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MINI REVIEW

Hyaluronan promotes the malignant phenotype

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Hyaluronan is a high-molecular-weight, negatively charged polysaccharide with unusual physical and interactive properties. Hyaluronan is localized in the extracellular matrix, at the cell surface, and inside cells. Its tissue distribution is ubiquitous, but it is particularly concentrated in pericellular matrices surrounding proliferating and migrating cells. Hyaluronan contributes to cell behavior in at least three ways. Its unique physical properties influence the biomechanical properties of extracellular and pericellular matrices; it is a template for assembly of other pericellular macromolecules; and it interacts directly with cell surface receptors that transduce intracellular signals. Experimental studies in animal models have documented a crucial role for hyaluronan in tumor growth and metastasis. Cellular manipulations have shown that hyaluronan promotes anchorage-independent growth and invasiveness, hallmarks of the malignant phenotype.

Key words: anchorage-independent growth/CD44/hyaluronan/ RHAMM/ tumor invasion

Introduction

Hyaluronan (also called hyaluronic acid or hyaluronate) is a linear glycosaminoglycan composed of repeating disaccharides of glucuronic acid and N-acetylglucosamine:[β1,4-GlcUA-β1,3-GlcNAc-]$_n$. It usually contains 2000–25,000 disaccharides, exhibits varying molecular weights ranging from 10^5 to 10^7 Da, and has an extended length of up to 25 μm, depending on tissue source and physiological conditions. In free solution under physiological conditions of pH and ionic strength, it forms a stiffened and expanded random coil. It can occupy a very large solvent domain, but at relatively high concentration individual molecules entangle and form continuous networks. Because, in tissues, hyaluronan interacts with numerous binding proteins (known as hyaladherins) (Toole, 1990), its conformation is likely to alter dramatically from that measured under free solution conditions. Hyaluronan is distributed ubiquitously in vertebrate tissues, both in the embryo and in the adult. It is also produced by some bacteria and viruses but is apparently absent in invertebrate metazoans.

The hyaluronan synthases (identified as *Has1*, *Has2*, and *Has3*) are integral plasma membrane proteins whose active sites are located at the intracellular face of the membrane (Weigel *et al.*, 1997; DeAngelis, 1999). Newly synthesized hyaluronan is extruded directly onto the cell surface; it is either retained there by sustained attachment to the synthase or by interactions with receptors, or it is released into pericellular and extracellular matrices. Hyaluronan is also found in several intracellular compartments. Regulation of targeting to these various locations is not understood at this time.

Hyaluronan has multiple physiological and cellular roles that arise from its unique biophysical and interactive properties. Its charge characteristics and polymeric properties contribute in several ways to tissue homeostasis and biomechanics. Its interactions with other extracellular macromolecules (especially aggregating proteoglycans) are crucial to the assembly and integrity of extracellular and pericellular matrices. Its interactions with cell surface receptors, such as CD44 and RHAMM, influence cell behavior in a variety of morphogenetic and physiological systems. Both RHAMM and CD44 exist as multiple, alternatively spliced forms that vary in their physiological functions. RHAMM is present both on the cell surface and intracellularly and most likely has different functions at these sites.

Several recent reviews discuss the properties and normal functions of hyaluronan and its binding partners in more detail (Toole, 2000, 2001; Lee and Spicer, 2000; Day and Prestwich, 2001; Tammi *et al.*, 2001; Turley *et al.*, 2001). There is also an excellent and extensive series of reviews on the Web (see "Science of Hyaluronan Today" at *http://www.glycoforum.gr.jp*).

Numerous studies performed over the past three decades have demonstrated a correlation between levels of hyaluronan production and malignancy in several types of tumor, both in animal models and in human patients (Toole *et al.*, 1979, 2001; Knudson *et al.*, 1989; Knudson, 1996). In the case of human breast, ovarian, and colon carcinomas, a high level of hyaluronan associated with cancer cells themselves or with the tumor stroma is a reliable prognostic indicator of patient morbidity (Ropponen *et al.*, 1998; Anttila *et al.*, 2000; Auvinen *et al.*, 2000). This review summarizes some of the experimental evidence demonstrating the important role of hyaluronan in tumor progression and probing the possible underlying mechanisms whereby hyaluronan influences the malignant phenotype.

Hyaluronan influences cell behavior by several different mechanisms

There are three fundamentally different ways in which hyaluronan can influence normal and abnormal cell behavior. First, due to its unique biophysical properties, free hyaluronan has a profound effect on the biomechanical properties of extracellular and pericellular matrices in which cells reside. Second, hyaluronan forms a repetitive template for specific interactions with other pericellular macromolecules, thus contributing to the assembly, structural integrity, and physiological properties of these matrices. Third, hyaluronan interacts with cell surface receptors that transduce intracellular signals and influence cellular form and behavior. A recent study of *Has2*-knockout mice (Camenisch *et al.*, 2000) highlights the various ways hyaluronan contributes to tissue structure and cell behavior: (1) extracellular matrices in *Has2*-null mice are more compact, i.e., less hydrated, than normal; (2) the organization of pericellular matrix components, especially versican, is altered; and (3) major defects occur in morphogenetic events, especially those involved in development of the cardiovascular system. With respect to the latter, cell migration and epithelial-mesenchymal transformation required for development of cardiac cushion tissues are inhibited. This is due at least in part to the absence of hyaluronan–cell interactions that regulate intracellular Ras signaling (Camenisch *et al.*, 2000).

Numerous studies of embryonic development and adult tissue remodeling have demonstrated that extracellular matrices surrounding proliferating and migrating cells are enriched in hyaluronan and are often highly hydrated. Hyaluronan has the ability to trap large amounts of water within its structure and to exert swelling pressure as a result of compressing hyaluronan molecules into a volume less than that occupied when fully extended. Thus, an early interpretation of the association of changes in hyaluronan concentration or organization with dynamic cell behavior was that hyaluronan creates fluid, malleable matrices in which cells can readily change shape during mitosis or penetrate tissues during migration (Toole, 1991, 2000). In agreement with this idea, it was shown in a recent study that addition of hyaluronan promotes glioblastoma cell migration within a fibrin gel by causing increased hydration and thus gel porosity (Hayen *et al.*, 1999). It also appears that extrusion of hyaluronan onto the cell surface at mitosis may create a hydrated microenvironment that promotes partial detachment and rounding of dividing cells. Hyaluronan synthase activity fluctuates with the cell cycle, peaking at mitosis, and inhibition of hyaluronan synthesis leads to cell cycle arrest at mitosis, just before cell rounding and detachment (Brecht *et al.*, 1986). Also, hyaluronan-dependent pericellular matrix forms around dividing cells immediately preceding mitosis; removal of this matrix by competitive displacement with hyaluronan oligosaccharides inhibits cell division (Evanko *et al.*, 1999). A similar hyaluronan-dependent matrix assembles around migrating cells, especially at the leading and trailing edges, and displacement of this matrix with hyaluronan oligomers reduces the rate of movement (Banerjee and Toole, 1992; Evanko *et al.*, 1999).

These observations suggest that the biomechanical properties of pericellular hyaluronan may facilitate regional detachment of a cell from its substratum, a necessary step in both mitosis and migration**,** by creating a hydrated zone around the cell. This hydrated zone, or pericellular matrix, would separate the cell from physical barriers to penetration or shape changes required for division or movement. However, to create this hydrated pericellular matrix, hyaluronan requires cooperative interactions with other matrix molecules, especially "aggregating" proteoglycans, such as aggrecan (Knudson and Knudson, 2001) or versican (Evanko *et al.*, 1999; Camenisch *et al.*, 2000). Interaction with these proteoglycans causes hyaluronan to take on a more linear conformation that extends out from the cell surface, where it is tethered either by binding to CD44 or by transmembrane interaction of "nascent" hyaluronan with hyaluronan synthase (Lee *et al.*, 1993; Toole, 2001). The resulting pericellular matrix also incorporates other agents, such as the hyaladherins, TSG-6, and inter-α-trypsin inhibitor (Carrette *et al.*, 2001; Mukhopadhyay *et al.*, 2001), which may contribute to the physical properties of the matrix or to cell surface interactions that lead to downstream cellular events.

In addition to its roles in the biomechanical and physiological properties of matrices, hyaluronan also interacts directly with the cell to influence intracellular signaling. Hyaluronan interacts with the plasma membrane in at least two ways, that is, by sustained attachment to hyaluronan synthase on the cytoplasmic side of the plasma membrane or by binding to cell such surface receptors as CD44 and RHAMM. As mentioned, hyaluronan synthase activity and extrusion of hyaluronan onto the cell surface peak at mitosis. In addition, however, hyaluronan is enriched within the cytoplasm of proliferating cells, especially around and between chromosomes prior to mitosis (Evanko and Wight, 1999). Also, hyaluronan internalized by *ras*-transformed cells is distributed to several intracellular sites and promotes cell motility (Collis *et al.*, 1998). Several intracellular hyaladherins that interact with kinases important in regulation of cell behavior have been characterized (Grammatikakis *et al.*, 1995; Entwistle *et al.*, 1996; Huang *et al.*, 2000; Turley *et al.*, 2001). Thus it has been proposed that intracellular hyaluronan interactions may be involved in coordination of hyaluronan synthase activity, intracellular hyaluronan concentrations, and cell behavior.

In a more conventional manner, binding of hyaluronan to its cell surface receptors leads to many downstream signaling events, the precise nature of which depends on cell type and physiology (Herrlich *et al.*, 2000; Turley *et al.*, 2001). This very active area of research, as it relates to the malignant phenotype, is discussed in the following sections.

Experimental manipulation of hyaluronan *in vivo* influences tumor progression

Experimental evidence has been obtained in animal models that directly implicates hyaluronan in tumor progression. Several approaches have been used, including manipulation of levels of hyaluronan and perturbation of endogenous hyaluronan interactions.

Experimental overexpression of the hyaluronan synthase, *Has2*, in HT1080 human fibrosarcoma cells gives rise to elevated hyaluronan production and causes increased tumor growth *in vivo* (Kosaki *et al.*, 1999). Similar results were obtained on overexpression of *Has3* in TSU human prostate tumor cells (Liu *et al.*, 2001a). In another study, mouse

mammary carcinoma cell lines were selected for high and low hyaluronan production and tested for growth and metastasis *in vivo*. In this case no effect on growth was observed, but decreased formation of metastatic nodules in the lung after intravenous injection occurred in lines selected for low hyaluronan production; the metastatic potential of these cells was rescued by increasing hyaluronan production via transfection with *Has1* (Itano *et al.*, 1999).

Three approaches that have been used to manipulate endogenous hyaluronan–protein interactions are overexpression of soluble hyaladherins, administration of hyaluronan oligosaccharides, and treatment with antibodies that block hyaluronan–CD44 binding. Soluble hyaladherins competitively displace hyaluronan from its endogenous cell surface receptors, for example, CD44 or RHAMM, thus inhibiting putative downstream events. Several studies have demonstrated inhibition of tumor progression by treatment with soluble forms of CD44 (e.g., Sy *et al.*, 1992; Bartolazzi *et al.*, 1994). Overexpression of soluble CD44 in mouse mammary carcinoma cells or in human malignant melanoma cells leads to inhibition *in vivo* of growth, local invasion, and metastasis (Yu *et al.*, 1997; Yu and Stamenkovic, 1999; Peterson *et al.*, 2000; Ahrens *et al.*, 2001b). No significant effects were obtained in these studies if the soluble CD44 was mutated such that hyaluronan binding was eliminated. Soluble RHAMM, another hyaladherin, also inhibits metastasis (Mohapatra *et al.*, 1996) and a hyaluronan-binding complex from cartilage, containing link protein and fragments of aggrecan, inhibits both tumor growth and metastasis (Liu *et al.*, 2001b). Hyaluronan oligomers compete for endogenous polymeric hyaluronan–receptor interactions, thus resulting in low-valency, low-affinity binding rather than polyvalent, high-affinity interactions with receptors (Underhill *et al.*, 1983). Oligomers containing 6–18 sugar residues are effectively monovalent in their interaction with CD44 (Lesley *et al.*, 2000). Thus displacement of endogenous polymeric hyaluronan with oligomers of this size could potentially lead to the loss of hyaluronan-induced signaling. In similar fashion to soluble hyaluronan-binding proteins, these oligomers inhibit growth of several tumor types in vivo (Zeng *et al.*, 1998; Ghatak *et al.*, unpublished data). Likewise, treatment with antibodies that block hyaluronan binding to CD44 inhibit tumor growth and invasion (Guo *et al.*, 1994; Zahalka *et al.*, 1995).

Although the cited data point clearly to a crucial role for hyaluronan in tumor progression *in vivo*, other data suggest that this involvement is complex. For example, methods used to perturb hyaluronan interactions, that is, overexpression of soluble hyaladherins or treatment with hyaluronan oligomers, could have manifold biochemical effects with differing downstream consequences. These include competition for binding of endogenous hyaluronan to cell surface receptors, as discussed (Ahrens *et al.*, 2001b), reduced hyaluronan endocytosis and degradation (Yu *et al.*, 1997), interference with CD44 clustering in the plasma membrane (Yu and Stamenkovic, 1999), and disruption of interactions within the pericellular matrix (Yu *et al.*, 1992; Knudson *et al.*, 1999; Knudson *et al.*, 2000). Also, it is possible that, in addition to interfering with downstream effects of hyaluronan polymer interactions, hyaluronan oligomers may interact with cells in a manner that directly induces signaling. Direct signaling by oligomers and small polymers of hyaluronan has been demonstrated with endothelial cells (Deed *et al.*, 1997; Slevin *et al.*, 1998), macrophages (Horton *et al.*, 1998), and other cell types (Turley *et al.*, 2001). In the case of endothelial cells, such signaling is mediated by RHAMM and CD44 (Lokeshwar and Selzer, 2000; Savani *et al.*, 2001) and leads to increased angiogenesis (West *et al.*, 1985; West and Kumar, 1989b; Montesano *et al.*, 1996). A related finding is that tumor cells often exhibit elevated levels not only of hyaluronan itself but also of hyaluronidase and the ability to internalize and degrade hyaluronan (Culty *et al.*, 1994; Liu *et al.*, 1996; Yu *et al.*, 1997; Lokeshwar *et al.*, 2001). It has been concluded from these studies that angiogenic hyaluronan oligomers are produced in aggressive tumors and would be expected to promote tumor progression (Liu *et al.*, 1996; Lokeshwar *et al.*, 2001). However, administration of such oligomers *in vivo* inhibits tumor growth (Zeng *et al.*, 1998; Ghatak *et al.*, unpublished data), and at least one of the several hyaluronidase genes corresponds to a previously mapped tumor suppressor (Csoka *et al.*, 1998). Further study is necessary to clarify these apparent contradictions.

Hyaluronan stimulates intracellular signaling pathways that promote anchorage-independent growth and invasiveness of tumor cells

Recent investigations indicate that hyaluronan is critically involved in anchorage-independent survival and growth, one of the fundamental attributes of the malignant phenotype. Overexpression of *Has2* in HT1080 human fibrosarcoma cells (Kosaki *et al.*, 1999) or Mero-25 mesothelioma cells (Li and Heldin, 2001) has been shown to stimulate anchorage-independent growth in soft agar. Other studies have demonstrated that perturbation of endogenous hyaluronan interactions, either by overexpression of soluble CD44 (Peterson *et al.*, 2000) or by addition of hyaluronan oligomers (Ghatak *et al.*, unpublished data), inhibits anchorage-independent growth of several tumor cell types, including breast carcinoma, colon carcinoma, and glioma cells. Such perturbations lead to cell cycle arrest or apoptosis of the tumor cells, both *in vivo* (Yu *et al.*, 1997; Peterson *et al.*, 2000) and under anchorage-independent conditions *in vitro* (Ghatak *et al.*, unpublished data). The underlying mechanism whereby hyaluronan oligomers inhibit anchorage-independent growth is suppression of the phosphoinositol-3-kinase/Akt survival pathway (Ghatak *et al.*, unpublished data). This is in agreement with other studies showing involvement of this pathway in signaling events arising from interactions of hyaluronan with CD44 (Kamikura *et al.*, 2000; Sohara *et al.*, 2001).

Although the evidence suggests a positive role for hyaluronan in tumor cell proliferation, *in vitro* studies of anchorage-dependent growth have produced variable results. One reason for this is that in many cases the effect of hyaluronan on cell proliferation in routine culture is highly dependent on concentration and molecular weight. High concentrations of high-molecular-weight hyaluronan are usually inhibitory, whereas low concentrations of lower-molecular-weight hyaluronan can be neutral or stimulatory (Goldberg and Toole, 1987); the molecular basis of these different effects is not known. A recent study has shown that hyaluronan-dependent interaction of CD44 with the tumor suppressor merlin mediates contact inhibition of growth of

several cell types (Morrison *et al.*, 2001). Apparently, however, many tumor cells have lost this inhibitory mechanism because hyaluronan–CD44 interactions clearly promote proliferation *in vitro* and growth *in vivo* in several tumor cell types, such as melanoma (Bartolazzi *et al.*, 1994; Ahrens *et al.*, 2001a,b), mammary carcinoma (Peterson *et al.*, 2000), and ovarian carcinoma (Bourguignon *et al.*, 1997). In the case of ovarian tumor cells, hyaluronan-stimulated proliferation has been shown to involve interaction of CD44 with and activation of the HER2/neu oncogene (Bourguignon *et al.*, 1997). Also, hyaluronan may influence growth by activating Ras signaling through interactions with CD44 and RHAMM (Zhang *et al.*, 1998; Camenisch *et al.*, 2000; Fitzgerald *et al.*, 2000).

As noted, another mechanism whereby hyaluronan may influence tumor growth is via stimulation of angiogenesis, a process that, again, is likely to be dependent on molecular weight and concentration (West and Kumar, 1989a; Banerjee and Toole, 1992). Recent studies have indicated that interactions of hyaluronan with both cell surface RHAMM and CD44 are involved in endothelial cell proliferation, migration, and assembly of endothelial tubules (Savani *et al.*, 2001) and that hyaluronan stimulates FAK and ERK kinase activities in these cells (Lokeshwar and Selzer, 2000).

A large body of work indicates that hyaluronan–CD44 and hyaluronan–RHAMM interactions promote tumor cell movement and invasion as well as growth. Interactions with src kinases, Rac and Ras signaling pathways, and cytoskeletal components have been implicated in the effects of hyaluronan. In breast carcinoma cells, hyaluronan-stimulated interaction of CD44 with Tiam1 leads to Rac1 signaling. This results in cytoskeletal rearrangements, membrane ruffling and increased cell migration (Bourguignon *et al.*, 2000). Local application of hyaluronan to the surface of mammary epithelial cells also causes Rac1 activation and lamellipodia formation via hyaluronan-CD44 interaction (Oliferenko *et al.*, 2000). However, a puzzling aspect of the latter study was the observation that unsaturated hyaluronan disaccharides, which presumably do not react with CD44 (Underhill *et al.*, 1983; Lesley *et al.*, 2000), also elicited this effect. Stimulation of ovarian carcinoma cell migration by hyaluronan involves interaction of CD44 with and activation of src kinase. This leads to phosphorylation of cortactin, rearrangement of actin filaments, and stimulation of migration (Bourguignon *et al.*, 2001). Hyaluronan–RHAMM interaction induces transient phosphorylation of p125FAK in concert with turnover of focal adhesions in ras-transformed cells, thus leading to initiation of locomotion. Suppression of hyaluronan–RHAMM interaction inhibits cell locomotion (Hall *et al.*, 1994, 1995). These signaling events lead to alterations in the cytoskeleton required for migration and invasion. The cytoplasmic tail of CD44 has been shown to interact with ankyrin (Zhu and Bourguignon, 2000) and the ezrin-radixin-moesin family (Morrison *et al.*, 2001; Yonemura *et al.*, 1998; Zohar *et al.*, 2000) and both types of interaction promote migration. RHAMM also interacts directly with the cytoskeleton (Entwistle *et al.*, 1996; Assmann *et al.*, 1999; Turley *et al.*, 2001).

Another event that promotes migration is shedding of CD44 from the cell surface, presumably allowing the cell to deadhere from hyaluronan during movement. CD44 shedding is mediated by a membrane-associated protease (Okamoto *et al.*, 1999), most likely membrane-type 1-matrix metalloproteinase (Kajita *et al.*, 2001). As a result of cleavage, the cytoplasmic region of CD44 is targeted to the nucleus, where it stimulates transcription of several genes, including CD44 itself (Okamoto *et al.*, 2001). In accord with the studies of signaling discussed previously, cleavage of CD44 is regulated by Ras and Rho family signaling and requires PI3-kinase activity (Kawano *et al.*, 2000). A crucial event in metastasis is invasion of extracellular matrices, a process that involves not only migratory activity but also remodeling of the matrix. Soluble CD44 (Yu *et al.*, 1997; Yu and Stamenkovic, 1999; Peterson *et al.*, 2000), antibody to CD44 (Koochekpour *et al.*, 1995), and hyaluronan oligomers (Ward *et al.*, unpublished data) inhibit tumor cell invasion of extracellular matrix, implicating hyaluronan in this process. An underlying mechanism, demonstrated in mammary carcinoma cells, is hyaluronan-mediated clustering of CD44 that leads to docking of matrix metalloproteinase-9 (gelatinase B) and, consequently, increased invasiveness (Yu and Stamenkovic, 1999). Docking of gelatinase B to CD44 also mediates processing of TGF-beta and stimulation of angiogenesis (Yu and Stamenkovic, 2000).

In conclusion, it is apparent that hyaluronan contributes in many ways to hallmark properties of the malignant phenotype, especially anchorage-independent growth and invasiveness. However, numerous questions remain unanswered. What is the basis for the different effects of various sized preparations of hyaluronan? Why do hyaluronan oligomers stimulate angiogenesis but inhibit tumor growth? What is the significance of active hyaluronan internalization by tumor cells? What are the interrelations between hyaluronan-induced signaling and integrin or growth factor signaling with respect to tumor progression? Are the *Has* genes oncogenes? Is hyaluronan involved in tumor initiation as well as progression? In what ways can manipulation of hyaluronan-induced events be applied therapeutically?

References

Ahrens, T., Assmann, V., Fieber, C., Termeer, C., Herrlich, P., Hofmann, M., and Simon, J.C. (2001a) CD44 is the principal mediator of hyaluronic-acid-induced melanoma cell proliferation. *J. Invest. Dermatol.*, **116**, 93–101.

Ahrens, T., Sleeman, J.P., Schempp, C.M., Howells, N., Hofmann, M., Ponta, H., Herrlich, P., and Simon, J.C. (2001b) Soluble CD44 inhibits melanoma tumor growth by blocking cell surface CD44 binding to hyaluronic acid. *Oncogene*, **20**, 3399–3408.

Anttila, M.A., Tammi, R.H., Tammi, M.I., Syrjanen, K.J., Saarikoski, S.V., and Kosma, V.M. (2000) High levels of stromal hyaluronan predict poor disease outcome in epithelial ovarian cancer. *Cancer Res.*, **60**, 150–155.

Assmann, V., Jenkinson, D., Marshall, J.F., and Hart, I.R. (1999) The intracellular hyaluronan receptor RHAMM/IHABP interacts with microtubules and actin filaments. *J. Cell Sci.*, **112**, 3943–3954.

Auvinen, P., Tammi, R., Parkkinen, J., Tammi, M., Agren, U., Johansson, R., Hirvikoski, P., Eskelinen, M., and Kosma, V.M. (2000) Hyaluronan in peritumoral stroma and malignant cells associates with breast cancer spreading and predicts survival. *Am. J. Pathol.*, **156**, 529–536.

Banerjee, S.D. and Toole, B.P. (1992) Hyaluronan-binding protein in endothelial cell morphogenesis. *J. Cell Biol.*, **119**, 643–652.

Bartolazzi, A., Peach, R., Aruffo, A., and Stamenkovic, I. (1994) Interaction between CD44 and hyaluronate is directly implicated in the regulation of tumor development. *J. Exp. Med.*, **180**, 53–66.

Bourguignon, L.Y., Zhu, H., Chu, A., Iida, N., Zhang, L., and Hung, M.C. (1997) Interaction between the adhesion receptor, CD44, and the oncogene product, p185HER2, promotes human ovarian tumor cell activation. *J. Biol. Chem.*, **272**, 27913–27918.

Bourguignon, L.Y., Zhu, H., Shao, L., and Chen, Y.W. (2000) CD44 interaction with tiam1 promotes Rac1 signaling and hyaluronic acid- mediated breast tumor cell migration. *J. Biol. Chem.*, **275**, 1829–1838.

Bourguignon, L.Y., Zhu, H., Shao, L., and Chen, Y.W. (2001) CD44 interaction with c-Src kinase promotes cortactin-mediated cytoskeletal function and hyaluronic acid-dependent cell migration. *J. Biol. Chem.*, **276**, 7327–7336.

Brecht, M., Mayer, U., Schlosser, E., and Prehm, P. (1986) Increased hyaluronate synthesis is required for fibroblast detachment and mitosis. *Biochem. J.*, **239**, 445–450.

Camenisch, T.D., Spicer, A.P., Brehm-Gibson, T., Biesterfeldt, J., Augustine, M.L., Calabro, A. Jr., Kubalak, S., Klewer, S.E., and McDonald, J.A. (2000) Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. *J. Clin. Invest.*, **106**, 349–360.

Carrette, O., Nemade, R.V., Day, A.J., Brickner, A., and Larsen, W.J. (2001) TSG-6 is concentrated in the extracellular matrix of mouse cumulus oocyte complexes through hyaluronan and inter-alpha-inhibitor binding. *Biol. Reprod.*, **65**, 301–308.

Collis, L., Hall, C., Lange, L., Ziebell, M., Prestwich, R., and Turley, E.A. (1998) Rapid hyaluronan uptake is associated with enhanced motility: implications for an intracellular mode of action. *FEBS Lett.*, **440**, 444–449.

Csoka, T.B., Frost, G.I., Heng, H.H., Scherer, S.W., Mohapatra, G., and Stern, R. (1998) The hyaluronidase gene HYAL1 maps to chromosome 3p21.2-p21.3 in human and 9F1-F2 in mouse, a conserved candidate tumor suppressor locus. *Genomics*, **48**, 63–70.

Culty, M., Shizari, M., Thompson, E.W., and Underhill, C.B. (1994) Binding and degradation of hyaluronan by human breast cancer cell lines expressing different forms of CD44: correlation with invasive potential. *J. Cell Physiol.*, **160**, 275–286.

Day, A.J. and Prestwich, G.D. (2001) Hyaluronan-binding proteins: tying up the giant. *J. Biol. Chem.*, in press.

DeAngelis, P.L. (1999) Hyaluronan synthases: fascinating glycosyltransferases from vertebrates, bacterial pathogens, and algal viruses. *Cell Mol. Life Sci.*, **56**, 670–682.

Deed, R., Rooney, P., Kumar, P., Norton, J.D., Smith, J., Freemont, A.J., and Kumar, S. (1997) Early-response gene signalling is induced by angiogenic oligosaccharides of hyaluronan in endothelial cells. Inhibition by non-angiogenic, high-molecular-weight hyaluronan. *Int. J. Cancer*, **71**, 251–256.

Entwistle, J., Hall, C.L., and Turley, E.A. (1996) HA receptors: regulators of signalling to the cytoskeleton. *J. Cell. Biochem.*, **61**, 569–577.

Evanko, S.P. and Wight, T.N. (1999) Intracellular localization of hyaluronan in proliferating cells. *J. Histochem. Cytochem.*, **47**, 1331–1342.

Evanko, S.P., Angello, J.C., and Wight, T.N. (1999) Formation of hyaluronan- and versican-rich pericellular matrix is required for proliferation and migration of vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.*, **19**, 1004–1013.

Fitzgerald, K.A., Bowie, A.G., Skeffington, B.S., and O'Neill, L.A. (2000) Ras, protein kinase C zeta, and I kappa B kinases 1 and 2 are downstream effectors of CD44 during the activation of NF-kappa B by hyaluronic acid fragments in T-24 carcinoma cells. *J. Immunol.*, **164**, 2053–2063.

Goldberg, R.L. and Toole, B.P. (1987) Hyaluronate inhibition of cell proliferation. *Arthritis Rheum.*, **30**, 769–78.

Grammatikakis, N., Grammatikakis, A., Yoneda, M., Yu, Q., Banerjee, S.D., and Toole, B.P. (1995) A novel glycosaminoglycan-binding protein is the vertebrate homologue of the cell cycle control protein, Cdc37. *J. Biol. Chem.*, **270**, 16198–16205.

Guo, Y., Ma, J., Wang, J., Che, X., Narula, J., Bigby, M., Wu, M., and Sy, M.S. (1994) Inhibition of human melanoma growth and metastasis *in vivo* by anti-CD44 monoclonal antibody. *Cancer Res.*, **54**, 1561–1565.

Hall, C. L., Wang, C., Lange, L.A., and Turley, E.A. (1994) Hyaluronan and the hyaluronan receptor RHAMM promote focal adhesion turnover and transient tyrosine kinase activity. *J. Cell Biol.*, **126**, 575–588.

Hall, C.L., Yang, B., Yang, X., Zhang, S., Turley, M., Samuel, S., Lange, L.A., Wang, C., Curpen, G.D., Savani, R.C., and others (1995) Overexpression of the hyaluronan receptor RHAMM is transforming and is also required for H-ras transformation. *Cell*, **82**, 19–28.

Hayen, W., Goebeler, M., Kumar, S., Riessen, R., and Nehls, V. (1999) Hyaluronan stimulates tumor cell migration by modulating the fibrin fiber architecture. *J. Cell Sci.*, **112**, 2241–2251.

Herrlich, P., Morrison, H., Sleeman, J., Orian-Rousseau, V., Konig, H., Weg-Remers, S., and Ponta, H. (2000) CD44 acts both as a growth- and invasiveness-promoting molecule and as a tumor-suppressing cofactor. *Ann. N. Y. Acad. Sci.*, **910**, 106–118; discussion 118–120.

Horton, M.R., McKee, C.M., Bao, C., Liao, F., Farber, J.M., Hodge-DuFour, J., Pure, E., Oliver, B.L., Wright, T.M., and Noble, P.W. (1998) Hyaluronan fragments synergize with interferon-gamma to induce the C-X- C chemokines mig and interferon-inducible protein-10 in mouse macrophages. *J. Biol. Chem.*, **273**, 35088–25094.

Huang, L., Grammatikakis, N., Yoneda, M., Banerjee, S.D., and Toole, B.P. (2000) Molecular characterization of a novel intracellular hyaluronan-binding protein. *J. Biol. Chem.*, **275**, 29829–29839.

Itano, N., Sawai, T., Miyaishi, O., and Kimata, K. (1999) Relationship between hyaluronan production and metastatic potential of mouse mammary carcinoma cells. *Cancer Res.*, **59**, 2499–2504.

Kajita, M., Itoh, Y., Chiba, T., Mori, H., Okada, A., Kinoh, H., and Seiki, M. (2001) Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J. Cell Biol.*, **153**, 893–904.

Kamikura, D.M., Khoury, H., Maroun, C., Naujokas, M.A., and Park, M. (2000) Enhanced transformation by a plasma membrane-associated met oncoprotein: activation of a phosphoinositide 3′-kinase-dependent autocrine loop involving hyaluronic acid and CD44. *Mol. Cell Biol.*, **20**, 3482–3496.

Kawano, Y., Okamoto, I., Murakami, D., Itoh, H., Yoshida, M., Ueda, S., and Saya, H. (2000) Ras oncoprotein induces CD44 cleavage through phosphoinositide 3-OH kinase and the rho family of small G proteins. *J. Biol. Chem.*, **275**, 29628–29635.

Knudson, C.B. and Knudson, W. (2001) Cartilage proteoglycans. *Sem. Cell Dev. Biol.*, **12**, 69–78.

Knudson, C.B., Nofal, G.A., Pamintuan, L., and Aguiar, D.J. (1999) The chondrocyte pericellular matrix: a model for hyaluronan-mediated cell-matrix interactions. *Biochem. Soc. Trans*, **27**, 142–147.

Knudson, W. (1996) Tumor-associated hyaluronan. Providing an extracellular matrix that facilitates invasion. *Am. J. Pathol.*, **148**, 1721–1726.

Knudson, W., Biswas, C., Li, X.Q., Nemec, R.E., and Toole, B.P. (1989) The role and regulation of tumour-associated hyaluronan. *Ciba Found. Symp.*, **143**, 150–159.

Knudson, W., Casey, B., Nishida, Y., Eger, W., Kuettner, K.E., and Knudson, C.B. (2000) Hyaluronan oligosaccharides perturb cartilage matrix homeostasis and induce chondrocytic chondrolysis. *Arthritis Rheum.*, **43**, 1165–1174.

Koochekpour, S., Pilkington, G.J., and Merzak, A. (1995) Hyaluronic acid/CD44H interaction induces cell detachment and stimulates migration and invasion of human glioma cells *in vitro*. *Int. J. Cancer*, **63**, 450–454.

Kosaki, R., Watanabe, K., and Yamaguchi, Y. (1999) Overproduction of hyaluronan by expression of the hyaluronan synthase Has2 enhances anchorage-independent growth and tumorigenicity. *Cancer Res.*, **59**, 1141–1145.

Lee, G.M., Johnstone, B., Jacobson, K., and Caterson, B. (1993) The dynamic structure of the pericellular matrix on living cells. *J. Cell Biol.*, **123**, 1899–1907.

Lee, J.Y. and Spicer, A.P. (2000) Hyaluronan: a multifunctional, megaDalton, stealth molecule. *Curr. Opin. Cell Biol.*, **12**, 581–586.

Lesley, J., Hascall, V.C., Tammi, M., and Hyman, R. (2000) Hyaluronan binding by cell surface CD44. *J. Biol. Chem.*, **275**, 26967–26975.

Li, Y. and Heldin, P. (2001) Hyaluronan production increases the malignant properties of mesothelioma cells. *Br. J. Cancer*, **85**, 600–607.

Liu, D., Pearlman, E., Diaconu, E., Guo, K., Mori, H., Haqqi, T., Markowitz, S., Willson, J., and Sy, M.S. (1996) Expression of hyaluronidase by tumor cells induces angiogenesis *in vivo*. *Proc. Natl Acad. Sci. USA*, **93**, 7832–7837.

Liu, N., Gao, F., Han, Z., Xu, X., Underhill, C.B., and Zhang, L. (2001a) Hyaluronan synthase 3 overexpression promotes the growth of TSU prostate cancer cells. *Cancer Res.*, **61**, 5207–5214.

Liu, N., Lapcevich, R.K., Underhill, C.B., Han, Z., Gao, F., Swartz, G., Plum, S.M., Zhang, L., and Gree, S.J. (2001b) Metastatin: a hyaluronan-binding complex from cartilage that inhibits tumor growth. *Cancer Res.*, **61**, 1022–1028.

Lokeshwar, V.B. and Selzer, M.G. (2000) Differences in hyaluronic acid-mediated functions and signaling in arterial, microvessel, and vein-derived human endothelial cells. *J. Biol. Chem.*, **275**, 27641–27649.

Lokeshwar, V.B., Rubinowicz, D., Schroeder, G.L., Forgacs, E., Minna, J.D., Block, N.L., Nadji, M., and Lokeshwar, B.L. (2001) Stromal and epithelial expression of tumor markers hyaluronic acid and HYAL1 hyaluronidase in prostate cancer. *J. Biol. Chem.*, **276**, 11922–11932.

Mohapatra, S., Yang, X., Wright, J.A., Turley, E.A., and Greenberg, A.H. (1996) Soluble hyaluronan receptor RHAMM induces mitotic arrest by suppressing Cdc2 and cyclin B1 expression. *J. Exp. Med.*, **183**, 1663–1668.

Montesano, R., Kumar, S., Orci, L., and Pepper, M.S. (1996) Synergistic effect of hyaluronan oligosaccharides and vascular endothelial growth factor on angiogenesis *in vitro*. *Lab. Invest.*, **75**, 249–262.

Morrison, H., Sherman, L.S., Legg, J., Banine, F., Isacke, C., Haipek, C.A., Gutmann, D.H., Ponta, H., and Herrlich, P. (2001) The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. *Genes Dev.*, **15**, 968–980.

Mukhopadhyay, D., Hascall, V.C., Day, A.J., Salustri, A., and Fulop, C. (2001) Two distinct populations of tumor necrosis factor-stimulated gene-6 protein in the extracellular matrix of expanded mouse cumulus cell-oocyte complexes. *Arch. Biochem. Biophys.*, **394**, 173–181.

Okamoto, I., Kawano, Y., Murakami, D., Sasayama, T., Araki, N., Miki, T., Wong, A.J., and Saya, H. (2001) Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. *J. Cell Biol.*, **155**, 755–762.

Okamoto, I., Kawano, Y., Tsuiki, H., Sasaki, J., Nakao, M., Matsumoto, M., Suga, M., Ando, M., Nakajima, M., and Saya, H. (1999) CD44 cleavage induced by a membrane-associated metalloprotease plays a critical role in tumor cell migration. *Oncogene*, **18**, 1435–1446.

Oliferenko, S., Kaverina, I., Small, J.V., and Huber, L.A. (2000) Hyaluronic acid (HA) binding to CD44 activates Rac1 and induces lamellipodia outgrowth. *J. Cell Biol.*, **148**, 1159–1164.

Peterson, R.M., Yu, Q., Stamenkovic, I., and Toole, B.P. (2000) Perturbation of hyaluronan interactions by soluble CD44 inhibits growth of murine mammary carcinoma cells in ascites. *Am. J. Pathol.*, **156**, 2159–2167.

Ropponen, K., Tammi, M., Parkkinen, J., Eskelinen, M., Tammi, R., Lipponen, P., Agren, U., Alhava, E., and Kosma, V.M. (1998) Tumor cell-associated hyaluronan as an unfavorable prognostic factor in colorectal cancer. *Cancer Res.*, **58**, 342–347.

Savani, R.C., Cao, G., Pooler, P.M., Zaman, A., Zhou, Z., and DeLisser, H.M. (2001) Differential involvement of the hyaluronan (HA) receptors CD44 and receptor for HA-mediated motility in endothelial cell function and angiogenesis. *J. Biol. Chem.*, **276**, 36770–36778.

Slevin, M., Krupinski, J., Kumar, S., and Gaffney, J. (1998) Angiogenic oligosaccharides of hyaluronan induce protein tyrosine kinase activity in endothelial cells and activate a cytoplasmic signal transduction pathway resulting in proliferation. *Lab. Invest.*, **78**, 987–1003.

Sohara, Y., Ishiguro, N., Machida, K., Kurata, H., Thant, A.A., Senga, T., Matsuda, S., Kimata, K., Iwata, H., and Hamaguchi, M. (2001) Hyaluronan activates cell motility of v-Src-transformed cells via Ras-mitogen-activated protein kinase and phosphoinositide 3-kinase-Akt in a tumor-specific manner. *Mol. Biol. Cell*, **12**, 1859–1868.

Sy, M.S., Guo, Y.J., and Stamenkovic, I. (1992) Inhibition of tumor growth *in vivo* with a soluble CD44-immunoglobulin fusion protein. *J. Exp. Med.*, **176**, 623–627.

Tammi, M.I., Day, A.J., and Turley, E.A. (2001) Hyaluronan and homeostasis: a balancing act. *J. Biol. Chem.*, in press.

Toole, B.P. (1990) Hyaluronan and its binding proteins, the hyaladherins. *Curr. Opin. Cell Biol.*, **2**, 839–844.

Toole, B.P. (1991) "Proteoglycans and hyaluronan in morphogenesis and differentiation." In Hey, E.D. (ed.), *Cell biology of extracellular matrix*. Plenum Press, New York. pp. 305–341.

Toole, B.P. (2000) "Hyaluronan." In Iozzo, R.V. (ed.), *Proteoglycans*. Marcel Dekker, New York, pp. 61–92.

Toole, B.P. (2001) Hyaluronan in morphogenesis. *Sem. Cell Dev. Biol.*, **12**, 79–87.

Toole, B.P., Biswas, C., and Gross, J. (1979) Hyaluronate and invasiveness of the rabbit V2 carcinoma. *Proc. Natl Acad. Sci. USA*, **76**, 6299–6303.

Toole, B.P., Wight, T.N., and Tammi, M. (2001) Hyaluronan-cell interactions in cancer and vascular disease. *J. Biol. Chem.*, in press.

Turley, E.A., Noble, P.W., and Bourguignon, L.Y. (2001) Signaling properties of hyaluronan receptors. *J. Biol. Chem.*, in press.

Underhill, C.B., Chi-Rosso, G., and Toole, B.P. (1983) Effects of detergent solubilization on the hyaluronate-binding protein from membranes of simian virus 40-transformed 3T3 cells. *J. Biol. Chem.*, **258**, 8086–8091.

Weigel, P.H., Hascall, V.C., and Tammi, M. (1997) Hyaluronan synthases. *J. Biol. Chem.*, **272**, 13997–14000.

West, D.C. and Kumar, S. (1989a) The effect of hyaluronate and its oligosaccharides on endothelial cell proliferation and monolayer integrity. *Exp. Cell Res.*, **183**, 179–196.

West, D.C. and Kumar, S. (1989b) Hyaluronan and angiogenesis. *Ciba Found. Symp.*, **143**, 187–201.

West, D.C., Hampson, I.N., Arnold, F., and Kumar, S. (1985) Angiogenesis induced by degradation products of hyaluronic acid. *Science*, **228**, 1324–1326.

Yonemura, S., Hirao, M., Doi, Y., Takahashi, N., Kondo, T., and Tsukita, S. (1998) Ezrin/radixin/moesin (ERM) proteins bind to a positively charged amino acid cluster in the juxta-membrane cytoplasmic domain of CD44, CD43, and ICAM-2. *J. Cell Biol.*, **140**, 885–895.

Yu, Q. and Stamenkovic, I. (1999) Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev.*, **13**, 35–48.

Yu, Q. and Stamenkovic, I. (2000) Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev.*, **14**, 163–176.

Yu, Q., Banerjee, S.D., and Toole, B.P. (1992) The role of hyaluronan-binding protein in assembly of pericellular matrices. *Dev. Dyn.*, **193**, 145–151.

Yu, Q., Toole, B.P., and Stamenkovic, I. (1997) Induction of apoptosis of metastatic mammary carcinoma cells in vivo by disruption of tumor cell surface CD44 function. *J. Exp. Med.*, **186**, 1985–1996.

Zahalka, M.A., Okon, E., Gosslar, U., Holzmann, B., and Naor, D. (1995) Lymph node (but not spleen) invasion by murine lymphoma is both CD44- and hyaluronate-dependent. *J. Immunol.*, **154**, 5345–5355.

Zeng, C., Toole, B.P., Kinney, S.D., Kuo, J.W., and Stamenkovic, I. (1998) Inhibition of tumor growth *in vivo* by hyaluronan oligomers. *Int. J. Cancer*, **77**, 396–401.

Zhang, S., Chang, M.C., Zylka, D., Turley, S., Harrison, R., and Turley, E.A. (1998) The hyaluronan receptor RHAMM regulates extracellular-regulated kinase. *J. Biol. Chem.*, **273**, 11342–11348.

Zhu, D. and Bourguignon, L.Y. (2000) Interaction between CD44 and the repeat domain of ankyrin promotes hyaluronic acid-mediated ovarian tumor cell migration. *J. Cell Physiol.*, **183**, 182–195.

Zohar, R., Suzuki, N., Suzuki, K., Arora, P., Glogauer, M., McCulloch, C.A., and Sodek, J. (2000) Intracellular osteopontin is an integral component of the CD44-ERM complex involved in cell migration. *J. Cell Physiol.*, **184**, 118–130.