Hyaluronan in adipose tissue: Beyond dermal filler and therapeutic carrier

Yi Zhu,1,2 Clair Crewe,1 Philipp E. Scherer1,3*

Adipose hyaluronan is increasingly recognized as an active player in adipose tissue fibrosis and metabolic dysfunction. However, this role poses as many challenges as opportunities for therapeutic targeting of adipose tissue dysfunction during nutrient oversupply.

THE EXTRACELLULAR MATRIX OF ADIPOSE TISSUE
The extracellular matrix (ECM) is an integral component for the process of adipogenesis and adipose tissue homeostasis however, excessive production of ECM components can result in local adipose tissue fibrosis as well, leading to adipocyte dysfunction. By acting as a scaffold for cell migration, a reservoir for cytokines and growth factors, and a binding site for various cellular receptors, the ECM modulates adipose tissue metabolism, immune responses, and cell behavior. The ECM is maintained and expanded by the adipocytes themselves as well as resident stromal cells, such as fibroblasts, which secrete ECM proteins, proteoglycans, and nonproteoglycan polysaccharides, along with a host of enzymes that control modifications and degradation of these structures. The high level of activity between buildup and breakdown allows the ECM to provide structural support while also maintaining the capacity to undergo dramatic remodeling.

Rapid tissue expansion during obesity alters this balance and induces local tissue hypoxia and activation of HIF1α. When adipose tissue expansion exceeds the HIF1α-induced angiogenic program, an alternate HIF1α-mediated transcriptional program is induced that enhances synthesis of ECM collagen proteins and enzymes involved in collagen cross-linking and stabilization. Hypoxic adipocytes become dysfunctional and prompt the infiltration of macrophages, neutrophils, lymphocytes, and mast cells by secreting various adipokines. These changes give rise to a local proinflammatory microenvironment, further exacerbating the accumulation of fibrotic proteins in adipose tissue. In humans, adipose tissue fibrosis, as quantified by total tissue hydroxyproline, or histologically by trichrome or picrosirius red staining, is inversely associated with the overall metabolic fitness of the individual. We have recently discussed the general implications of fibrosis for the pathophysiology of adipose tissue (1).

HYALURONIC ACID: HIGHLY ABUNDANT, HIGHLY NEGLECTED
The ECM proteins collagen and fibronectin have been widely studied for their roles in obesity-associated adipose tissue dysfunction, but much less is known about the participation of other macromolecules such as proteoglycans and nonproteoglycan polysaccharides. Of particular recent interest is hyaluronic acid (HA, also known as hyaluronan). Historically, HA has been understudied as an ECM component in the context of obesity, mostly because of the lack of easily accessible assay protocols and histological methods for its visualization. HA is a nonsulfated glycosaminoglycan (GAG) polymer consisting of repeating disaccharide units of D-glucuronic acid (GlcUA) and N-acetyl-d-glucosamine (GlcNAc) and is energetically stable, with high abundance in connective, epithelial, and dermal tissues. HA is synthesized at the level of the plasma membrane by hyaluronan synthases (HAS1 to HAS3), with HAS2 being the major isoform in adult adipose tissues; HA degradation is mediated by hyaluronidases (HYAL1-4, PH20, and HYALP1) (Fig. 1). HA polymers vary widely in size, ranging from kilodaltons to megadaltons, and each isoform of HA synthesized and hyaluronidase displays enzymatic specificity toward HA within a given molecular weight range. HAS are hydrophilic and influence the hydration and biomechanical properties of many tissues, including adipose tissue. Additionally, successful morphogenesis commonly relies on the physical properties of HA, which regulate the interaction of HA with many proteoglycans that are important for ECM maturation (2).

HYALURONIC ACID: FROM A NEW GENERATION OF DERMAL FILLERS TO POTENTIALLY A NEW GENERATION OF THERAPEUTIC CARRIERS
The cosmetic industry has long used the hydrophilic and nonimmunogenic properties of HA for the development of cosmetic dermal fillers with no adverse side effects. Unlike previous generations of fillers, HA can be injected into deeper tissue layers of the face to bring a subtle, yet definitive, rejuvenation rather than just simply “filling” wrinkles or scars (3). HA fillers also last longer owing to slower absorption. Recently, HA has also been studied as a potential therapeutic carrier for human adipose-derived stem cell (hASC) transplantation. A HA gel containing hASCs promoted in vivo growth of new adipocytes, acting as a long-lasting soft tissue filler, although the occurrence of bona fide adipogenesis and adipose progenitor recruitment needs further verification (4). This technique has also been applied to a promising therapeutic strategy in combating metabolic syndrome. Transplantation of adipose tissue–derived multipotent stem cells (ADMSCs) with HA-based hydrogels has led to in vivo differentiation of lipid-accumulating, UCPI-expressing beige adipose tissue (5). Implant recipient mice exhibited enhanced respiration rates and improved glucose homeostasis. This study demonstrated the therapeutic potential of this potentially translatable approach for humans. HA has also been used in delivering many other U.S. Food and Drug Administration–approved drugs. For example, a relevant application in the context of whole-body metabolism is the use of HA in oral delivery of insulin. An HA-insulin complex was prepared in the laboratory and was shown to be effective after oral administration in lowering blood glucose in diabetic rats (6, 7), although testing the efficacy in humans is pending.

HYALURONIC ACID: BEYOND MERE STRUCTURAL COMPONENT IN THE ADIPOSE TISSUE
HA function stretches beyond inert structural carrier properties. It also binds to many ECM proteins and cell membrane receptors to activate downstream signaling pathways that affect cell migration, apoptosis, tumorigenesis, and inflammation (Fig. 1) (8, 9). Recent studies have provided evidence that HA-mediated...
signaling is altered in major tissues in obesity. Total HA content is increased in insulin-resistant skeletal muscle and adipose tissue in a mouse model of diet-induced obesity (DIO) (10) possibly through multiple mechanisms (11). Treatment of these mice with a serum-stable, recombinant hyaluronidase PH20 (PEGPH20) reduced HA accumulation and preserved whole-body insulin sensitivity (10). Interestingly, treatment with PEGPH20 resulted in up to 35% reduction of adipose tissue mass, with simultaneous reduction of adipocyte size (10). The mechanism for the PEGPH20-mediated reduction in adipose tissue mass is unknown, although a recent study may offer insight. Ji et al. demonstrated that HA is a positive regulator of adipogenesis (12). During adipogenesis, HA synthesis is increased, whereas experimental inhibition of HA synthesis in 3T3-L1 adipocytes resulted in suppressed peroxisome proliferator–activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα) expression, which are critical mediators for adipogenesis as well as lipid droplet formation and accumulation. Because adipogenesis and lipid deposition are important elements of adipose tissue expansion, pharmacological modulation of HA levels may offer an opportunity to control fat mass gain. HA-mediated signaling in DIO mice may also promote inflammation, a hallmark of late-stage adipose tissue dysfunction and possible cause of adipose tissue fibrosis.

There is also a likely connection between HA and excessive ECM accumulation. Clinically, subcutaneous adipose tissue fibrosis is the major negative predictor for bariatric surgery–mediated weight loss (13). However, the interplay between fibrosis and hyaluronan content was not investigated further in this particular study.

Patient-matched dermal and oral mucosal fibroblasts used as models of scarred versus scar-free healing showed that HA expression was much higher in dermal fibroblasts, in which HA was implicated in tumor growth factor–β1 (TGF–β1)–mediated induction of proliferation and fibrotic protein deposition (14), highlighting the involvement of HA in fibroblast proliferation and TGF–β1–mediated fibrosis. Kang et al. showed that PEGPH20 decreased gene expression of proinflammatory markers in adipose tissue, whereas the expression of the anti-inflammatory markers and total macrophage markers was unchanged. The authors concluded that macrophages with the classical activation state (M1) were decreased by PEGPH20 treatment and thus resulted in a decreased inflammatory profile in adipose tissue during high-fat diet (HFD) exposure. This effect is suspected to be modulated through CD44, the major cell-surface HA-binding protein. HA binds to CD44, triggering phosphorylation of the CD44 cytoplasmic tail and activation of downstream signaling cascades, regulating inflammation, T cell recruitment, and activation. CD44-deficient mice exhibit a substantially reduced WAT–associated inflammation but an increased lipid accumulation during an HFD challenge (15), which complicates the interpretation of the role of the interaction between HA and CD44 in WAT during DIO.

Expression levels of several collagen genes were greatly diminished in CD44-deficient mice, suggesting HA–CD44 interactions may promote a buildup of collagen and lead to the development of fibrosis.

HYALURONIC ACID: FUNCTIONAL IMPLICATIONS OF SIZE

A major question remaining is whether the size distribution of HA is important for the regulation of adipogenesis. Interestingly, both HAS2 and HYAL2 are up-regulated during adipogenesis. The net result is an overall increase in HA production, associated with a high degree of HA turnover. The HYAL2 enzyme hydrolyzes only HA of high molecular mass, yielding intermediate-sized HA fragments of ~20 kilodaltons, which can be further hydrolyzed to small oligosaccharides by PH20. Therefore, it is possible that induction of HAS2 and HYAL2 leads to a net increase of both high-molecular-weight and intermediate-molecular-weight HAs. Yet, how the potential size distribution of HA polymers affects adipogenic signaling remains to be determined. Smaller HA fragments produced by hyaluronidases can induce angiogenesis, an important component of adipose tissue expansion. However, a recent study showed medium-molecular-weight HA inhibits adipogenesis in cultured 3T3-L1 cells (16), further complicating the roles of different molecular weight HAs in adipogenesis. Furthermore, HA interacts with collagen VI and promotes its assembly in vitro (17). Whether this process is physiologically relevant in vivo and whether.

Fig. 1. Illustration depicting an adipocyte and its extracellular matrix focusing on hyaluronic acid. Collagen I fibrils form the main structural component and provide the physical support of the adipose tissue. Collagen VI is the major isofibril of collagen that surrounds each adipocyte. HAs are synthesized by HAS2 and exported during the synthesis. HA fibrils provide an anchor for the proteoglycan core protein aggrecan. HYAL2 processes HAs into small fragments, which have a different binding dynamics compared with high-molecular-weight HA, and may promote angiogenesis and attract macrophages, eosinophils, and other cells.
it is involved in pathological collagen VI deposition in WAT needs to be investigated further. Last, it is unknown whether there is a reciprocal interaction between HA and fibrosis. We therefore need to be careful to pay attention to the possibility that the changes in HA may be secondary to changes in fibrosis.

OUTLOOK

HA has been extracted from rooster combs and studied for many decades, but the molecular regulation of its synthesis and degradation in adipose tissue and its physiological and pathological roles in adipose tissue expansion are still largely unknown. In order to use HA as a pharmacological target so as to reduce adipose tissue fibrosis and metabolic disease, many questions remain to be answered: Is there an optimal size distribution of HA in adipose tissue? Is this profile altered in obese patients through changes in the expression of synthesis and/or degradation enzymes? Does HA accumulation in obesity play a causative role in the development of fibrosis? Answering these questions will help us assess whether hyaluronidase-based interventions aimed at a reduction of adipose HA content can lead to an improvement in the metabolic profile in obese individuals. With the advancement of transgenic animal techniques, we can start to dissect these pathways in adipose tissue itself and also elucidate a possible crosstalk between multiple metabolically active tissues. Hopefully, these preclinical studies will pave the way for clinically applicable approaches that target HA turnover, with the goal to ameliorate metabolic disease sequelae.

REFERENCES AND NOTES


Funding: P.E.S. is funded by U.S. National Institutes of Health grants R01-DK55758, R01-DK099110, and P01-DK088761 as well as a grant from the Cancer Prevention and Research Institute of Texas (CPRIT RP140412). Y.Z. is funded by a Lilly Innovation Fellowship Award (LIFA).

Competing interests: The authors declare they have no competing interests.

Hyaluronan in adipose tissue: Beyond dermal filler and therapeutic carrier
Yi Zhu, Clair Crewe and Philipp E. Scherer

Sci Transl Med 8, 323ps4323ps4.
DOI: 10.1126/scitranslmed.aad6793