

Peri-adipocyte ECM remodeling in obesity and adipose tissue fibrosis

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Adipocytes differentiate and function in environments rich in extracellular matrix (ECM) proteins. The phenotypes of genetically modified mice have aided in recognizing the importance of ECM proteins and their modifiers, e.g., proteinases, in the regulation of obesity and metabolism. Most of the molecular mechanisms through which ECM proteins and modifiers regulate adipogenesis or adipocyte function have not been fully defined. Adipose tissue fibrosis may be a factor that links obesity to diabetes or cardiovascular disease risk in conjunction with tissue inflammation. Defining the molecular mechanisms through which the ECM environment regulates adipogenesis and adipocyte function should provide us with a better understanding of the disease link between obesity and diabetes or cardiovascular diseases.

Adipose Tissue Fibrosis and Inflammation

Obesity increases the risk of diabetes, cardiovascular diseases and cancer. The molecular mechanisms underlying the link between obesity and the risk of developing metabolic and cardiovascular diseases, however, are still enigmatic. In addition to obesity, the loss of fat tissue (lipodystrophy) increases the risk of diabetes and cardiovascular diseases. The U- or J-curve relationship between body mass index (BMI) and the risk of diabetes and cardiovascular diseases^{1,2} suggests the important role played by “balanced adiposity” in maintaining metabolic health. Why do the two opposite extremes of adiposity similarly increase the risk of diabetes and cardiovascular diseases? Some hints may be found in the biological processes common to both obesity and lipodystrophy. Among an array of biological processes, inflammation and fibrosis are two candidate biological processes that may explain the metabolic consequences of obesity and lipodystrophy.

In adipose tissues, mature adipocytes and their progenitor cells (preadipocytes) exist within a three-dimensional (3D) network of ECM proteins. Adipose tissue function is regulated by the physiological interaction between cells and a variety of ECM proteins. The collagen family is the largest group of ECM proteins.³ The density and structure of collagens in organs are tightly regulated. The excess deposition of collagens in individuals with pathological conditions is defined as fibrosis, a hallmark of

chronic tissue damage. Unlike the existence of fibrosis in the skin, lungs, liver or kidneys, the existence of fibrosis in adipose tissue has not been fully recognized. Recent reports, however, suggest that obese individuals display excess collagen deposition in adipose tissues.^{4–6} Notably, the excess collagen deposition in adipose tissues was observed along with inflammatory tissue damage, which is characterized by the infiltration of neutrophils, lymphocytes and macrophages. Thus, fibrotic tissue damage is perceived by many as a process secondary to tissue inflammation, whose pathological impact on obesity and metabolism has been extensively studied in recent years.^{7–9} Adding a layer of complexity to the comprehensive understanding of adipose tissue biology, inflammatory tissue damage characterized by macrophage infiltration can also be found in lipodystrophy.¹⁰ Likewise, adipose tissue fibrosis has been observed in individuals with congenital lipodystrophy and in the dystrophic interscapular fat pads of partially lipodystrophic patients.^{11,12} Of note, *LMNA* mutations reported in the latter group lead to the paradoxical expansion of interscapular fat pads, which display fibrotic changes but not evidence of ongoing inflammatory processes, suggesting the possibility that fibrosis and inflammation are not always coupled.¹² The causal and temporo-spatial relationships between adipose tissue fibrosis and inflammation remain to be defined in the contexts of obesity and lipodystrophy. Despite these unresolved questions, a series of studies using gene targeting in rodents and mechanistic experiments at the cellular level have helped elucidate some of the genetic and molecular mechanisms involved in the regulation of adipose tissue ECM remodeling and function.

MMP-Dependent Type I Collagen Turnover during Adipose Tissue Development

Adipocytes differentiate and function in vivo within a 3D environment surrounded by a number of extracellular matrix (ECM) proteins (Figs. 1 and 2). In the adipose tissue primordium, preadipocytes progressively change cell shape and accumulate lipid droplets in a space juxtaposed to collagen bundles.¹³ Among the ECM proteins, collagens are the most abundant proteins that constitute interstitial fibers and pericellular basement membranes.³ Type I collagen molecules, which exist mainly as $[\alpha 1(I)]_2\alpha 2(I)$ heterotrimers in a triple helix, are staggered and interwoven with each other to form thick collagen bundles.¹⁴ Type I collagen bundles provide the major ECM framework necessary to sustain the structure and function of mesenchymal tissues. Although triple-helical type I collagen is highly resistant to proteolytic degradation, it can be

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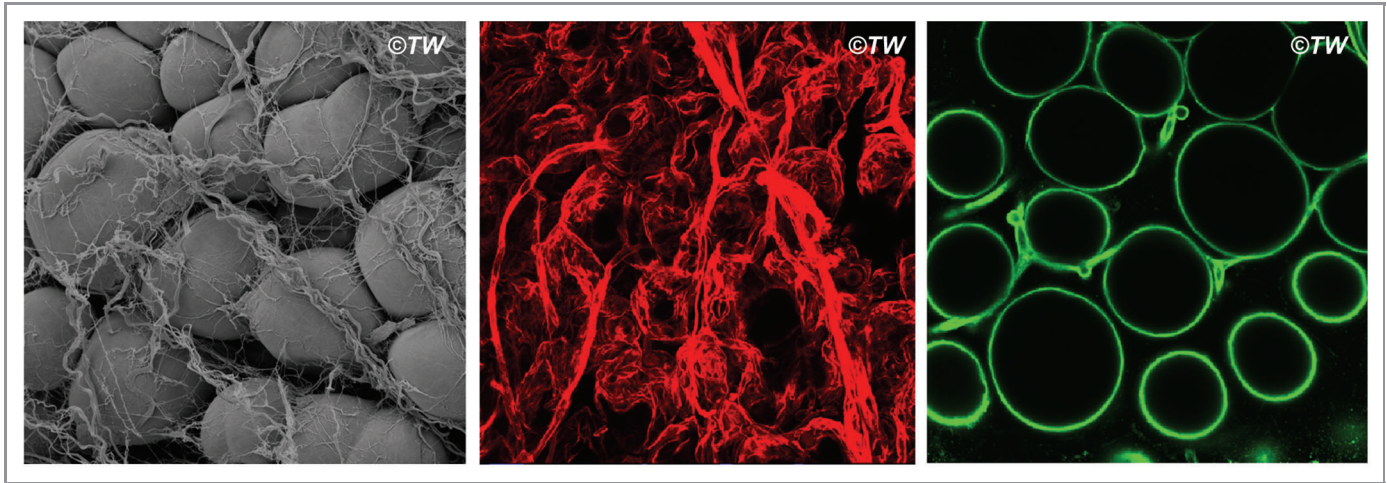


Figure 1. Peri-adipocyte collagens. Left: the scanning electron micrograph of mouse inguinal fat pads; the group of round adipocytes are surrounded with collagen bundles. Middle: immunofluorescent staining of type I collagen (red); thick bundles of type I collagen surrounding the group of adipocytes as well as thinner fibers of type I collagen wrapping individual adipocyte is displayed. Right: immunofluorescent staining of type IV collagen (green); type IV collagen is found as a component of basement membrane that wraps each adipocytes; type IV collagen can also be found as the basement membrane underneath the layer of vascular endothelial cells.

cleaved and degraded by a set of matrix metalloproteinases (MMPs) in certain stages of development and chronic disease progression.¹⁵ Of the 28 members of the MMP family,¹⁶ MMP14 (membrane type 1 matrix metalloproteinase, MT1-MMP) plays a major role in the postnatal development of mesenchymal tissues, particularly bones and adipose tissues.^{17,18} The dominant role played by MMP14 is attributable to its collagenolytic activity tethered to the cell surface. Although the soluble collagenases of rodents, i.e., MMP2, MMP8, MMP13 and Mcol1a (an ortholog of human MMP1), are secreted

as inactive zymogens, MMP14 is expressed as an active enzyme on the cell surface, as its inhibitory pro-domain is intracellularly removed by furin or furin-like proprotein convertases.^{19,20}

The complete loss of *Mmp14* in mice leads to postnatal lipodystrophy and leptin-null status.¹⁸ In vitro studies suggest that MMP15 (MT2-MMP) possesses pericellular collagenase activity similar to that of MMP14;²¹⁻²³ however, the lipodystrophic phenotype of *Mmp14*-null mice is not rescued by *Mmp15* because *MMP15* is rarely expressed in adipose tissues. Cell-autonomous

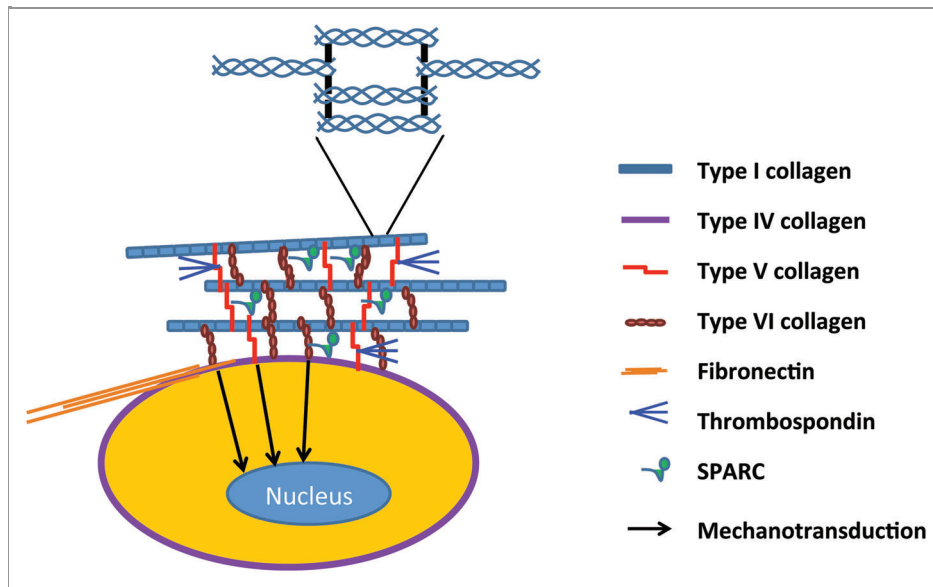


Figure 2. Peri-adipocyte ECM proteins. Each adipocyte is surrounded by basement membrane whose framework is defined by type IV collagen. The most abundant fibrillar structure is provided by the cross-linking of triple-helical type I collagen molecules (enlarged in the inset). Types V and VI collagens form micro-fibers that exist between type I collagen fibers as well as between cell surface and type I collagen fibers. Fibronectin is the key ECM protein that defines cell shape and contractility in close association with type I collagen. Matricellular proteins, i.e., thrombospondins and SPARC, regulate the fibrillogenesis of type I collagen and interact with multiple ECM proteins.

defects in adipogenic potential were confirmed by the observations of impaired *in vitro* adipogenesis within a 3D collagen environment and defective *in vivo* adipogenesis when *Mmp14*-null preadipocytes were transplanted into an *MMP14*-sufficient host.¹⁸ Of note, *Mmp14* is largely dispensable for adipogenesis under 2D culture conditions, suggesting that the interaction of MMP14 with type I collagen, particularly in a 3D environment, plays a central role in regulating adipocyte maturation. As such, MMP14 can be defined as an *in vivo* factor that is necessary for adipocyte maturation (Fig. 3).

The inextricable relationship between the MMP family proteins and type I collagen has been further underscored by the developmental defects observed in mice that harbor an MMP-resistant *Col1a1* gene mutation.^{24,25} This knock-in mutation (*Col1a1^{rr}*) renders triple-helical type I collagen molecules resistant to MMP-dependent hydrolysis and impairs anabolic bone remodeling.^{25,26} Although the gene targeting of *Mmp2*, which encodes the most abundant collagenase/gelatinase found in tissues and the circulation, results in only a subtle skeletal growth defect,²⁷ *Mmp2* gene targeting in mice harboring the MMP-resistant mutant *Col1a1* gene leads to a profound defect in postnatal development; this effect is similar to that observed with *Mmp14*-null mice.²⁸ MMP2 is activated by MMP14 on the cell surface²⁹ in a manner that depends on the physical interactions between MMP2, MMP14 and an endogenous MMP inhibitor, TIMP2.³⁰ MMP2 can also be activated by the other membrane-type MMPs, e.g., MMP15 (MT2-MMP) and MMP16 (MT3-MMP); indeed, the activation of MMP2 under normal conditions is barely affected by the *Mmp14*-null state in rodents.³¹ These complex results suggest that the phenotypes

observed in *Mmp14*-null mice are mostly independent of *Mmp2*; however, the proteolytic activity mediated by MMP2 may play a qualitatively distinct role, beyond that of collagenolysis, in certain stages of development and disease. Consistent with the non-overlapping roles played by MMP2 and MMP14, *Mmp2* and *Mmp14* double-knockout mice die at birth, which is a significantly more severe and lethal phenotype than that of *Mmp14*-null mice.³² It is conceivable, however, that the roles played by MMPs in cleaving substrates other than ECM proteins, particularly chemokines and cytokines, may complicate the phenotypic consequences of targeting each MMP gene.³³⁻³⁵ *Mmp2*-deficient mice are more resistant to high-fat diet-induced weight gain than littermate wild-type mice³⁶; however, the direct role of MMP2 in regulating adipose tissue ECM remodeling and function is undefined.

Type V and VI Collagens in Adipose Tissue Development and Obesity

Type V collagen molecules exist in the form of $[\alpha 1(V)]_2\alpha 2(V)$ or $\alpha 1(V)\alpha 2(V)\alpha 3(V)$ heterotrimers.³⁷ These molecules constitute micro-fibers that are closely associated with thick type I collagen bundles.³⁸ Genetic mutations in *Col5a1* and *Col5a2* are found in families with Ehlers-Danlos syndrome,³⁹ underscoring the role played by type V collagen in regulating the elastic resilience of connective tissues in association with type I collagen. A previous study has shown that the loss of *Col5a3*, which is highly expressed in adipose tissues, muscles and pancreatic islets, leads to a reduced size of pancreatic islets and adipose tissues.⁴⁰ In that study, *Col5a3*-null adipocytes and skeletal muscles displayed

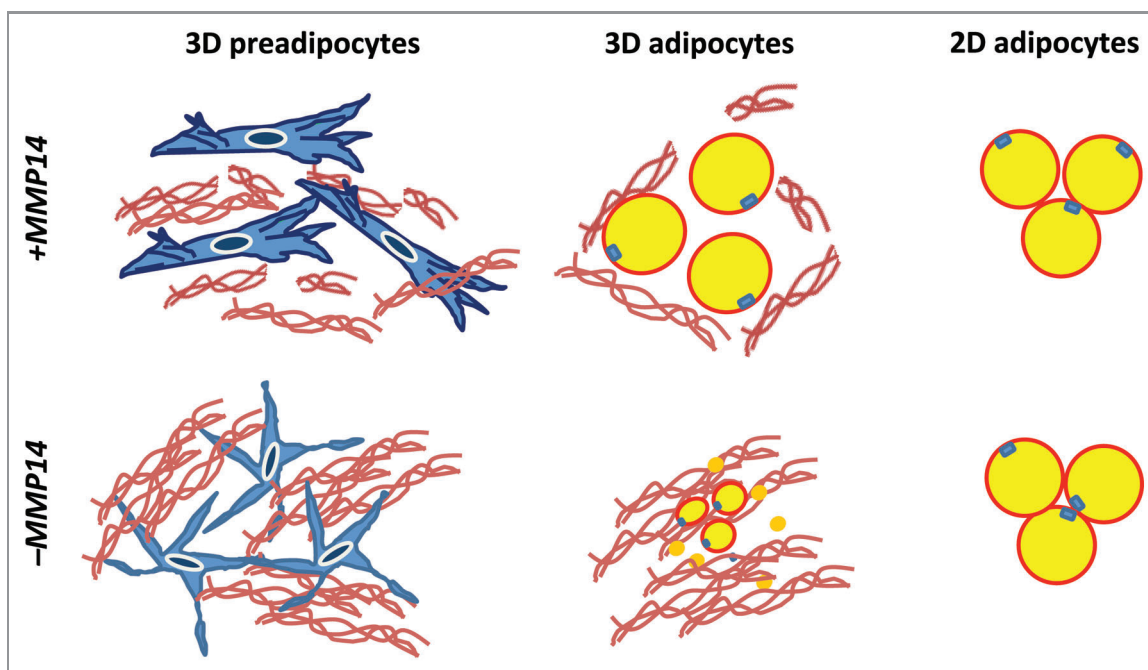


Figure 3. MMP14 (MT1-MMP) as a 3D factor. MMP14 is required for the cytoskeletal rearrangement of preadipocytes in a 3D collagen environment (3D preadipocytes). MMP14-dependent cell shape regulation is closely linked to the adipogenic potential of preadipocytes in a 3D environment (3D adipocytes). The loss of MMP14 activity leads to the aberrant cell shape of preadipocytes and impaired 3D adipogenesis, which is coupled with the excess accumulation of undigested collagen fibers. MMP14 is not required for adipogenesis under 2D culture conditions (2D adipogenesis).

insulin resistance due to the suppression of insulin-induced Glut4 translocation. The molecular mechanism through which the loss of *Col5a3* negatively impacts adipocyte size and function, however, is undefined. In addition to modifying the tensile strength of type I collagen fibers, type V collagen also binds to thrombospondin 1, a matricellular ECM protein that is abundant in connective tissues and the circulation.⁴¹ It is conceivable that type V collagen heterotrimers regulate the composition and density of ECM proteins in adipose tissues through their interactions with multiple ECM proteins.

Type VI collagen heterotrimers form microfibrils in the interface between the basement membrane and thick bundles of type I collagens.⁴²⁻⁴⁴ *Col6a1*-null mice are born and develop normally with no gross abnormalities.⁴⁵ As observed in Bethlem myopathy, which is linked to 2q37 or *Col6a* mutations in humans,^{46,47} these mice display a mild form of necrotizing myopathy. When *Col6a1*-deficient mice were crossed with *ob/ob* mice, *Col6a1* deficiency was demonstrated to protect mice from the high-fat diet-induced impairment of glucose metabolism.⁴⁸ Although *Col6a1*-deficient *ob/ob* mice display a lower body weight relative to wild-type mice in the early stages of postnatal development, the fat mass of *Col6a1*-deficient *ob/ob* mice increases faster and catches up with that of *ob/ob* mice at 12 weeks of age. Although the consequence of whole-body *Col6a1* deficiency is complex, affecting multiple organs and displaying reduced food intake and energy expenditure, it has been suggested that type VI collagen is a fibrotic component that restricts adipose tissue expandability. In humans, *Col6a3* gene expression in adipose tissues was found to correlate with visceral adipose tissue mass and pro-inflammatory gene expression.⁴⁹ The causal roles played by type VI collagen in regulating adipogenesis, adipocyte hypertrophy, or adipocyte function, however, have not been fully addressed in vitro.

Non-Collagen ECM Proteins in Adipocyte Biology

Fibronectin inhibits 3T3 adipogenesis in conjunction with the regulation of cell shape and stretch.⁵⁰ The pericellular assembly of fibronectin molecules dictates cytoskeletal and ECM organization.⁵¹ Fibronectin plays a critical role in embryonic mesoderm development and cardiovascular morphogenesis.^{52,53} Recently, Wang et al. demonstrated that soluble Pref-1 (DLK1) released from the cell surface interacts with fibronectin and inhibits adipogenesis by activating the Rac1-Erk1/2 pathway.⁵⁴ Pref-1 was initially identified as a member of the EGF-like family of proteins, which is highly expressed in preadipocytes and inhibits adipocyte differentiation.⁵⁵ The novel interaction identified between Pref-1 and fibronectin,⁵⁶ which is the major ligand of $\alpha 5\beta 1$ integrin,⁵⁷ is intriguing in understanding the molecular mechanisms through which the interaction between cells and pericellular ECM proteins regulates adipogenesis and adipocyte function. Moreover, fibronectin assembly plays a key role in maintaining the fibrillar organization of type I collagen and thrombospondin 1.⁵⁸ Fibronectin can be cross-linked to an $\alpha 1(I)$ collagen chain through factor XIIIa.⁵⁹ This close molecular interaction between fibronectin and type I collagen may play a major role in the ECM interactomes that may regulate adipocyte function. The most recent

study from Spiegelman's group suggests a role of a myokine, termed Irisin, in inducing the "browning" of subcutaneous fat and thermogenesis.⁶⁰ Irisin is a proteolytically cleaved fragment of Fndc5 mostly comprising a type III fibronectin domain (Fn3), which is commonly found in ECM proteins and cell surface receptors.⁶¹ Fn3 promotes cell adhesion via activated $\beta 1$ integrin.⁶² Although the molecular mechanism through which Irisin induces the "browning" of adipose tissues remains undefined, it is intriguing to speculate that this molecule may regulate adipocyte function by fine-tuning cell-cell or cell-ECM interactions.

Secreted protein, acidic and rich in cysteine (SPARC) is a matricellular ECM protein⁶³ that indirectly contributes to the formation of the structural framework of mesenchymal tissues. *Sparc*-null mice display increased adipose tissue size and higher expandability in response to the consumption of a high-fat diet.^{64,65} The phenotype is attributable, at least partly, to the role played by SPARC in regulating the density and structure of type I collagen fibrils.^{66,67} Conversely, the loss of *Colla1* gene expression in fibroblasts (embryonic fibroblasts isolated from *Mov-13* mice) impairs the extracellular accumulation of SPARC but not the extracellular accumulation of fibronectin or type III collagen.⁶⁷ Moreover, the loss of SPARC interferes with the cell-mediated contraction of collagen gels, which can be rescued with exogenously added recombinant SPARC protein, suggesting that SPARC plays paracrine and endocrine roles. SPARC was found to suppress adipogenesis and promote osteogenesis by regulating the intra-nuclear content of β -catenin.⁶⁸ Thus, SPARC, in association with type I collagen, can be a potent modifier of Wnt signaling in adipocytes, which is a major pathway in the regulation of adipogenesis and adipocyte gene expression.^{69,70}

Thrombospondin 1 and 2 (THBS1 and THBS2) belong to the group of matricellular ECM proteins.⁶³ THBS1 is a large glycoprotein that contains multiple functional domains, including a coiled-coil domain for oligomerization, an N-terminal laminin G-like domain (LG), a thrombospondin type 1 repeat (TSR), an epidermal growth factor-like domain, type 3 repeats and a C-terminal lectin-like domain. THBS1 binds to a number of ECM proteins, cell surface proteins, and growth factors. THBS1 binds to collagens, particularly type V collagen⁴¹; however, the metabolic significance of this interaction is undefined. Given the positive role of type V collagen in adipose tissue development,⁴⁰ the specific interaction between THBS1 and type V collagen could be a biological modifier of adipocyte function. *THBS1* expression in adipose tissue is significantly elevated in obese diabetic humans, suggesting its role in the progression of metabolic syndrome.⁷¹ The loss of *Thbs1* protects mice from diet-induced obesity⁷²; however, the role of *Thbs1* in regulating peripheral insulin resistance remains elusive in light of the complex effects exerted by this protein on multiple organs and tissues, including pancreatic islets.⁷³ The effect of the *Thbs1* gene on obesity and metabolism appears to be variable depending on genetic and environmental factors; another group reported a subtle effect or almost no effect of *Thbs1* gene targeting on adipose tissue size.⁷⁴ THBS2 shares most of the functional domains found in THBS1 except for the N-terminal domain, which displays distinct sequences. *Thbs2*-null mice have fragile skin and an abnormal thickening of cortical bones in association with

dysregulated type I collagen fibrillogenesis.⁷⁵ A previous study showed that *Thbs2*-null mesenchymal cells displayed better lipid accumulation than wild-type cells during adipogenesis despite the gene expression of adipocyte markers being almost identical to that of wild-type cells.⁷⁶ In this study, increased fat mass was selectively found in female mice fed the control diet but not those fed a high-fat diet, and no significant difference was found in male mice under any nutritional conditions. Another group demonstrated that *Thbs2*-null male mice are modestly protected from high-fat diet-induced obesity, but no metabolic benefit was observed.⁷⁷ Despite these studies, the roles played by either THBS1 or THBS2 in the regulation of adipogenesis and adipocyte function remain to be defined at the molecular and cellular levels.

Matrix Elasticity and Geometry in Adipocyte Function

Recently, the role of geometrical constraints in regulating cell fate and differentiation has been highlighted. The differentiation of human mesenchymal cells into the cells of adipocyte or osteoblast lineage can be controlled by cell shape.⁷⁸ Stretched cells with a flattened cell shape differentiate more easily into osteoblasts, whereas small and round-shaped cells differentiate into adipocytes. The study showed that the cell shape-dependent regulation of lineage commitment was reversed by dominant-negative or active RhoA, suggesting that the effects of cell shape serve as upstream initiators of RhoA-dependent mechanotransduction. Nonetheless, it is difficult to determine the specific effect of

matrix elasticity or 3D geometry in the *in vivo* environment that is intricately regulated by ECM protein composition and density.⁷⁹ Despite the importance of physical constraints imposed by a 3D meshwork of collagen, the layer of collagen itself can exert inhibitory effects on the induction of a set of adipocyte genes in conjunction with suppressed pro-transcriptional histone mark modification.⁸⁰ The use of non-ECM nanomaterials that may bypass the interaction between cells and ECM proteins may aid in dissecting the signaling pathways that are regulated by physical parameters, such as cell shape, force, and pressure.

Future Directions

The critical role played by ECM proteins and their modifying enzymes (proteinases) in the regulation of adipogenesis and adipocyte function has been recognized in animal models. Gene targeting or knockdown has been used to determine the biological role of ECM proteins and proteinases. The pericellular ECM composition and its remodeling are physically linked to the cytoskeleton, which is further linked to the nuclear structure. This hard-wired interaction between ECM proteins and nuclear structure is considered to play a central role in the gene regulation and phenotype switching of cells during their adaptation to the tissue environment. Due to the multiple players and interactions in ECM biology, we must advance our understanding of the complex cell-ECM interactome by combining the evolving system biology and discovery-oriented reductionist approaches. The comprehensive understanding of the genetic architecture that underlies the interactomes of ECM proteins and their modifiers should help us define the pathogenesis of adipose tissue fibrosis and inflammation, which are closely linked to diabetes and cardiovascular diseases (Fig. 4).

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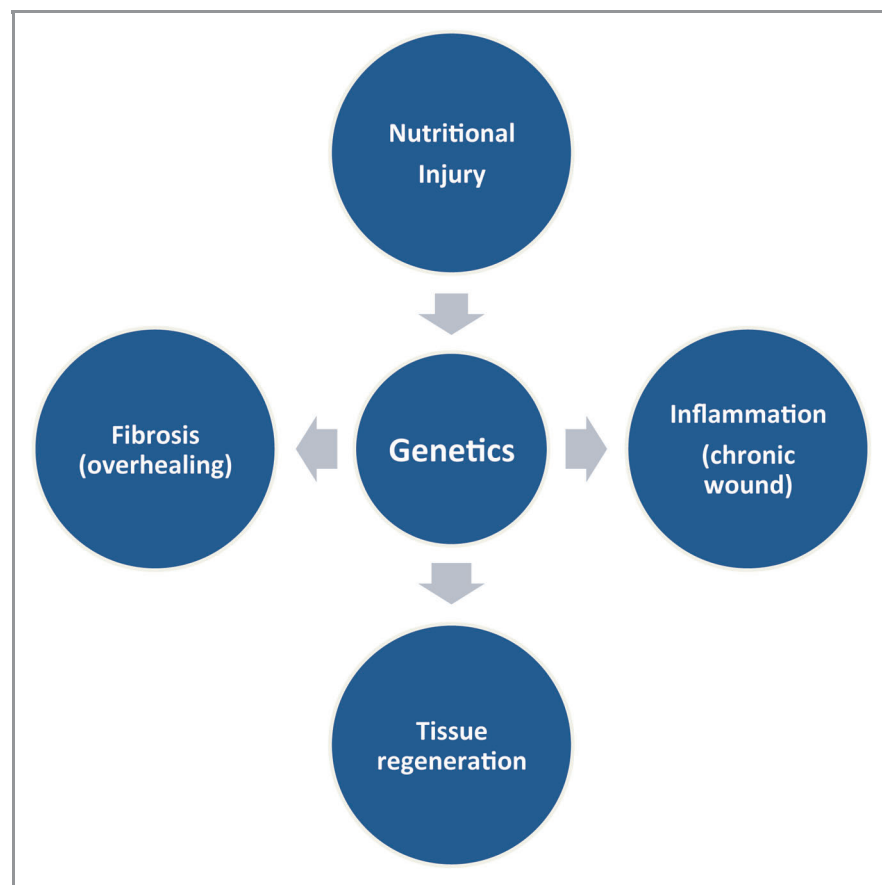


Figure 4. Nutritional injury and genetics in adipose tissue pathology. Nutritional injury, such as high-fat diet, unravels genetic predispositions of individuals. Adipose tissues can maintain healthy tissue function through physiological tissue regeneration in response to nutritional injury; however, in certain individuals, adipose tissues are genetically predisposed to fibrosis (overhealing) or inflammation (chronic wound).

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