[Frontiers in Bioscience 3, d604-615, July 1, 1998]

THE ROLE OF CD44 AS A CELL SURFACE HYALURONAN RECEPTOR DURING TUMOR INVASION OF CONNECTIVE TISSUE

Warren Knudson

Department of Biochemistry, Department of Pathology, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612-3864

Received 4/27/98, Accepted 5/15/98

TABLE OF CONTENTS

1. ABSTRACT

Tumor progression involves a series of complex interactions between infiltrating malignant cells and adjacent normal tissues. The cell surface receptor CD44 has been implicated as an active participant in a number of these interactions. Although assigned a variety of functions, it is the role of CD44 as a receptor for the glycosaminoglycan hyaluronan that is likely to be of most importance. The matrix macromolecule hyaluronan often becomes deposited in the tissue spaces immediately surrounding invasive tumors. As such, hyaluronan may function as a ligand for CD44-mediated locomotion or assemble into a protective matrix coat surrounding the tumor cells. Alternatively, the adjacent hyaluronan-rich matrices may serve as a barrier to migration, breached in part by aggressive cell types exhibiting a capacity for CD44-mediated hyaluronan endocytosis. The significance of tumor-associated hyaluronan accumulation as well as potential functions of CD44 – hyaluronan interactions are reviewed.

2. INTRODUCTION

The articles in this series focus on the role and significance of CD44 in tumor progression and metastasis. CD44 is a cell surface, single-pass transmembrane glycoprotein. CD44 is expressed by a wide variety of cell types; from dermal fibroblasts to transitional epithelial cells, lymphocytes to macrophages, articular chondrocytes to neural glial cells (1, 2). As with the diversity of CD44-expressing cell types, the purported functions for CD44 are also diverse. Through an interaction with the lining adhesion molecule *mucosal vascular addressin*, CD44 functions as a homing receptor, targeting lymphocytes to the high endothelial venules of the peripheral lymph node (3, 4). As such, CD44 has been designated by other names including; In[Lu]-related p80, Pgp-1, Hermes antigen, HUTCH-1 and H-CAM (5). In other cells, CD44 functions as a "part time" proteoglycan expressing a chondroitin sulfate or heparan sulfate glycosaminoglycan side chain (6, 7). Although intriguing, it is not clear whether the critical aspect of the glycosaminoglycan addition is to modify a functional aspect of CD44 or provide a unique glycosaminoglycan-related role. By far the greatest interest in CD44 has been in relation to its function as a binding protein (i.e., "receptor") for the extracellular matrix macromolecule hyaluronan (a.k.a., hyaluronic acid, hyaluronate). The distal extracellular domain of CD44 contains a B loop motif, homologous to the functional binding domain present on other hyaluronan binding proteins e.g., aggrecan and link protein (1, 7). Although hyaluronan is considered the principal CD44 ligand, other macromolecules have been described that exhibit specific binding to CD44 including; chondroitin, chondroitin sulfate and collagen types I and VI (5, 8). These observations have yielded at least one additional designation for CD44 as the *extracellular matrix receptor type III* (ECM-III). The interaction of CD44 with the latter ligands have been considered of low affinity (and thus of less importance). This may be somewhat misleading as, in actual fact, the monovalent interaction of CD44 with hyaluronan is also of somewhat low affinity. In studies where it has been carefully examined, the binding affinity (K_m) of solubilized hyaluronan receptor derived from SV-3T3 cells (representative of monovalent binding of hyaluronan to CD44) was 7.5 times lower than the K_m observed on intact membranes (conditions that allow for multivalent interactions) (9). Data such as these led to the suggestion

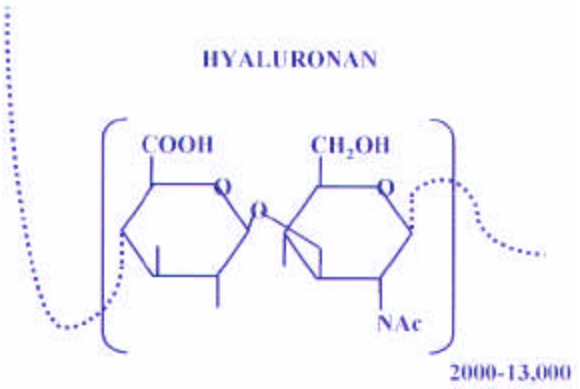

Figure 1. Disaccharide structure of hyaluronan. Hyaluronan is a linear, non-branching polysaccharide chain consisting of the disaccharide unit beta-1,4-N-acetylglucosamine linked beta-1,3 to residues of glucuronic acid. This disaccharide unit is repeated from approximately 2000-13000 times resulting in hyaluronan chains with molecular mass ranging from 1 - 6 x 10^6 Dalton.

that the apparent high affinity for CD44/hyaluronan binding (~10^{-9} M) is due to multivalent binding of hyaluronan to several CD44 receptor sites (together with the high molecular mass and regular repeating structure of hyaluronan). Thus, hyaluronan/CD44 interactions have been considered to be of most importance physiologically. This physiological significance has been confirmed from studies suggesting hyaluronan/CD44 interactions are directly involved in various hyaluronan-dependent cellular functions such as cell migration, matrix assembly, matrix:cell signaling and matrix endocytosis (1).

The question remains, why the interest in CD44 in tumor progression and metastasis? There are perhaps two reasons. First, using monoclonal antibodies to screen for epitopes present on a metastatic pancreatic carcinoma cell line (but not present on the non-metastatic cell line), Gunthert *et al.*, isolated a novel isoform of CD44--an alternatively spliced isoform termed CD44v6 (10). When an antibody specific to the variant CD44 isoform (CD44v6) was co-injected with the metastasizing cells, metastatic growth of the pancreatic carcinoma was inhibited and host survival was prolonged (11). Interest in CD44 variant isoforms peaked when it was found that non-metastatic tumor cells transfected with CD44v6 metastasized efficiently to lymph nodes (10). Since these, now seminal observations, numerous studies have documented the prevalence as well as diagnostic/prognostic value of CD44 variant isoforms in human cancer (e.g., (12-14)).

A second line of interest in CD44 relates more to the ligand hyaluronan. Several tumors, particularly those of human solid cancers as will be discussed below, are often enriched in hyaluronan (15). There has always been a question of whether the increase in tumor-associated hyaluronan provides an advantageous role in tumor progression or an inhibitory role. As data became available that tumor cell expression of CD44 was also upregulated as compared to cells of their tissue of origin (as illustrated in an immunostained section of human breast carcinoma, Figure 2A), a possible link began to emerge. Further, tumor cells not only express a receptor capable of interacting with hyaluronan but also exhibit a capacity to stimulate or direct the *neo*-synthesis of hyaluronan. In this review, the focus will be on this linkage, namely, between tumor cell expression of CD44 and the aberrant levels of hyaluronan that accumulate within tumor tissue. Are they related and do they serve to facilitate tumor progression and metastasis?

3. TUMOR-ASSOCIATION HYALURONAN

In order to assess the significance of CD44 as a hyaluronan receptor during tumor progression, it is first necessary to understand the relationship between hyaluronan itself and tumor progression.

3.1. Structure and synthesis of tumor-associated hyaluronan

All evidence to date suggests that there is no difference in the basic structure of tumor-associated hyaluronan and hyaluronan synthesized by normal tissues. Hyaluronan is a member of the glycosaminoglycans--long nonbranching aminosugar polysaccharides that reside and function primarily within the extracellular space (7). Several properties distinguish hyaluronan as a unique member of the glycosaminoglycan family including: 1) It is the only member that does not contain esterified sulfate residues; 2) Individual chains of hyaluronan are of very high molecule mass, in the range of 1-6 x 10^6 Daltons; 3) Hyaluronan is not synthesized via attachment to a protein core, nor is it synthesized within intracellular organelles and; 4) Hyaluronan is the only glycosaminoglycan synthesized by eukaryotes as well as some prokaryotes. As shown in figure 1, hyaluronan contains a typical glycosaminoglycan repeating disaccharide structure comprised of beta-1,4-N-acetylglucosamine residues linked beta-1,3 to residues of glucuronic acid. Hyaluronan polysaccharide chains containing thousands of these disaccharide units are believed to be synthesized by a single enzyme termed "hyaluronan synthase," a multipass protein localized within the plasma membrane (16-18). As the hyaluronan is synthesized from individual UDP-sugar precursors, the elongated chain is simultaneously extruded through the plasma membrane and eventually released into the extracellular space (18, 19). Hyaluronan is synthesized, to varying degrees, by nearly all cell types (20). Some of the more avid hyaluronan-synthesizing cell types include fibroblasts and related fibrosarcoma, glioma and mesothelioma cells (15).

3.2. Preferential enrichment of tumor tissue with hyaluronan

Recent studies, as will be discussed below, have made attempts to correlate levels of tumor-associated hyaluronan to other parameters of tumor progression, i.e., its use as a prognostic indicator. With this, an inherent assumption has already been made in these studies that hyaluronan is preferentially enriched within tumor extracellular matrix, i.e., it is the glycosaminoglycan of

choice to examine. Is this a valid assumption? Is hyaluronan enriched in tumors over other glycosaminoglycans such as chondroitin sulfate or heparan sulfate? That an enrichment in tumor tissues of some form of polysaccharide-like substance, has long been recognized. A pathology textbook from 1907 describes a "mucin-like" substance commonly associated with malignant breast carcinoma (21). The mucin was described in the text as; acid in reaction, to have an affinity for basic dyes, and to be analogous to the mucinous substance present in the umbilical cord. Although well before the chemical characterization of hyaluronan in 1934 (22), the mucinous substance described was almost certainly hyaluronan. As better techniques became available, investigators re-investigated the composition of tumor-associated extracellular matrix. From the early use of chemical and spectroscopic analysis evolved methods such as cellulose acetate electrophoresis, differential reactivity to basic dyes, differential susceptibility to enzymes (i.e., the advent of purified hyaluronidases, chondroitinases and heparinases) and, HPLC separation of individual glycosaminoglycan disaccharides. Current approaches incorporate the use of specific RIAs, ELISAs and morphological probes. As these techniques evolved, so did the accuracy/validity of the detection. Nonetheless, the overall conclusions remained the same. Many tumors, both of epithelial and connective tissue origins, appear to be selectively or preferentially enriched in hyaluronan. Example tumors include human carcinomas (i.e., mammary, lung, pancreatic, parotid, prostatic, hepatic, esophageal, gastric and colonic carcinoma), as well as human gliomas, nephroblastomas, and mesotheliomas (23). Animal models of chicken sarcoma, fibrosarcoma, melanona and lymphosarcoma have also been described as enriched in hyaluronan (23). Three tumor types in particular within this listing, human mesotheliomas, nephroblastomas (Wilms' tumor) and, to a somewhat lesser extent, breast carcinomas, are considered to have the highest enrichment in hyaluronan. In these tumors, significant increases in hyaluronan are not only present within the primary tumor, but also readily detected in serum and other body fluids using current ELISA and RIA techniques (24-28). Although an increase in the proportion of hyaluronan to other glycosaminoglycans is common to most solid human tumors (as compared to the uninvolved normal tissue), there are notable exceptions. For example, studies on colon and bladder cancer have shown no change, or even a reduction in tumor-associated hyaluronan as compared to corresponding normal tissues (29, 30). However, even in these tumors there are reports to the contrary (25, 31).

Current methodologies rely on ELISAs, RIAs and morphological probes that specifically detect deposition of hyaluronan *in situ*, as a protein would be detected and quantified using monoclonal antibodies. Because hyaluronan is essentially non-immunogenic, the use of anti-hyaluronan antibodies is not available. Thus, indirect methods have been developed using connective tissue proteins such as the major proteoglycan of cartilage, aggrecan, and link protein, that naturally bind hyaluronan with high affinity and specificity via a hyaluronan binding region (HABR) of the protein. Using a biotinylated, partially digested aggrecan / link protein complex (HABR complex), De la Torre *et al.* demonstrated intense hyaluronan staining within sections of human breast carcinoma (32). Most of the hyaluronan was found deposited within the tumor-associated stromal elements. The nests of malignant infiltrating cells were essentially negative. Normal breast connective tissue was totally negative as was the uninvolved connective tissue adjacent to the carcinoma margin. That is, a clear diminution in staining was observed at the tumor margin. As shown in figure 2, panel C, we observed a similar staining pattern in one example of human breast carcinoma (using a similar biotinylated HABR probe). The tumor-involved areas are richly stained for hyaluronan. The tumor cells appear to be essentially unstained, with most reactivity occurring within the stroma. Panel D of figure 2 depicts a higher power micrograph of a different, more cellular, human breast carcinoma. Again, it appears that the predominant deposition of hyaluronan is within the residual stromal connective tissue. Staining within the tumor mass can also be seen however, but at this stage of tumor progression, the tumor stroma and parenchyma become more highly intermixed. In another study, hyaluronan distribution within normal and cancerous human gastrointestinal tissues was examined, again utilizing a biotinylated HABR probe (33). In the normal tissues, only the stratified squamous epithelia of the esophagus exhibited prominent hyaluronan staining, the simple epithelia of the stomach and large intestine were completely negative. Like the mammary cancers described above, the stroma of all of the gastrointestinal tumors displayed intense positive staining for hyaluronan stained as compared to normal connective tissue. Although hyaluronan deposition is often associated within stromal elements within the tumor tissue, there are notable exceptions. For example, in Wilm's tumor (nephroblastoma) hyaluronan was localized within the epithelial blastemal cells, with little reaction in the adjacent stromal compartment (34).

Thus, a somewhat generalized conclusion can be made that hyaluronan is elevated in most solid tumor tissues, at least with respect to comparable normal tissues. Typically, but not always, this elevation is at the expense of other glycosaminoglycans (35, 36). However, in cases where other glycosaminoglycans are upregulated, hyaluronan is often upregulated as well. The particular tumor-association glycosaminoglycan composition may be dependent on the particular host connective tissue in which the tumor resides (especially if the source of the glycosaminoglycan is the host connective tissue cells). For example, when rabbit V2 carcinoma invades into rabbit muscle there is a nine-fold increase in total glycosaminoglycan composed predominately of (71%) hyaluronan (37). However, when the same V2 carcinoma cells invades into rabbit mesenteric tissue, there is a nine-fold increase in chondroitin sulfate but only a three-fold increase in hyaluronan, resulting in a tumor-associated matrix composed of approximately equal concentrations of hyaluronan and chondroitin sulfate (38).

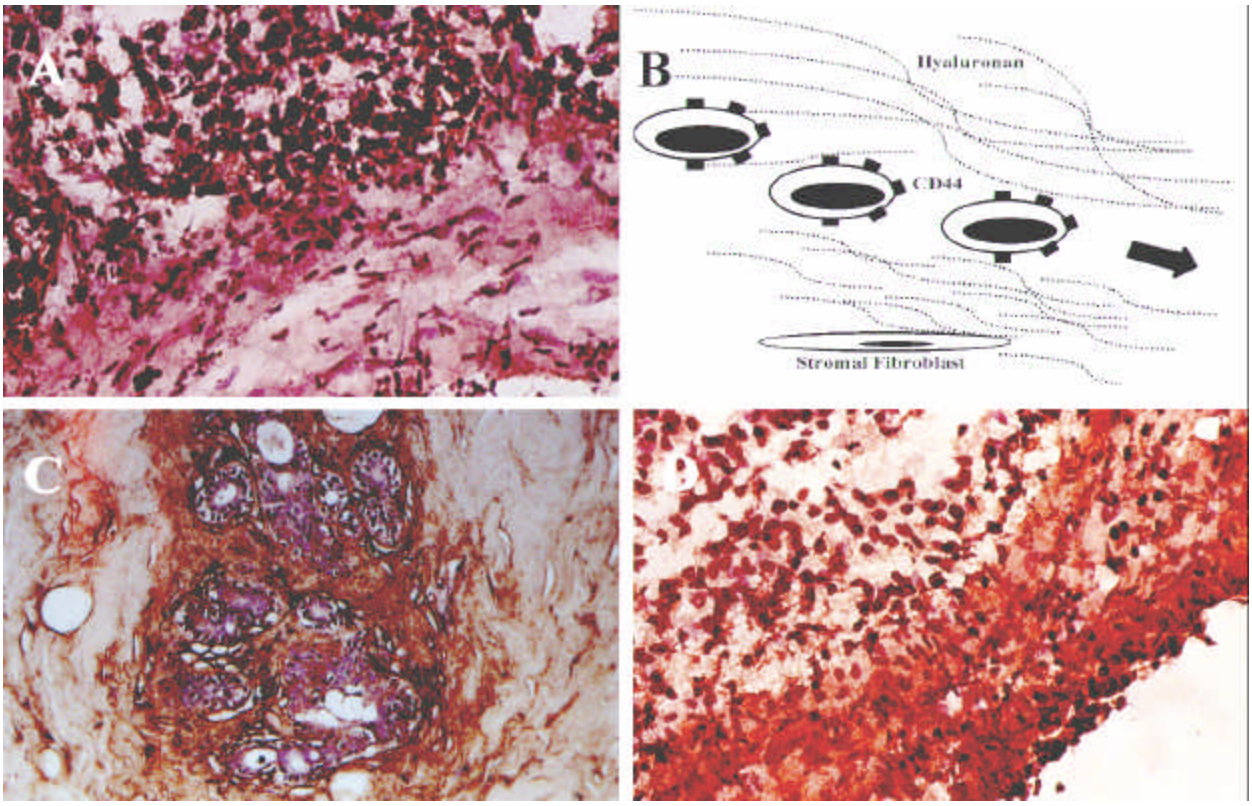

Figure 2. Localization of hyaluronan and CD44 within sections of human breast carcinoma. Sections were made from two different samples of formaldehyde-fixed, paraffin-embeded human breast carcinoma. The samples were deparaffinized and incubated with biotinylated anti-human CD44H antibody (A3D8, Panel A) or biotinylated HABR complex (Panels C and D). Following incubation the sections were washed and processed using a *Vectastain* ABC kit (Vector laboratories). Following development of peroxidase reaction (brownish color) the sections were counterstained with Mayer's hematoxylin. Malignant cells invading into adjacent stroma stain positively for CD44 expression (Panel A). Sections from the same tumor display prominent staining for hyaluronan (Panel D) with the majority of the staining associated with the stroma. Sections from a different tumor also display prominent staining for hyaluronan (Panel C). In the lower power view depicted in Panel C, clear demarcation of hyaluronan from more distant uninvolved mammary connective tissue can clearly be seen. Panel B represents an idealized cartoon of the interactions that are occurring in Panels A, C and D.

3.3. Is the level of tumor hyaluronan content of diagnostic or prognostic value?

As discussed above, in some cancers such as mesothelioma and nephroblastoma, hyaluronan levels are elevated to such an extent that the elevation can be detected in the serum (27) or urine (39). In the study of mesothelioma patients, elevated serum hyaluronan was found in all patients at time of presentation. Patients that presented with serum hyaluronan at, or above, 250 µg/l showed significant likelihood to fall into the progressive disease group whereas, those with lower values typically fell into a group that responded to therapy. It was concluded that serum hyaluronan in this malignancy was predictive of progressive disease. In a study of Wilm's tumor patients, urine hyaluronan was significantly elevated in 74% of the patients pre-operatively as compared to normal control volunteers (39). Post-operatively, urine hyaluronan values were significantly reduced, returning to near normal in disease-free patients. In post-operative relapse patients, urine hyaluronan levels began to again increase. Thus, for Wilm's tumor there also appears to be a close correlation between urine hyaluronan levels and disease progression.

Serum hyaluronan was also monitored in malignant breast carcinoma. Initial results from one group of investigators suggested a significant elevation in serum hyaluronan in patients with disseminated metastatic disease as compared to patients with malignant disease without metastasis or benign disease of the breast (26). However, a subsequent study, with a larger population of patients, showed no prognostic significance of serum hyaluronan in breast cancer (40). In the latter study, serum hyaluronan levels were similar to those of control patients.

In a small number of studies, attempts have been made to quantify hyaluronan content within the primary tumor and compare these values to tumor grade or stage. In a study of 35 cases of malignant brain tumors (astrocytomas, gliomas and meningiomas) the investigators

demonstrated that hyaluronan levels within tumor tissues were substantially elevated as compared to normal brain tissue (36). However, the elevated hyaluronan levels showed little statistical change with tumor grade. The hyaluronan levels in brain tumors grades II through IV were all elevated (similar to the concentrations present in fetal brain). A similar finding was obtained in a study of breast carcinoma (41). Although the hyaluronan levels were substantially elevated in the breast carcinoma tissue as compared to normal tissues, there was little change in hyaluronan in malignancies between grades I-III. A more recent study examined the significance of hyaluronan in colorectal cancer (42). In this study of 202 samples of patients with colorectal adenocarcinoma, all adjacent normal intestinal epithelium, in all samples, was negative for hyaluronan. However, 93% of the tumor tissues analyzed were positive for hyaluronan (i.e., 7% of the cases were hyaluronan negative). It was also observed that high grade tumors had moderate to strong hyaluronan staining intensity while lower grades were typically weakly stained. Patients where the tumor-associated hyaluronan was absent or weakly stained were noted to have a higher survival rate than patients with moderate or strong hyaluronan staining. However, as will be discussed below, a better prognostic indicator that was revealed from this study was the association of hyaluronan with tumor epithelium versus tumor stroma (i.e., the relative distribution of hyaluronan within tumor tissue). The authors speculated that the tumor epithelial-association of hyaluronan could have been the result of an interaction with tumor cell CD44. When more studies are performed it may be found that tumor-associated hyaluronan levels, although elevated, are not varying appreciably with tumor grade or stage. Instead, what may be of more prognostic value are the expression levels of proteins that interact with the hyaluronan, either hyaluronan-binding proteins within the matrix or, the expression of hyaluronan receptors such as CD44.

4. The function of tumor-associated hyaluronan

Although hyaluronan concentrations are elevated in many tumors and, may even be prognostic for some, it remains to be determined whether this hyaluronan is important or serves any relevant function. Does the presence of hyaluronan facilitate tumor progression and if so, by what mechanism(s)? Alternatively, is the hyaluronan synthesized as a host response to tumor invasion, i.e., a desmoplastic response to present a matrix barrier or even to "wall-off" or encapsulate the growing tumor? Although no definitive answers are available, considerable research and speculation have been directed toward these questions. Whatever, the function, the association of hyaluronan with embryonic development, wound healing and regeneration suggests that the accumulation of this glycosaminoglycan is a common feature of tissue remodeling in physiologic as well as pathologic settings.

4.1. Hyaluronan and cell migration: A function by association

The potential importance of hyaluronan to aspects of cellular function, were first noted by Toole and his co-workers in embryonic systems. Hyaluronan was observed to be actively synthesized and deposited nearly coincident with the onset of migration of particular embryonic cells (43). Furthermore, the cessation of migration was tightly correlated with a dramatic reduction in the levels of hyaluronan within the connective tissue. This pattern was documented in the migration of dorsal trunk and cranial neural crest cells (e.g., presumptive corneal fibroblasts) as well as the migration of endocardial cushion cells of the developing heart (44-46). Subsequent to these early observations, increasing hyaluronan deposition was also found in regenerating and wound healing systems in adult tissues. For example, large concentrations of hyaluronan are deposited precisely coincident with the extensive migration of blastemal cells during limb regeneration in the newt (47). Results such as these led to the suggestion that the presence of large concentrations of extracellular hyaluronan were, in some way, functioning to facilitate cell migration. However, the evidence for such a role was still circumstantial. One attempt at a direct approach was the injection of *Streptomyces* hyaluronidase into the sub-blastodisc of stage-8 chick embryos, a region known to be involved in the migration of dorsal neural crest cells. Although the hyaluronidase treatment did not totally prevent the migration of the neural crest cells, the migration pathway was significantly truncated (48). In still another system, high concentrations of hyaluronan were also expressed in a temporal fashion within the developing embryonic chick limb. However, little overt cell migration is occurring at this developmental stage—being a stage better characterized by extensive cell proliferation. So, the original model for hyaluronan was expanded to include facilitation of cell division as well as migration (49).

When investigators re-examined tumor tissues and found an enrichment in hyaluronan, a function was proposed based on the association of hyaluronan during development, wound healing and regeneration discussed above, i.e., to provide an embryonic-like extracellular environment, conducive to cell migration and/or proliferation. An early attempt to validate this role was made by Toole and co-workers using a highly invasive rabbit carcinoma. When inoculated at either a subcutaneous or intramuscular site in the nude-mouse, non-invasive tumors were established (37). These well-encapsulated benign tumors contained 3-4 fold less hyaluronan than the same tumor grown in the rabbit (the natural host), where the cells behaved as an aggressively invasive and metastatic tumor. Thus, an accumulation in hyaluronan appeared to be associated with the active invasion of cells into adjacent tissue. In a more recent work, a time-dependent increase in stromal staining for hyaluronan was observed in lining mesentery tissue following metastatic attachment and growth of murine ovarian carcinoma cells (50). Similar accumulations were observed in the metastatic seeding of murine breast carcinoma cells (50). Hyaluronan concentration increased each day coordinate with the invasion and growth of these cells at the metastatic site. The mesenteric cells were suggested as the source of the

hyaluronan as neither the ovarian nor the mammary carcinoma cells exhibited a capacity to synthesize hyaluronan *in vitro*. Thus it appeared that the host tissue was *responding* to the attachment, proliferation and invasion of the malignant cells. The stromal association of hyaluronan, in the human tumors described above, as well as those illustrated in figure 2C and D, suggest that there is a host response to the presence of actively migrating cells. The question remains whether this is an effort to mount a "protective" response, induced by the host tissue cells or, a process of "matrix engineering," or tissue remodeling, directed by the infiltrating tumor cells, i.e., an effort to establish a more conducive extracellular environment. Unfortunately, observations again appear to point in both directions. In the benign rabbit V2 carcinoma tumor, established in the nude mouse, large concentrations of hyaluronan were apparently not necessary for the host to mount an effective response to the presence of a growing tumor mass. Second, several studies have demonstrated that tumor cells have the capacity *in vitro* to stimulate hyaluronan synthesis by normal connective tissue cells such as fibroblasts (50-53). Some of the tumor-directed hyaluronan stimulatory activities, especially those involving soluble factors, have been defined (e.g., tumor cell-derived PDGF) (54), while others remain to be elucidated (55). Many of the same tumor cells that exhibit a capacity to induce increases in hyaluronan synthesis by adjacent fibroblasts also exhibit a capacity to induce fibroblast production of matrix metalloproteinases (56). Thus, these malignant cell types have a (potentially) tremendous capacity for matrix engineering or directing the remodeling of adjacent extracellular matrix. Such remodeling would be predicted to result in an increase in tissue hydration, likely reminiscent of the embryonic tissue environment—an environment conducive to cell migration and proliferation.

Alternatively, some investigators have correlated the cellulular capacity for CD44-mediated endocytosis and degradation of hyaluronan (assayed *in vitro*), to tumor metastatic aggressiveness (53, 57). That is, the malignant cells that are best equipped to internalize and degrade hyaluronan appear to be the most efficient at metastasis. One interpretation of these results is that tumor-associated hyaluronan presents a barrier to invasion, that is breached effectively by cells with a capacity to bind, internalize and degrade the glycosaminoglycan. However, others have suggested binding and endocytosis of a substratum ligand as part of the mechanism for cell locomotion (58). Thus, the relationship between endocytosis capacity and its involvement in tumor progression remain to be better defined. This may require methods to selectively inhibit CD44-mediated endocytosis and determine whether tumor cells maintain hyaluronan-mediated locomotion, both *in vivo* and *in vitro*.

4.2. Proposed mechanisms for the involvement of hyaluronan in cell migration and proliferation

One mechanism proposed to explain how hyaluronan could potentially facilitate cell migration and proliferation was based on the intrinsic physicochemical nature of hyaluronan, i.e., deposition of high concentrations of hyaluronan result in an expansion of, or "loosening," of spaces within the connective tissue (59). In the embryonic systems described above, the deposition of hyaluronan was associated with an overall expansion of the tissue (43). Conversely, the removal of hyaluronan was associated with a dramatic shrinkage of the tissue and coincident with loss of tissue hydration (43). The injection of *Streptomyces* hyaluronidase into the chick embryo described above resulted in a complete collapse of tissue spaces between somites and somitomeres as well as the overlying ectoderm, neural tube and endoderm (48). Thus, the hydrodynamic effects of hyaluronan, i.e., the hydration and subsequent expansion that occurs in the presence of a high molecular mass, negatively charged polysaccharide, were often suggested as the primary mechanism for how hyaluronan may facilitate cell migration and/or proliferation. Again however, this model was based on evidence of coincidence. To address this question directly, investigators established model systems for cell migration. Nonetheless, the results obtained were ambiguous. For example, the migration of neutrophils is completely inhibited in gels highly enriched in hyaluronan (60). On the other hand, the migration in hyaluronan gels of other cell types such as fibroblasts, appears to be enhanced (61, 62). This suggested that hyaluronan involvement in cell migration was somehow cell-specific, serving as a favorable milieu for some cell types, an impediment for others. This also led investigators to look for the nature of these cell-specific differences, possibly due to the presence or absence of cell surface hyaluronan receptors (e.g., CD44), or perhaps again, to differences in CD44-mediated capacity for hyaluronan endocytosis.

The role of direct tumor cell - hyaluronan interactions in facilitating cell migration and/or cell proliferation fall primarily into two categories, i) hyaluronan as a matrix support for haptotaxic cell locomotion as depicted in figure 2B and, ii) hyaluronan serving as a cell surface-bound, or pericellular, coating. Concerning a role of hyaluronan as cell locomotion substratum, Thomas *et al*. demonstrated that the stable transfection of human melanoma cells with pCD44 resulted in an enhanced motility of the cells on hyaluronan-coated surfaces as compared to the parental, CD44-negative, cells (63). This enhanced motility was inhibited by anti-CD44 antibodies or, by the presence of a CD44 ligand competitor, soluble CD44-immunoglobulin fusion protein. Thus, hyaluronan could function as an insoluble matrix ligand support for the locomotion of tumor cells, mediated via CD44. However, regardless of this *in vitro* potential the question remains whether CD44-hyaluronan interactions participate in cell adhesion, migration or proliferation *in vivo* In a recent study, Yu *et al*., attempted to answer this question by transfecting a CD44-positive murine mammary carcinoma cell line (TA3/St) with cDNA encoding soluble CD44 (53). The transfectants spontaneously release soluble CD44 that, in turn, competes with the activity of endogenous cell surface-localized CD44. Whereas, following tail vein injection into syngeneic mice, control cells formed massive lung metastases, metastases were

reduced to near zero in the transfectants expressing soluble CD44. When lung metastases did become established, stimulated deposition of hyaluronan was observed. These data serve to support the suggestion that, *in vivo*, some form of tumor cell CD44-mediated interaction is important for the stabile establishment and growth of distant metastases. That the critical partner in this interaction with CD44 is hyaluronan, although highly likely, still remains to be definitively determined.

Tumor cells expressing CD44 have the capacity to bind hyaluronan and, in the presence of other hyaluronan binding proteins (i.e., proteoglycans such as aggrecan, versican or hyaluronectin), assemble the components into a pericellular matrix shell or coat (64). Analogous to a bacterial glycocalyx, this pericellular coat may serve to cocoon the malignant cells, or serve as a shield, to prevent undesirable cell-cell interactions. Undesirable cell-cell interactions would included homotypic as well as heterotypic cell-cell interactions. Providing a homotypic cell-cell barrier may help in facilitating cell proliferation. Providing a heterotypic barrier may help malignant cells evade immune surveillance or inhibit T-cell mediated cell lysis. Some cells such as synovial fibroblasts synthesize sufficient hyaluronan and hyaluronan binding-proteins to exhibit an endogenous pericellular matrix. Early studies demonstrated that the pericellular coat exhibited by synovial fibroblasts served as an effective barrier to lymphocyte-mediated cell lysis *in vitro* (65). Following treatment with hyaluronidase, this barrier was removed and the lymphocytes were allowed direct cell-cell contact with the synovial cells (65, 66). Many tumor cells, particularly those derived from carcinomas, do not make the levels of extracellular matrix components necessary to establish a pericellular coat such as that of a synovial lining cell (64). However, these same tumor cells do express CD44 and can bind hyaluronan synthesized and secreted by other cell types. Given the appropriate extracellular macromolecules, epithelial tumor cells can assemble a pericellular matrix nearly identical to that of synoviocytes (64). An example of such is illustrated in figure 3. Panel A depicts anti-CD44 staining of the MCF-7 human mammary carcinoma cell line in co-culture with a rat fibroblast-like cell line that synthesizes copious amounts of hyaluronan. This *in vitro* system was designed to represent a simplified model of the tumor tissue shown in figure 2. As can be seen, the MCF-7 cells display a rich immunostaining for CD44. However, in the process of plating the cells in co-culture an unfortunate accident occurred—the cells became contaminated with bacteria. As shown in panel B, the MCF-7 cells cultured separately were completely encompassed by contaminating bacteria. However, in the co-cultures (panel C), the fibroblast-like cells exhibited a pericellular matrix barrier that prevented bacterial encroachment. Even more interesting was that the tumor cells within the culture (the more polygonal to rounded cells) also exhibited a protective pericellular coat. Similar results were obtained in co-cultures of human colon carcinoma cells (HT29) and fibroblasts (panel D). Addition of 1 U/ml of *Streptomyces* hyaluronidase for 30 minutes, resulted in the complete disillusionment of this pericellular matrix and now, the bacteria were able to come into direct contact with both the tumor cells and the fibroblasts (panel E). Considering that these tumor cell lines have little capacity to synthesize hyaluronan or, at least enough to establish an endogenous pericellular matrix (panel B), a most likely explanation is CD44-mediated assembly of fibroblast-derived matrix macromolecules. We have demonstrated previously that COS-7 cells transfected with pCD44, or tumor cells already exhibiting a high level of CD44 expression, have the capacity to assemble similar pericellular matrices, in the presence of exogenously added hyaluronan and proteoglycan (64, 67). These pericellular matrices were visualized by the exclusion of uniform particles (i.e., glutaraldehyde-fixed, horse red blood cells). The ability of the cells in figure 3 to exclude live bacteria serves to demonstrate the barrier capacity of such hyaluronan-rich cell coats. Whether this mechanism occurs *in vivo*, as with other CD44:hyaluronan-mediated activities, remains to be determined. Nonetheless, the potential for establishing a glycosaminoglycan-rich protective cocoon exists.

It should also be noted that elaborate pericellular coats may function to reduce cell-matrix interactions and work to inhibit migration, i.e., masking receptors interactions necessary for cell locomotion. For example, we have shown previously that, in the presence of hyaluronan alone, the hyaluronan becomes bound via CD44 but is insufficient to establish a pericellular coat capable of excluding particles (64, 68). However, when aggrecan proteoglycan is added to the cells in addition to hyaluronan, pericellular matrices form within two hours of culture. Why is this important? Addition of aggrecan or versican proteoglycan to cultures of neural crest cells inhibited their migration on fibronectin and/or laminin-coated substrata (69). The authors suggested that the deposition of hyaluronan-binding proteoglycans into the extracellular matrix may serve as natural matrix cues to signal a migration stop during development. Putting the two ets of data together, we hypothesized that it is the assembly of a proteoglycan plus hyaluronan pericellular matrix cocoon that functions to prevent migration (1). Taken further, this model would predict that during development (and possibly tumor invasion), cells expressing CD44 migrate along hyaluronan-containing matrix tracts until they encounter areas rich in hyaluronan as well as proteoglycan. After encountering the proteoglycan-enriched matrix, the cells become coated in a pericellular matrix and their migration ceases. Taken another step, this would predict that in healthy, adult connective tissues (containing hyaluronan and proteoglycans such as versican), cell migration is actively inhibited by these natural stop signals. However, following proteolytic activity and subsequent removal of the proteoglycan, coupled with elevated hyaluronan synthesis, a migratory-favorable extracellular environment would be established. In the study of brain tumors described above, sthe tumor-associated hyaluronan levels were elevated, but varied little with tumor grade (36). However, the level of the hyaluronan-binding proteoglycan hyaluronectin (related to versican) dropped precipitously with tumor grade. The ratio of hyaluronectin to hyaluronan was reduced from 1.3–6.5 in normal adult brain tissue

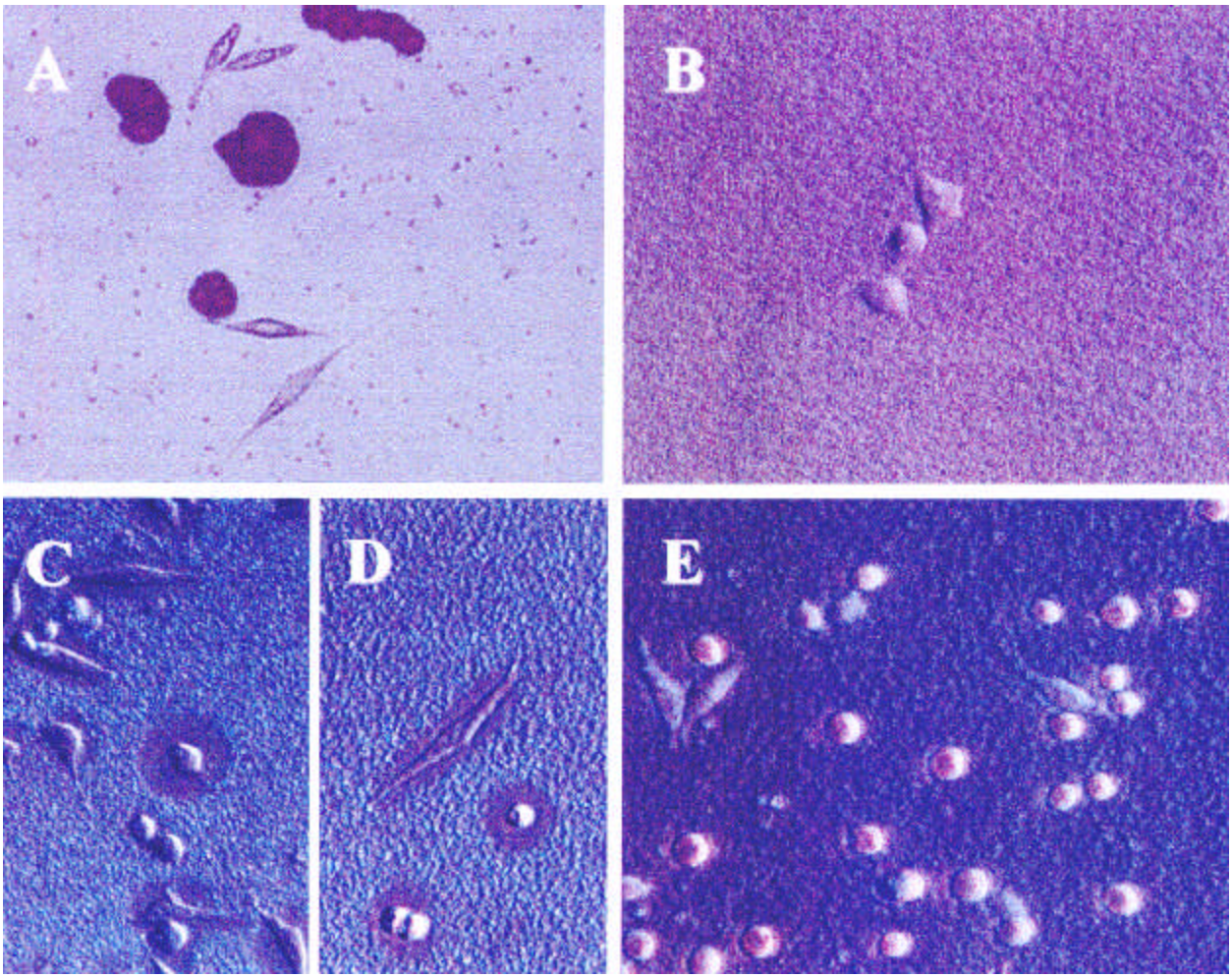

Figure 3. Formation of hyaluronan-rich pericellular matrices around CD44-positive tumor cells *in vitro*. Co-cultures of MCF-7 human mammary carcinoma cells (round-polygonal shaped cells) together with cells derived from a rat fibrosarcoma cell line (slender, elonged cells, (64)) were fixed and incubated with a biotinylated anti-human CD44H monoclonal antibody (A3D8). Following incubation the cells were washed and processed using a *Vectastain* ABC kit (Vector laboratories). The human tumor cells displayed prominent staining for CD44 (Panel A). All of these cultures then became heavily contaminated with bacteria. Panel B depicts cultures of MCF-7 cells grown independently. The cells are completely engulfed with bacteria. However, the same cells in co-culture with fibroblasts display excluded zone of pericellular matrix to which the bacteria cannot penetrate (Panel C). Co-cultures of human colon carcinoma cells, HT-29 display similar barrier matrices (Panel D). Following treatment of MCF-7 containing co-cultures with 1U/ml *Streptomyces* hyaluonidase (Panel E), the protective barrier is lost and the bacteria are allowed direct contact with both cell types within the co-culture.

to 0.8 – 4.3 in grade II and III tumors, to 0.01–0.4 in grade IV tumors. The grade IV ratios were similar to ratios present in fetal brain tissues.

5. THE ROLE OF CD44 AS A HYALURONAN RECEPTOR DURING TUMOR PROGRESSION

Thus far, several potential functions for CD44 interaction with hyaluronan have been discussed. As illustrated in figure 2B, hyaluronan could serve as a ligand support for CD44-mediated cell locomotion. This hypothesis is attractive because many of the same types of tumor cells expressing CD44, also express hyaluronan-stimulatory-activity, i.e., a capacity to induce hyaluronan synthesis in advance of the cell migration. Together with a capacity to stimulate fibroblast collagenase activity, these induction mechanisms could function to prepare a pathway for migration. Alternatively, CD44-mediated endocytosis may function to remove adjacent stromal hyaluronan that acts as a barrier, or again, represent part of the "rolling" mechanism of endocytosis-mediated locomotion (58, 70). CD44– hyaluronan interactions may result in the assembly of a protective pericellular matrix. Such matrices may serve as a protective barrier or function to reduce homotypic cell-cell adhesion or even cell-matrix interactions. Do any of these potential CD44-mediated interactions participate in tumor progression *in vivo*? CD44 is upregulated in many tumors, including CD44H as well as other CD44 variant isoforms. Hyaluronan synthesis and deposition are also

upregulated. In other words, the two critical components are present but, does this represent two independent activities or, do CD44 – hyaluronan interactions occur?

In a recent study by Auvinen *et al.*, human benign breast lesions displayed hyaluronan localized within the stroma, with all epithelial elements being negative (71). In cases of malignant mammary carcinoma, hyaluronan staining intensity was significantly elevated. However, in addition to intense staining within the tumor-associated stroma, hyaluronan was also observed associated with some of the malignant adenocarcinoma cells, both at the tumor cell surface as well as within intracellular sites. If, the adenocarcinoma cells are not synthesizing hyaluronan (as is often the case (50, 72)), this data would suggest that the more aggressive cells are binding and/or internalizing tumor-associated hyaluronan. Somewhat similar results were obtained in a recent study of human colorectal cancer (42). Again, there was a trend for elevation in hyaluronan staining intensity associated with increase in tumor grade. However, analysis of patients with hyaluronan associated with, or bound to, cells of the tumor epithelium was correlated with a low survival rate. Furthermore, multivariate analysis demonstrated that, in patients without metastases, only intensity of hyaluronan associated with tumor epithelium was a predictive indicator of survival. Hints of tumor cell binding of hyaluronan can also be observed in mouse mammary carcinoma following metastatic colonization of mesenteric tissue (50) and lung epithelium (53) described above, and possibly, the infiltrating malignant cells depicted in figure 2D. Thus, the more aggressive and invasive the tumor, the more likely that cells of the tumor parenchyma will be observed staining strongly for hyaluronan. That this epithelial-asssociated hyaluronan is bound or internalized via CD44 interactions is likely as epithelial-derived tumor cells typically synthesize little hyaluronan. CD44-mediated endocytosis has been shown to be primarily mechanism responsible for intracellular accumulation of hyaluronan in malignant as well as normal cell types (57, 73, 74). Lastly, as described above, inhibiting CD44 interaction with ligands, via the secretion of soluble CD44, resulted in the near complete inhibition of lung metastases (53).

In summary, tumor progression is associated with an increase in expression of CD44 as well as the principal CD44 ligand, hyaluronan. Further, progression also appears associated with malignant cells staining strongly for hyaluronan, likely bound to the tumor cell surface via CD44. Although CD44 has the potential to participate in tumor progression via a variety of mechanisms (lymphocyte homing receptor, a proteoglycan, receptor for other ligands, etc), its function as a hyaluronan receptor is likely its critical role in this disease. Development of methods to selectively inhibit CD44 – hyaluronan interactions will go a long way in definitively establishing the functional link between these interactions and their role in tumor progression.

6. ACKNOWLEDGEMENTS

Many thanks to Dr. Cheryl B. Knudson for her comments on this manuscript; to Fadwa Nayfeh for her exceptional technical assistance and; to Drs. Raija and Markkku Tammi for the gift of some of their biotinylated HABR/link protein probe. This work supported in part by grants P50-AR39239 and RO1-AR43384 from the National Institutes of Health.

7. REFERENCES

1. Knudson, C. B., and W. Knudson: Hyaluronan-binding proteins in development, tissue homeostasis and disease. *FASEB J.* 7, 1233-41 (1993)

2. Hughes, H. E., D. M. Salter, and R. Simpson: CD44 expression in human bone: a novel marker of osteocytic differentiation. J *Bone and Mineral Res.* 9, 39-44 (1994)

3. Jalkanen, S. T., R. F. Bargatze, J. Toyos, and E. C. Butcher: Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85-95 kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal or synovial endothelial cells. *J Cell Biol.* 105, 983-90 (1987)

4. Stamenkovic, I., M. Amiot, J. M. Pesando, and B. Seed: A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. *Cell.* 56, 1057-62 (1989)

5. Haynes, B. F., L. Hua-Xin, and K. L. Patton: The transmembrane hyaluronate receptor (CD44): Multiple functions, multiple forms. *Cancer Cells.* 3, 347-50 (1991)

6. Jackson, D. G., J. I. Bell, R. Dickinson, J. Timans, J. Shields, and N. Whittle: Proteoglycan forms of the lymphocyte homing receptor CD44 are alternatively spliced variants containing the v3 exon. *J Cell Biol.* 128, 673-85 (1995)

7. Knudson, W., and K. E. Kuettner: Proteoglycans. In: Primer on the Rheumatic Diseases. R. L. Wortmann, editor. Arthritis Foundation, Atlanta, GA, 33-38 (1997).

8. Chi-Rosso, G., and B. P. Toole: Hyaluronate - binding protein of simian virus 40 - transformed 3T3 Cells: membrane distribution and reconstitution into lipid vesicles. *J Cell Biochem.* 33, 173-83 (1987)

9. Underhill, C. B., G. Chi-Rosso, and B. P. Toole: Effect of detergent solubilization on the hyaluronanate-binding protein from membranes of Simian virus 40-transformed 3T3 cells. *J Biol Chem*. 258, 8086-91 (1983)

10. Gunthert, U., M. Hofmann, W. Rudy, S. Reber, M. Zoller, I. Haussmann, S. Matzku, A. Wenzel, H. Ponta, and P. Herrlich: A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell.* 65, 13-24 (1991)

11. Reber, S., S. Matzku, U. Gunthert, H. Ponta, P. Herrlich, and M. Zoller: Retardation of metastatic tumor growth after immunization with metastasis-specific

monoclonal antibodies. *Internatl J Cancer.* 46, 919-27 (1990)

12. Salmi, M., K. Gron-Virta, P. Sointu, R. Grenman, H. Kalimo, and S. Jalkanen: Regulated expression of exon v6 containing isoforms of CD44 in man: downregulation during malignant transformation of tumors of squamocellular origin. *J Cell Biol.* 122, 431-42 (1993)

13. Tanabe, K. K., L. M. Ellis, and H. Saya: Expression of CD44R1 adhesion molecule in colon carcinomas and metastases. *Lancet.* 341, 725-6 (1993)

14. Muller, W., A. Schneiders, K. H. Heider, S. Meier, G. Hommel, and H. E. Habbert: Expression and prognostic value of the CD44 splicing variants v5 and v6 in gastric cancer. *J Pathol.* 183, 222-7 (1997)

15. Knudson, W., and C. B. Knudson: Overproduction of hyaluronan in the tumor stroma. In: Tumor Matrix Biology. R. Adany, editor. CRC Press, Boca Raton, FL. 55-79. (1995).

16. Weigel, P. H., V. C. Hascall, and M. Tammi: Hyaluronan synthases. *J Biol Chem*. 272, 13997-4000 (1997)

17. Spicer, A. P., M. L. Augustine, and J. A. McDonald: Molecular cloning and characterization of a putative mouse hyaluronan synthase. *J Biol Chem.* 271, 23400-6 (1996)

18. Prehm, P.: Hyaluronate is synthesized at plasma membranes. *Biochem J.* 220, 597-600 (1984)

19. Prehm, P.: Release of hyaluronate from eukaryotic cells. *Biochem J.* 267, 185-9 (1990)

20. Laurent, T. C., and R. E. Fraser: Hyaluronan. *FASEB J.* 6, 2397-404 (1992)

21. Wells, H. G.: The Chemistry of Tumors. In: Chemical Pathology. W.B. Sanders, Philadelphia, PA. 411-30. (1907).

22. Meyer, K., and J. W. Palmer: The polysaccharide of the vitreous humor. *J Biol Chem.* 107, 629-34 (1934)

23. Knudson, W., C. Biswas, X. Q. Li, R. E. Nemec, and B. P. Toole: The role and regulation of tumour-associated hyaluronan. In: The Biology of Hyaluronan, Ciba Foundation Symposium 143. D. Evered and J. Whelan, editors. John Wiley and Sons, Chichester, U.K. 150-69. (1989).

24. Roboz, J., A. P. Chahinian, J. F. Holland, D. Silides, and L. Szrajer: Early diagnosis and monitoring of transplanted human malignant mesothelioma by serum hyaluronic acid. *J Natl Cancer Inst.* 81, 924-8 (1989)

25. Delpech, B., P. Bertrand, and C. Maingonnat: Immunoenzymeassay of the hyaluronic acid-hyaluronectin interaction: Application to the the detection of hyaluronic acid in serum of normal subjects and cancer patients. *Anal Biochem.* 149, 555-65 (1985)

26. Delpech, B., B. Chevalier, N. Reinhardt, J. P. Julien, C. Duval, C. Maingonnat, P. Bastit, and B. Asselain: Serum hyaluronan (hyaluronic acid) in breast-cancer patients. *Internatl J Cancer.* 46, 388-90 (1990)

27. Dahl, I. M. S., O. P. Solheim, B. Erikstein, and E. Muller: A longitudinal study of the hyaluronan level in the serum of patients with malignant mesothelioma under treatment. *Cancer.* 64, 68-73 (1989)

28. Kumar, S., D. C. West, J. M. Ponting, and H. R. Gattamaneni: Sera of children with renal tumours contain low-molecular-mass hyaluronic acid. *Internatl J Cancer.* 44, 445-8 (1989)

29. De Klerk, D. P.: The glycosaminoglycans of human bladder cancers of varying grade and stage. *J Urology.* 134, 978-81 (1985)

30. Iozzo, R. V., R. P. Bolender, and T. N. Wight: Proteoglycan changes in the intercellular matrix of human colon carcinoma. *Lab Invest.* 47, 124-37 (1982)

31. Bouziges, F., P. Simon-Assman, C. Leberquier, J. Marescaux, J. P. Bellocq, K. Haffen, and M. Kedinger: Changes in glycosaminoglycan synthesis and heparan sulfate deposition in human colorectal adenocarcinomas. *Internatl J Cancer.* 46, 189-97 (1990)

32. De la Torre, M., A. F. Wells, J. Bergh, and A. Lindgren: Localization of hyaluronan in normal breast tissue, radial scar, and tubular breast carcinoma. *Human Path.* 24, 1294-7 (1993)

33. Wang, C., M. Tammi, H. Guo, and R. Tammi: Hyaluronan distribution in the normal epithelium of esophagus, stomach and colon and their cancers. *Am J Pathol.* 148, 1861-9 (1996)

34. Longaker, M. T., N. S. Adzick, D. Sadigh, B. Hendin, S. E. Stair, B. W. Duncan, M. R. Harrison, R. Spendlove, and R. Stern: Hyaluronic acid-stimulating activity in the pathophysiology of Wilms' tumors. *J Natl Cancer Inst .* 82, 135-8 (1990)

35. Sylven, B.: Ester sulphuric acids in stroma connective tissue. *Acta Radiology.* 59, 11-6 (1949)

36. Delpech, B., C. Maingonnat, N. Girand, C. Chauzy, R. Maunoury, A. Olivier, J. Tayot, and P. Creissard: Hyaluronan and hyaluronectin in the extracellular matrix of human brain tumour stroma. *Eur J Cancer.* 29A, 1012-7 (1993)

37. Toole, B. P., C. Biswas, and J. Gross: Hyaluronate and invasiveness of the rabbit V2 carcinoma. *Proc Natl Acad Sci USA*. 76, 6299-303 (1979)

38. Iozzo, R. V., and W. Muller-Glauser: Neoplastic

modulation of extracellular matrix: Proteoglycan changes in the rabbit mesentery induced by V2 carcinoma cells. *Cancer Res.* 45, 5677-87 (1985)

39. Lin, R. Y., P. A. Argenta, K. M. Sullivan, R. Stern, and N. S. Adzick: Urinary hyaluronic acid is a Wilm's tumor marker. *J Pediatric Surgery.* 30, 304-8 (1995)

40. Ponting, J., A. Howell, D. Pye, and S. Kumar: Prognostic relevance of serum hyaluronan levels in patients with breast cancer.*Intl J Cancer.* 52, 873-6 (1992)

41. Bertrand, P., N. Girard, B. Delpech, C. Duval, J. D'Anjou, and J. P. Dauce: Hyaluronan (hyaluronic acid) and hyaluronectin in the extracellular matrix of human breast carcinomas: comparison between invasive and non-invasive areas.*Intl J Cancer.* 52, 1-6 (1992)

42. Ropponen, K., M. Tammi, J. Parkkinen, M. Eskelinen, R. Tammi, P. Lipponen, U. Agren, E. Alhava, and V. M. Kosma: Tumor cell-associated hyaluronan as an unfavorable prognostic factor in colorectal cancer. *Cancer Res.* 58, 342-7 (1988)

43. Toole, B. P.: Developmental role of hyaluronate. *Connect Tissue Res.* 10, 93-100 (1982)

44. Toole, B. P., and R. L. Trelstad: Hyaluronate production and removal during corneal development in the chick. *Dev Biol.* 26, 28-35 (1971)

45. Markwald, R. R., F. M. Funderberg, and D. H. Bernanke: Glycosaminoglycans: Potential determinants in cardiac morphogenesis. *Texas Rep Biol and Med.* 39, 253-70 (1979)

46. Orkin, R. W., and B. P. Toole: Hyaluronidase activity and hyaluronate content of the developing chick embryo heart. *Dev Biol.* 66, 308-20 (1978)

47. Smith, G. N., B. P. Toole, and J. Gross: Hyaluronidase activity and glycosaminoglycan synthesis in the amputated newt limb. *Dev Biol.* 43, 221-32 (1975)

48. Anderson, C. B., and S. Meier: The effect of hyaluronidase treatment on the distribution of cranial neural crest cells in the chick embryo. *J Exp Zoology.* 221, 329-35 (1982)

49. Toole, B. P.: Proteoglycans and hyaluronan in morphogenesis and differentiation. In: Cell Biology of Extracellular Matrix. E. D. Hay, editor. Plenum Press, NY. 305-39. (1991).

50. Yeo, T.-K., J. A. Nagy, K.-T. Yeo, H. F. Dvorak, and B. P. Toole: Increased hyaluronan at sites of attachment to mesentary by CD44-positive mouse ovarian and breast tumor cells. *Am J Path.* 148, 1733-40 (1996)

51. Knudson, W., C. Biswas, and B. P. Toole: Interaction between human tumor cells and fibroblasts simulate hyaluronate synthesis. *Proc Natl Acad Sci USA.* 81, 6767-71 (1984)

52. Asplund, T., M. A. Versnel, T. C. Laurent, and P. Heldin: Human mesothelioma cells produce factors that stimulate the production of hyaluronan by mesothelial cells and fibroblasts. *Cancer Res.* 53, 388-92 (1993)

53. Yu, B. Q., B. O. Toole, and I. Stamenkovic: Induction of apoptosis of metastatic mammary carcinoma cells in vivo by disruption of tumor cell surface CD44 function. *J Exp Med.* 186, 1985-96 (1997)

54. Heldin, P., T. Asplund, D. Ytterberg, S. Thelin, and T. C. Laurent: Characterization of the molecular mechanism involved in the activation of hyaluronan synthetase by platelet-derived growth factor in human mesothelioma cells. *Biochem J.* 283, 165-70 (1992)

55. Knudson, W., and B. P. Toole: Membrane association of the hyaluronate stimulatory factor from LX-1 human lung carcinoma cells. *J Cell Biochem.* 38, 165-77 (1988)

56. Biswas, C., and B. P. Toole: Modulation of the extracellular matrix by tumor cell-fibroblast interactions. In: Cell Membranes. E. Elson, W. Frazier, and L. Glaser, editors. Plenum Publishing Corporation. NY, 341-63. (1987).

57. Culty, M., M. Shizari, E. W. Thompson, and C. B. Underhill: Binding and degradation of hyaluronan by human breast cancer cell lines expressing different isoforms of CD44: Correlation with invasive potential. *J Cell Physiol.* 160, 275-86 (1994)

58. Bretscher, M. S.: Endocytosis and recycling of the fibronectin receptor in CHO cells. EMBO J. 8, 1341-8 (1989)

59. Meyer, F. A.: Macromolecular basis of globular protein exclusion and of swelling pressure in loose connective tissue (umbilical cord). *Biochem Biophys Acta.* 755, 388-99 (1983)

60. Forrester, J. V., and P. C. Wilkinson: Inhibition of leukocyte locomotion by hyaluronic acid. *J Cell Sci.* 48, 315-31 (1981)

61. Doillon, C. J., and F. H. Silver: Collagen-based wound dressing: Effects of hyaluronic acid and fibronectin on wound healing. *Biomaterial.* 7, 3-10 (1995)

62. Turley, E. A., P. Bowman, and M. A. Kytryk: Effects of hyaluronate and hyaluronate-binding proteins on cell motile and contact behaviour. *J Cell Sci.* 78, 133-45 (1985)

63. Thomas, L., H. R. Byers, J. Vink, and I. Stamenkovic: CD44H regulates tumor cell migration on hyaluronate-coated substrate. *J Cell Biol.* 118, 971-7 (1992)

64. Knudson, W., and C. B. Knudson: Assembly of a chondrocyte-like pericellular matrix on non- chondrogenic

cells. *J Cell Sci.* 99, 227-35 (1991)

65. Fraser, J. R., and B. J. Clarris: On the reactions of human synovial cells exposed to homologous leucocytes in vitro. *Clin Exp Immun.* 6, 211-25 (1970)

66. McBride, W. H., and J. B. L. Bard: Hyaluronidase sensitive halos around adherent cells. J Exp Med. 149, 507-15 (1979)

67. Knudson, W., E. Bartnik, and C. B. Knudson: Assembly of pericellular matrices by COS-7 cells transfected with CD44 homing receptor genes. *Proc Natl Acad Sci USA.* 90, 4003-7 (1993)

68. Knudson, C. B.: Hyaluronan receptor-directed assembly of chondrocyte pericellular matrix. *J Cell Biol.* 120, 825-34 (1993)

69. Perris, R., D. Perissinotto, Z. Pettway, M. Bronner-Fraser, M. Morgelin, and K. Kimata: Inhibitory effects of PG-H/aggrecan and PG-M/versican on avian neural cHTrest cell migration. *FASEB J.* 10, 293-301 (1996)

70. Bretscher, M. S.: Endocytosis, the sorting problem and cell locomotion in fibroblasts. Ciba Foundation Symp. 92, 266-81 (1982)

71. Auvinen, P. K., J. J. Parkkinen, R. T. Johansson, U. M. Agren, R. H. Tammi, M. J. Eskelinen, and V. M. Kosma: Expression of hyaluronan in benign and malignant breast lesions. *Internatl J Cancer.* 74, 477-81 (1997)

72. Knudson, W., C. Biswas, and B. P. Toole: Stimulation of glycosaminoglycan production in murine tumors. *J Cell Biochem.* 25, 183-96 (1984)

73. Hua, Q., C. B. Knudson, and W. Knudson: Internalization of hyaluronan by chondrocytes occurs via receptor-mediated endocytosis. *J Cell Sci.* 106, 365-75 (1993)

74. Kaya, G., I. Rodriguez, J. L. Jorcano, P. Vassalli, and I. Stamenkovic: Selective suppression of CD44 in keratinocytes of mice bearing an antisense CD44 transgene driven by a tissue-specific promoter disrupts hyaluronate metabolism in the skin and impairs keratinocyte proliferation. *Genes & Develop.* 11, 996-1007 (1997)

Key words: CD44, Hyaluronan, Tumor, Invasion, Receptor, Connective tissue

---

Send correspondence to: Warren Knudson, Ph.D., Department of Biochemistry, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, 1653 West Congress Parkway, Chicago, IL 60612, Tel: (312) 942-7837, Fax: (312) 942-3053, E-mail: wknudson@rush.edu