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Review

Hyaluronan: Biosynthesis and signaling ☆

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ABSTRACT

Background: Hyaluronan is a critical component of extracellular matrix with several different roles. Besides the contribution to the tissue hydration, mechanical properties and correct architecture, hyaluronan plays important biological functions interacting with different molecules and receptors.
Scope of review: The review addresses the control of hyaluronan synthesis highlighting the critical role of hyaluronan synthase 2 in this context as well as discussing the recent findings related to covalent modifications which influence the enzyme activity. Moreover, the interactions with specific receptors and hyaluronan are described focusing on the importance of polymer size in the modulation of hyaluronan signaling.
Major conclusions: Due to its biological effects on cells recently described, it is evident how hyaluronan is to be considered not only a passive component of extracellular matrix but also an actor involved in several scenarios of cell behavior.
General significance: The effects of metabolism on the control of hyaluronan synthesis both in healthy and pathologic conditions are critical and still not completely understood. The hyaluronan capacity to bind several receptors triggering specific pathways may represent a valid target for new approach in several therapeutic strategies. This article is part of a Special Issue entitled Matrix-mediated cell behaviour and properties.

1. Introduction

Extracellular matrix (ECM) is a complex network of macromolecules that surrounds cells not only to mechanically support cells and tissue structure, but also to control nutrient and waste exchanges, cell–cell and cell–matrix interactions, and signaling molecular diffusion. Thus, ECM is critical to regulate cell behavior and increasing body of evidences supports the ECM involvement in both physiological as well as pathological processes. The cell microenvironment has a pivotal role in several pathologies as tumors and cardiovascular diseases, in which alterations of cell functions can be exacerbated by external stimuli and altered ECM composition [1–4].

Glycosaminoglycans (GAGs) differ from the other ECM components as they are complex polysaccharides which can carry many chemical possible modifications (*i.e.*, sulfation, acetylation and epimerization) and are covalently linked to a core protein forming proteoglycans (PGs). Such variability permits to these macromolecules to finely modulate protein–protein interactions, enzymatic activity or diffusion of signalling molecules.

Hyaluronan is an atypical and relatively simple GAG, in fact it is an unsulfated and unbranched polysaccharide not linked to any PG core proteins. Hyaluronan is ubiquitously expressed in the ECM of mammals and is composed D-glucuronic acid (GlcUA) and N-acetyl-D-glucosamine (GlcNAc) bound together through β1,3 and β1,4 glycosidic bonds, respectively [5]. This disaccharide moiety is repeated thousands of times generating a linear polymer with a molecular mass ranging from 5×10^5 to $4–5 \times 10^6$ Da and more. Due to its hydrophilic properties, hyaluronan is very hydrated causing the ECM an ideal environment in which cells can move and proliferate. Hyaluronan is an important space filling molecule as it is evident in humor vitreous, derma and at joint level. Besides its molecular sieving properties related to the chemical and bio-mechanical characteristics of hyaluronan, this polymer interacting with specific proteins called hyaladerins, such as TSG6, and membrane receptors like CD44, RHAMM, HARE and toll like receptor (TLR) 4/2, modulates the development, the morphogenesis, the tumorigenesis, the migration, the apoptosis, the cell survival and the inflammation [6–9]. This review focuses on hyaluronan biosynthesis regulation and signaling. On these bases the knowledge of how hyaluronan is produced by the cells and how its metabolism is regulated is of great interest. Growing mass of data related to the control of hyaluronan synthesis is reported in literature in various models and pathologies, and these aspects will be discussed in this work.

2. Hyaluronan synthesis regulation

Although the relatively simple structure, hyaluronan possesses many physiological roles. Surprisingly, hyaluronan appeared late during evolution and can be typically found from chordates. Interestingly, some pathogenic bacteria acquired the capability of synthesize hyaluronan that use as a mimetic shield against the host immune system. How bacteria acquired the ability to synthesize hyaluronan is still

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debated, but *Streptococcus* bacteria need three different genes to produce hyaluronan capsule. Interestingly these three genes, which encode UDP-Glucose pyrophosphorylase (*hasC*), UDP-Glucose dehydrogenase (*hasB*) and hyaluronan synthase (*hasA*), are located in an operon, suggesting the critical role of precursors in hyaluronan biosynthesis [10–12].

In mammals, hyaluronan synthesis occurs on the cellular plasma membrane (contrary to the other GAG synthesis that is in the Golgi apparatus) by means of three hyaluronan synthase isoenzymes (HAS1, 2, and 3) that utilize the uridine diphosphate (UDP) glucuronic acid (UDP-GlcUA) and UDP N-acetylglucosamine (UDP-GlcNAc) as substrates. These two sugar nucleotides have critical functions to regulate hyaluronan synthesis through the modulation of gene expression of HASes and by altering HAS enzymatic activity and stability. UDP-Glucose pyrophosphorylase and UDP-Glucose dehydrogenase mediating the synthesis of UDP-glucose and UDP-GlcUA, respectively, have a positive effect on hyaluronan synthesis increasing the availability of one of the HAS substrate [13,14]. Whether or not the activity of HAS enzymes during the sorting to the plasma membrane is still unclear, but it seems that these proteins could be functional in endoplasmic reticulum and Golgi [15]. Interestingly, UDP-GlcUA is able to influence the accumulation of HAS2 and 3 transcripts, although the molecular mechanism of such regulation is still not known.

The physiological role of HAS isoenzymes is not yet understood; *in vitro* HASes have different biochemical properties in terms of size of hyaluronan synthesized and catalytic efficiency [16]. Hyaluronan chain size is critical in many pathophysiological conditions [17,18]. Due to the action of hyaluronidases [19] or the action of oxygen radicals [20], hyaluronan can be fragmented in short oligosaccharides that can trigger inflammation or metalloproteinase activation [21,22]. Alternatively, low molecular weight hyaluronan could be produced by HAS3 [23]. Other mechanisms to alter hyaluronan synthesis can directly involve HAS enzymes by, for instance, post-translational modifications or by alteration of the UDP-sugar pool as already discussed [24,25].

Increased hyaluronan levels are often described in the presence of elevated activities of growth factors and cytokines that are released in rapidly remodeling tissues as during embryonic development and would healing or during certain pathological situations, such as inflammation, tumor progression, and vessel thickening.

Smooth muscle cells (SMCs) have a critical role in cardiovascular pathologies as they strongly contribute to neointima formation. Hyaluronan has pro-atherosclerotic properties controlling SMC proliferation, migration and contributing to immune cell recruitment. Several experiments confirmed this effect by using CD44 knockout animals [26], HAS2 transgenic mice [27], by blocking HA/CD44 interaction or inhibiting hyaluronan synthesis. On these bases, many efforts have been done to study the regulation of hyaluronan synthesis in SMCs. From these studies it is now clear that HAS2, the most abundant and active HAS isoenzymes, has several levels of regulation to coordinate hyaluronan production with the cellular metabolism [28].

Although HASes do not use directly ATP, the synthesis of UDP-GlcUA and UDP-GlcNAc precursors requires ATP, UTP and other critical metabolic molecules as glucose, glutamine, glucosamine and acetyl-CoA, in order to perform the GAG production, an energy consuming process. It is well known that energy charge of the cell has a central role in the integration of the entire metabolism. Although, many enzymes can directly use ATP, ADP and AMP as allosteric modulators, the adenosine monophosphate activated protein kinase (AMPK) has a pivotal role as metabolic sensor and regulator. AMPK is a heterotrimeric protein formed by catalytic α subunit and two regulatory subunits. When the ATP:AMP ratio is high, AMPK is not active, but, when ATP:AMP ratio decreases, the catalytic subunit can be phosphorylated by AMPK upstream kinases triggering AMPK activation. In this active form, AMPK is able to phosphorylate several target proteins that lead to the inhibition of anabolic processes and, in the same time, increasing catabolic pathways with the results to restore ATP levels [29]. AMPK specifically inhibited hyaluronan synthesis without altering other GAGs in human SMC and in mouse embryonic fibroblasts [30]. The reduction of hyaluronan accumulation mediated a decrement of SMC proliferation and migration highlighting as the blocking of hyaluronan can be a new candidate to develop vasoprotective drugs. From a molecular point of view, AMPK phosphorylates HAS2 at threonine 110 (T110); this residue is located on a cytosolic loop of the enzyme which is known to be critical for the enzymatic activity. Interestingly, the action of AMPK could be more complex and tissue specific as in human dermal fibroblasts the activation of AMPK induces HAS2 messenger accumulation [31].

HAS2 can also undergo to the regulation by O-GlcNAcylation. This latter is an intracellular glycosylation mediated by O-GlcNac transferase (OGT) which catalyzes the β-*O*-linkage of one residue of N-acetylglucosamine (GlcNAc) to serine or threonine of proteins [32]. The donor of GlcNAc is UDP-GlcNAc, which is synthesized by the hexosamine biosynthetic pathway (HBP) (Fig. 1). HBP is now considered one of the main nutrient sensor as, not only is able to directly recognize the availability of glucose (the more glucose, the more flux though the HBP), but the synthesis of UDP-GlcNAc depends on the metabolism of fatty acids, amino acids, and nucleotides. The pathological relevance of HBP is well known in diabetes, in fact the hyperglycemic condition leads to an increase of UDP-GlcNAc that, in turn, leads to an increase of O-GlcNAcylation [33]. High blood glucose is the cause of diabetic complications (*i.e.*, angiopathies, neuropathies, nephropathies, and retinopathies) which can dramatically affect the patient's lifestyle. However, which molecular mechanisms modified by the excess of glucose are still debated, but O-GlcNAcylation seems to have a central role. Hyaluronan accumulates in the aortas of diabetic subjects [34] and in hyperglycemic animal models hyaluronan increased in vessels [35]. HAS2 can be modified by O-GlcNAc at serine 221 (S221) and such glycosylation induced a great stability of the enzyme in cellular plasma membrane inducing an enhanced hyaluronan synthesis [36]. HAS2 is rapidly degraded by the 26S proteasome and O-GlcNAcylation prevented HAS2 degradation. The molecular mechanism of such protection against proteasomal digestion is not understood although it is known that O-GlcNAcylation can directly affect proteasomal activity and ubiquitination. Interestingly, HAS2 can form dimers or oligomers and is subjected to regulatory ubiquitination *i.e.* is mono-ubiquitinated at lysine residue 190 (K190) which has a key role for its activity and dimerization [37] (Fig. 2A).

HAS2 represents the main hyaluronan synthetic enzyme in adult cells and is reasonable that its activity is finely regulated. On the other hand, HAS1 and HAS3 seem to have peculiar roles even if not completely clarified. HAS1 seems to be important in hyperglycemic conditions having a low affinity for hyaluronan precursors, whereas HAS3 is involved in the formation of particular microvilli structures [38,39]. Although HAS3 has been described to be phosphorylable when over expressed in cells, the effects of AMPK and O-GlcNAcylation are specific for HAS2 [30,36]. Interestingly, ERK increased the activity of all the three HASes indicating that protein phosphorylation in residues different from that modified by AMPK, can lead to hyaluronan accumulation [40]. The regulation of HASes is also at transcriptional level, as previously reviewed [41] and, recently, HAS2 and 3 have been demonstrated to be similarly upregulated by oxidized LDL and 22-oxysterol which is the agonist of Liver X Receptor agonist [42]. Although not deeply investigated in mammals, bacterial HASes need a particular lipid microenvironment (*i.e.*, cardiolipin) [43] and, thus, eukaryotic HASes could have another point of regulation through lipid metabolism and/or the presence of lipid rafts.

Interestingly, the genomic locus of HAS2 can generate a natural antisense transcript (NAT) of HAS2, named HAS2-AS1 and exon 2 of HAS2-AS1 NAT is complemental to exon 1 of HAS2 mRNA. Although several years ago HAS2-AS1 was described that the overexpression of HAS2-AS1 decreased HAS2 mRNA [44], recently it has been described that HAS2-AS1–HAS2 duplex at RNA level stabilized HAS2 messenger leading to HAS2 accumulation [45] (Fig. 2B).

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