissue vascular endothelial growth factors and metalloproteinase levels, *BMC Physiol.* 12 (2012) 4.
- [38] G. Derosa, I. Ferrari, A. D'Angelo, C. Tinelli, S.A. Salvadeo, L. Ciccarelli, M.N. Piccinni, A. Gravina, F. Ramondetti, P. Maffioli, A.F. Cicero, Matrix metalloproteinase-2 and -9 levels in obese patients, *Endothelium* 15 (4) (2008) 219–224.
- [39] S.S. Signorelli, G. Malaponte, M. Libra, L. Di Pino, G. Celotta, V. Bevelacqua, M. Petrina, G.S. Nicotra, M. Indelicato, P.M. Navolanic, G. Pennisi, M.C. Mazarino, Plasma levels and zymographic activities of matrix metalloproteinases 2 and 9 in type II diabetics with peripheral arterial disease, *Vasc. Med.* 10 (1) (2005) 1–6.
- [40] E. Hopps, R. Lo Presti, M. Montana, D. Noto, M.R. Averna, G. Caimi, Gelatinases and their tissue inhibitors in a group of subjects with metabolic syndrome, *J. Investig. Med.* 61 (6) (2013) 978–983.
- [41] E. Maquoi, C. Munaut, A. Colige, D. Collen, H.R. Lijnen, Modulation of adipose tissue expression of murine matrix metalloproteinases and their tissue inhibitors with obesity, *Diabetes* 51 (4) (2002) 1093–1101.
- [42] C. Chavey, B. Mari, M.N. Monthouel, S. Bonnafous, P. Anglard, E. Van Obberghen, S. Tartare-Deckert, Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation, *J. Biol. Chem.* 278 (14) (2003) 11888–11896.
- [43] E. Maquoi, D. Demeulemeester, G. Voros, D. Collen, H.R. Lijnen, Enhanced nutritionally induced adipose tissue development in mice with stromelysin-1 gene inactivation, *Thromb. Haemost.* 89 (4) (2003) 696–704.
- [44] R. Agnihotri, H.C. Crawford, H. Haro, L.M. Matrisian, M.C. Havrda, L. Liaw, Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin), *J. Biol. Chem.* 276 (30) (2001) 28261–28267.
- [45] H.R. Lijnen, H.B. Van, L. Frederix, M.C. Rio, D. Collen, Adipocyte hypertrophy in stromelysin-3 deficient mice with nutritionally induced obesity, *Thromb. Haemost.* 87 (3) (2002) 530–535.
- [46] H.R. Lijnen, B. Van Hoef, J.A. Rodriguez, J.A. Paramo, Stromelysin-2 (MMP-10) deficiency does not affect adipose tissue formation in a mouse model of nutritionally induced obesity, *Biochem. Biophys. Res. Commun.* 389 (2) (2009) 378–381.
- [47] M. Van Hul, H.R. Lijnen, A functional role of gelatinase A in the development of nutritionally induced obesity in mice, *J. Thromb. Haemost.* 6 (7) (2008) 1198–1206.
- [48] M. Van Hul, H. Piccard, H.R. Lijnen, Gelatinase B (MMP-9) deficiency does not affect murine adipose tissue development, *Thromb. Haemost.* 104 (1) (2010) 165–171.

- [49] V. Bourlier, A. Zakaroff-Girard, A. Miranville, S. De Barros, M. Maumus, C. Sengenès, J. Galitzky, M. Lafontan, F. Karpe, K.N. Frayn, A. Bouloumié, Remodeling phenotype of human subcutaneous adipose tissue macrophages, *Circulation* 117 (6) (2008) 806–815.
- [50] M. Van Hul, H.R. Lijnen, Matrix metalloproteinase inhibition impairs murine adipose tissue development independently of leptin, *Endocr. J.* 58 (2) (2011) 101–107.
- [51] T.-H. Chun, F. Sabeh, I. Ota, H. Murphy, K.T. McDonagh, K. Holmbeck, H. Birkedal-Hansen, E.D. Allen, S.J. Weiss, MT1-MMP-dependent neovessel formation within the confines of the three-dimensional extracellular matrix, *J. Cell Biol.* 167 (4) (2004) 757–767.
- [52] T.H. Chun, K.B. Hotary, F. Sabeh, A.R. Saltiel, E.D. Allen, S.J. Weiss, A pericellular collagenase directs the 3-dimensional development of white adipose tissue, *Cell* 125 (3) (2006) 577–591.
- [53] H. Sato, T. Takino, Y. Okada, J. Cao, A. Shinagawa, E. Yamamoto, M. Seiki, A matrix metalloproteinase expressed on the surface of invasive tumour cells, *Nature* 370 (6484) (1994) 61–65.
- [54] J. Oh, R. Takahashi, E. Adachi, S. Kondo, S. Kuratomi, A. Noma, D.B. Alexander, H. Motoda, A. Okada, M. Seiki, T. Itoh, S. Itohara, C. Takahashi, M. Noda, Mutations in two matrix metalloproteinase genes, MMP-2 and MT1-MMP, are synthetic lethal in mice, *Oncogene* 23 (29) (2004) 5041–5048.
- [55] L. Szabova, M.-Y. Son, J. Shi, M. Sramko, S.S. Yamada, W.D. Swaim, P. Zerfas, S. Kahan, K. Holmbeck, Membrane-type MMPs are indispensable for placental labyrinth formation and development, *Blood* 116 (25) (2010) 5752–5761.
- [56] J.S. Choi, B.S. Kim, J.Y. Kim, J.D. Kim, Y.C. Choi, H.J. Yang, K. Park, H.Y. Lee, Y.W. Cho, Decellularized extracellular matrix derived from human adipose tissue as a potential scaffold for allograft tissue engineering, *J. Biomed. Mater. Res. A* 97 (3) (2011) 292–299.
- [57] S. Filippov, I. Caras, R. Murray, L.M. Matrisian, H.A. Chapman, S. Shapiro, S.J. Weiss, Matrilysin-dependent elastolysis by human macrophages, *J. Exp. Med.* 198 (6) (2003) 925–935.
- [58] J.T. Lee, N. Pamir, N.C. Liu, E.A. Kirk, M.M. Averill, L. Becker, I. Larson, D.K. Hagman, K.E. Foster-Schubert, B. van Yserloo, K.E. Bornfeldt, R.C. LeBoeuf, M. Kratz, J.W. Heinecke, Macrophage metalloelastase (MMP12) regulates adipose tissue expansion, insulin sensitivity, and expression of inducible nitric oxide synthase, *Endocrinology* 155 (9) (2014) 3409–3420.
- [59] G. Martinez-Santibanez, K. Singer, K.W. Cho, J.L. DelProposto, T. Mergian, C.N. Lumeng, Obesity-induced remodeling of the adipose tissue elastin network is independent of the metalloelastase MMP-12, *Adipocyte* 4 (4) (2015) 264–272.
- [60] D. Bauters, M. Van Hul, H.R. Lijnen, Macrophage elastase (MMP-12) in expanding murine adipose tissue, *Biochim. Biophys. Acta* 1830 (4) (2013) 2954–2959.
- [61] K. Brew, D. Dinakarandian, H. Nagase, Tissue inhibitors of metalloproteinases: evolution, structure and function, *Biochim. Biophys. Acta* 1477 (1–2) (2000) 267–283.
- [62] I. Gerin, G.W. Louis, X. Zhang, T.C. Prestwich, T.R. Kumar, M.G. Myers Jr., O.A. Macdougald, W.B. Nothnack, Hyperphagia and obesity in female mice lacking tissue inhibitor of metalloproteinase-1, *Endocrinology* 150 (4) (2009) 1697–1704.
- [63] H. Jiang, H. Zhu, X. Chen, Y. Peng, J. Wang, F. Liu, S. Shi, B. Fu, Y. Lu, Q. Hong, Z. Feng, K. Hou, X. Sun, G. Cai, X. Zhang, Y. Xie, TIMP-1 transgenic mice recover from diabetes induced by multiple low-dose streptozotocin, *Diabetes* 56 (1) (2007) 49–56.
- [64] D.M. Jaworski, O. Sideleva, H.M. Stradecki, G.D. Langlois, A. Habibovic, B. Satish, W.G. Tharp, J. Lausier, K. Larock, T.L. Jetton, M. Peshavaria, R.E. Pratley, Sexually dimorphic diet-induced insulin resistance in obese tissue inhibitor of metalloproteinase-2 (TIMP-2)-deficient mice, *Endocrinology* 152 (4) (2011) 1300–1313.
- [65] V. Kandalam, R. Basu, T. Abraham, X. Wang, P.D. Soloway, D.M. Jaworski, G.Y. Oudit, Z. Kassiri, TIMP2 deficiency accelerates adverse post-myocardial infarction remodeling because of enhanced MT1-MMP activity despite lack of MMP2 activation, *Circ. Res.* 106 (4) (2010) 796–808.
- [66] R. Menghini, S. Menini, R. Amoroso, L. Fiorentino, V. Casagrande, V. Marzano, F. Tornei, P. Bertucci, C. Iacobini, M. Serino, O. Porzio, M.L. Hribal, F. Follì, R. Khokha, A. Urbani, R. Lauro, G. Pugliese, M. Federici, Tissue inhibitor of metalloproteinase 3 deficiency causes hepatic steatosis and adipose tissue inflammation in mice, *Gastroenterology* 136 (2) (2009) 663–672 e664.
- [67] R. Menghini, V. Casagrande, S. Menini, A. Marino, V. Marzano, M.L. Hribal, P. Gentileschi, D. Lauro, O. Schillaci, G. Pugliese, P. Sbraccia, A. Urbani, R. Lauro, M. Federici, TIMP3 overexpression in macrophages protects from insulin resistance, adipose inflammation, and nonalcoholic fatty liver disease in mice, *Diabetes* 61 (2) (2012) 454–462.
- [68] G. Murphy, Tissue inhibitors of metalloproteinases, *Genome Biol.* 12 (11) (2011) 233.
- [69] R.O. Hynes, Integrins: bidirectional, allosteric signaling machines, *Cell* 110 (6) (2002) 673–687.
- [70] L. Kang, J.E. Ayala, R.S. Lee-Young, Z. Zhang, F.D. James, P.D. Neuffer, A. Pozzi, M.M. Zutter, D.H. Wasserman, Diet-induced muscle insulin resistance is associated with extracellular matrix remodeling and interaction with integrin alpha2beta1 in mice, *Diabetes* 60 (2) (2011) 416–426.
- [71] H. Zong, C.C. Bastie, J. Xu, R. Fassler, K.P. Campbell, I.J. Kurland, J.E. Pessin, Insulin resistance in striated muscle-specific integrin receptor beta1-deficient mice, *J. Biol. Chem.* 284 (7) (2009) 4679–4688.
- [72] P.J. Meakin, V.L. Morrison, C.C. Sneddon, T. Savinko, L. Uotila, S.M. Jaliczy, J.L. Gabriel, L. Kang, M.L. Ashford, S.C. Fagerholm, Mice lacking beta2-integrin function remain glucose tolerant in spite of insulin resistance, neutrophil infiltration and inflammation, *PLoS ONE* 10 (9) (2015) e0138872.
- [73] K. Takahashi, S. Mizuarai, H. Araki, S. Mashiko, A. Ishihara, A. Kanatani, H. Itadani, H. Kotani, Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice, *J. Biol. Chem.* 278 (47) (2003) 46654–46660.
- [74] H. Wu, X.D. Perrard, Q. Wang, J.L. Perrard, V.R. Polsani, P.H. Jones, C.W. Smith, C.M. Ballantyne, CD11c expression in adipose tissue and blood and its role in diet-induced obesity, *Arterioscler. Thromb. Vasc. Biol.* 30 (2) (2010) 186–192.
- [75] A.P. Thomas, T.N. Dunn, P.J. Oort, M. Grino, S.H. Adams, Inflammatory phenotyping identifies CD11d as a gene markedly induced in white adipose tissue in obese rodents and women, *J. Nutr.* 141 (6) (2011) 1172–1180.
- [76] C.N. Lumeng, J.L. Bodzin, A.R. Saltiel, Obesity induces a phenotypic switch in adipose tissue macrophage polarization, *J. Clin. Invest.* 117 (1) (2007) 175–184.
- [77] C. Zheng, Q. Yang, C. Xu, P. Shou, J. Cao, M. Jiang, Q. Chen, G. Cao, Y. Han, F. Li, W. Cao, L. Zhang, L. Zhang, Y. Shi, Y. Wang, CD11b regulates obesity-induced insulin resistance via limiting alternative activation and proliferation of adipose tissue macrophages, *Proc. Natl. Acad. Sci. U.S.A.* 112 (52) (2015) E7239–E7248.
- [78] J.M. Wentworth, G. Naselli, W.A. Brown, L. Doyle, B. Phipson, G.K. Smyth, M. Wabitsch, P.E. O'Brien, L.C. Harrison, Pro-inflammatory CD11c+CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity, *Diabetes* 59 (7) (2010) 1648–1656.
- [79] S. Liu, W.B. Kiosses, D.M. Rose, M. Slepak, R. Salgia, J.D. Griffin, C.E. Turner, M. A. Schwartz, M.H. Ginsberg, A fragment of paxillin binds the alpha 4 integrin cytoplasmic domain (tail) and selectively inhibits alpha 4-mediated cell migration, *J. Biol. Chem.* 277 (23) (2002) 20887–20894.
- [80] S. Ghosh, E. Goldin, F.H. Gordon, H.A. Malchow, J. Rask-Madsen, P. Rutgeerts, P. Vyhnaek, Z. Zadorova, T. Palmer, S. Donoghue/Natalizumab Pan-European Study G, Natalizumab for active Crohn's disease, *N. Engl. J. Med.* 348 (1) (2003) 24–32.
- [81] D.H. Miller, O.A. Khan, W.A. Sheremata, L.D. Blumhardt, G.P. Rice, M.A. Libonati, A.J. Willmer-Hulme, C.M. Dalton, K.A. Miszkiel, P.W. O'Connor/International Natalizumab Multiple Sclerosis Trial G, A controlled trial of natalizumab for relapsing multiple sclerosis, *N. Engl. J. Med.* 348 (1) (2003) 15–23.
- [82] C.C. Feral, J.G. Neels, C. Kummer, M. Slepak, J.M. Olefsky, M.H. Ginsberg, Blockade of alpha4 integrin signaling ameliorates the metabolic consequences of high-fat diet-induced obesity, *Diabetes* 57 (7) (2008) 1842–1851.
- [83] D. Naor, R.V. Sionov, D. Ish-Shalom, CD44: structure, function, and association with the malignant process, *Adv. Cancer Res.* 71 (1997) 241–319.
- [84] M. Zoller, CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? *Nat. Rev. Cancer* 11 (4) (2011) 254–267.
- [85] H. Ponta, L. Sherman, P.A. Herrlich, CD44: from adhesion molecules to signalling regulators, *Nat. Rev. Mol. Cell Biol.* 4 (1) (2003) 33–45.
- [86] K. Kodama, M. Horikoshi, K. Toda, S. Yamada, K. Hara, J. Irie, M. Sirota, A.A. Morgan, R. Chen, H. Ohtsu, S. Maeda, T. Kadowaki, A.J. Butte, Expression-based genome-wide association study links the receptor CD44 in adipose tissue with type 2 diabetes, *Proc. Natl. Acad. Sci. U.S.A.* 109 (18) (2012) 7049–7054.
- [87] A. Bertola, V. Deveaux, S. Bonnafous, D. Rousseau, R. Anty, A. Wakkach, M. Dahman, J. Tordjman, K. Clement, S.E. McQuaid, K.N. Frayn, P.M. Huet, J. Gugenheim, S. Lotersztajn, Y. Le Marchand-Brustel, A. Tran, P. Gual, Elevated expression of osteopontin may be related to adipose tissue macrophage accumulation and liver steatosis in morbid obesity, *Diabetes* 58 (1) (2009) 125–133.
- [88] L.F. Liu, K. Kodama, K. Wei, L.L. Tolentino, O. Choi, E.G. Engleman, A.J. Butte, T. McLaughlin, The receptor CD44 is associated with systemic insulin resistance and proinflammatory macrophages in human adipose tissue, *Diabetologia* 58 (7) (2015) 1579–1586.
- [89] H.S. Kang, G. Liao, L.M. DeGraff, K. Gerrish, C.D. Bortner, S. Garantzios, A.M. Jetten, CD44 plays a critical role in regulating diet-induced adipose inflammation, hepatic steatosis, and insulin resistance, *PLoS ONE* 8 (3) (2013) e58417.
- [90] R. Nergiz-Unal, T. Rademakers, J.M. Cosemans, J.W. Heemskerk, CD36 as a multiple-ligand signaling receptor in atherothrombosis, *Cardiovasc. Hematol. Agents Med. Chem.* 9 (1) (2011) 42–55.
- [91] K.C. Hames, A. Vella, B.J. Kemp, M.D. Jensen, Free fatty acid uptake in humans with CD36 deficiency, *Diabetes* 63 (11) (2014) 3606–3614.
- [92] M. Febbraio, N.A. Abumrad, D.P. Hajjar, K. Sharma, W. Cheng, S.F. Pearce, R.L. Silverstein, A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism, *J. Biol. Chem.* 274 (27) (1999) 19055–19062.
- [93] T. Hajri, X.X. Han, A. Bonen, N.A. Abumrad, Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice, *J. Clin. Invest.* 109 (10) (2002) 1381–1389.
- [94] H.T. Nicholls, G. Kowalski, D.J. Kennedy, S. Risis, L.A. Zaffino, N. Watson, P. Kanellakis, M.J. Watt, A. Bobik, A. Bonen, M. Febbraio, G.I. Lancaster, M.A. Febbraio, Hematopoietic cell-restricted deletion of CD36 reduces high-fat diet-induced macrophage infiltration and improves insulin signaling in adipose tissue, *Diabetes* 60 (4) (2011) 1100–1110.
- [95] M. Heni, K. Mussig, F. Machicao, J. Machann, F. Schick, C.D. Claussen, N. Stefan, A. Fritsche, H.U. Haring, H. Staiger, Variants in the CD36 gene locus determine



- whole-body adiposity, but have no independent effect on insulin sensitivity, *Obesity* (Silver Spring) 19 (5) (2011) 1004–1009.
- [96] Y.K. Yang, M. Chen, R.H. Clements, G.A. Abrams, C.J. Aprahamian, C.M. Harmon, Human mesenteric adipose tissue plays unique role versus subcutaneous and omental fat in obesity related diabetes, *Cell. Physiol. Biochem.* 22 (5–6) (2008) 531–538.
- [97] E. Berger, S. Heraud, A. Mojallal, C. Lequeux, M. Weiss-Gayet, O. Damour, A. Geloën, Pathways commonly dysregulated in mouse and human obese adipose tissue: FAT/CD36 modulates differentiation and lipogenesis, *Adipocyte* 4 (3) (2015) 161–180.
- [98] L. Daviet, E. Malvoisin, T.F. Wild, J.L. McGregor, Thrombospondin induces dimerization of membrane-bound, but not soluble CD36, *Thromb. Haemost.* 78 (2) (1997) 897–901.
- [99] K.J. Strissel, Z. Stancheva, H. Miyoshi, J.W. Perfield 2nd, J. DeFuria, Z. Jick, A.S. Greenberg, M.S. Obin, Adipocyte death, adipose tissue remodeling, and obesity complications, *Diabetes* 56 (12) (2007) 2910–2918.
- [100] Y. Yeh, B. Sheu, Matrix metalloproteinases and their inhibitors in the gastrointestinal cancers: current knowledge and clinical potential, *Metalloproteinases Med.* 1 (1) (2014) 3–13.
- [101] N.J. Nasser, Heparanase involvement in physiology and disease, *Cell. Mol. Life Sci.* 65 (11) (2008) 1706–1715.
- [102] J.S. Desgrosellier, D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities, *Nat. Rev. Cancer* 10 (1) (2010) 9–22.
- [103] N. Halberg, T. Khan, M.E. Trujillo, I. Wernstedt-Asterholm, A.D. Attie, S. Sherwani, Z.V. Wang, S. Landskroner-Eiger, S. Dineen, U.J. Magalang, R.A. Brekken, P.E. Scherer, Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue, *Mol. Cell. Biol.* 29 (16) (2009) 4467–4483.
- [104] H.K. Sung, K.O. Doh, J.E. Son, J.G. Park, Y. Bae, S. Choi, S.M. Nelson, R. Cowling, K. Nagy, I.P. Michael, G.Y. Koh, S.L. Adamson, T. Pawson, A. Nagy, Adipose vascular endothelial growth factor regulates metabolic homeostasis through angiogenesis, *Cell Metab.* 17 (1) (2013) 61–72.
- [105] M.C. Brahim-Horn, J. Chiche, J. Pouyssegur, Hypoxia and cancer, *J. Mol. Med. (Berl.)* 85 (12) (2007) 1301–1307.
- [106] J.S. Bonner, L. Lantier, C.M. Hasenour, F.D. James, D.P. Bracy, D.H. Wasserman, Muscle-specific vascular endothelial growth factor deletion induces muscle capillary rarefaction creating muscle insulin resistance, *Diabetes* 62 (2) (2013) 572–580.
- [107] L. Kang, W.H. Mayes, F.D. James, D.P. Bracy, D.H. Wasserman, Matrix metalloproteinase 9 opposes diet-induced muscle insulin resistance in mice, *Diabetologia* 57 (3) (2014) 603–613.
- [108] Z. Arany, S.Y. Foo, Y. Ma, J.L. Ruas, A. Bommi-Reddy, G. Girnun, M. Cooper, D. Laznik, J. Chinsomboon, S.M. Rangwala, K.H. Baek, A. Rosenzweig, B.M. Spiegelman, HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha, *Nature* 451 (7181) (2008) 1008–1012.
- [109] J. Chinsomboon, J. Ruas, R.K. Gupta, R. Thom, J. Shoag, G.C. Rowe, N. Sawada, S. Raghuram, Z. Arany, The transcriptional coactivator PGC-1alpha mediates exercise-induced angiogenesis in skeletal muscle, *Proc. Natl. Acad. Sci. U.S.A.* 106 (50) (2009) 21401–21406.
- [110] L. Leick, Y. Hellsten, J. Fentz, S.S. Lyngby, J.F. Wojtaszewski, J. Hidalgo, H. Pilegaard, PGC-1alpha mediates exercise-induced skeletal muscle VEGF expression in mice, *Am. J. Physiol. Endocrinol. Metab.* 297 (1) (2009) E92–E103.
- [111] G.C. Rowe, C. Jang, I.S. Patten, Z. Arany, PGC-1beta regulates angiogenesis in skeletal muscle, *Am. J. Physiol. Endocrinol. Metab.* 301 (1) (2011) E155–E163.
- [112] J.X. Rong, Y. Qiu, M.K. Hansen, L. Zhu, V. Zhang, M. Xie, Y. Okamoto, M.D. Mattie, H. Higashiyama, S. Asano, J.C. Strum, T.E. Ryan, Adipose mitochondrial biogenesis is suppressed in db/db and high-fat diet-fed mice and improved by rosiglitazone, *Diabetes* 56 (7) (2007) 1751–1760.
- [113] S. Kleiner, R.J. Mepani, D. Laznik, L. Ye, M.J. Jurczak, F.R. Jornayvaz, J.L. Estall, D. Chatterjee Bhowmick, G.I. Shulman, B.M. Spiegelman, Development of insulin resistance in mice lacking PGC-1alpha in adipose tissues, *Proc. Natl. Acad. Sci. U.S.A.* 109 (24) (2012) 9635–9640.
- [114] S.M. Weis, D.A. Cheresh, Tumor angiogenesis: molecular pathways and therapeutic targets, *Nat. Med.* 17 (11) (2011) 1359–1370.
- [115] Y. Cao, Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity, *Cell Metab.* 18 (4) (2013) 478–489.
- [116] E. Brakenhielm, R. Cao, B. Gao, B. Angelin, B. Cannon, P. Parini, Y. Cao, Angiogenesis inhibitor, TNP-470, prevents diet-induced and genetic obesity in mice, *Circ. Res.* 94 (12) (2004) 1579–1588.
- [117] M.A. Rupnick, D. Panigrahy, C.Y. Zhang, S.M. Dallabrida, B.B. Lowell, R. Langer, M.J. Folkman, Adipose tissue mass can be regulated through the vasculature, *Proc. Natl. Acad. Sci. U.S.A.* 99 (16) (2002) 10730–10735.
- [118] K. Sun, I. Wernstedt Asterholm, C.M. Kusminski, A.C. Bueno, Z.V. Wang, J.W. Pollard, R.A. Brekken, P.E. Scherer, Dichotomous effects of VEGF-A on adipose tissue dysfunction, *Proc. Natl. Acad. Sci. U.S.A.* 109 (15) (2012) 5874–5879.
- [119] C.T. Luk, S.Y. Shi, E.P. Cai, T. Sivasubramaniam, S.A. Schroer, M. Woo, Adipocyte-specific FAK deletion in mice leads to insulin resistance but divergent adipose tissue remodelling under lean and obese conditions, *Can. J. Diabetes* 37 (Suppl. 4) (2013) S8–S9.
- [120] K. Sun, C.M. Kusminski, P.E. Scherer, Adipose tissue remodeling and obesity, *J. Clin. Invest.* 121 (6) (2011) 2094–2101.
- [121] K. Sun, J. Tordjman, K. Clement, P.E. Scherer, Fibrosis and adipose tissue dysfunction, *Cell Metab.* 18 (4) (2013) 470–477.
- [122] K.J. Moore, M.W. Freeman, Scavenger receptors in atherosclerosis: beyond lipid uptake, *Arterioscler. Thromb. Vasc. Biol.* 26 (8) (2006) 1702–1711.