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Hyaluronan in Adipose Tissue: Beyond Dermal Filler and Therapeutic Carrier

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Abstract

Adipose hyaluronan is increasingly recognized as an active player in adipose tissue fibrosis and metabolic dysfunction, posing 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) of adipose tissue is an important regulator of cellular homeostasis of the adipocyte and adipose tissue function. 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, by secreting ECM proteins, proteoglycans, and non-proteoglycan polysaccharides, along with a host of enzymes controlling modifications and the degradation of these structures. There is a high level of activity between build-up and break-down, allowing the ECM to provide structural support, while also maintaining the capacity to undergo dramatic remodeling. Although the ECM is an integral component for the process of adipogenesis and adipose tissue homeostasis, excessive production of ECM components can result in local adipose tissue fibrosis as well, leading to adipocyte dysfunction. Rapid tissue expansion during obesity induces local tissue hypoxia and activation of HIF1 α . When adipose tissue expansion exceeds the HIF1 α -induced angiogenic program, an alternate HIF1 α -mediated transcriptional program is induced that enhances synthesis of ECM collagen proteins and enzymes involved in collagen crosslinking and stabilization. Hypoxic adipocytes become dysfunctional and prompt the infiltration of macrophages, neutrophils, lymphocytes, and mast cells by secreting various adipokines, giving rise to a local proinflammatory microenvironment, further exacerbating the

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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

While ECM proteins collagen and fibronectin have been widely studied for their roles in obesity-associated adipose tissue dysfunction, much less is known about the participation of other macromolecules such as proteoglycans and non-proteoglycan polysaccharides. Of particular recent interest is hyaluronic acid (HA, also known as hyaluronan), a historically understudied ECM component in the context of obesity, mostly due to the lack of easily accessible assay protocols and histological methods for its visualization. HA is a non-sulfated glycosaminoglycan (GAG) polymer consisting of repeating disaccharide units of D-glucuronic acid (GlcUA) and N-acetyl-D-glucosamine (GlcNAc). HA 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 (HAS1–3), with HAS2 being the major isoform in adult adipose tissues while HA is degraded by hyaluronidases (HYAL1–4, PH20, and HYALP1). HA polymers vary widely in size ranging from kilodaltons to megadaltons. Each isoform of HA synthase and hyaluronidase displays enzymatic specificity towards 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 been utilizing these hydrophilic and non-immunogenic properties of HA for the development of cosmetic dermal fillers with no adverse side effects. HA differs from previous generations of fillers that it 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). Compared to traditional fillers, HA fillers also last longer due to slower absorption. Recently, HA is also studied as a potential therapeutic carrier for human adipose-derived stem cells (hASCs) transplantation. A study using a HA gel containing hASCs displayed *in vivo* growth of new adipocytes, thereby 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) and *in vivo* differentiation into lipid-accumulating, UCP1-expressing beige adipose tissue has been achieved in mice with HA-based hydrogels (5). Implant recipient mice demonstrated enhanced respiration rates and improved glucose homeostasis. For the first time, this study demonstrated the

therapeutic potential of this potentially translatable approach for humans. HA has also been used in delivering many other FDA approved drugs. For example, a relevant application in the context of whole body metabolism is the utilization of HA in oral delivery of insulin. An HA–insulin complex was prepared in the lab and was shown effective after oral administration in lowering blood glucose in diabetic rats (6, 7), although the 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 (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). Mechanisms that drive HA synthesis up in diabetes were discussed previously (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 adipocytes 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 results in suppressed PPAR γ and C/EBP α 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. 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 (14). However, the interplay between fibrosis and hyaluronan content was not investigated further in this particular study.

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. An earlier study used patient-matched dermal and oral mucosal fibroblasts as models of scarred versus scar-free healing and showed that HA was much higher in dermal fibroblasts, where HA was implicated in TGF- β (1)-mediated induction of proliferation and fibrotic protein deposition (13), highlighting the involvement of HA in fibroblast proliferation and TGF- β mediated fibrosis. Kang et al. showed that PEGPH20 decreased gene expression of pro-inflammatory markers in adipose tissue, while the expression of the anti-inflammatory markers and total macrophage markers were unchanged. The authors concluded that macrophages with the classical activation state (M1) were decreased by PEGPH20 treatment and thus result in a lower 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, thereby 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 significantly 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 build-up of collagen and lead to the development of fibrosis in adipose tissue.

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 upregulated during adipogenesis. The net result is an overall increased 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 approximately 20 kilodaltons, which can be further hydrolyzed to small oligosaccharides by PH20. So it is possible that induction of HAS2 and HYAL2 leads to a net increase of both high molecular weight and intermediate weight HAs. 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 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 utilize HA as a pharmacological target 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.

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Figure 1.

Cartoon 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 collagen type surrounding each adipocyte. HAs are synthesized by HAS2 and exported during the synthesis. HA fibrils offer an anchor function for the proteoglycan core protein aggrecan. HYAL2 processes HAs into small fragments, which have a different binding dynamics compared to high molecular weight HA, and may promote angiogenesis and attract macrophages, eosinophils, and other cells. (Cartoon drawing instructions: both HAS2 and HYAL2 are membrane-anchored proteins, they should stay close to cell membrane.)