Review

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The extracellular matrix and insulin resistance

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The extracellular matrix (ECM) is a highly-dynamic compartment that undergoes remodeling as a result of injury and repair. Over the past decade, mounting evidence in humans and rodents suggests that ECM remodeling is associated with diet-induced insulin resistance in several metabolic tissues. In addition, integrin receptors for the ECM have also been implicated in the regulation of insulin action. This review addresses what is currently known about the ECM, integrins, and insulin action in the muscle, liver, and adipose tissue. Understanding how ECM remodeling and integrin signaling regulate insulin action may aid in the development of new therapeutic targets for the treatment of insulin resistance and type 2 diabetes (T2D).

Overview of the ECM and integrins

The ECM (see Glossary) is composed of a diverse network of proteins and proteoglycans [1]. It provides a scaffold for cells and modulates biological processes including differentiation, cell migration, repair and development [2,3]. The interaction between cells and the ECM is important for all organs. The ECM communicates with cells through transmembrane cell surface receptors termed integrins [4]. Integrins bind to the ECM and transduce signals through the plasma membrane to activate intracellular signaling. Integrins themselves lack kinase activity. Thus, they are reliant on scaffolding proteins and downstream kinases for signal transduction. Integrins signal through various proteins including focal adhesion kinase (FAK) and integrin-linked kinase (ILK) (Box 1). The detailed structure and function of integrins have been reviewed elsewhere [1,4,5].

The ECM is a dynamic structure that remodels during times of injury and repair [6]. Pathological states are associated with ECM remodeling and alterations in integrin expression. In obese conditions the expression of ECM proteins increases several-fold, while a shift appears to occur from low-density ECM proteins to more fibril-forming proteins. Several recent lines of evidence suggest that ECM remodeling and changes in integrin signaling in the diet-induced obese (DIO) state are associated with insulin resistance [7–16]. The potential mechanisms whereby this occurs are represented in Figure 1. Herein we discuss recent findings related to the emerging link between ECM remodeling, integrin signaling, and insulin resistance in the skeletal muscle, liver, and adipose tissue.

Glossary

Cirrhosis: late-stage fibrosis of the liver as a result of different liver diseases and conditions such as hepatitis and chronic ethanol ingestion.
Collagen: the most abundant structural protein, consisting of three α polypeptide chains folded into a triple-helix formation. Collagen proteins are divided into subgroups depending on their organization and/or molecular size; these include the fibril-forming collagens type I and III, the basement membrane-associated collagen type IV, and collagen type V, a minor ECM component.
Endothelial dysfunction: deleterious alterations in endothelial physiology characterized by impaired endothelium-dependent vasodilation due to decreased availability of vasodilators such as NO and/or an increase in endothelium-derived contracting factors.
Extracellular matrix (ECM): the space outside the cell composed of a complex meshwork of different proteins, proteoglycans, glycoproteins, polysaccharides, and other structural proteins.
Glycosaminoglycans: large linear polysaccharides containing repeating disaccharide units with an amino sugar (either *N*-acetyl glucosamine, GlcNAc; or *N*-acetyl galactosamine, GalNAc) and an uronic acid. Five glycosaminoglycan chains have been identified: hyaluronan, dermatan, keratin, chondroitin, and keratan.
Homeostatic model assessment of insulin resistance (HOMA-IR): a method to assess insulin resistance and β cell function from basal (fasting) glucose and insulin or C-peptide concentrations.
Hyaluronan: an anionic, nonsulfated glycosaminoglycan. It is a major component of the ECM and has multiple functions, including creating space between cells and facilitating cell migration.
Hyperinsulinemic–euglycemic clamp (insulin clamp): the gold standard for assessing insulin action *in vivo*. During the insulin clamp, insulin is infused at a constant rate and glucose is infused at a variable rate to maintain euglycemia. The amount of glucose that is infused reflects the insulin sensitivity. The insulin clamp can be combined with tracer techniques to determine sites of insulin resistance.
Interstitial space: the narrow, fluid-filled areas that surround the cells of a tissue.
Myofibroblasts: cells in a state between a fibroblast and a smooth muscle cell. Fibrogenic cells are not part of the normal tissue and are only present following cellular injury. Often characterized by the presence of ruffled membranes and a highly-active endoplasmic reticulum.
Nonalcoholic fatty liver disease (NAFLD): also known as fatty liver disease, refers to the accumulation of excess lipids in liver cells that can induce inflammation and fibrosis.
Oxidative stress: the imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses that may result in tissue damage.
Relaxin: a protein hormone that acts through two G-protein-coupled receptors, RXFP1 and RXFP2, and has effects on the cardiovascular system. The vascular effects of relaxin include vasodilation and a decrease in systemic vascular resistance.
Space of Disse: the sinusoidal endothelium is separated from hepatocytes by the space of Disse which all metabolites from the bloodstream must pass through to reach the hepatocytes. The surface area of hepatocytes exposed to the space of Disse is greatly enhanced by the presence of microvilli. Under normal conditions, the space of Disse is filled with loosely assembled, low-density ECM proteins.
Stellate cells: previously known as Ito cells, these are quiescent vitamin A-rich cells. Following liver injury they transform into activated proliferative and fibrogenic myofibroblasts. This process is initiated by autocrine and paracrine stimuli including inflammatory cytokines and growth factors.

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Box 1. Integrin signaling molecules

Focal adhesion kinase (FAK)
FAK is a cytoplasmic tyrosine kinase that localizes with integrin receptors at sites where cells attach to the ECM [89]. FAK undergoes rapid autophosphorylation at Tyr397 upon integrin-mediated cell adhesion [90], and this is associated with increased catalytic activity. In addition, FAK can be regulated by the growth factor receptors EGFR (epidermal growth factor receptor), FGFR (fibroblast growth factor receptor) and the insulin receptor [63,91]. This results in the activation of several downstream signaling cascades including the mitogen-activated protein kinase (MAPK) and PI3K signaling pathways [63,91]. In addition to its signaling properties, FAK is important for cytoskeletal stabilization and focal adhesion turnover [92].

Integrin-linked kinase and insulin action
Integrin-linked kinase (ILK) is a highly-conserved intracellular scaffolding protein. It interacts with the β1, β2, and β3-integrin cytoplasmic domains, and with numerous cytoskeleton-associated proteins. It is composed of three distinct domains: an N-terminus that contains five ankyrin repeats, a pleckstrin homology-like domain, and a pseudokinase domain at the C-terminus. Considering that it is a scaffolding protein, it has been proposed that ILK modulates intracellular signaling through its ability to recruit a kinase or multiple kinases into a multiprotein complex. This complex then facilitates the activation of downstream signaling molecules upon insulin stimulation. The pseudokinase domain of ILK is an essential domain for the recruitment of adaptor proteins and/or signaling molecules, including several proteins involved in insulin action such as Akt/PKB, pyruvate dehydrogenase kinase, isozyme 1 (PDK1), and GSK-3β. Overexpression of ILK or insulin treatment results in increased GSK-3 and Akt phosphorylation [93]. Cotransfection of Akt with wild type ILK in 293 cells resulted in an enhancement of phosphorylation of Akt Ser473 [93]. Several studies have shown that the ablation of ILK results in decreased Akt Ser473 phosphorylation [94–96]. Moreover, ILK is connected to growth factor receptors through the adaptor protein NCK2 [97]. Therefore, although ILK lacks intrinsic kinase activity, it has been shown to regulate the activation of numerous intracellular growth factor signaling cascades [98–100].

The skeletal muscle

Mechanisms of high-fat diet (HFD)-induced ECM remodeling in the skeletal muscle

Inflammation and elevated transforming growth factor (TGF) β signaling are associated with muscle ECM remodeling in obese mice and humans [17]. Mice fed a HFD exhibit increased infiltration of proinflammatory M1-activated ($CD11c^+$) macrophages in muscle [18]. In addition, $CD68^+$ macrophages are elevated in obese individuals [19]. The association between ECM remodeling and inflammation was further shown in a study by Kang *et al.* [9]. In this study, 20 weeks of HFD feeding in mice led to increased muscle collagen content associated with increased gene expression of the proinflammatory marker tumor necrosis factor (TNFα) and the macrophage marker F4/80. Importantly, gene expression for these inflammatory markers was diminished in mouse models of improved insulin sensitivity and decreased muscle collagen deposition. It is possible that increased recruitment of proinflammatory macrophages may lead to ECM remodeling via TGFβ-mediated Smad (from SMA, *small*; and MAD, mothers against decapentaplegic) activation [20]. Smad3 activation is elevated in skeletal muscle biopsies of obese individuals compared to lean controls [17]. Collectively, this suggests that ECM remodeling in obese skeletal muscle occurs as a result of increased inflammation.

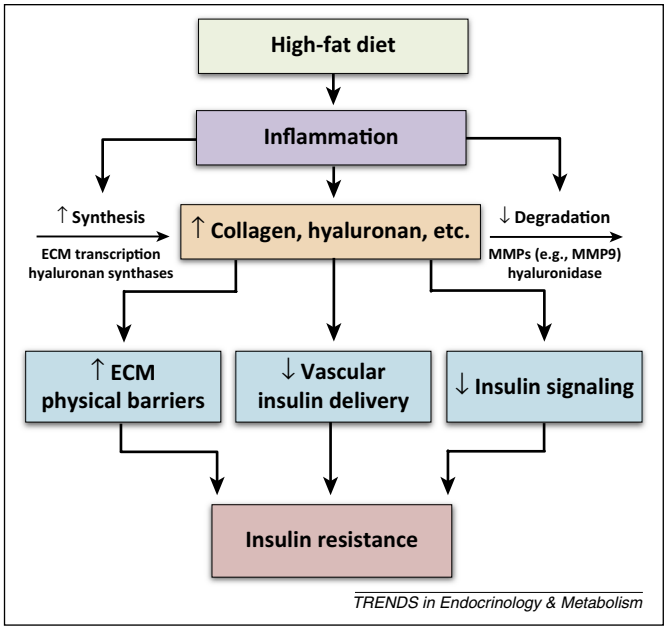

Figure 1. A link between extracellular matrix (ECM) remodeling and insulin resistance. A diet high in fat generates a state of chronic inflammation. This inflammatory response leads to increased ECM synthesis and decreased ECM degradation, resulting in increased deposition and remodeling of ECM. Increased levels of ECM lead to increased physical barriers for insulin and glucose transport, decreased vascular insulin delivery, and reduced insulin signaling. The combination of all of these factors then culminates in insulin resistance. Abbreviation: MMP, matrix metalloproteinase.

The skeletal muscle ECM and glucose metabolism

Insulin-resistant muscle in obese and T2D humans is characterized by increased collagen deposition [7,8]. Rapid weight gain in healthy young males resulted in impaired insulin sensitivity and the upregulation of several muscle ECM genes [21]. There was no evidence of local adipose tissue or systemic inflammation despite weight gain, suggesting a key role for muscle ECM in the regulation of glucose homeostasis rather than secondary effects due to adipose tissue inflammation.

Muscle collagen content is also increased in DIO insulin-resistant mice [9]. Studies by Kang *et al.* showed that increased collagen deposition in the DIO state is due in part to decreased muscle matrix metalloproteinase 9 (MMP9) activity [9], and that the genetic deletion of MMP9 in mice increases collagen deposition in the muscle and exacerbates muscle insulin resistance in HFD-fed mice [15].

Hyaluronan is an anionic, nonsulfated glycosaminoglycan. As a major component of the ECM, hyaluronan has multiple functions, including creating space between cells [22]. Serum hyaluronan is increased in T2D [23]. Insulin-resistant animals have increased hyaluronan in muscles [16], aorta [24], and kidneys [25]. Elevated muscle hyaluronan levels are associated with muscle insulin resistance in the obese state. A reduction of muscle hyaluronan by intravenous injection of pegylated human recombinant hyaluronidase PH-20 (PEGPH20) results in a dose-dependent increase in glucose infusion rate and muscle glucose uptake during a hyperinsulinemic–euglycemic clamp [16]. This study showed for the first time that whole-body depletion of an ECM polysaccharide rescues insulin sensitivity in C57BL/6J HFD-fed mice.