Hyaluronan

A Master Switch Between Vascular Homeostasis and Inflammation

Maria Grandoch, Paul L. Bollyky, Jens W. Fischer

Hyaluronan (HA) matrix responds quickly to changing conditions during atherogenesis and atheroprogression. Our evolving understanding of HA's specific functions at the interface of inflammatory responses and phenotypic control of mesenchymal cells in the vessel wall points to its importance in inflammation and atherosclerosis. Additionally, the enzymes that mediate HA biosynthesis and degradation are potential target molecules for novel therapies, as are signal-transducing HA receptors: deciphering the HA matrix, thus, has significant translational possibilities.

HA Matrix Plasticity

HA is a glycosaminoglycan polymer without covalent binding to a core protein. A unique feature of HA is its de novo synthesis at the plasma membrane by HA synthases (HAS1–3) from UDP (uridine diphosphate)-sugar precursors. The chemical structure of HA is simple and comprises repeating disaccharides of β(1,4)-*N*-acetyl-D-glucosamine and β(1,3)-D-glucuronic acid that form an unbranched and unmodified polymer. This structure is conserved across all mammalian species.

The biological functions of HA are complex and highly context-specific because as soon as HA is extruded to the extracellular space, it is subject to complex interactions with HA-binding proteins (hyaladherins). The function of the pericellular HA matrix depends on the particular hyaladherins present and their engagement with HA receptors, such as CD44, RHAMM (receptor of HA-mediated motility), and Lyve-1. The resulting interactions modulate the adhesion, migration, and proliferation of smooth muscle cells (SMC), fibroblasts, and other cell types present in vascular tissues. In parallel, HA complexes also influence important aspects of immune cell polarization, differentiation, and function. Beyond the immediate pericellular microenvironment, HA is also integrated into structures such as basement membranes, is present in circulation, and can function as a signaling molecule to activate HA receptors.

The molecular weight of HA has critical functional importance in vivo. In the steady state, longer, high-molecular-weight HA polymers support homeostasis and immune surveillance. On injury, however, HA degradation and de novo synthesis occur acutely and promote inflammatory responses. HA breakdown is mediated by hyaluronidases and nonenzymatically by reactive oxygen species. This catabolism of HA engages alternative HA receptors, such as TLR (toll-like receptors), present on both immune cells and resident cardiovascular cells, such as SMC, fibroblasts, and endothelial cells. Thus, the high-molecular-weight HA, which functions as a noninflammatory matrix in healthy vessels, undergoes transformation into an inflammatory matrix microenvironment.

What else makes the HA matrix so special? As the features mentioned above clearly indicate the HA matrix and HA's specific interactions are structurally and functionally distinct from other elements of the ECM (extracellular matrix) that involve integrin signaling, matrix metalloproteinases, and matrix cross-linking enzymes. Furthermore, HA synthesis is a rapid process that can build an HA-rich microenvironment within hours of stimulation and is responsive to growth factors, prostaglandins, and cytokines such as PDGF (platelet-derived growth factor)-BB, prostaglandin E2, and interleukin 1β.[1,2] Time-lapse videos of SMC have shown the rapid formation of HA- and versican-rich pericellular matrix coats to be essential for cell migration and mitosis.

The speed of HA biosynthesis and its degradation and modifications by HA–protein interaction enables rapid responses to pathophysiological stimuli. Recently, it became evident that immune cells such as macrophages and T cells also elaborate HA-rich matrices.[3] This new insight stimulated the hypothesis that mobile immune cells shape HA-rich microenvironments by de novo synthesis at their destination and that this may be essential for specific immune functions. Importantly, in this context, the HA receptor CD44 can serve as a coreceptor together with, for example, TLRs or MHC (major histocompatibility complex) class II receptors.

Despite these pivotal discoveries regarding its roles and functions, HA's organization in inflammatory contexts and contributions to disease pathogenesis remain incompletely understood. Why has HA not yet received more attention within cardiovascular research? Indeed, the contributions of the HA matrix and associated molecules have likely been underestimated because the HAS isoenzymes are made from low-copy transcripts and are transmembrane proteins and because HA has a pure carbohydrate structure. Consequently, HAS isoenzymes and HA have escaped genomic and proteomic analysis in many past approaches.

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HA-Driven Inflammation and Atherosclerosis

In this section, we note key findings on HA's functions in other systems and highlight specific observations on inflammation regulation by HA in atherosclerosis.

Under homeostatic conditions, most immune cells have low HA-binding capacity. However, in inflammatory settings, HA binding via CD44 specifically increases in activated immune cell subsets, as shown for B and T cells.[4,5] During inflammation, both HA synthesis by HAS isoenzymes (HAS1–3) and HA catabolism increase. This leads to a preponderance of low-molecular-weight, fragmentary HA which may serve as a danger signal that can activate macrophages via TLR or change engagement of other HA receptors, such as CD44. Small HA fragments have also been demonstrated to activate dendritic cells (DC) and promote their maturation.

HA contributes to the propagation of inflammation at sites of tissue damage as shown, for example, in lung injury and inflammatory bowel disease. Specifically, *Has2* and *Tlr* knockout protects against lung injury and fibrosis, respectively.[6] The use of 4-methylumbelliferone, which inhibits HA synthesis, prevents, for example, T cell–mediated β-cell death in type 1 diabetes mellitus,[7] in line with HA's proinflammatory role in autoimmunity. Therefore, HA contributions to tissue injury and inflammation likely depend on context.

The HA matrix also modulates cellular adhesion and trafficking. Studies of inflammatory bowel disease have shown that HA can associate with other matrix molecules, likely CD44, inter alpha trypsin inhibitor I, tumor necrosis factor-α–stimulated gene 6, and HA-binding proteoglycans, to form supramolecular structures that are highly adhesive for not only monocyte/macrophages but also T cells. This unique, highly adhesive HA matrix has been termed HA cables by de la Motte et al.[8]

Although the aforementioned effects of HA occur locally, there is evidence that HA has important immune modulatory effects on a systemic level as well. An important example is the HA function in T cell responses. CD44 on T cells is important for adhesion and recruitment of T cells. CD44 potentiates T-cell activation, expansion, and maintenance of memory cells. In the T-cell immune synapse, DC-synthesized HA promotes T-cell binding to DC via CD44 and supports antigen presentation[9] and Th1 (type1 T helper) cell polarization. In addition, in a feed-forward loop, Th1 cytokines directly influence HA production by DCs, which then in turn affects T cell–DC binding. This role of HA in T cell–mediated diseases is important in multiple sclerosis and diabetes mellitus type 1.[5,7] From these and other studies, a critical role for HA in immune governance emerges; this also applies to cardiovascular disease.

Specifically, 2 aspects of HA function are important for balancing chronic inflammation in atherosclerosis: (1) homeostatic function in the glycocalyx and (2) promoting macrophage-driven inflammation and Th1 cell polarization. These effects are paralleled by the promotion of hyperplastic SMC phenotypes. Therefore, HA matrix has a strategically important and unique position to modulate both vascular inflammation and SMC-driven aspects of atherosclerosis, as detailed below.

In healthy vessels, HA is present as part of the endothelial glycocalyx. The function of HA as part of the vascular glycocalyx is both anti-inflammatory and anti-thrombotic.[10] Consequently, inhibiting HA synthesis and HA shedding from the glycocalyx in response to inflammation, smoking, or diabetes mellitus increases platelet activation and macrophage-driven inflammation.[11] Within the glycocalyx, then, HA is atheroprotective. In contrast, the interstitial HA matrix has been shown to promote neointimal hyperplasia and atherosclerosis in vivo.[12] Consistent with this, *Has3* knockout in C57BL/6 mice inhibited neointimal hyperplasia and atherosclerosis, and HAS2 overexpression in SMC of apolipoprotein E–deficient mice increased atherosclerosis.[2,13,14]

In contrast to SMC-driven neointimal hyperplasia, the effects of interstitial HA on inflammatory cell circuits and HA's function at nonvascular sites are less well studied. The work on HA-mediated stimulation of TLR2 in lung injury suggests that HA likely contributes to TLR-based inflammation in atherosclerosis. Furthermore, it is conceivable that HA cables promote atherosclerosis, similarly to the above mentioned importance of HA cables in enteric wall inflammation. In line with this hypothesis, several in vitro studies in human and rodent SMC suggest that HA-mediated TLR signaling and HA cables take part in monocyte/macrophage retention within vascular disease. Accordingly, HA-mediated TLR signaling and HA cables could play an important role in controlling the function and kinetics of macrophage traffic in atherosclerotic lesions.

Further, the HA-rich immune synapse data described above support the idea that HA influences T-cell responses during atherosclerosis. This might be therapeutically important because the T-cell response in atherosclerosis is thought to occur early in the disease initiation process and would therefore be a promising target for therapeutic interventions. In line with this assumption, we have shown that deleting HAS3 inhibits murine atherosclerosis by reducing Th1 cell polarization and, subsequently, macrophage-driven inflammation.[2] Indirect evidence for the importance of HA in the progression of atherosclerosis also comes from studies investigating the HA receptors CD44 and RHAMM in vascular disease. RHAMM was found to be crucial for SMC migration and proliferation, while absence of *Cd44* inhibited macrophage-driven inflammation in apolipoprotein E–deficient mice, as we have shown for *Has3* deletion.

Future Directions and Open Questions

In human atherosclerosis, HA accumulation has been shown in primary and secondary lesions, as well as in restenosis. Interestingly, while eroded human plaques are devoid of some matrix components, such as biglycan, they are rich in HA and versican, an HA-binding proteoglycan. Therefore, it might be considered that either the accumulation of HA and versican or the lack of other ECM molecules such as biglycan modulates thrombogenicity of the eroded plaque matrix.

HAS isoenzyme-specific knockout studies are needed to reveal how individual HAS isoenzymes contribute to disease progression and to identify specific isoenzymes that could therapeutically be targeted to inhibit intramural HA synthesis without disturbing the endothelial gylcocalyx.

Further, investigations into the importance of HA in atherosclerosis could likely be extended to other cardiovascular diseases. Particularly promising areas are aortic aneurysms and myocardial infarction because both conditions generate extensive inflammatory responses and cause substantial remodeling, including both matrix degradation and de novo synthesis. With respect to myocardial infarction, we know cardiac fibroblasts upregulate HA synthesis via IL (interleukin)-6 and that *Cd44* knockout severely changes both the inflammatory response and the overall healing response.[15,16] However, the specific roles of HASs postinfarction await clear mechanistic studies.

In summary, HA-rich ECM plays a pivotal role in orchestrating immune responses both locally and systemically. At the same time, HA provides important cues to direct the SMC and fibroblast phenotype. The opportunity to modulate immune responses and mesenchymal cell phenotypes simultaneously by interfering with the HA system may offer unique opportunities and novel therapeutic classes in cardiovascular disease.

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P.L. Bollyky is a founder of Hyalos, Inc., a company developing novel molecules targeting hyaluronan (HA) synthesis. The other authors report no conflicts.

References

1. Sussmann M, Sarbia M, Meyer-Kirchrath J, Nüsing RM, Schrör K, Fischer JW. Induction of hyaluronic acid synthase 2 (HAS2) in human vascular smooth muscle cells by vasodilatory prostaglandins. *Circ Res*. 2004;94:592–600. doi: 10.1161/01.RES.0000119169.87429.A0.
2. Homann S, Grandoch M, Kiene LS, Podsvyadek Y, Feldmann K, Rabausch B, Nagy N, Lehr S, Kretschmer I, Oberhuber A, Bollyky P, Fischer JW. Hyaluronan synthase 3 promotes plaque inflammation and atheroprogression. *Matrix Biol*. 2018;66:67–80. doi: 10.1016/j.matbio.2017.09.005.
3. Chang MY, Chan CK, Braun KR, Green PS, O'Brien KD, Chait A, Day AJ, Wight TN. Monocyte-to-macrophage differentiation: synthesis and secretion of a complex extracellular matrix. *J Biol Chem*. 2012;287:14122–14135. doi: 10.1074/jbc.M111.324988.
4. Hathcock KS, Hirano H, Murakami S, Hodes RJ. CD44 expression on activated B cells. Differential capacity for CD44-dependent binding to hyaluronic acid. *J Immunol*. 1993;151:6712–6722.
5. Kuipers HF, Rieck M, Gurevich I, Nagy N, Butte MJ, Negrin RS, Wight TN, Steinman L, Bollyky PL. Hyaluronan synthesis is necessary for autoreactive T-cell trafficking, activation, and Th1 polarization. *Proc Natl Acad Sci USA*. 2016;113:1339–1344. doi: 10.1073/pnas.1525086113.
6. Liang J, Zhang Y, Xie T, Liu N, Chen H, Geng Y, Kurkciyan A, Mena JM, Stripp BR, Jiang D, Noble PW. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. *Nat Med*. 2016;22:1285–1293. doi: 10.1038/nm.4192.
7. Nagy N, Kaber G, Johnson PY, Gebe JA, Preisinger A, Falk BA, Sunkari VG, Gooden MD, Vernon RB, Bogdani M, Kuipers HF, Day AJ, Campbell DJ, Wight TN, Bollyky PL. Inhibition of hyaluronan synthesis restores immune tolerance during autoimmune insulitis. *J Clin Invest*. 2015;125:3928–3940. doi: 10.1172/JCI79271.
8. de la Motte CA, Hascall VC, Drazba J, Bandyopadhyay SK, Strong SA. Mononuclear leukocytes bind to specific hyaluronan structures on colon mucosal smooth muscle cells treated with polyinosinic acid:polycytidylic acid: inter-alpha-trypsin inhibitor is crucial to structure and function. *Am J Pathol*. 2003;163:121–133.
9. Bollyky PL, Evanko SP, Wu RP, Potter-Perigo S, Long SA, Kinsella B, Reijonen H, Guebtner K, Teng B, Chan CK, Braun KR, Gebe JA, Nepom GT, Wight TN. Th1 cytokines promote T-cell binding to antigen-presenting cells via enhanced hyaluronan production and accumulation at the immune synapse. *Cell Mol Immunol*. 2010;7:211–220. doi: 10.1038/cmi.2010.9.
10. Nieuwdorp M, Holleman F, de Groot E, Vink H, Gort J, Kontush A, Chapman MJ, Hutten BA, Brouwer CB, Hoekstra JB, Kastelein JJ, Stroes ES. Perturbation of hyaluronan metabolism predisposes patients with type 1 diabetes mellitus to atherosclerosis. *Diabetologia*. 2007;50:1288–1293. doi: 10.1007/s00125-007-0666-4.
11. Nagy N, Freudenberger T, Melchior-Becker A, Röck K, Ter Braak M, Jastrow H, Kinzig M, Lucke S, Suvorava T, Kojda G, Weber AA, Sörgel F, Levkau B, Ergün S, Fischer JW. Inhibition of hyaluronan synthesis accelerates murine atherosclerosis: novel insights into the role of hyaluronan synthesis. *Circulation*. 2010;122:2313–2322. doi: 10.1161/CIRCULATIONAHA.110.972653.
12. Riessen R, Wight TN, Pastore C, Henley C, Isner JM. Distribution of hyaluronan during extracellular matrix remodeling in human restenotic arteries and balloon-injured rat carotid arteries. *Circulation*. 1996;93:1141–1147.
13. Kiene LS, Homann S, Suvorava T, et al. Deletion of hyaluronan synthase 3 inhibits neointimal hyperplasia in mice. *Arterioscler Thromb Vasc Biol*. 2016;36:e9–e16. doi: 10.1161/ATVBAHA.115.306607.
14. Chai S, Chai Q, Danielsen CC, Hjorth P, Nyengaard JR, Ledet T, Yamaguchi Y, Rasmussen LM, Wogensen L. Overexpression of hyaluronan in the tunica media promotes the development of atherosclerosis. *Circ Res*. 2005;96:583–591. doi: 10.1161/01.RES.0000158963.37132.8b.
15. Müller J, Gorressen S, Grandoch M, Feldmann K, Kretschmer I, Lehr S, Ding Z, Schmitt JP, Schrader J, Garbers C, Heusch G, Kelm M, Scheller J, Fischer JW. Interleukin-6-dependent phenotypic modulation of cardiac fibroblasts after acute myocardial infarction. *Basic Res Cardiol*. 2014;109:440. doi: 10.1007/s00395-014-0440-y.
16. Huebener P, Abou-Khamis T, Zymek P, Bujak M, Ying X, Chatila K, Haudek S, Thakker G, Frangogiannis NG. CD44 is critically involved in infarct healing by regulating the inflammatory and fibrotic response. *J Immunol*. 2008;180:2625–2633.

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