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Hyaluronan: A simple polysaccharide with diverse biological functions ☆

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ABSTRACT

Hyaluronan (HA) is a linear polysaccharide with disaccharide repeats of D-glucuronic acid and *N*-acetyl-D-glucosamine. It is evolutionarily conserved and abundantly expressed in the extracellular matrix (ECM), on the cell surface and even inside cells. Being a simple polysaccharide, HA exhibits an astonishing array of biological functions. HA interacts with various proteins or proteoglycans to organize the ECM and to maintain tissue homeostasis. The unique physical and mechanical properties of HA contribute to the maintenance of tissue hydration, the mediation of solute diffusion through the extracellular space and the lubrication of certain tissues. The diverse biological functions of HA are manifested through its complex interactions with matrix components and resident cells. Binding of HA with cell surface receptors activates various signaling pathways, which regulate cell function, tissue development, inflammation, wound healing and tumor progression and metastasis. Taking advantage of the inherent biocompatibility and biodegradability of HA, as well as its susceptibility to chemical modification, researchers have developed various HA-based biomaterials and tissue constructs with promising and broad clinical potential. This paper illustrates the properties of HA from a matrix biology perspective by first introducing the principles underlying the biosynthesis and biodegradation of HA, as well as the interactions of HA with various proteins and proteoglycans. It next highlights the roles of HA in physiological and pathological states, including morphogenesis, wound healing and tumor metastasis. A deeper understanding of the mechanisms underlying the roles of HA in various physiological processes can provide new insights and tools for the engineering of complex tissues and tissue models.

1. Introduction

Hyaluronan(HA) was first purified from the vitreous humor of bovine eyes by Karl Meyer in 1934 [1]. He named the molecule "hyaluronic acid" because of the hyaloid appearance of the substance when swollen in water and the probable presence of hexuronic acid as one of the components. In the 1950s, Meyer and colleagues determined that HA was a linear polysaccharide composed of repeating β-1,4-linked D-glucuronic acid (GlcA) and β-1,3-linked *N*-acetyl-D-glucosamine (GlcNAc) disaccharide units (Fig. 1A) [2]. The various names of HA reflect the properties of the molecule under various conditions. When first isolated, HA behaved like a mild acid; therefore, Meyer named it "hyaluronic acid" [1]. Under physiological conditions, HA exists as a polyelectrolyte with associated cations, frequently as a sodium salt; therefore, the name sodium hyaluronate. The name was later amended to "hyaluronate" in reference to its salt form or "hyaluronan," a term used to encompass all forms of the molecule [3].

HA is found ubiquitously in the extracellular matrix (ECM) of all vertebrate tissues, although its concentration and binding partners vary. In bodily fluids, the concentration of HA ranges from 0.01–0.1 μg g^{-1} in blood serum to 1400–3600 μg g^{-1} in synovial fluid; HA content in soft connective tissues ranges from 8.5–18 μg g^{-1}

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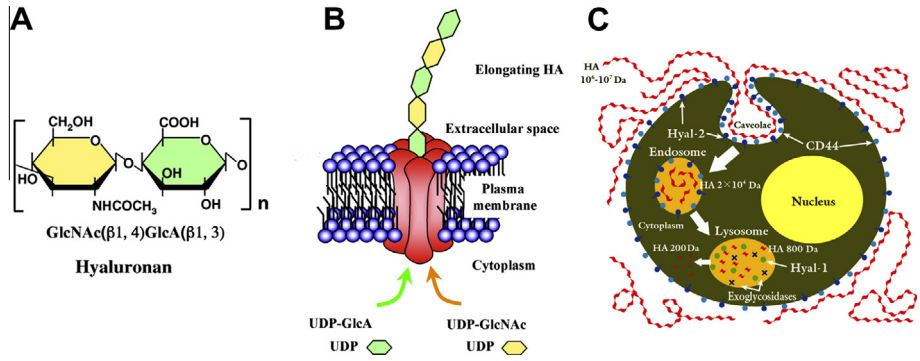

Fig. 1. (A) Chemical structure of HA and schematic illustration of (B) HA biosynthesis and (C) biodegradation. (A) HA is a linear polysaccharide with disaccharide repeats of D-glucuronic acid and *N*-acetyl-D-glucosamine. (B) HA is synthesized by transmembrane proteins HAS1, 2 and 3 and is extruded into the extracellular space as the polymerization proceeds. (C) HA is internalized by binding to HA receptors and Hyal2 within caveolae. Hyal2 cleaves HA into intermediate fragments that are transported to lysosomes. Lysosomal Hyal1 cleaves HA into tetramers that are finally cleaved into HA monomers by exoglycosidases. (A, B) Reproduced with permission [125], Copyright 2008, The Japanese Biochemical Society. (C) Reproduced with permission, Copyright 2013, Glycoforum.

in the thoracic lymph to 140–338 μg g^{-1} in the vitreous body [4]. HA is also present on some cell surfaces as a pericellular sugary coat, a feature thought to be involved in cell differentiation and morphogenesis. In the cumulus cell–oocyte complex, the HA concentration can be as high as 0.5–1.0 mg ml^{-1} [5,6]. Classically considered an extracellular molecule, the presence of HA in the cytoplasm and the nucleus was suggested as early as the 1970s [7,8] and was convincingly confirmed in the 1990s [9–12]. Although intracellular HA has been suggested to play important roles in inflammation, its intracellular functions remain largely unknown [13].

HA is not branched, nor does it contain any sulfate groups [14]. Despite its simple chemical composition, HA fulfills several distinct molecular functions that contribute not only to the structural and physiological characteristics of tissues, but also to the mediation of cell behaviors during morphogenesis, tissue remodeling, inflammation and diseases. Owing to its unique biophysical properties, HA contributes directly to the maintenance of tissue homeostasis and biomechanics. Through its interactions with proteoglycans and link proteins, HA organizes and maintains the structural integrity of extracellular and pericellular matrices. As a signaling molecule, HA interacts with a variety of cell surface receptors and HA-binding proteins to activate intracellular events to mediate cell functions [15].

After more than two decades of intense study, the molecular details of the role of HA in normal and pathophysiological processes are finally emerging. The fascinating characteristics of HA have motivated two distinct groups of scientists to investigate HA-related phenomena and applications. While biologists continue to unravel the complex biological functions of HA and its receptors in various cell signaling processes, biomedical engineers are creating a range of HA-based hydrogel materials with increasing complexity and diverse functions for tissue regeneration purposes [16–18]. This paper highlights the essential biological functions of HA, with the goal of motivating the biomaterials community to investigate HA as both a synthetic building block and a biological signaling motif. This is not an all-inclusive review, and readers are referred to in-depth reviews in an edited book for further reading [15].

2. Biosynthesis and degradation

Unlike other glycosaminoglycan (GAG) molecules that are synthesized in the Golgi apparatus, HA is synthesized at the plasma membrane by a group of highly specialized membrane proteins, HA synthases (HASs) [19]. There are three well-conserved HAS isozymes present in mammalian species: HAS1, HAS2 and HAS3 [20], each possessing two distinct binding domains for UDP-sugars (Fig. 1B). Polymerization of HA occurs at the inner face of the plasma membrane, where HAS alternatively adds UDP-GlcA and UDP-GlcNAc monomers to the reducing end of the growing polymer. As the polymerization is occurring, the non-reducing end of the sugar chain is translocated into the extracellular space through a pore in the HAS structure [21]. An intriguing question is why nature uses three different isozymes for the synthesis of HA with such a simple repeating unit. Although these three enzymes share a structural identity of ~55–70%, they differ in terms of their ability to synthesize HA. HAS1 has a significantly higher Michaelis constant (K_m) value, the substrate concentration where the reaction rate is half of its maximum, for both UDP-GlcA and UDP-GlcNAc compared with HAS2 and HAS3, suggesting that HAS1 has a slower rate of HA synthesis compared with the other synthases [22,23]. As discussed below, HA of different sizes exhibits distinctly different, sometimes conflicting biological functions. Therefore, the expression of various HAS isozymes is likely to be a fine control system critical for the effective mediation of diverse cell behaviors. While HAS1 and HAS2 are able to produce large-sized HA (up to 2000 kDa), HA produced by HAS3 is of a lower molecular mass (100–1000 kDa) [22,24]. McDonald and coworkers were the first to recognize the isoform specificity for HA production in embryogenesis; they discovered that HAS2 (but not HAS1 or HAS3) knock-out mice died at day 9.5 from incomplete atrioventricular septum formation [25].

The expression levels of HAS isozymes differ during morphogenesis and in disease states [26]. Thus, the differential distribution of HA in tissues varies at individual developmental stages and in pathological conditions, and is controlled by the spatio-temporally regulated transcription of the three different synthases. HA is abundant in fetal tissues, but is partially replaced by collagen fibers and proteoglycans during development, so that the mature tissues can fulfill more stringent mechanical tasks [27]. For example, the newborn vocal fold is composed of a loose connective tissue rich in HA. As the vocal fold develops and matures, HA content is reduced, and the fibrous proteins are deposited across the lamina propria in a gradient fashion. Overall, HA is indispensable for vocal fold development and maturation [28], and its presence in vocal fold is evolutionarily beneficial for the tissue to cope with constant trauma [29]. As discussed below, HA is enriched in tumors and tumor-associated stromal tissues, possibly as a result of increased expression or activity of HAS isozymes.

The diverse functions of HA originate from its primary and secondary structures [30,31]. Connected by glycosidic links, individual