REVIEW

Hyaluronic Acid in Inflammation and Tissue Regeneration

Authors

Malgorzata Litwiniuk
Alicja Krejner
Tomasz Grzela

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Abstract

Hyaluronic acid (HA), the main component of extracellular matrix, is considered one of the key players in the tissue regeneration process. It has been proven to modulate via specific HA receptors, inflammation, cellular migration, and angiogenesis, which are the main phases of wound healing. Studies have revealed that most HA properties depend on its molecular size. High molecular weight HA displays anti-inflammatory and immunosuppressive properties, whereas low molecular weight HA is a potent proinflammatory molecule. In this review, the authors summarize the role of HA polymers of different molecular weight in tissue regeneration and provide a short overview of main cellular receptors involved in HA signaling. In addition, the role of HA in 2 major steps of wound healing is examined: inflammation and the angiogenesis process. Finally, the antioxidative properties of HA are discussed and its possible clinical implication presented.

Introduction

Hyaluronic acid (HA) is a member of a large family of glycosaminoglycans (GAGs), which are the main components of the extracellular matrix (ECM). Unique features that distinguish HA from other GAGs are its simple structure and large molecular size. The HA molecule is composed of D-glucuronic acid and N-acetyl-D-glucosamine bound with β-glycosidic linkages. This simple molecular unit repeated thousands of times forms a structure of a very long linear polymer, with molecular weight reaching 5 x 106 kDa.[1,2] Hyaluronic acid is synthesized by HA synthases on the inner surface of the cellular membrane and translocated into extracellular space along with the elongation of the polymeric chain. This is a unique method of synthesis, different from other GAGs that are synthesized at the intracellular space. Hyaluronic acid is also the only GAG not linked to a core protein, and it does not undergo any postsynthetic modifications. Long hyaluronan polymers have the ability to bind large amounts of water. Hygroscopic and viscoelastic properties of HA make it a perfect component of vitreous fluid, joint fluid, and derma. Hyaluronan in its native form of a very long polymer is known as high molecular weight (HMW) hyaluronan. However, in certain conditions, it can be decomposed into small fragments referred to as low molecular weight HA (LMWHA).[3] Hyaluronan turnover is a rapid process, as the half-life of HA molecules in the bloodstream is only about 2-5 minutes.[4]Fragmentation of hyaluronan is controlled by enzymes called hyaluronidases. Hyaluronidase-1 (Hyal-1) and hyaluronidase-2 (Hyal-2) are responsible for HA degradation in somatic tissues. At first Hyal-2, which is a cell-membrane linked enzyme, degrades hyaluronan to fragments with molecular weight reaching 20 kDa. These HA molecules are subsequently endocytosed and delivered to lysosomes, where further digestion is performed by Hyal-1.[5] In injured tissue, free radicals are also able to decompose HA polymers into smaller fragments. [6]
It has been shown that HA fragments of different molecular sizes can display different, sometimes opposing properties. For example, it has been well documented that HMWHA displays anti-inflammatory and immunosuppressive properties, whereas LMWHA is a potent proinflammatory molecule. A proposed hypothesis suggests LMWHA may have a different biological function than HMWHA, such as to inform cells about stress conditions.[7]

Wound healing is a complex biological process, comprised of a series of sequential events aimed at repairing injured tissue. Extracellular matrix components play an important role in regulation of all phases of tissue repair, including cellular migration, inflammation, angiogenesis, remodeling, and scar formation. Studies have revealed that not only do they provide a wound microenvironment but are also involved in various signaling pathways that are activated in the wound bed during the healing process. Hyaluronic acid is a basic ECM component with some unique properties that make it a key player in tissue regeneration.

Hyaluronic Acid Signaling

CD44. The CD44 antigen, a type 1 transmembrane glycoprotein, is the main receptor for hyaluronan. It is present on cell membranes of almost all human cells,[8] and consists of 10 constant and 10 variant exons inserted in various combinations at a single extramembrane site (Figure 1). That gives a great number of different splicing variants, which may vary in function and properties.[9] A specific function of CD44 is the capability to bind and internalize HA; however, it can interact with other ligands, such as fibronectin, collagens, osteopontin, and matrix metalloproteinases (MMPs). It has proven to work as a docking agent for MMP[9] and as a growth factor reservoir.[10] In a wound environment, CD44 is responsible for cellular internalization of HA degradation products. This CD44 function has been demonstrated in lung inflammation, where blocking CD44 on the surface of macrophages resulted in impaired clearance and delay in the healing process.[11] Therefore, CD44 is involved in the control of exaggerated inflammatory response. The other role of CD44 signaling in the wound environment is to induce fibroblast migration from surrounding tissue into the wounded area. It is noteworthy that neither CD44 nor HA alone can induce cell migration and promote wound healing; an interaction between HA and CD44 is essential to activate this process.[12] The ability of hyaluronan particles to bind with the CD44 receptor depends on its molecular size. It has been proven that avidity of binding of hyaluronan oligomers to CD44 increases with an oligomer size of up to 38 sugars.[13]

Smaller hyaluronan fragments can also interact with CD44, but the effects they induce in target cells are different than those caused by HMWHA. The current hypothesis that could explain this phenomenon is that HMWHA is able to cluster the receptors on the cell membrane surface to modulate the receptor activity.[14] It has also been proven that HA binding to the CD44 receptor provides the effect of coating the cell membrane. This protective layer of hyaluronan on a cell surface is able to mask cell death receptors and consequently prevent the cell from reaching apoptosis.[15] Small HA fragments do not display these kinds of properties.

Stimulation of CD44 triggers a signaling cascade associated with 2 tyrosine kinases: p185 human epidermal growth factor receptor 2 (HER2)[16] and c-Src kinase.[17] Activation of p185HER2 leads to increased cell growth, whereas c-Src kinase activity is responsible for phosphorylation of cytoskeleton proteins and induction of cell motility. In addition to phosphoinositide-3 kinase/phosphoinositide-dependent kinase 1/protein kinase B pathway, Ras protein signaling pathways are also involved in CD44 cytoplasmic signaling[18,19] (Figure 2).

Receptor for hyaluronan-mediated motility. Receptor for hyaluronan-mediated motility (RHAMM), also known as CD168, is present in several cell types including endothelial cells and various tumor cell lines.[20] Similar to CD44, RHAMM also exists in several isoforms, produced by alternative splicing, that can be distributed on the cell surface, or intracellularly, within the cytoplasm or in the nucleus[21] (Figure 1). Several kinases, such as Src kinase, focal adhesion kinase, extracellular-signal-regulated kinases (ERK) 1/2 and protein kinase C, are involved in RHAMM signaling. This receptor is also associated with the Ras protein and the Ras signaling pathway[22,23] (Figure 2). Intracellular RHAMM interacts with cytoskeletal proteins, such as actin filaments and microtubules, and activates the previously mentioned protein kinases, which result in cell movement stimulation.[24] These properties of RHAMM-HA complex are important in tissue repair and the inflammation process. It has been demonstrated that the blocking of hyaluronan-mediated RHAMM signaling in smooth muscle cell cultures inhibited cell ability of migration.[25] The receptor for hyaluronan-mediated motility is also highly expressed in fibroblasts, and it has been proven that activation of this receptor stimulates fibroblast proliferation in vitro.[26] Tolg and colleagues[26] have shown in a murine model that RHAMM-deficient fibroblasts display reduced migration capacity and healing potential. At the same time, activation of RHAMM induces an inflammatory response in the wound environment which can have a negative influence on the wound healing process. Zaman and coauthors[24] have shown RHAMM signaling was critical for the activation of macrophages during incubation with HA. These results are consistent with the findings of Tolg and coauthors,[27] which have proven that blockage of HA signaling with a RHAMM-mimetic peptide was able to reduce inflammation and fibrogenesis in an excisional skin wound model.[27] The implication of RHAMM and RHAMM signaling in the wound healing process requires further investigation.

Recent studies have shown that cell surface RHAMM can interact with CD44 while regulating signal transduction, thus may influence cell motility and wound healing.[22] The receptor for hyaluronan-mediated motility expression has been shown to play a key role in the translocation of CD44 to the cell surface, formation of CD44-ERK1/2 complexes, and subsequent ERK1/2 activation. Hamilton et al[28] observed elevated levels of ERK1/2 activation in invasive breast cancer cells upon growth factor/motogenic stimulation when cell surface CD44 and RHAMM were coexpressed and coassociated with each other. It is postulated that while CD44 and

RHAMM can independently regulate cell behavior via HA signaling, in some cases they cooperate or at least have overlapping functions. However, all of the cooperative mechanisms between RHAMM and CD44 are not clearly understood and require further investigation.[29]

Toll-like receptors. Toll-like receptors (TLRs) are a group of highly conserved molecules that allow the immune system to sense common bacteria and viruses and to coordinate an early host defense against these pathogens. The human TLR family consists of 10 receptors comprised of different pathogen-associate molecular patterns (PAMPs) that are characteristic of various microbial classes. For example, TLR4 recognizes lipopolysaccharide (LPS), which is an integral component of the outer membranes of gram-negative bacteria, whereas TLR2 recognizes the peptidoglycan and lipopeptide, which are cell membrane components of gram-positive bacteria. Recent studies[30] suggest TLRs can be activated not only by PAMPs but also by some endogenous molecules, called damage associated molecular patterns (DAMPs).[30] They include heat shock proteins, members of the S100 protein family, high mobility group box-1 (HMGB1) and ECM breakdown products such as low-molecular weight hyaluronan fragments (Figure 2). To date, it has been confirmed that LMWHA is recognized by TLR2 and TLR4.[30]

Hyaluronan receptor for endocytosis and lymphatic vessel endothelial receptor 1. Hyaluronan receptor for endocytosis (HARE), also known as stabilin 2, is responsible for clearance of HA, as well as other GAGs, from systemic circulation.[31,32] Hyaluronan receptor for endocytosis is present on the inner surface of endothelial cells in vascular and lymphatic vessels (Figure 1). Interaction of HA with HARE leads to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), signaling pathway activation[33] (Figure 2).

Lymphatic vessel endothelial receptor 1 (LYVE-1) typically occurs in the lymphatic system and has been detected in lymphatic endothelial cells, liver sinusoidal endothelial cells, and in reticular cells of the lymph nodes.[34,35] The biological function of LYVE-1 is the absorption of HA from tissue to the lymph, that helps to regulate tissue hydration level.[36]

The involvement of HARE and LYVE-1 in tissue regeneration has not yet been well documented and requires further study.

Hyaluronic Acid and Inflammation

It has been proven that HMWHA displays anti-inflammatory activity, whereas low molecular weight degradation products of HA can induce inflammation.[36] It has been reported that LMWHA may elicit various proinflammatory responses such as activation of murine alveolar macrophages,[37] or induction of irreversible phenotypic and functional maturation of human dendritic cells.[38,39] Further studies have shown that small hyaluronan fragments increase the expression and protein production of several cytokines such as MMP-12, plasmogen activator inhibitor-1,[40,41] macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractrant-1, keratinocyte chemoattractant, interleukin (IL)-8, and IL-12 by macrophages.[42-44] The obvious candidate to activate this series of events in immune system cells is CD44, the main receptor for hyaluronan. Nevertheless, macrophages from CD44 null mice still respond to small HA fragments treatment, which is consistent with the fact that other receptors, such as TLRs, are also involved in HA signaling.[45]

Several studies have concentrated on TLRs and TLR signaling as a key player responsible for proinflammatory properties of LMWHA. Several authors have confirmed, using in vitro studies, that LMWHA is able to bind to TLR receptors and consequently initiate the signaling cascade, leading to the production of proinflammatory cytokines and chemokines in various types of cells.[46] In immune cells from injured tissues, TLR2 and TLR4 activation by LMWHA leads to initiation of MyD88-dependent NFκB signaling cascade and pro-inflammatory cytokine gene expression.[14,47] Iwata and coauthors[48] have shown that LMWHA stimulates B lymphocytes via TLR4 receptor to IL-6 and TGF beta production. Induction by LMWHA TLR-related myeloid differentiation primary response gene 88 (MyD88)/NFκB signaling was also confirmed in breast tumor cells. Bourguignon et al[49] have shown that small HA fragments stimulate CD44 association with TLR2, TLR4, and MyD88, leading to NF-κB-specific transcriptional activation and the expression of proinflammatory cytokines IL-1β and IL-8 in the human breast cell line. Taken together, these reports suggest that LMWHA induces inflammation via activation of TLR receptors and initiation of MyD88/NFκB signaling which leads to production of proinflammatory cytokines and chemokines. In physiological conditions, the activation of immune system cells is crucial for proper wound healing. In acute wounds, small hyaluronan fragments accumulating at the site of injury activate the immune system to manage rupture in tissue integrity; however, in chronic wounds a constant excessive inflammatory response proves to be a negative phenomenon that actually prevents that wound from healing.

Unlike small HA fragments, hyaluronan in its native form of a large polymer displays anti-inflammatory and immunosupresive properties. The anti-inflammatory potential of HMWHA has been well documented in osteoarthritis. Since HA is a basic component of normal synovial fluid and the concentration of HA is decreased in joints affected with osteoarthritis, intra-articular injection of HMWHA has been used to treat this condition. Therapeutic effects of such supplementation have been well documented in a number of clinical studies. Several authors investigated the influence of HMWHA on the expression of proinflammatory and anti-inflammatory cytokines by synoviocytes. High molecular weight HA was able to inhibit IL-1β expression in synoviocytes in a rabbit model of osteoarthritis.[50] Also the IL-1 dependent expression of MMP-1 and MMP-3 was reduced in human synoviocytes by HMWHA treatment. In a large study, Wang and coauthors[51] analyzed the influence of HMWHA on gene expression of various inflammatory cytokines by human fibroblast-like synoviocytes (FLS) in patients with early-stage of osteoarthritis. They reported the downregulation of IL-8 and iNOS gene expression in unstimulated FLS and aggrecanase-2, and tumor necrosis factor alpha (TNFα) gene expression in IL-1-stimulated FLS. Blocking the CD44 receptor with anti-CD44 antibody inhibited the down-regulatory effects of HMWHA on gene expression.[49] This may suggest the important role of CD44 signaling pathway in the regulation of inflammation by HMWHA. Campo et al[52] studied the involvement of TLR receptors in this process. They have reported that HMWHA was able to significantly diminish TLR4, TLR2, MyD88 and NF-kB expression and protein synthesis in synoviocytes in murine model of osteoarthritis. The have also observed reduced mRNA expression and protein production for TNFα, IL-1β, IL-17, MMP-13 and inducible nitrous oxide synthase gene in arthritic mice treated with HMWHA.[52] Interestingly, all of the effects mentioned above were present only when HMWHA was administered in an early inflammatory phase of osteoarthritis. This may suggest that along with disease progression, signaling pathways apart from the TLR-dependent ones could be involved in the chronic inflammation state. The exact mechanism in which HMWHA interacts with TLR receptors, leading to inhibition of inflammatory cascade is not known. Campo et al[52] have proposed that HA polymers of high molecular mass may mask TLR2 and TLR4 via its polymerized structure and subsequently prevent the stimulation of these receptors.

Antioxidant properties of hyaluronic acid. Oxidative stress is caused by the imbalance between the amount of reactive oxygen species (ROS) and antioxidant capacity. When present in excess, ROS has noxious effects on cellular proteins, lipids, and DNA. It is postulated that especially high molecular weight forms of HA can protect from the effects of ROS.

Different antioxidant effects of HMWHA include the decrease in ultraviolet B-induced apoptosis and ethylenediaminetetraacetic acid-induced oxidative damage of DNA.[15,53] Moreover, HMWHA diminishes apoptosis and oxidative stress triggered with benzalkonium chloride and sodium lauryl sulfate detergents, which are widely used in ophthalmic preparations. It is suggested that HMWHA should be proposed to patients treated with ocular medications with the ingredients aforementioned to reduce the risk of ocular surface impairment.[54,55] What is more, it has been demonstrated that treatment with eye drops containing HA reduces oxidative stress in the conjunctiva of patients with dry eye disease.[56] Despite numerous studies, the HMWHA mechanisms of oxidative stress reduction are still not completely understood. High molecular weight HA possesses hydroxyl functional groups, which can presumably absorb ROS. Furthermore, HMWHA interacts with the CD44 receptor. It is suggested that due to this interaction, HMWHA may activate pathways involved in the regulation of cellular redox status and intracellular ROS generation.[57] Moreover, HMWHA forms a cytoprotective coat on a cell membrane and, as a result, protects the cell from apoptosis. The antioxidant function of HMWHA contributes to the attenuation of DNA damage in human leukocytes during oxidative burst. It is postulated that HA binds to the CD44 receptor on the surface of monocytes and granulocytes and is endocytosed. Subsequently, HA neutralizes intracellular ROS and attenuates the DNA damage. Polyanionic HA molecules chelate Fe2+ and Cu2+ ions, which are required in Fenton's reaction; in the absence of these ions, hydroxyl radical, which is highly reactive with DNA, cannot be generated. Furthermore, HA may also neutralize ROS outside the leukocytes and protect neighboring cells.[58]

Using an intra-articular injection of HA in the therapy of osteoarthritis is not a novel idea. Hyaluronic acid determines elastic properties and viscosity of synovial fluid. Recently, antioxidant properties of HA have also been taken into consideration in various pathologies, including osteoarthritis.[59] According to Yu et al,[60] HA intra-articular injection reduces ROS levels in synovial fluid of patients suffering from osteoarthritis. In addition, HA suppresses hydrogen peroxide-induced cell death in human chondrocytes through an intracellular signaling pathway. The proteomic analysis of human osteoarthritis chondrocytes cultured in stress conditions have revealed the up-regulated expression of proteins involved in stress response and apoptotic pathways: transaldolase, annexin A1, and elongation factor 2. This effect was suppressed by adding HA to the culture medium.[60]

As previously mentioned, the majority of HA biological actions are strongly associated with its molecular size. Therefore, when it comes to its antioxidative potential, the integrity of the polymeric structure of HA should always be considered. Most studies focus on the underlying antioxidant properties of HMWHA or just HA.[54-57] However, Ke and colleagues[61] demonstrated strong antioxidant functions of LMWHA, which has shown protective effects against ROS both in vitro and in vivo, and inhibits lipid peroxidation and scavenges free radicals. Moreover, LMWHA raises total antioxidant capacity in immunosuppressed mice.[62] The mechanisms of the LMWHA antioxidant effect remain unknown and need further study.

The effects of the antioxidant properties of HA are also of interest in different research fields. Najafi et al[63] have proposed to add HA to semen extenders used in cryopreservation of animal sperm. Major medical applications include the treatment of dry eye disease and osteoarthritis.[56,59]

Hyaluronan in angiogenesis. Angiogenesis is an essential part of the proliferation phase of the wound healing process. During this phase, endothelial cells (EC) migrate from established vessels into surrounding tissues where they proliferate and create new cell-to-cell attachments as well as tubule structures of new capillaries.[7] Interactions between EC and ECM components are crucial in the regulation of new vessel formation.

Numerous studies have shown that HA signaling plays an important role in angiogenesis regulation, mainly by influencing EC behavior. Both HMWHA and LMWHA are potent regulators of angiogenesis, however they display opposite effects on EC proliferation and motility. It has been proven that LMWHA stimulates vascular EC proliferation, migration, and tubule formation in vitro, as well as in various in vivo models of angiogenesis.[63] At the same time HMWHA displays antiangiogenic properties by inhibiting EC proliferation, motility, and sprout formation.[63]

The exact molecular mechanisms determining the proangiogenic or antiangiogenic effects of different HA forms have not been fully elucidated. CD44 as well as RHAMM, 2 main receptors for HA, are present on the surface of the endothelial cells. In vitro experiments demonstrated both anti-RHAMM and anti-CD44 antibodies blocked the EC ability to form tubule-like structures in matrigel.[64] Blocking the CD44 signaling by anti-CD44 antibody inhibited EC proliferation in vitro.[65] Cao and coauthors[66] have reported angiogenesis was significantly affected in CD44 knockout mice. The earliest studies concentrated on the HA-mediated mitogenesis via the MAPK/ERK signaling pathway activation. It has been shown that CD44 and RHAMM stimulated by HA oligomers create a complex with ERK 1/2, that leads to constant ERK 1/2 activation and increases cell motility of invasive breast cancer cell lines.[67] Similar ERK 1/2 pathway activation by LMWHA has also been reported in other tumor cell lines, but also in various types of ECs, such as human umbilical vein ECs (HUVECs), human micro vessels endothelial cells, or human pulmonary artery ECs.[68]

Interestingly, recent reports suggest that the promotion of EC proliferation caused by LMWHA may also be associated with expression of other signaling molecules, such as ezrin. This protein, also known as cytovillin or villin-2 belongs to the ERM protein family,which also includes moesin, radixin, and merlin. Ezrin is a linkage protein between the plasma membrane and actin skeleton, thus it plays a key role in cell surface adhesion and migration. Ezrin can interact with cellular C-terminus of CD44 receptor. Studies have shown that ezrin promotes cell adhesion and migration, whereas merlin inhibits cell proliferation. Mo and coauthors[68] have shown that LMWHA promoted the proliferation of HUVECs. At the same time, expression of ezrin mRNA and protein was significantly increased in HUVECs pretreated with LMWHA. In contrast, no difference in ezrin and merlin expressions have been observed in HUVECs incubated with HMWHA.[69]

The proangiogenic effects of LMWHA and antiangiogenic properties of HMWHA have been reported in numerous studies. Surprisingly, in several types of tumor xenografts, injection of hyaluronan oligomers inhibited rather than stimulated tumor growth. This may suggest a context-dependent response to different forms of HA and a possible role of the microenvironment in this process. Fuchs et al[70] have investigated the influence of HMWHA and LMWHA on chemokine (C-X-C motif) ligand 12/chemokine receptor type 4 (CXCR4) signaling. Chemokine (C-X-C motif) ligand 12 (CXCL12) can stimulate angiogenesis by activating CXCR4 present on the EC surface.[70] In wound closure assays, adding the CXCL12 to the culture medium significantly increased cell migration and induced faster wound closure. This effect was statistically augmented when cells were preincubated with HMWHA. In vitro studies have shown that CXCR4 activation by CXCL12 was significantly increased in HUVECs pretreated with HMWHA, whereas preincubation with LMWHA blocked CXCL12 signaling in these cells.

A new, interesting way of CD44 signaling regulation has been reported by Yang and coauthors.[71]Using several renal and breast cancer cell lines, the researchers demonstrated that HMWHA was able to stimulate clustering of CD44 into large conglomerates, whereas LMWHA disrupted these structures.[72] It is postulated that clusters may influence CD44 signaling by stabilizing complexes; this receptor forms with various signaling molecules. Nevertheless, the possible impact of CD44 clustering on its biological function requires further investigation.

Conclusions

Hyaluronic acid is present through all steps of the wound healing process not only as a component of the wound environment, but as a factor that actively modulates tissue regeneration. Along with studies that revealed the unique properties of HA, some attempts have been made to apply it in clinical practice, especially in chronic wound treatment. Extensive literature can be found concerning exogenous applications of HA to wounds. Various HA sources, formulations, and delivery systems have been used in clinical trials.[73] A rich natural source of HA are fetal membranes, especially the jelly substance from the umbilical cord and the amnion. The amniotic membrane has been used in traditional medicine, and also in the form of a commercially available dressing in wound treatment.[72] Studies have revealed the amniotic membrane, even after various processing and preservation procedures, contains high amounts of HMWHA. This component of the amnion is one of the factors responsible for its beneficial actions observed in chronic wound therapy.[74] An effective wound healing therapy remains one of the greatest challenges of modern clinical medicine. Hyaluronic acid as a biologically active molecule that regulates tissue repair process on multiple levels should be considered as a safe and effective option to be used in skin repair.

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From the Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland