Minireview

Signaling Properties of Hyaluronan Receptors*

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In 1979, hyaluronan was demonstrated to bind specifically and with high affinity to intact cells (1), and in 1980, it was shown to enhance cell motility on two-dimensional culture surfaces where the hydrodynamic properties of hyaluronan were not necessarily too open to spaces for cells to move into (2). These two demonstrations raised the possibility that hyaluronan had the potential to directly modify cell behavior. In 1989, hyaluronan was shown to promote protein tyrosine phosphorylation cascades (3) that were later proven to be required for hyaluronan-mediated motility on planar culture surfaces (4). Since then, small amounts (nanograms) of hyaluronan have been shown to activate a variety of protein tyrosine and serine/threonine kinases. These include the non-receptor protein tyrosine kinase Src (5, 6), HER2/Neu receptor (7), focal adhesion kinase (4, 5–10), protein kinase C (11, 12), and MAP kinases (9, 10). Likely as a consequence of regulating these kinases, hyaluronan promotes expression of specific cytokines and proteins involved in extracellular matrix remodeling (e.g. Ref. 13).

The study of murine cardiac cells derived from hyaluronan synthase 2 (HAS2) knockout mice has provided the most convincing evidence for a signaling capability of hyaluronan (14). HAS2–/– cardiac cells do not undergo an endothelial-mesenchymal transformation associated with migration from tissue explants whereas wild-type cells do (14). However, the addition of nanogram amounts of exogenous hyaluronan “rescues” knockout cells. Furthermore, a dominant negative mutant of the small GTPase, Ras, blocks the effects of exogenous hyaluronan (14). These results suggest that hyaluronan signals through Ras to regulate motility and are consistent with previous studies showing that exogenous hyaluronan-receptor interactions regulate Ras signaling (4, 8). This ability of hyaluronan to activate intracellular signaling cascades requires interactions with cell-associated hyaluronan-binding proteins or hyaladherins (15) but is additionally modified by the amount and size of hyaluronan present in the environment of the cell. Further, not all cell types activate signaling cascades in response to hyaluronan (11), indicating that cell background is also an important determinant. Here, we review current understanding of the mechanisms by which hyaluronan signals.

Role of Hyaluronan Receptors in Signaling

The first cell-associated hyaladherin, RHAMM, whose cell surface form is now designated CD168, was isolated from embryonic heart cells (16). Later CD44 was identified as the first integral hyaluronan “receptor.” Both RHAMM and CD44 mediate hyaluronan signaling and participate in growth factor-regulated signaling. However, they likely regulate signaling by different mechanisms because they are not homologous proteins, are compartmentalized differently in the cell (17), and differ in the mechanisms by which they bind to hyaluronan (18) (Figs. 1 and 2). Additional cellular hyaladherins have been identified (19–22), but their role in cell signaling has not yet been reported. Therefore, this review focuses upon the signaling cascades that RHAMM and CD44 regulate.

CD44

CD44 is an integral protein that is subject to extensive alternative splicing (23–26). All CD44 isoforms contain a link module hyaluronan-binding site in their extracellular domain (see minireview by Day and Prestwich (18) in this series). The binding of CD44 isoforms to hyaluronan affects cell adhesion to extracellular matrix components and is implicated in the stimulation of aggregation, proliferation, migration, and angiogenesis (23–25, 27, 28). The intracellular domain of CD44 isoforms selectively interacts with cytoskeletal proteins and regulates specific signaling (27). Therefore, CD44 isoforms likely provide a direct association between hyaluronan and the cytoskeleton. The mechanisms by which CD44 achieves this association and the signaling cascades that it regulates are summarized in Fig. 1.

CD44 Interaction with Tyrosine Kinases—CD44 is tightly coupled with at least two tyrosine kinases, p185HER2 (7) and c-Src kinase (6). CD44 and p185HER2 are physically linked to each other via interchain disulfide bonds; and hyaluronan can stimulate CD44-associated p185HER2 tyrosine kinase activity that leads to increased tumor cell growth (7). The cytoplasmic domain of CD44 binds to c-Src kinase at a single site with high affinity (6, 29). Importantly, hyaluronan interaction with CD44 stimulates c-Src kinase activity, increasing tyrosine phosphorylation of the cytoskeletal protein, cortactin. This attenuates the ability of cortactin to cross-link filamentous actin in vitro (Fig. 1) (6). Most Src family kinases are modified with specific lipids that direct them to sub-domains of the cell membrane called “rafts” that have high cholesterol and glycolipid content. The Src kinases, Lck and Fyn, associate with CD44 in glycosphingolipid-rich plasma membrane domains of human peripheral blood lymphocytes (29). Thus, direct binding of CD44 to c-Src kinase in the membrane “rafts” may facilitate hyaluronan-mediated stimulation of the catalytic activity of c-Src kinase and induce cytoskeleton-regulated tumor cell migration. Therefore, the binding of hyaluronan to CD44 isoforms, which complex with p185HER2 and c-Src kinase, likely trigger direct “cross-talk” between two tyrosine kinase-linked signaling pathways during tumor progression.

CD44-specific Activation of Rho-like GTPhases—Rho GTPhases such as RhoA and Rac1 participate in the interaction between CD44 and cytoskeletal proteins. In particular, RhoA is non-covalently linked to a CD44 alternate isoform (e.g. CD44v3,8–10) in breast tumor cells (30). When complexed with CD44v3, RhoA stimulates Rho kinase (ROK) to phosphorylate several cellular proteins including CD44v3,8–10. This phosphorylation promotes binding of the CD44 variant to ankyrin (Fig. 1). Overexpression of the Rho-binding domain can act as a dominant negative inhibitor of ROK and reverse tumor cell-specific phenotypes (30). Therefore, it has been proposed that CD44v3,8–10 and RhoA-mediated signaling are involved in the up-regulation of ROK and that this is necessary for membrane-cytoskeleton interactions and tumor cell migration during the progression of breast cancers (30).

Binding of hyaluronan to some CD44-expressing cells also activates Rac1 signaling, a pathway known to regulate actin assembly that is associated with membrane ruffling, cellular projections, cell

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¶ The abbreviations used are: MAP, mitogen-activated protein; HAS2, hyaluronan synthase 2; ROK, Rho kinase; ERK, extracellular-regulated kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PDGF, platelet-derived growth factor; RHAMM, receptor for hyaluronan-mediated motility.

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Motility, and cell transformation (31, 32). In particular, the cytoplasmic domain of CD44 binds to guanine nucleotide exchange factors such as Tiam1 and Vav2 that have been shown to catalyze the GDP-GTP exchange leading to hyaluronan-mediated tumor cell migration (Fig. 1) (32–34). The fact that both Tiam1-Rac1 activation during hyaluronan signaling (Fig. 1) also promotes the association of CD44 forms with Vav2. Hyaluronan (HA) also promotes the association of CD44 forms with cytoskeletal proteins such as ankyrin and ERM proteins. Activation of these signaling pathways together leads to tumor behavior such as migration and invasion. Our current model suggests that the close interactions between CD44 and its selected binding partners play a pivotal role in coordinating “cross-talk” among various intracellular signaling pathways (e.g., Rho/Ras signaling and receptor-linked (p185HER2)/non-receptor-linked (c-Src) tyrosine kinase pathways) leading to the concomitant onset of multiple functions such as tumor cell adhesion, proliferation/growth, migration, and invasion. MLC, myosin light chains.

RHAMM

Like CD44, RHAMM is alternatively spliced. Truncated RHAMM forms are also expressed in cells following injury (36), in tumors, and in some mutant active Ras-transformed cell lines (9, 37–39). RHAMM distributes into multiple compartments including the cell surface (40), cytoskeleton (41), mitochondria (42), and cell nucleus (41, 43). The RHAMM gene does not encode a traditional leader sequence to permit secretion via the traditional Golgi/endoplasmic reticulum export pathway, thus, resembling proteins such as bFGF, HIV Tat protein, the homeobox protein engrailed (44), heat shock proteins (45, 46), and epimorphin (47).

The binding of exogenous hyaluronan to cell surface RHAMM plays a key role in activating signaling cascades, probably as a co-receptor for integral membrane proteins. Although the role(s) of intracellular RHAMM protein forms are not yet known, their ability to associate with kinases (5, 9), calmodulin (41, 42), and the cytoskeleton (41, 43) predicts that they play key roles in cytoskeletal assembly. The presence of intracellular hyaladherins typified by RHAMM also raises the interesting possibility that intracellular hyaluronan (48–50) plays a role in signaling. If so, the separation of cell surface RHAMM from the intracellular RHAMM forms provides the potential for a modular association among hyaluronan, cytoskeleton-signaling complexes, and the cell nucleus (Fig. 2). In this case, hyaladherins such as RHAMM could contribute to the basal and flow switch between the cell surface and extracellular environment, a phenomenon that has been termed “dynamic reciprocity” (51). For instance, RHAMM modules could represent a modified version of “inside-outside” signaling characteristic of integrin receptors (52).

RHAMM Interaction with Tyrosine and Serine/Threonine Kinases (see Fig. 2)—Cell surface RHAMM-hyaluronan interactions mediate activation of the protein tyrosine kinases, Src (5) and focal adhesion kinase (4, 8, 10), as well as Erk kinases (10) and protein kinase C (11, 12). Additionally, cell surface RHAMM is required for activation of Erk kinases through PDGF (9), nerve growth factor...
UTES WITH contactin. Exogenous hyaluronan initially promotes RHAMM occurs in cell lamellae and podosomes where it co-distrib-

ution (8). This is consistent with a requirement for Ras in the different points along the signaling pathways (e.g. face and intracellular forms of RHAMM may regulate Ras but at

(55), and transformation by oncogenic Ras (8), as determined by

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RhAMM because blocking RHAMM antibodies inhibit, whereas agonist RHAMM antibodies mimic this effect (6, 7). The rapid formation and then disassembly of focal adhesions precede a hya-

luronan-induced increase in cell motility (4). These effects are possibly mediated, either directly or indirectly, through integrin receptors. For example, cell surface RHAMM co-regulates migration of thymocytes on fibronectin substrata in concert with the integrin fibronectin receptors, αβ1 or αβ5 (56).

Intracellular RHAMM forms associate with the actin cytoskele-
ton and both interphase and mitotic spindle microtubules (41). The ability of RHAMM to form coiled coils as well as its limited homol-
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potentially the cytoskeleton and the nucleus.

RHAMM Regulation of Ras GTPase—Several studies have indi-
cated that RHAMM regulates Ras (4, 5, 8, 9, 37, 55), and this regulation likely involves both cell surface and intracellular RHAMM forms. Cell surface RHAMM is required for random motility (4, 5), progression through the G2M boundary of cell cycle

(55), and transformation by oncogenic Ras (8), as determined by

antibody blocking and soluble protein competition. Both cell sur-

defice in the absence of CD44 or RHAMM. Phenotypes for CD44–/– have been described (Ref. 64 and see below).

Potential Role of Hyaluronan Size in Signaling: Importance in Response to Injury

In physiologic conditions, hyaluronan is a high average molecular mass polymer in excess of 10^6 Da. However, following tissue injury, hyaluronan fragments of lower molecular mass accumulate. A potential functional significance for hyaluronan fragments has been suggested by in vitro studies (e.g. Refs. 65–67). Oligomers of 8–16 disaccharides prepared by enzymatic digestion of native hya-
luronan induce angiogenesis in a chick corneal assay whereas the native hyaluronan molecules do not (68). Small amounts of high molecular weight hyaluronan can activate protein-tyrosine kinase cascades in endothelial cells and Ras-transformed fibroblasts (4, 10), although at lower levels than smaller fragments (10). Studies with inflammatory macrophages have shown that fragmented hya-
luronan with an average molecular mass of 250,000 Da, but not native hyaluronan from which it was prepared, can induce the expression of inflammatory genes (64, 65–69). Similar results have been shown with renal tubular epithelial cells (70), T-24 carcinoma cells (71), and eosinophils (72). Smaller hyaluronan oligosaccha-

des in the 6–20 kDa size range (but not the 250,000 Da or higher molecular mass hyaluronan) induce inflammatory gene expression in
dendritic cells (73). Controls for excluding contaminating sub-

cances that can be present in even medical grade hyaluronan preparations are often lacking, as noted in Ref. 75. Nevertheless, biological relevance is suggested by reports showing that frag-


ted hyaluronan that induces inflammatory gene expression in vitro is in the same size range as hyaluronan that accumulates under inflammatory conditions in vivo (74). A common theme ap-

pears to be that low (but not high) molecular weight hyaluronan can initiate gene transcription geared toward cell proliferation and migration. Generation of hyaluronan fragments under conditions of inflammation, tumorigenesis, or tissue injury as a result of hyaluronidases (76) or oxidation (77) may then signal the host that normal homeostasis has been profoundly disturbed.

Role of Hyaladherins in Hyaluronan Internalization and Host Response to Injury

Cellular hyaladherins also bind and internalize hyaluronan (1). Depending on the cell type, the binding affinity and rate of internal-

ization varies. Elegant studies have recently shown that the avidity of binding of hyaluronan oligomers to CD44 increases with an oligomer size of up to 38 sugars (78). Labeled hyaluronan is also observed in the cytoplasm as a diffuse network and in vesicles, in lamellae and the nucleus of smooth muscle cells and fibroblasts (49, 50). This unusual pattern of uptake is most obvious following stimulation of serum-starved 3T3 cells or in subconfluent, mutant active Ras-transfected fibroblasts. Hyaluronan uptake into these novel compartments is associated with enhanced cell motility (49). CD44-deficient mice develop normally but exhibit abnormalities in hematopoiesis and lymphocyte recirculation (79, 80). Induction of inflammatory gene expression in response to hyaluronan is ob-

served in the absence of CD44 in cultures of bone marrow and dendritic cells (73, 81). These data suggest that there are CD44-
dependent mechanisms for induction of gene expression by hya-
luronan. In contrast, CD44-deficient mice challenged in models of tissue injury have revealed essential roles for CD44 in regulating pathogenesis of host injury (82, 83). Depending on the mechanism of pathogenesis and the predominant cell types that mediate the host injury, differing effects of CD44 have been observed. In a model of endothelial cell injury mediated by interleukin-2, CD44-
deficient mice were protected from endothelial injury (82). This protection may be because of a decrease in interleukin-2-induced lymphocyte-activated killer cell activity. However, in a model of hepatocellular injury due to administration of concanavalin A, CD44-deficient mice exhibited enhanced hepatitis (83). The increased susceptibility to hepatocyte injury correlated with the ob-
servation that T cells from CD44-deficient animals were resistant to activation-induced cell death. The challenge of future studies

3 C. Toelg, S. Hamilton, and E. A. Turley, unpublished results.
will be to sort out the CD44-dependent as well as the independent roles in mediating hyaluronan-cell interactions.

Conclusions

Hyaluronan signaling involves cellular hyaladherins such as CD44 and RHAMM that are selectively coupled with their particular downstream signaling pathway(s) leading to the onset of hyaluronan-dependent functions in various cell types and tissues. A signaling response to hyaluronan may be strongly influenced by both the size of hyaluronan and the cell background. Additional studies with defined preparations of hyaluronan of varying size that utilize hyaladherin null mice are clearly required to sort out the cellular conditions that permit hyaluronan-mediated signaling.

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