Hyaluronan-Cell Interactions in Cancer and Vascular Disease*

Published, JBC Papers in Press, November 20, 2001, DOI 10.1074/jbc.R100039200

Bryan P. Toole§§, Thomas N. Wight¶¶, and Markku I. Tammi

From the §Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, Massachusetts 02111, ¶The Hope Heart Institute, Seattle, Washington 98104, and the ¶Department of Anatomy, University of Kuopio, Savilahdentie 9, Fin-70211 Kuopio, Finland

Other articles in this series concentrate on normal physiological and cellular functions of hyaluronan. In this article we discuss the influences of hyaluronan on disease progression. Alterations in hyaluronan metabolism, distribution, and function have been documented in many diseases, e.g. arthritis, immune and inflammatory disorders, pulmonary and vascular diseases, and cancer (see Refs. 1 and 2). In this article we will concentrate on cancer and vascular disease because our knowledge in these areas has advanced rapidly over the past several years and because work in these areas has highlighted the importance of hyaluronan-cell interactions in cell behavior.

Hyaluronan in Cancer

High Hyaluronan Levels Are Associated with Many Human Cancers—Studies on histological sections from various tumors, using a specific hyaluronan affinity probe, have indicated that virtually all human epithelial tumors are surrounded by a connective tissue matrix (stroma) enriched in hyaluronan. Although this was anticipated from earlier reports, it is quite striking that the extent of stromal hyaluronan accumulation is a strong, independent, negative predictor of patient survival, particularly sensitive in tissues such as breast (3) and ovaries (4) where there is a low basal level of hyaluronan in normal tissue. Hyaluronan is thus a central component of the distinct stroma that surrounds and probably supports the tumor. The tumor extracellular matrix (ECM)3 is also enriched in hyaluronan-binding proteoglycans such as versican (5).

Furthermore, some breast, stomach, and colon carcinomas show ectopic expression of hyaluronan associated with the malignant cells themselves, whereas the corresponding normal epithelia give virtually no signal for hyaluronan (3, 6, 7). Again, a high number of hyaluronan-positive cells predicts unfavorable outcome. For instance, the recurrence rate of colon carcinoma after an operation increases from 20 to 80% with increasing levels of hyaluronan associated with the carcinoma cells (6). Likewise, elevated levels of hyaluronan and hyaluronidase in the urine form a clinically reliable marker for the presence and grade of bladder cancer (8).

Malignancies originating in stratified squamous epithelial cells that normally express hyaluronan within their epithelial layers, like those in the epidermis, esophagus, larynx, and uterine cervix, usually continue to express hyaluronan. However, the clinical significance of hyaluronan levels appears different from those of simple epithelia because local decreases in the cell-associated hyaluronan signal in these tumors correlate with poor histologic differentiation and prognosis (9, 10). Nevertheless, hyaluronan synthesis stimulates, and appears necessary for, the migration of epidermal keratinocytes,2 supporting the idea that synthesis of hyaluronan also promotes the spreading of cancers from squamous epithelia.

Hyaluronan-Cell Interactions Influence Cell Behavior—At least three major molecular characteristics of hyaluronan contribute to normal and tumor cell behavior. These are its unique hydrodynamic properties, its interactions with various hyaluronan-binding macromolecules (hyaladherins) in the assembly of organized pericellular and extracellular matrices, and its instructive effects on cell signaling and behavior.

Studies of embryonic development, regeneration, and healing, as well as of cancer and vascular disease, have demonstrated that extracellular matrices surrounding proliferating and migrating cells are highly enriched in hyaluronan (11). An early interpretation of this finding was that, as a consequence of its effect on hydration of tissues and associated swelling pressures, hyaluronan creates fluid, malleable matrices in which cells can readily change shape during mitosis or penetrate tissues during migration. In agreement with this idea, a recent study showed that hyaluronan promotes glioblastoma cell migration within a fibrin gel by increasing hydration and consequently the porosity of the gel (12).

Hyaluronan synthases (termed Has1, Has2, and Has3) are integral plasma membrane proteins with their active sites located at the intracellular face of the plasma membrane (13). Synthase activity fluctuates with the cell cycle and peaks at mitosis (14) at which time hyaluronan is enriched in intracellular compartments of the cell, notably around the nucleus (15), as well as in the pericellular matrix (16). Hyaluronan extruded onto the cell surface at mitosis provides an essential template for assembly of a multicomponent pericellular matrix that most likely serves both signaling and structural functions. Interactions of hyaluronan with CD44, versican, aggrecan, TSG-6, and other hyaladherins in this matrix create a complex, hydrated microenvironment that supports and promotes the cellular characteristics of dividing and migrating cells. For example, hyaluronan-dependent pericellular matrix formation increases around dividing cells immediately preceding mitosis, and removal of this matrix by competitive displacement with hyaluronan oligosaccharides inhibits cell division (16). Likewise, inhibition of hyaluronan synthesis leads to cell cycle arrest at mitosis, just before cell rounding and detachment (14). A hyaluronan-dependent matrix also assembles around migrating cells, especially at the leading and trailing edges (16). Removal

2 K. Rilla, M. Lammi, R. Sironen, K. Torrönén, M. Luukkonen, V. C. Hascall, R. J. Midura, M. Hyttinen, M. I. Tammi, and R. Tammi, submitted for publication.
of this matrix by displacement with hyaluronan oligomers reduces the rate of cell movement (16, 17).

In addition to its functions in tissue hydration and assembly of matrices, hyaluronan exerts influences on cell behavior by interacting directly with the cell surface, leading to signal transduction and cytoskeletal rearrangements. Hyaluronan interacts with the cell in at least two ways: by binding to cell surface receptors, such as CD44 and RHAMM, or by sustained attachment to hyaluronan synthase across the plasma membrane (11, 13, 18, 19). The biochemical mechanisms whereby hyaluronan-receptor interactions are transduced into intracellular signals that regulate cell growth, survival, and movement are being intensely studied by several groups, and these studies are reviewed in a separate article in this series (19). In addition to these receptor-mediated events, a relatively unexplored area is the potential role of hyaluronan synthases in intracellular signaling. As noted above, newly synthesized hyaluronan is extruded from the cell while still attached to the synthases but may also be deposited in the cytoplasm. Several intracellular hyaladherins, e.g. Cdc37, IHABP4, and an intracellular form of RHAMM, have been characterized. Each of these proteins interacts with kinases important in regulation of the cell cycle and thus may be involved in coordination of hyaluronan synthase activity, intracellular versus pericellular hyaluronan concentrations, and the cell cycle (11, 13, 15, 19).

**Hyaluronan-Cell Interactions Are Crucial in Cancer Progression**—A large body of experimental evidence from animal models directly implicates hyaluronan in the progression of several tumor types (20–29). Two major approaches have been used to probe the involvement of hyaluronan. First, it has been shown that overexpression of Has promotes growth of fibrosarcoma and prostate carcinoma (20, 21) and metastasis of mammary carcinoma (22) in vivo. Second, perturbation of endogenous hyaluronan interactions inhibits growth, invasion, and metastasis in several tumor types in vivo (23–28). Several approaches have been used to manipulate hyaluronan interactions, the most common of which has been overexpression of soluble hyaladherins, e.g. soluble CD44 (24–28), RHAMM (19), or hyaladherins from cartilage extracts (23). Soluble hyaladherins act as an interactive sink for displacement of endogenous hyaluronan from its receptors, e.g. CD44, thus inhibiting putative downstream events. For example, overexpression of soluble CD44 in mammary carcinoma or melanoma cells inhibits tumor growth and metastasis, but these effects are not obtained if the soluble CD44 is mutated such that hyaluronan binding does not occur (24–28). Cellular effects of soluble CD44 include induction of G1 arrest (24) or apoptosis (25) in the tumor cells and inhibition of MMP-mediated invasion (26). Administration of hyaluronan oligosaccharides also inhibits growth of several tumor types in vivo, including mammary and lung carcinomas and melanoma (29). Hyaluronan oligomers compete for endogenous polymeric hyaluronan-receptor interactions, resulting in monovalent rather than polyvalent interactions with receptors. Recent results have shown that hyaluronan oligomers also induce G1 arrest or apoptosis in tumor cells.

An exciting new development is the finding that hyaluronan is critical for anchorage-independent growth in culture, one of the most reliable indicators of tumorigenicity in vivo. Overexpression of Has in fibrosarcoma cells stimulates both tumor growth in vivo and anchorage-independent growth in soft agar (20). Likewise, perturbation of endogenous hyaluronan interactions, either by overexpression of soluble CD44 (24) or by addition of hyaluronan oligomers, inhibits anchorage-independent growth. Hyaluronan oligomers act via inhibition of the phosphoinositol 3-kinase-Akt survival pathway.

Another consequence of treatment with soluble hyaladherins is the loss of hyaluronan-induced clustering of CD44 in the plasma membrane. Clustering of CD44 in the membrane leads to docking of gelatinase B (MMP-9) on the surface of mammary carcinoma and melanoma cells (26). This phenomenon results in promotion of tumor cell invasiveness and angiogenesis (26, 27), both of which are important events in tumor progression. Overexpression of membrane-bound CD44 can also disrupt clustering (26), possibly explaining some of the apparently contradictory findings concerning the relationship of CD44 levels to tumorigenesis, e.g. inhibition (30) versus promotion (31) of tumor progression by overexpression of intact CD44. Thus, there is likely a fine balance between the amount and organization of CD44 and its ability to respond to interaction with high molecular weight hyaluronan.

Although it is clear that hyaluronan interactions directly influence various intracellular signaling pathways important for cell behavior, binding of hyaluronan to CD44 also leads to internalization and degradation. In this regard it is significant that some tumor cells exhibit elevated levels of hyaluronidase and the ability to internalize and degrade hyaluronan (25, 32). Thus penetration of hyaluronan-rich stroma (25) or production of angiogenic breakdown products of hyaluronan (32) may also promote tumor progression.

Hyaluronan-RHAMM interactions have also been implicated in tumor cell behavior in vitro and in vivo. RHAMM is involved in the Ras and extracellular signal-regulated kinase signaling pathways and associates with the cytoskeleton (19). Hyaluronan-RHAMM interactions induce transient phosphorylation of p125FAK in concert with turnover of focal adhesions in Ras-transformed cells, thus leading to initiation of locomotion (33). Suppression of this interaction inhibits both cell locomotion and proliferation in vitro and leads to inhibition of tumor growth in vivo, whereas overexpression of RHAMM leads to enhanced tumor growth and metastasis (19, 33). The involvement of RHAMM in cell behavior is discussed more fully in another review in this series (19).

**Hyaluronan in Vascular Disease**

**Hyaluronan Increases in Atherosclerotic and Restenotic Lesions**—Atherosclerosis and restenosis are characterized by marked changes in the content and distribution of hyaluronan. Early biochemical studies showed that the hyaluronan concentration of human atherosclerotic plaques generally decreases with increasing severity of atherosclerosis (34–36). However, morphological studies indicate that hyaluronan is present throughout both early and late human atherosclerotic lesions in defined locations (37, 38). It is difficult to document the involvement of hyaluronan in the early phase of human atherosclerosis because this stage is rarely detected and the disease is only recognized when clinical symptoms appear. Thus, the bulk of information on the involvement of hyaluronan in early atherosclerosis comes from experimental animal studies in which lesion development can be closely monitored. Hyaluronan is dramatically increased in early experimental vascular lesions in response to balloon catheter injury (39, 40). In the early lesions, hyaluronan is especially enriched around proliferating and migrating arterial smooth muscle cells (ASMCs) (41–44). The accumulation of hyaluronan in early atherosclerotic lesions is often accompanied by increases in molecules that associate with hyaluronan, such as versican (45–47), TSG-6 (48), and CD44 (49).

Thus, it is well documented from experimental animal studies that injury to blood vessels induces a hyaluronan response.

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3. S. Ghatak, S. Misra, J. Ward, and B. P. Toole, submitted for publication.
that likely contributes to lesion growth following vascular injury. Hyaluronan also increases when human vessels are subjected to balloon angioplasty during surgical procedures to open blocked arteries and is a prominent component of ASMC-rich human restenotic arteries (43). Like the experimental lesions, hyaluronan-binding molecules such as versican accumulate in these lesions (50, 51). Tissues enriched in hyaluronan have the tendency to trap water and swell. The rapid expansion of restenotic lesions could in large part be due to edematous changes created by hyaluronan and associated molecules. Interestingly, in other examples of tissue edema such as occurs in myocardial infarcts, hyaluronan plays a significant role (52). Removal of hyaluronan from infarcted hearts with hyaluronidase reduces tissue damage (53, 54). Loss or breakdown of hyaluronan as restenotic lesions remodel, however, could lead to expulsion of water and tissue shrinkage with reduction in arterial circumference, a condition often seen in restenotic lesions. Thus, this conversion may involve a waterlogged ECM becoming a cicatrix that shrinks and contracts the artery, causing loss of lumen diameter. On the other hand, hyaluronan may promote vessel shrinkage following angioplasty by influencing the contraction of the ECM by the ASMCs. For example, collagen gels impregnated with hyaluronan show CD44-dependent enhanced contraction when populated by ASMCs (55). Thus, hyaluronan may play significant roles in both the hyperplastic and remodeling phases of human restenosis. It is clear that this molecule could be a useful target in attempts to modify therapeutically the events associated with restenotic lesion progression.

**Hyaluronan Is a Component of the Inflammatory Phase of Vascular Disease**—Hyaluronan is also present in regions of atherosclerotic lesions that contain inflammatory cells such as macrophages and lymphocytes (37, 56). Consistent with this, the extravasation of leukocytes from the blood into the vascular wall involves hyaluronan anchored to the surface of the endothelial cells by CD44 (57) or RHAMM (58) and is mediated by CD44 on the surface of the leukocytes (59–61). These findings place hyaluronan at the beginning of the inflammatory response that is thought to be a critical step in the formation of the atherosclerotic lesion (62). Not only is hyaluronan important in the initial stages of leukocyte extravasation, but its accumulation in the early lesions may promote inflammatory cell retention by serving as a substrate for these cells! The presence of hyaluronan in macrophage-rich regions of the plaque (37, 56) supports this possibility. Macrophages are present in hyaluronan-rich regions in other inflammatory tissues such as in ulcerative colitis through associations with CD44 (63). In fact, early studies identified hyaluronan as an agglutinating factor for macrophages (64). The importance of the hyaluronan-CD44 connection in developing atherosclerotic lesions is further highlighted by studies that show that blocking CD44 receptors on monocytes and lymphocytes by the exogenous administration of hyaluronan prevents their accumulation in developing lesions and markedly reduces the severity of experimental atherosclerosis (65).

Hyaluronan is also present in areas of atherosclerotic lesions that contain extracellular lipid deposits (37, 56). In fact, lipoprotein-hyaluronan complexes have been isolated from human atherosclerotic lesions (66), and in vitro studies have shown that hyaluronan does interact with phospholipids through hydrophobic interactions (67). It is clear that lesions that contain excess lipid are usually rich in hyaluronan. Such a concentration of molecules that soften and swell the tissue could very well weaken the plaque and predispose the plaque to rupture.

**Hyaluronan Influences Vascular Cell Phenotype**—The enrichment of hyaluronan in early atherosclerotic lesions around proliferating and migrating ASMCs suggests that hyaluronan may have a role in these cellular events. The mitogen, platelet-derived growth factor, stimulates hyaluronan synthesis by ASMCs (68) and promotes the formation of pericellular coats as these cells divide and migrate (16). Interference with the binding of hyaluronan to the surface of ASMCs by using either competitive oligosaccharides (16) or blocking antibodies to hyaluronan receptors such as RHAMM (69) blocks ASMC proliferation and migration. Hyaluronan is also present inside proliferating ASMCs (15), which suggests an intracellular role for hyaluronan in this process. The fact that there are multiple intracellular proteins that exhibit hyaluronan binding characteristics supports this possibility.

Hyaluronan also influences the behavior of the vascular endothelial cells. Fragments of hyaluronan stimulate CD44-mediated endothelial migration, proliferation, and ECM synthesis of macromolecules associated with new blood vessel formation in vitro (70–72). In fact, hyaluronan fragments can promote the formation of new blood vessels in vivo (73, 74). Neovascularization of atherosclerotic lesions is a critical event in determining the severity of the lesions.

Hyaluronan may also influence the phenotype of macrophages within the atherosclerotic plaques. In addition to potentially serving as a substrate for the macrophage as described above, hyaluronan degradation products induce cytokine and chemokine expression by macrophages (75, 76). Thus, hyaluronan may drive the inflammatory response not only by retaining macrophages but also by partly regulating macrophage activation!

In summary, hyaluronan is a critical “player” in blood vessel physiology and pathology in a similar fashion to its central role in cancer. Its role as a structural component regulating the biomechanical properties of blood vessels is well established.

## Conclusion

Over the past decade there has been a paradigm shift in research on hyaluronan. Numerous studies, both old and recent, have supported the concept that hyaluronan is a biopolymer with extraordinary biophysical properties that contribute to extracellular matrix structure and interstitial homeostasis (1, 2). Recent work, however, has highlighted the equally important role of hyaluronan in cell behavior. A particularly striking example of this is the failure in epithelial-mesenchymal transition and altered ras signaling during cardiac development in the Has-2 knockout mouse (77). Thus, it is not surprising that disease processes that exhibit aberrant cell behavior, such as cancer and atherosclerosis, involve altered hyaluronan-cell interactions. Although much progress has been made in our understanding of these areas in recent years, our understanding of the detailed mechanisms whereby hyaluronan influences cell behavior is still very incomplete. Many current investigations are focused on transduction of signals arising from hyaluronan-CD44 and RHAMM interactions (see Ref. 19), and interesting new information is accumulating in this area at a rapid rate. However, other areas of potentially equal importance may require an unusually imaginative investigation to determine their biological significance. For example, does hyaluronan act as a polyvalent template for efficient pericellular interactions that promote cell division and migration?
Do hyaluronan-cell interactions contribute to mechanical regulation of signaling in a similar manner to integrin-mediated interactions (78)? Is hyaluronan deposited directly into the cytoplasm, and what is its function therein? What is the role of hyaluronan internalization and degradation in cell behavior? We look forward to very exciting developments in the near future.

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