Commentary

Metalloproteases and Adipogenesis: A Weighty Subject

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Obesity has become a prevalent health hazard in industrialized countries and is closely associated with a number of pathological disorders such as cancer, cardiovascular disease, hypertension, and non-insulin-dependent diabetes.¹ Even with such serious health implications there is still a great deal to learn before we can fully understand the mechanisms controlling adipocyte differentiation during normal development as well as in pathological conditions. Adipose tissues are aggregates of specially differentiated mesenchymal cells capable of storing large amounts of triglycerides in periods of energy excess and subsequently releasing those triglycerides during energy deprivation. Kawaguchi and colleagues² in this issue of *The American Journal of Pathology* introduce a mouse model that, together with several other recent studies,^{3,4} may lead to greater understanding of the events in the extracellular microenvironment that mediate mesenchymal fate decisions and adipocyte differentiation.

The adipose lineage arises from a multipotent stem cell of mesenchymal origin from which muscle, bone, and cartilage cells are also derived, although the exact combination of regulatory genes that determine adipocyte fate in development is yet to be determined.⁵ In most species, white adipose tissue formation begins before birth and expands rapidly after birth as a result of increased fat cell size as well as an increase in fat cell number. It is not clear whether the increased adipose tissue mass of obesity is because of increases in adipocyte size or number, or a combination thereof.⁶ The availability of established preadipocyte cell lines, such as 3T3-L1 and 3T3-F442A, has made it possible to investigate the adipocyte differentiation program under controlled conditions. Through *in vitro* studies the transcriptional mechanisms that control the transition from the undifferentiated, fibroblast-like preadipocyte into mature, round fat cells are now well understood, particularly with respect to the identification of key transcription factors such as those of the C/EBP and PPAR families.^{5,7} Although most studies have been devoted to the hormonal and transcriptional regulation of adipocyte differentiation,

new attention is being given to the role of extracellular matrix (ECM) organization in adipogenesis.

During adipogenesis ECM remodeling defines the onset of the differentiation process; this is characterized by the conversion from the fibronectin-rich stromal matrix of the preadipocyte to the basement membrane of an adipocyte (Figure 1).^{3,8,9} The expression of ECM components is highly regulated during the process of adipocyte differentiation: types I and III collagen, fibronectin, and β 1-integrins are down-regulated, whereas type IV collagen and entactin are up-regulated.⁵ This ECM remodeling is a key event in the adipogenesis program. Growth of preadipocytes on a fibronectin matrix inhibits adipocyte differentiation, and this effect is overcome by the addition of cytochalasin D, which disrupts actin filaments and promotes rounding-up of cells.¹⁰ This indicates that cell shape change is crucial to differentiation, and that this change in shape is modulated by ECM components. The interplay between the changing ECM, cytoskeleton, and cell shape during adipocyte differentiation was observed 20 years ago, however, the molecular relationship between these events and the transcription factors that activate adipogenic genes is not yet understood. The requirement for a cell shape change during adipocyte differentiation suggests the crucial factors may be the major modifiers of extracellular environment—the ECM-degrading proteases.

During ECM remodeling adipogenic cells release their cell-ECM adhesion and change their morphology and volume. The morphological and cytoskeletal reorganizations in the preadipocyte seem to be required for induction of expression of mRNAs for lipogenic proteins. Therefore, if ECM remodeling is prerequisite to the differentiation of preadipocytes into mature adipocytes, it is conceivable that the remodeling acts as a trigger of differentiation. What molecular events does this trigger

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Figure 1. Summary of adipocyte differentiation. **a:** Preadipocytes grow in stromal ECM rich in fibronectin and fibrillar collagens. **b:** Once confluent, the cells are committed to differentiate. **c:** Proteases then extensively remodel the ECM; notably fibronectin is degraded. **d:** The cytoskeleton is also remodeled and transcription factors initiate changes in gene expression. **e:** The maturing adipocytes produce lipogenic proteins and deposit basement membrane. **f:** At the end of differentiation, the adipocytes are round and filled with lipid.

bring about? Besides providing an appropriate milieu for cell survival, ECM also binds and sequesters growth factors. It is possible that the ECM, through altering cell spreading, can expose the cell surface to growth factors and hormones that are present in the environment. Proteases may therefore control adipocyte differentiation through regulating bioavailability of signaling molecules that bind to the ECM. Proteases could play a dual role: degrading the fibronectin-rich matrix and regulating factors regulating growth and differentiation.

Focalized proteolysis of the ECM is an important regulator of various physiological and pathological processes.¹¹ Several classes of proteases including metalloproteases, cysteine proteases, and serine proteases play an important role in these events. We are beginning to understand that this is also true in the process of adipocyte differentiation. Several classes of metalloproteases have intriguing and opposing effects in the adipogenic process, suggesting that they have distinct roles and substrates during fate decisions and/or terminal differentiation. For example, the secretion of matrix metalloproteases (MMPs)-2 and -9 increases during adipocyte differentiation in both human adipocytes and the 3T3-F442A cell line, and inhibition of MMP activity inhibits differentiation of confluent preadipocytes.¹² Another MMP seems to have an inhibitory effect on adipogenesis: MMP-3-deficient mice show accelerated adipogenesis during mammary gland involution, suggesting an ordinarily negative regulatory role for MMP-3 in adipocyte differentiation.¹³ Furthermore, MMP-3 expression is up-regulated during 3T3-L1 differentiation into adipocytes,¹³ so that whereas MMP-2 and MMP-9 may be promoting ECM remodeling in favor of adipocyte differentiation, MMP-3 is acting to slow down the adipogenic process. Accordingly, as MMP activity acts to regulate adipose formation, then it not surprising that MMP regulators are also involved. For example, the tissue inhibitor of metalloproteinases-3 (TIMP-3) is an ECM-bound molecule that inhibits MMPs, as well as some members of

the related protease family of ADAMs (a disintegrin and metalloprotease, to be discussed below).¹⁴ Like the MMP-3 $-/-$ mice, mice deficient in *Timp-3* also show accelerated adipose reconstitution during mammary involution.¹⁵ In contrast, overexpression of TIMP-1 results in enhanced involutional adipogenesis.¹³ Taken together, it is clear that MMPs and their inhibitors function to regulate adipogenesis.

The study by Kawaguchi and colleagues² presents a novel mechanism of *in vivo* adipogenesis promoted by the transgenic expression of an ADAM. ADAMs are either transmembrane or soluble molecules that contain a metalloprotease domain as well as an integrin-binding domain. The catalytically active members of the ADAMs family function in mediating cell-cell adhesion, sperm-egg fusion, the ectodomain shedding of growth factors, and intracellular signaling through their cytoplasmic domains.¹⁶ As such, there are potentially many ways in which ADAMs might be involved in the ECM remodeling that allows adipogenesis. The study by Kawaguchi and colleagues² describes an unexpected effect of ADAM 12 (meltrin- α) overexpression on fat differentiation in a transgenic mouse. Previously, these authors and others have demonstrated a role for ADAM 12 in myogenesis,^{17–19} and generated transgenic mice expressing the secreted form of ADAM 12 (ADAM 12S) under the control of the muscle creatine kinase promoter.

Interestingly, these mice develop perivascular adipocytes in their skeletal muscle and accumulate fat tissue in and around skeletal muscle. Furthermore, female ADAM 12S-overexpressing mice developed significantly more abdominal and total body fat than their wild-type littermates; this effect was not as severe in male transgenic mice. The mechanism by which ADAM 12S unexpectedly causes adipogenesis is yet to be identified. Modification to a pro-adipogenic ECM may attract circulating mesenchymal precursors or direct local precursors to an adipocyte fate, or in the case of the skeletal muscle fat, initiate transdifferentiation of resident muscle cells. However, this effect of ADAM 12S overexpression is dependent on the metalloprotease domain, suggesting a proteolytic role for ADAM 12S in adipogenesis.

Other metalloproteinases may function in adipogenesis as well. Analysis of a transgenic mouse null for a related soluble metalloprotease family protease ADAMTS1 (ADAM with thrombospondin-like repeats-1) shows that, among other deficiencies, these mice are leaner, having less epididymal fat.⁴ Because MMPs, ADAMs, and ADAMTSs are highly related and thus are structurally similar (Figure 2), these observations suggest that extracellular proteolysis by metalloproteinases is a significant regulator of a number of events in the program of mesenchymal differentiation to the adipogenic lineage.

The plasminogen cascade of serine proteases also is involved in adipogenesis. Plasmin directly cleaves various ECM molecules, such as fibronectin and laminin, releases bound cytokines such as insulin-like growth factor (IGF)-I,²⁰ and activates latent transforming growth factor (TGF)- β .²¹ Plasminogen is necessary for development of mammary gland lactational competence and postlactational involution.²² Furthermore, adipogenesis is

Figure 2. Schematic comparison of the protein structure of metalloproteases. Pre, amino-terminal signal sequence; Pro, propeptide with a zinc-interacting thiol group; H, hinge region; Dis, disintegrin-like domain; Cys, cysteine-rich domain; TSP, thrombospondin-1-like motif. (Adapted from Egeblad and Werb³³ and Tang³⁴.)

suppressed during involutional repopulation of adipocytes in plasminogen-deficient mice and during culture of 3T3-L1 preadipocytes in plasminogen-depleted medium.³ Plasminogen can be activated to plasmin by urokinase-type plasminogen activator, tissue-type plasminogen activator, and plasma kallikrein. Intriguingly, the dominant plasminogen activator for adipogenesis *in vivo* and in culture for adipogenesis is neither urokinase nor tissue-type plasminogen activator, but instead is plasma kallikrein.³ However, as plasma kallikrein and plasminogen are circulating factors, further research is required to identify how these proteases regulate (and are regulated by) extracellular proteolysis in adipogenesis.

Proteases could regulate adipocyte differentiation through controlling the bioavailability of differentiation-promoting or differentiation-inhibiting growth factors from the stromal ECM. IGF-I stimulates the proliferation of 3T3-L1 cells and primary cultures of preadipocytes *in vitro*, but when cells reach confluence, IGF-I stimulates differentiation.²³ The bioavailability of IGF-I is modulated by specific, high-affinity IGF-binding proteins (IGFBPs). Transgenic mice with increased IGFBP concentration show impaired adipogenesis *in vivo*.²⁴ Plasmin and other serine proteases can cleave IGFBPs and release active IGF²⁵ with the potential to stimulate preadipocytes to differentiate.

TGF- β , on the other hand, is a potent inhibitor of adipocyte differentiation in most cell culture models. It has been suggested that this inhibition is because of an increased synthesis of ECM components such as fibronectin as a result of TGF- β stimulation,²⁶ potentially inhibiting insulin signaling. Additionally, TGF- β is expressed in adipose tissue as well as in cultured adipocytes, and TGF- β receptor availability decreases during 3T3-F442A differentiation,²⁷ suggesting that adipocytes are capable of autoregulation.

The secreted signaling molecules of the Wnt family also inhibit adipogenesis, perhaps by regulating fate decisions in mesenchymal stem cells. Wnt proteins maintain adipocytes in the undifferentiated state through inhibition of the adipogenic transcription factors C/EBP α and PPAR γ ; inhibition of Wnt pathway induced spontaneous differentiation of 3T3-L1 preadipocytes.²⁸ Intriguingly, knowing that Wnt signaling also regulates myogenesis, the same authors inhibited Wnt signaling in two myoblast cell lines and showed that in both cases these cells transdifferentiated into adipocytes. A potential mechanism

by which ECM remodeling could affect the Wnt signaling pathway involves heparan sulfate proteoglycans. In epithelium for example, Wnt signaling is mediated by binding to the heparan sulfate proteoglycan syndecan-1.²⁹ These heparan sulfate proteoglycans could be a target for ECM-remodeling proteases, thereby releasing Wnts and elevating the negative block on adipocyte differentiation.

It is important to remember that different adipose tissues are very likely differentially regulated, depending on the constituents of the ECM in which the preadipose cells are situated. For example, abdominal fat pad ECM is different from that of the mammary fat pad. Differences between species in their ability to develop intramuscular fat may depend on differences in ECM collagen composition.³⁰ Thus, for fat to develop, competent proteases and ECM components must be present or producible.

Knowing what we do of the extracellular remodeling events that mediate adipocyte differentiation, we can hypothesize numerous ways in which ADAM 12S could affect adipogenesis. ADAM 12-integrin-syndecan interactions can mediate cell adhesion and spreading.³¹ Also, ADAM 12 cleaves IGFBP-3 and -5, possibly modulating IGF availability for stimulating differentiation.³² Further studies should provide greater insight as to the mechanism of ADAM 12S regulation of adipogenesis. ADAM 12S could be affecting the bioavailability of differentiation-inducing or differentiation-inhibiting growth factors, or helping to modify the ECM via degradation, or affecting cell migration and adhesion, or activating other protease pathways. In any case, proteases are now firmly established as critical regulators of the differentiation of adipocytes, acting upstream of the much more intensely investigated transcriptional regulators of the adipogenic program.

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