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Hyaluronan and hyaluronidase in genitourinary tumors

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1. ABSTRACT

Genitourinary cancers are the most frequently diagnosed cancers in men and the fifth most common in women. Management of disease through accurate and cost effective early diagnostic markers, as well as identification of valid prognostic indicators, has contributed significantly to improved treatment outcomes. In this review, we will discuss the function, regulation and clinical utility of hyaluronan (HA), genes encoding its metabolic enzymes and receptors that mediate its cellular effects. Specific HA synthase (HAS) and hyaluronidase (HAase) genes encode the enzymes that produce HA polymers and oligosaccharides, respectively. Differential effects of these enzymes in progression of genitourinary tumors are determined by the relative balance between HAS and HAase levels, as well as the distribution of receptors. The genes are regulated in a complex fashion at the transcriptional and post-translational levels, but also by epigenetic events, alternative mRNA splicing, and subcellular localization. Importantly, the major tumor-derived HAase enzyme, HYAL-1, either alone or together with HA, is an accurate diagnostic and prognostic marker for genitourinary tumors.

2. INTRODUCTION

Genitourinary cancers (i.e.; prostate, bladder, renal, testicular, penile and Wilms' tumor) account for severe cancer-related morbidity and mortality in the United States. Approximately 30,000 deaths per year result from prostate cancer, and another 25,000 per year from cancers of the bladder and kidney. Early diagnosis is critical for effective treatment of these diseases, and morbidity is both an enormous clinical problem and a serious quality of life issue. Beyond emotional and psychological cost to the patient and family, the average cost for diagnosis and treatment is estimated at $15,000-$25,000 per year, whether in the first year after diagnosis or the final year of metastatic disease, and costs may escalate depending on the aggressiveness of treatment. Identification of convenient, inexpensive diagnostic tools, and more importantly, evaluation of their prognostic value, has been instrumental in minimizing the effects of these cancers.

It has been over 50 years since the first study was published implicating accumulation of hyaluronan, or hyaluronic acid (HA), in the diagnosis of cancer (1). Hundreds of reports have now described correlation of HA

with various types of epithelial and circulating cancers, and investigated the expression and regulation of HA metabolic enzymes for specific roles in cancer initiation and progression. The HA synthase (HAS) genes encode the enzymes that produce and deposit HA, while the hyaluronidase (HAase or HYAL) genes code for enzymes that degrade HA. Expression of both HAS and HYAL enzyme family members is dysregulated in genitourinary tumors as a result of transcriptional and epigenetic changes that accompany progression. In particular, the HAase Hyal1 has shown diagnostic and prognostic potential for genitourinary tumors. In this review, we summarize the functions of HA and HAase in cancer, with special emphasis on their roles in genitourinary tumor growth and progression. We will also discuss their clinical utility for diagnosis and treatment of these cancers and their associated pathologies.

3. HYALURONAN

HA is an unsulfated anionic linear glycosaminoglycan polymer comprised of a repeating glucuronic acid and N-acetylglucosamine disaccharide motif. Synthesis and deposition of this secreted product is normally associated with cell division, motility, transformation and vascular development in embryogenesis (reviewed in (2, 3)). These normal functions continue into adulthood for maintenance of cell and tissue turnover. Additional utility of HA as an adhesion substrate is important for recruitment of circulating cells during wound healing and immune system function. Mechanisms underlying these normal processes are frequently exploited by cancerous cells, so it is not surprising that the enzymes and receptors which synthesize, process and signal cellular responses to HA are valuable diagnostic tools, as well as predictors of cancer aggressiveness

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The biophysical properties of large HA polymers are well suited to maintenance of cell-free space. Matrices rich in HA tend to be comparatively deficient in covalently cross-linked fibrous protein networks (4), and are therefore more gel-like and less organized, also ideal for providing a hydrated cushion or lubricant in joint tissues, skin, eyes, etc. Production of this fluid matrix within organ tissues such as prostate, however, alters the normal architecture of the tissue matrix by increasing its permeability. This undermining of tissue structural integrity may be permissive to pathological cell proliferation and movement, particularly in cancer (5). Furthermore, its role as an adhesion and migration substrate for cells in development may translate to enhanced metastatic potential of cells bearing surface associated HA.

3.1. Correlation with genitourinary cancer

Many studies have demonstrated the strong correlation between HA accumulation and presence of malignancy in numerous solid tumor types, including genitourinary cancers. Early reports from prostate histopathology first indicated increased HA content of the stroma in benign prostatic hyperplasia (BPH) relative to normal tissue (6), and found the magnitude of the increase directly mirrored level of dedifferentiation within the tumor (7). Subsequent studies have investigated the diagnostic and prognostic potential of HA detection in tumors. In general, HA is detected in tumor stroma if Gleason score is $\geq$5 and does not indicate severity. Tumor cell-associated HA is detected at high Gleason scores and in metastases, each of which correlates with poor prognosis (8, 9). In retrospective studies, detection of strong stromal HA staining in radical prostatectomy specimens was found to correlate with invasive primary tumors and recurrence of elevated PSA after tumor resection (10). However, similar investigations with minimum 5-year follow-up found that high HA levels in radical prostatectomy specimens is not an independent predictor of PSA biochemical recurrence (i.e.; a predictor of local or distant metastasis) (11, 12). Nonetheless, as discussed in section 6, HA appears to increase the accuracy of HAase (HYAL-1) in predicting biochemical recurrence. At the present time, no studies have been conducted on prostate biopsy specimens to assess the value of HA detection for disease prognosis following treatment.

Quantitative analysis of urinary HA, with or without concurrent measurement of HAase activity (i.e.; the HA-HAase test), has proven to be a sensitive and specific noninvasive diagnostic test for bladder cancer detection and surveillance (13-16). Analysis of urinary HA in transitional cell carcinoma patients before and after bladder tumor resection further showed the prognostic value of this test, since it is effective in identifying both primary and residual tumor (17). Measurement of urinary HA levels has also been effective in initial diagnosis of Wilms' tumor (18), a childhood renal cancer, as well as detection of its relapse after surgery (19).

Genetic determinants for genitourinary carcinomas are relatively few and have not correlated well with key aspects of disease etiology. Recently, chromosome locus 8q24 was found to be overrepresented in human prostate cancer and prostate tumor cell lines, as a result of its duplication and translocation to another chromosome (20). This locus contains, among others, the gene encoding the essential isozyme of the HA biosynthetic enzyme family (HAS2, section 5.3 below). Extra gene copies of the biosynthetic enzyme may account for elevation of HA with the progression of prostate cancer in patients with this genetic lesion. Interestingly, single nucleotide polymorphisms in untranslated DNA at chromosomal locus 8q24 were also newly confirmed by three independent groups simultaneously as a genetic correlate of up to 40% of prostate cancers, in particular accounting for early appearance of the disease in certain European lineages and African American men (21-23).

3.2. Functional roles in genitourinary cancer

3.2.1. Proliferation, transformation and cell cycle control

Targeted disruption of the genes encoding HA biosynthetic enzymes in mice has illustrated crucial roles for HA in embryonic heart development, where it is an essential signal for epithelial to mesenchymal transition that precedes atrioventricular septum formation (24). This function of HA as a transformation signal has been

demonstrated in cancer progression as well, in which case neoplastic transformations may be promoted by HA-mediated activation of receptor tyrosine kinases such as ErbB2 (25-27).

Despite its documented activation of growth factor receptors, many studies have shown that large HA quantities of high molecular mass (i.e.; from ≈ 100 kDa to ≈ 2 MDa) are antiproliferative and antiangiogenic (28, 29), capable of suppressing growth of both tumor cells (30, 31) and vascular endothelial cells (29). These responses are dependent upon both size and concentration of HA. Constitutive engagement of HA receptors by small amounts of newly synthesized HA polymers was actually shown to stimulate cell cycling through assembly of protein complexes including PI3-kinase, cdc37, and the cytoskeletal adaptor ezrin (27, 32). In addition, HA responses are context dependent with respect to two-dimensional versus three-dimensional culture conditions (5). Anchorage independent growth of prostate tumor cells requires HA polymer ligation of cell surface receptors to stabilize cytoskeletal architecture by intracellular association with ankyrin, ezrin, cytoskeletal proteins and other adaptors (32, 33). These interactions have been successfully disrupted and shown to induce tumor apoptosis *in vitro* and *in vivo* by administration of HA oligosaccharides (32, 34).

In renal cancers, renal proximal tubular epithelial cells deposit excessive newly synthesized collagen during the characteristic fibrotic response. Stimulation of collagen synthesis occurs via smad dependent and independent intracellular pathways upon stimulation by TGFβ1 (35). This effect is blocked by exposure to HA polymers, which attenuate TGFβ1 signaling in general by stimulating endocytosis of its receptors (36), suggesting elevated HA synthesis may be an early response in neoplasia, intended to suppress renal injury.

3.2.2. Adhesion, motility and metastasis

HA is a substrate for cell adhesion, as originally determined by its ability to arrest circulating lymphocytes in high endothelial venules, and confirmed by *in vitro* adhesion assays (37, 38). Retention of high molecular weight pericellular HA by prostate tumor cell lines correlates with their metastatic potential in mice and promotes adhesion specifically to bone marrow endothelial cells *in vitro* (39). This was proposed to be one mechanism underlying the skeletal metastatic frequency of clinical prostate cancer. Increased HA synthesis and pericellular retention by non-adherent prostate tumor cells rendered them adherent to bone marrow-derived endothelial cells and inhibition of HA synthesis in adherent cells by stable antisense RNA expression diminished their adhesion potential (40). HA accumulation in cable structures on renal proximal tubular cells was additionally shown to recruit monocyte infiltration by facilitated arrest (41, 42). Thus, blocking of HA-mediated adhesion may be prophylactic for prostate cancer bone metastasis and for renal fibrosis in kidney cancer.

In part because of its adhesive function but also as a result of receptor engagement, HA has a prominent role in promoting cell migration. Pericardial explants from HA deficient mice, which did not undergo endothelial cell migration needed for cardiac morphogenesis, were shown to have restored motility in the presence of exogenous HA or dominant negative ras (24). Thus, HA serves as a ras-dependent signal for cell migration. A similar role for HA in genitourinary development was shown by monitoring prostate ductal branching in embryonic explants, where HA mediated androgen-induced prostate morphogenesis (43). Tumor cells may also exhibit enhanced motility in the presence of HA polymers. In renal proximal tubular epithelial cells, HA directly stimulates migration through MAP kinase activation (44) and also suppresses the anti-migratory TGFβ1 signal normally produced by the cells (35). Pericellular HA synthesis and retention similarly promotes migration of prostate tumor cells (45). Time lapse videomicroscopy revealed that treatment of tumor cells with prostate fibroblast conditioned media, which caused formation of HA matrices on the tumor cells, also increased their motility. HA was most densely deposited at the trailing edge of the polarized motile cells, also consistent with a potential role in de-adhesion.

Increased adhesive potential and motility of HA-producing tumor cells translates into their enhanced metastatic potential (46). Prostate tumor cells that produce endogenous large quantities of HA polymer are more metastatic to lymph nodes following intraprostatic injection in mice, and this can be increased by overexpressing the HA processing enzyme HYAL-1 (47). The potential value of HA inhibition in metastasis prevention has been demonstrated both by gene silencing and chemotherapeutic techniques in mice. Yoshihara, et al, found that treatment with a HA precursor scavenger, 4-methylumbelliferone, inhibited HA synthesis, and concomitantly diminished cell adhesion, migration and metastasis (48). Stable antisense inhibition of HA synthesis abrogated ≈90% of spontaneous lymph node metastasis from orthotopic injections of prostate tumor cells (49). Finally, overexpression of a soluble form of the cell surface HA receptor by tumor cells successfully antagonized HA-mediated arrest and subsequent colonization of lung tissue upon intravenous injection (53). Thus, HA on the tumor cell surface is likely functioning in metastasis both at the level of facilitated arrest/adhesion to endothelial surfaces in remote tissues and also signaling of cell survival.

3.3.3. Angiogenesis

HA oligomers, specifically of 4-25 disaccharides, have been shown to stimulate angiogenesis (50), despite the antiproliferative effect of larger HA polymers on endothelial cells that suppresses angiogenesis (28, 29). Furthermore, the antiangiogenic effect of HA polymers on endothelial cells is irreversible once engaged except in the sustained presence of antagonistic HA oligosaccharides (51). Thus, this normal function of HA has the potential to allow tumor cells to directly signal their own vascular development. Numerous reports have implicated HA in tumor angiogenesis, including several studies in genitourinary models. For example, inhibition of HA polymer synthesis suppressed growth kinetics of prostate tumor cells *in vitro* and *in vivo* at subcutaneous (52) and

orthotopic (49) sites, but also diminished vascular density within the resultant tumors. Seemingly contrary to this finding, excess deposition of HA has also been shown to suppress angiogenesis of prostate tumors (53). This suggests HA is required for angiogenesis but modification or further metabolism of the polymeric form is critical for the angiogenic response. In support of this hypothesis, angiogenic HA fragments (10-15 disaccharide units) have been detected in high-grade prostate cancer tissues and in the urine of patients with high-grade bladder cancer (9, 14). Similarly, angiogenic HA fragments have also been detected in the sera of children with either Wilms' tumor or bone metastasizing renal tumors (54). Interestingly, HA production in relatively low quantities has a potently stimulatory effect on angiogenesis in both prostate (31) and bladder (55, 56) tumors, which is consistent with motility experiments described above that show low HA concentrations stimulate, while high levels inhibit, migration.

4. CELL AND TISSUE HYALURONAN RETENTION

HA is a secreted product that is not covalently attached to any cellular components so its retention at the cell surface and within matrices is mediated by extracellular matrix HA binding proteins and HA receptors. Although a number of these have been identified and reviewed, their expression is usually tissue restricted, so we will focus here on those that have been characterized in the context of genitourinary cancers.

4.1. Hyaluronan binding proteins

Versican is related to the family of large secreted proteoglycans that include also aggrecan, brevican, glypican, and others (57). These proteins non-covalently bind and crosslink HA into a network in the extracellular and/or pericellular spaces. The function of this network may be for cushioning or hydration, but versican levels do rise as a function of prostate cancer severity (58) and are independently prognostic. Versican/HA complexes have also been implicated in the mediation of prostate stromal cell motility. In fact, TGFβ1 secreted by tumor cells induces stromal fibroblast secretion of versican (59). The combined accumulation of versican and HA matrix is required for prostate tumor cell remodeling of the extracellular matrix and acquisition of intrinsic motility (45).

Additional HA binding proteins have been identified but their functions in genitourinary cancer are not known. For example, the protein TSG-6 (TNF-stimulated gene 6) is a member of the link module domain containing family of HA receptors, like CD44, and has been shown to catalyze covalent crosslinking of HA polymers in complex extracellular matrices required for inflammatory resolution (reviewed in (60)) and mammalian fertility (61). A related protein, LYVE-1, is primarily expressed on lymphatic endothelial cells and responsible for HA adhesion of lymphocytes, as well as mediation of HA clearance through the lymphatic system. Expression of LYVE-1 is used as a marker for lymphangiogenesis and lymphatic content of tumors, and has been used specifically to show that lymph vessels are destroyed in the transition from benign to malignant prostate cancer, rather than created (62).

4.2. Hyaluronan receptors

CD44 is the most well-characterized cell surface receptor for HA (37). The CD44 gene product is regulated at many levels: promoter methylation, mRNA transcription, post-translational glycosylation and phosphorylation, variable splicing, and binding of a ligand (reviewed in (63, 64)). Its regulatory status dictates response of a cell to the presence of HA. Thus, CD44-mediated responses are very complex, as it has been shown to function as a tumor suppressor, but activated signaling through CD44 promotes proliferation, motility and metastasis. Herrlich, et al (65), have proposed that its activity as a tumor suppressor is mediated by the local equilibrium of HA polymers and oligomers, whereas its signaling activities are the result of CD44 having the alternative HA independent function of growth factor presentation to relevant surface receptors.

Several splice variants of CD44 have been reported in prostate carcinomas (66), but there are conflicting reports on its relation to carcinoma stage. For example, levels of CD44 standard isoform have been reported to decrease with increasing cancer grade (67, 68) due to hypermethylation of the promoter (69), and loss of this form is an independent predictor of clinical recurrence (10, 70). However, levels of CD44 variant isoforms, CD44v3 and CD44v6, have been shown to increase with Gleason sum and T-stage (10). Expression of CD44v3, but not CD44v6, independently predicts PSA biochemical recurrence. Among cultured prostate tumor cell lines, poorly aggressive androgen sensitive LNCaP cells do not express CD44, whereas metastatic androgen independent cell lines express standard and variant isoforms of CD44 (66, 71). In transitional cell carcinoma of the bladder, one study found that the expression of CD44 standard form correlated with a shorter disease free survival while another correlated it inversely with tumor stage (72, 73). However, evaluation of CD44v8-10 and CD44 standard isoform demonstrated that in cells exfoliated to the urine, it was actually the ratio of variant to standard CD44 which correlated with disease-free survival (74). In renal cell carcinoma, CD44 expression increases with grade but it is not an independent prognostic indicator (75, 76). As an additional point of interest, tumor stem cells were recently implicated in prostate cancer progression (77). Prostate cancer stem cells were isolated and characterized by their capacity for self renewal, expressing among other markers, CD44 (78).

Certainly one important function of CD44 is to mediate the effects of HA polymers and oligosaccharides on cell signaling and response. Polymers and oligomers have opposite responses, thought to result from multivalent clustering of receptors bound to polymers that transduces intracellular signals differently from the monovalent unclustered CD44 monomer. For example, Culty, et al (79), and Harada, et al (80), have shown CD44 is required for uptake and turnover of HA polymers. CD44 is localized to lipid rafts in which surface anchored hyaluronidase enzyme is locally associated, possibly through simultaneous binding of both to the same HA strand, and activated by CD44-mediated acidification through a Na+/H+ exchange pump (81). CD44-mediated

HA polymer adhesion is at least partially responsible for stabilization of cell-extracellular matrix contacts through interaction of its C-terminal tail with cytoskeletal proteins (71) such as ankyrin (33) and ezrin (82), disruption of which can occur by HA-oligomer-mediated activation of merlin, an inhibitor of cytoskeletal complex formation (83). Misra, et al (84), have shown that CD44 ligation with HA polymers can induce multidrug resistance and disruption of signaling by oligomeric HA induces apoptosis (32), inhibits anchorage independent growth and reduces *in vivo* tumorigenesis (34).

Another well-characterized HA receptor involved in cancer progression is RHAMM, the receptor for HA-mediated motility. RHAMM is an intracellular protein that translocates to and from the cell surface in response to HA binding and triggers motility of ras-transformed fibroblasts (85). There are several isoforms of RHAMM, and its expression is a prognostic indicator for breast cancer lymph node metastasis (86). RHAMM mediates ras activation and its expression correlates with MAP kinase activity. Prognostic value may also exist for bladder cancer but needs to be assessed in a larger group of patients (87). Lin and Chang (88) recently showed that siRNA knock-down of Rho kinase (ROK) could reverse HA-RHAMM malignant properties of androgen-insensitive prostate tumor cell lines, so antagonism of this signaling pathway has potential therapeutic value.

It has been proposed that CD44 may be the receptor mediating HA-facilitated arrest on microvascular endothelial cells, since anti-CD44 blocks adhesion, while RHAMM is essential for migration in vitro and angiogenesis *in vivo* (89). Draffin, et al (90), have further demonstrated the involvement of CD44 in HA-mediated adhesion of prostate and breast tumor cells to bone marrow derived endothelial cells.

5. HYALURONAN SYNTHASES

HA is synthesized by a family of three membrane embedded HA synthase enzymes: HAS1, HAS2 and HAS3. The enzymes each catalyze the polymerization of HA from intracellular UDP-esterified precursor sugars and concurrently extrude the polymers to the extracellular space (reviewed in (91, 92)). Each isozyme is temporally and tissue specifically expressed but only HAS2 is an essential gene (24). All three isozymes synthesize the same polymeric chemical product. Some controversy exists about the absolute size of HA polymers, but the average reported molecular mass of HAS1 and HAS2 synthesized HA is ≈2 million Daltons, whereas the average for HAS3 polymers is ≈200 kDa (93).

5.1. Expression in genitourinary tumors

HAS1 has been detected in bladder tumors and correlated directly with levels of HA accumulation in those tumors. For this reason, HAS1 is being tested for its additional potential prognostic value (94). HAS2 was randomly identified and characterized as one of 27 genes in a Wilms' tumor expression profile developed by genomic array screening. HAS2 and HAS3 are upregulated ≈3-fold and ≈30-fold, respectively, in metastatic prostate tumor cell lines (39). Suppression of HAS2 and/or HAS3 expression by stable antisense RNA reduced the synthesis and cell surface retention of HA (40), and inhibited primary subcutaneous (52) or intraprostatic growth and metastasis to lymph nodes, as well as growth in bone (49). Reduced primary tumor growth was associated with comparable apoptotic and proliferative fractions in culture and in tumors, but virtually no vascularization of tumors. These results implicate HA, and specifically HAS2 and HAS3, in tumor angiogenesis, as well as intrinsic growth rate modulation (52). Interestingly, HA exogenous addition to knock-down cells upon injection restored subcutaneous tumor growth and angiogenesis, suggesting a tumor or stromal factor was expressed that could modulate effects of HA in trans, with the same malignant outcome. HAS2 overexpression in prostate cells impacted tumor take but did not significantly affect size of tumors that formed subcutaneously, despite increased angiogenesis (31). In contrast, overexpression of HAS3 suppressed growth and angiogenesis in subcutaneous tumors (53). On the other hand, HAS3 overexpression in TSU bladder carcinoma cells enhanced primary tumor growth (55). Two possible alternatives may explain this dichotomy: HAase coexpression with HA synthases may be required for maximum tumorigenesis; or HA synthesis may be dose responsive with enhanced tumorigenesis at low synthesis amounts and inhibited tumorigenesis at high amounts. In the case of HAS2, both alternatives have been functionally demonstrated.

5.2. Roles in cancer

Rapidly stimulated HA synthesis and deposition is associated with cell motility in some cell contexts, and appears to facilitate this dynamic process by mechanically releasing cells from their adhesion constraints (95, 96). However, excess HA bound at the cell surface has also been shown to mediate contact inhibited growth (83). Selection of cells for HA retention at the cell surface was correlated with metastatic potential in mouse models with no effect on tumor growth (46), but overexpression of HAS2 and HAS3 has provided evidence for both tumor promoting and tumor suppressing activities of HAS-synthesized HA polymers. This apparent contradiction was resolved by Itano, et al (97), who selected a series of HAS1 and HAS2 overexpressing cell lines for increasing production of HA polymers and found a dose response curve with respect to tumorigenic potential. That is, at relatively low levels, HAS expression promoted tumor growth while at high levels, tumor growth was suppressed. Thus, the phenotype of HA overproduction in cancer is dependent upon levels of HAS activity, as well as expression of HA receptors and HAases. The bottom line is that knock down or inhibition of HA synthesis consistently inhibits tumor growth and metastasis, which pinpoints this enzyme class for therapeutic targeting. Since HA is also detected in elevated amounts in lymph node metastasis of some tumors relative to the primary site, therapeutic value may extend to metastatic intervention.

5.3. Regulation of hyaluronan production

5.3.1. Epigenetic and mechanical regulation

Cells experiencing mechanical pressure are induced to synthesize HA. Spheroidal and non-spheroidal Dunning rat prostate carcinoma cells differ by HA production. Highly metastatic non-spheroidal cells can be pressurized in three-dimensional culture to form spheroids, and in doing so, they begin secreting HA. In addition, HAS2 may become overexpressed through its location in a DNA translocation hot spot. HAS2 is located on chromosome 8q24, a newly confirmed prostate cancer genetic risk factor, estimated to be significant in at least 25-50% of cancers. This region is overamplified in many prostate cancer patients and may be one way in which production of HA becomes elevated inappropriately (20-23). Functional analysis of HAS promoters has not been rigorously performed. In addition, no evidence for alternative splicing or other modes of epigenetic regulation have been uncovered so HAS is thought to be controlled largely by transcriptional mechanisms.

5.3.2. Transcriptional regulation

Many factors have now been shown to stimulate deposition of HA. Rats treated long term with androgens developed neoplastic prostate tissue in the stroma of which HA accumulation was elevated (98). Castration, on the other hand, reduced levels of HA in the rat prostate, and these were stimulated subsequently up to 10-fold by androgen administration (99). Monslow, et al (100-102) cloned, sequenced, and identified the major transcriptional control elements of the HAS2 promoter in renal cells, finding the major constitutive regulators were Sp1 and Sp3. Other predicted transcription factor binding sites for NFκB etc. were not functionally bound by regulatory proteins in these cells.

Growth factors and proinflammatory cytokines are particularly significant for HA production in genitourinary tumors. Notably, IGF-1, bFGF (103), PDGF (104), IL-1β and TNFα (105), have all been shown to trigger HA synthesis. PDGF in particular has been shown to stimulate HAS2 expression and HA production in prostate stromal fibroblasts during the transition from normal to BPH. IL-1β specifically elevates HAS1 in synoviocytes through NFκB signaling, and can be inhibited by pyrrolidine dithiocarbamate to eliminate HA production (106), suggesting this may also be effective for bladder tumor cells that express HAS1. Also, TGFβ1 signaling and BMP7, which stimulates HAS2 expression and HA cable formation that recruits monocyte infiltration to the renal cortex, influence HA synthesis in renal proximal tubule cells (107). IL-1β is stimulatory for HA synthesis and HAS expression, but not CD44-mediated monocyte adhesion or cable formation (42). HAS3 overexpression in renal cells also induced HA cable formation, while HAS2 stimulated cell migration and was dependent on TSG-6 protein for crosslinking through transfer of the HA-binding inter-alpha-trypsin inhibitor (108). However, overexpression of HAS has also been reported to suppress motility (109), suggesting components of this system may be cell type specific.

Suramin is a chemotherapeutic agent in androgen unresponsive prostate cancer (110). Suramin inhibits HA synthesis in stromal fibroblasts, but proliferation can still be stimulated by serum. Thus, the suramin effect on HA synthesis does not suppress tumor growth through anti-proliferative signaling in the tumor cells and its mechanism of action remains to be determined.

Finally, UDP-glucose dehydrogenase controls cellular HA deposition through precursor availability and is androgen stimulated. Inhibition of HA synthesis is achieved through 4-methylumbelliferone use, which scavenges the precursors (111) and effectively halts cell cycling. Treatment of pancreatic cancer cells with this agent sensitized them to gemcitabine treatment *in vitro* and *in vivo*, but its efficacy as a chemosensitizer in genitourinary cancer has not been tested (112). Derivatives of this chemical have been characterized and found to have increased cytotoxicity *in vitro,* but again, these remain to be evaluated *in vivo* (113).

In summary, HA accumulation is a pronounced feature of genitourinary tumors that has significant diagnostic value. Deposition of HA is controlled via regulation of three differentially expressed HA synthase genes. Of these, HAS1 overexpression has been functionally implicated in bladder cancer, while HAS2 and HAS3 expression and function contribute significantly to prostate cancer progression. HAS2 is epigenetically elevated in many prostate tumors and is part of a gene expression signature for Wilms' tumor. Thus, the common tumor-associated element, HA, may have several underlying genetic determinants, each of which has potential value as a diagnostic marker and/or a therapeutic target. Receptors such as CD44 and RHAMM provide additional complexity to the array of cellular responses to HA, and a more systematic study of the receptor isoform expression patterns in combination with HAS and HAase analysis is needed for a better understanding of the roles these genes play in cancer.

6. HYALURONIDASE

Originally termed as the "spreading factor", HAases are present in a variety of toxins and venoms. For example, HAase is the virulent factor of β-hemolytic *Streptococci* and it is also present in the venoms of snake, bee, wasp, scorpion, etc, where it aids in the spread of these venoms in the body (114-118). In mammals, testicular HAase present in the sperm acrosome is necessary for the fertilization of the ovum (119). Despite a lot of work on bacterial, invertebrate and testicular HAases, a connection between HAase and cancer was unequivocally established just over a decade ago and the functional significance of HAases in cancer was demonstrated just about a year ago (31, 120-124). In this part of the review, we will focus on the recent advances in our understanding of the role of HAases in genitourinary carcinomas.

6.1. Hyaluronidase gene and protein structure

HAases are a class of enzymes that predominantly degrade HA. However, HAases can also

degrade chondroitin sulfate and chondroitin, albeit at a slower rate (125). HAases are endoglycosidases, as they degrade the ß-N-acetyl-D-glucosaminidic linkages in the HA polymer. Six HAase genes are present in the human genome and these occur in two linked triplates. HYAL-1, -2 and -3 genes are clustered in the chromosome 3p21.3 locus, whereas HYAL-4, HYAL-P1 and PH20 (encodes testicular HAase) reside in the chromosome 7q31.3 locus (126). It is likely that the six mammalian HAase genes must have arisen through gene duplication events, since they share a significant amino acid identity. For example, HYAL-1, -2, -3, -4 and PH20 share ~ 40% amino acid identity (125). Based on their pH activity profiles, HAases are divided into two categories. HYAL-1, -2 and -3 are considered as acidic HAases because they are active at acidic pH. For example, HYAL-1 has a pH optimum around 4.0 – 4.2 and the enzyme is inactive above pH 5.0 (9). On the contrary, PH20 is a neutral active HAase as it is active at pH 7.0 (pH activity profile 3.0 – 9.0) (127).

Among the six mammalian HAases, HYAL-1, -2 and PH20 are well characterized. As described above, PH20 is necessary for ovum fertilization and several natural and synthetic HAase inhibitors have been tested for their use as contraceptives (128-131). PH20 and HYAL-2 are glycosyl phosphatidyl-inositol (GPI)-linked proteins. HYAL-2 degrades HA into ~ 20 kDa oligosaccharides (~ 25 disaccharide units). HYAL-1 is the serum HAase and is expressed in several somatic tissues (125, 132-134). HYAL-1 has also been purified from human urine, where it is expressed as two molecular forms (135). Although, HYAL-1 has high specific activity for degrading HA, its concentration in human serum is low (60 ng/ml) (125).

The active site and amino acid residues important for HA degradation are conserved among HAases, as revealed by site directed mutagenesis of PH20, identification of naturally occurring mutations in HYAL-1 and alternatively spliced variants of HYAL1 and HYAL3, and the crystal structure of bee venom HAase (115, 136-139). In all six mammalian HAases, a conserved glutamate and aspartate are believed to be responsible for the substrate cleavage. In addition to the active site, a 30 amino acid sequence that is conserved in all 6 mammalian HAases and also in the bee HAase, appears to be necessary for HAase activity (138), probably because it provides an integral part of one of the walls of the substrate binding groove. It is noteworthy that in HYAL-1 and HYAL-3 transcripts, this 30 amino acid sequence is encoded by a separate exon that is alternatively spliced (138).

Among the six mammalian HAases, HYAL-1 is the major tumor-derived HAase and is expressed by a variety of tumor cells. HYAL-1 was initially purified from the urine of patients with high-grade bladder cancer and was shown to be expressed in epithelial cells of bladder, and prostate tumors and in head and neck squamous cell carcinoma cells (9, 124, 127).

6.2. Hyaluronidase expression in genitourinary tumors

Detection and measurement of HAase activity in tissues, body fluids and cell conditioned media became possible because of an HAase ELISA-like assay developed by Stern and Stern (145). A modified version of this assay was used by Lokeshwar et al to measure HAase levels in prostate and bladder carcinoma tissues, cells, and the urine of bladder cancer patients (9, 124, 127, 138, 140-144). The modified HAase ELISA-like assay is called the HAase test, which involves incubation of tissue extracts, urine or cell conditioned media on HA-coated microtiter well plates in an HAase assay buffer. Following incubation at 37^0 C for ~ 16 hours, the degraded HA is washed off and the HA remaining on the HA-coated plate is detected using a biotinylated bovine nasal cartilage HA-binding protein. The HAase present in biological specimens is determined from a standard graph, plotted as HAase (mU/ml) versus $O.D._{405\ nm}$. The HAase activity is then normalized to total protein concentration (mg/ml) or to cell number (if assaying cell conditioned media). Using the HAase test and also a substrate (HA)-gel assay, Lokeshwar et al found that HAase levels are elevated in prostate cancer tissues, when compared to normal prostate and BPH tissues (141). This study also linked for the first time, HAase levels to tumor progression. In that study, HAase was found to be elevated 3-7-fold in high-grade (Gleason ≥ 7) prostate cancer tissues when compared to low-grade (Gleason 5 -7) prostate cancer tissues. Metastatic prostate cancer lesions were found to have even higher HAase levels than the high-grade primary tumor (9, 141). HAase levels are also elevated in high-grade bladder tumor tissues and in the urine of patients with high-grade bladder cancer. HAase levels in low-grade bladder tumor tissues and urine are comparable to those found in normal bladder tissues and urine (15, 140, 142-144). These studies have established a link between HAase and the tumor invasive/metastatic phenotype. In addition to bladder and prostate carcinomas, HAase levels have also been shown to be elevated in the urine of children with Wilms' tumor (145). In addition to genitourinary tumors, HAase levels are elevated in head and neck squamous cell carcinoma, breast tumors, metastatic tumors and glioma cells (30, 127, 146-156).

RT-PCR and cDNA cloning, protein purification, immunoblotting, pH activity profile and immunohistochemistry have revealed that HYAL-1 is the major tumor-derived HAase expressed in prostate and bladder carcinoma cells. HYAL-1 is a ~ 55 – 60 kDa protein consisting of 435 amino acids. In fact HYAL-1 was the first HAase to be recognized as being expressed by tumor cells and its expression correlates with their invasive/metastatic potential (9, 124). No HYAL-1 expression is observed in the tumor-associated stroma, although HYAL1 expression appears to correlate and perhaps induce HA production in the tumor-associated stroma (122, 123).

Contrary to the findings regarding elevated expression of one or more HAases in tumors, it has been shown that the chromosome locus 3p21.3, where HYAL-1, -2 and -3 genes are clustered, is deleted in lung and some breast carcinomas at a higher frequency. However, the tumor suppressor gene in this region is actually RASSF1 and not a HAase gene (152, 157, 158). Nonetheless, it was previously believed that HYAL-1 is a tumor suppressor gene (157, 159, 160). Interestingly, again based on the real

time RT-PCR studies, Bertrand, et al, reported that HYAL-2 expression correlates with lymphoma diagnosis, but the expression actually decreases in high-grade lymphomas, when compared to low-grade lymphomas (147).

Taken together, HAase expression appears to be elevated in many carcinomas and the expression correlates with tumor invasiveness. However, in some carcinomas HAase expression depends on the status of the chromosome 3p21.3 locus and may inversely correlate with tumor grade.

6.3. Hyaluronidase functions in genitourinary tumors

6.3.1. Hyaluronidase as a tumor promoter

Extensive digestion of HA by HAase generates tetrasaccharides, whereas limited digestion generates HA fragments, some of which are angiogenic (4 – 25 disaccharide units). HA fragments of 10 – 15 disaccharide units have been shown to stimulate endothelial cell proliferation, adhesion and capillary formation (161, 162). Such angiogenic HA fragments are found in the urine of patients with high-grade bladder cancer, in the tissue extracts of high-grade prostate tumors, and in the saliva of patients with head and neck squamous cell carcinoma, suggesting that the HA-HAase system is active in high-grade invasive tumors (9, 14, 127).

Recent evidence based on cDNA transfection studies shows that HYAL-1 is involved in tumor growth, muscle infiltration by tumor and tumor angiogenesis (31, 121-123). Lokeshwar et al have shown that blocking HYAL-1 expression in bladder and prostate cancer cells decreases tumor cell proliferation by ~ 4-fold, due to cell cycle arrest in the G2/M phase and decreases their invasive activity. In xenografts, inhibition of HYAL1 expression resulted in a decrease in tumor growth by 9 – 17-fold. While HYAL-1 expressing tumors infiltrated muscle and blood vessels, tumors lacking HYAL-1 expression resembled benign neoplasm and had 4 – 9-fold less microvessel density and smaller capillaries (122, 123). The contribution of HYAL-1 expression to muscle invasion by a bladder tumor has been observed in bladder cancer patients. Aboughalia has shown that HYAL-1 expression in tumor cells exfoliated in urine correlates with tumor invasion into the bladder muscle and beyond (163). It is noteworthy that patients with muscle invasive bladder cancer have poor prognosis, as 60% of the patients with muscle invasive bladder cancer will have metastasis within two years and two-thirds will die within five years. Interestingly, HA production by the tumor stroma correlates with HYAL-1 levels in tumor cells, suggesting crosstalk between the tumor and the tumor-associated stroma (122, 123). Such crosstalk between HA and HYAL1, with respect to tumor growth and angiogenesis, was recently confirmed by Simpson, who tested tumor growth and angiogenesis following the expression of HAS2 and HYAL-1, either individually or together, in a non-invasive prostate cancer cell line. While HAS2 or HYAL-1 when expressed individually in a prostate cancer cell line, increased tumor growth and angiogenesis their co-expression had a synergistic effect on this increase (31). Expression of HYAL-1 in a human prostate cancer cell line also causes a slight increase in its ability to form lung metastasis in xenografts (47), and induced spontaneous metastasis to lymph node following orthotopic implantation (121).

6.3.2. Hyaluronidase as a tumor suppressor

Contrary to the tumor promoting effects of HYAL-1, a prevalent concept has been that, in general, HAases are tumor suppressors (157, 159, 160). The origin of this concept lies in the observation that in some epithelial carcinomas, the 3p21.3 locus is deleted and although the tumor suppressor gene in this locus was shown not to be a HYAL gene (i.e., HYAL-1, -2, or -3), the concept continued (125, 152, 157, 160). Perhaps this concept became popular because HA is known to promote tumor metastasis, and therefore, conceptually it was easier to explain that an enzyme that degrades HA was a tumor suppressor. In support of this concept, Jacobson et al reported that while HAS2 expression in a rat colon carcinoma line promoted tumor growth, the over-expression of HYAL-1, at levels (220 – 360 mu/10^6 cells) that are not found in tumor tissues and tumor cells, inhibited tumor growth and generated necrotic tumors (120). Furthermore, Shuster et al showed that administration of very high concentrations of bovine testicular HAase (300 units) caused a ~ 50% regression in breast tumor xenografts (164). The controversy surrounding HAase as a tumor promoter or a suppressor was recently resolved, by Lokeshwar et al. Selection of cells for expression of different HYAL-1 levels showed that cells expressing amounts found in tumor tissues and cells promote tumor growth, invasion and angiogenesis. In contrast, cells with HAase levels exceeding 100 milliunits/10^6 cells, (i.e.; levels that are not naturally expressed by tumor cells) exhibit reduced tumor incidence and growth due to induction of apoptosis (122). Therefore, the function of HAase as a tumor promoter or a suppressor is a concentration-dependent phenomenon and levels in genitourinary tumors are consistent with tumor cell-derived HAase acting mainly as a tumor promoter.

6.4. Epigenetic Regulation of HAase activity

At the present time, not much information is available about the genetic regulation of HAases, because except for HYAL-2, the promoter regions for the HAases have not been mapped. However, HAase expression appears to be regulated by certain epigenetic mechanisms. One of the mechanisms to control cellular HAase expression is the loss of the chromosome 3p21.3 locus, which occurs at a higher frequency in some epithelial tumors (165-167). Alternative mRNA splicing is another mechanism by which HAase activity is regulated. A common internal splicing event occurs in the 5' untranslated region present in exon 1 (152, 159). This splicing event joins nucleotides 109 and 597. Frost et al and Junker et al reported that HYAL-1 protein levels and HAase activity in tumor cells correlate with a HYAL-1 transcript in which this 5' untranslated region is spliced. Furthermore, HYAL-1 protein is not detected in tumor cells which express a HYAL-1 transcript that retains the 5' untranslated region. Based on these findings, both groups concluded that the HYAL-1 transcript containing the 5' untranslated region is not translated and this incomplete

splicing of HYAL-1 pre-mRNA is of epigenetic nature (152, 159). However, it is unclear how and why splicing of the 5'-untranslated region in the HYAL-1 mRNA prevents translation, as the translation start site for HYAL-1 (nucleotide 617; GenBank accession # HSU03056) is downstream of the spliced region. Using normal and bladder tumor tissues and bladder and prostate cancer cells, Lokeshwar et al have reported several alternatively spliced variants of HYAL-1 and HYAL-3 transcripts. These variants are generated by alternative splicing occurring in the coding regions of HYAL-1 and HYAL-3 transcripts which encode truncated proteins lacking HAase activity (138). Five alternatively spliced variants of the HYAL-1 transcript that affect the coding region have been reported. HYAL1-v1 protein lacks a 30 amino acid stretch between amino acids 300 and 301 and is generated by alternative splicing of exon 2. The HYAL1-v2 protein sequence from amino acids 183 to 435 is identical to HYAL-1 and the HYAL1-v3 protein contains the first 207 amino acids of the HYAL-1 wild type protein. HYAL1-v4 and HYAL1-v5 proteins consist of amino acids 260 – 435 and 340 – 435, respectively, relative to the wild type protein. Among the HYAL-3 splice variants, HYAL3-v1 lacks a 30 amino acid sequence present in the wild type protein and this truncation joins amino acid 298 to 329. HYAL3-v1 is generated by alternative splicing of exon 3. HYAL3-v2 encodes a 168 amino acid protein, and this is identical to amino acids 249 – 417 in the HYAL-3 wild type protein. HYAL3-v3 protein encodes a 138 amino acid protein that is 100% identical to amino acids 249 – 417 except that it also lacks the 30 amino acid sequence from 299 to 328. As discussed above, although various splicing events maintain the open reading frame of the HYAL-1 and HYAL-3 proteins, none of these variants are functionally active (138).

Recent data on one of the HYAL-1 variants, HYAL1-v1, shows that the expression of HYAL1-v1 is higher in normal bladder tissues than in bladder tumor tissues. Furthermore, HYAL1-v1 expression reduces HAase activity secreted by bladder cancer cells because of a complex formation between HYAL-1 and HYAL1-v1. HYAL1-v1 expression induces apoptosis in bladder cancer cells and reduces tumor growth, infiltration and angiogenesis (168). This suggests that a critical balance between the levels of HYAL-1 and HYAL-1 variants may regulate HYAL-1 function in cancer.

6.5. Hyaluronidase and signaling

6.5.1. Hyaluronidase and cell cycle progression

As discussed above, blocking HYAL-1 expression in bladder and prostate cancer cells induces cell cycle arrest in the G2/M phase. G2/M arrest results from the down-regulation of the positive regulators of G2/M transition. For example, stable HYAL-1 antisense transfectants show downregulation of cdc25c, cyclin B1 and cdk1 levels, as well as cdk1 kinase activity (122, 123). In HSC3 oral carcinoma cells, HYAL-1 expression caused a 145% increase in the S-phase fraction, with a concomitant decrease in the G0/G1 phase (169).

The mechanism by which HYAL-1 induces cell cycle transition and upregulates the levels of positive regulators of G2/M transition is unknown. However, testicular HAase has been shown to induce phosphorylation of c-jun N-terminal kinases (JNK)-1 and -2 and p42/44 ERK in L929 murine fibroblast cells (170). ERK is required for G2/M and G1/S transitions (171). Lokeshwar et al have previously shown that cell surface interaction between HA oligosaccharides and RHAMM stimulates phosphorylation (and activation) of p42/p44 ERK and focal adhesion kinase (FAK) in human endothelial cells (161). RHAMM co-immunoprecipitates with src and ERK and contains recognition sequences for these kinases, suggesting a direct interaction (172, 173). Activated FAK also activates ERK through Grb2 and Shc, and PI3 kinase through a direct interaction (174, 175). It is noteworthy that angiogenic HA fragments are detected in high-grade tumor tissues and in body fluids (e.g., urine and saliva) of cancer patients (9, 14, 127).

6.5.2. Hyaluronidase and apoptosis

As discussed above, very high expression of HYAL-1 induces apoptosis in prostate cancer cells. The apoptosis induction by HYAL-1 involves mitochondrial depolarization and induction of a pro-apoptotic protein, WOX1. WOX1 is a ww-domain containing oxidoreductase that contains a nuclear localization signal, a mitochondrial localization signal and an alcohol dehydrogenase domain (178). Chang has shown that transient transfection of the murine fibroblast line L929, by HYAL-1 or HYAL-2 cDNA or ectopic addition of bovine testicular HAase (100 U/ml) enhances TNF-induced cytotoxicity, which is mediated by increased WOX1 expression and prolonged NFκB activation (170, 179, 180). WOX1 is known to induce apoptosis in a p53 independent manner, which involves WOX1 activation (i.e., WOX1-^{P}Tyr33), WOX1 translocation to mitochondria, and down-regulation of anti-apoptotic proteins bcl2 and $bclx_L$ (179).

Recently, Lokeshwar et al have shown that the expression of HYAL1-v1 in bladder cancer cells that express wild type HYAL-1, induces G2/M arrest and apoptosis. HYAL-1 and HYAL1-v1 form a non-covalent complex, which is enzymatically inactive. The HYAL1-v1 induced apoptosis involves the extrinsic pathway, since HYAL1-v1 expression induces activation of caspases -8, -9 and -3, Fas and FADD (Fas associated death domain) upregulation and BID activation. Moreover, inhibiton of Fas expression by Fas siRNA inhibits HYAL1-v1 induced apoptosis (168). These reports suggest that HYAL-1 and its variants are capable of inducing apoptotic pathways, the understanding of which has only recently begun.

6.6. Hyaluronidase as a diagnostic and prognostic indicator

The diagnostic potential of HAase, either alone or together with HA has been extensively explored in bladder cancer. For example, urinary HAase levels, measured using the HAase test, have been shown to be 3-7-fold elevated among patients with intermediate (G2) and high (G3)-grade bladder cancer when compared to normal individuals, patients with one of the many benign urologic conditions, patients with a history of bladder cancer, and patients with low-grade bladder cancer (143). In a study of 513 urine

specimens, the HAase test had 81.5% sensitivity, 83.8% specificity and 82.9% accuracy to detect G2/G3 patients. When the HAase test was combined with the HA test, which measures urinary HA levels, the combined HA-HAase test had higher sensitivity (91.2%) and accuracy (88.3%), and comparable specificity (84.4%) to detect bladder cancer, regardless of the tumor grade and stage (142). In another study, where 70 bladder cancer patients were prospectively followed for a period of 4 years to monitor bladder cancer recurrence, the HA-HAase test had 91% sensitivity and 70% specificity to detect bladder cancer recurrence (15). More importantly, a patient with a false-positive HA-HAase test had a 10-fold increased risk for developing bladder cancer within 5 months. In a side-by-side comparison, the HA-HAase test was also superior to a variety of FDA-approved bladder tumor markers (140, 144). Hautmann et al have shown a correlation between increased tumor-associated HYAL-1 and HA in tumor tissues and a positive HA-HAase test (183). This suggests that tumor-associated HYAL-1 and HA are released into the urine when it comes in contact with a tumor in the bladder. In addition to urinary HAase levels, measurement of HYAL-1 mRNA levels in exfoliated cells found in urine also appears to be a marker for bladder cancer. Eissa et al found that HYAL-1 mRNA expression determined by RT-PCR has > 90% accuracy in detecting bladder cancer (184). Furthermore, HYAL-1 mRNA levels measured in exfoliated cells are elevated in patients with invasive and poorly differentiated carcinoma (163). These studies show that HAase is a highly accurate marker for detecting high-grade bladder cancer, and when it is combined with HA, it detects both low-grade and high-grade bladder cancer with ~ 90% accuracy.

The prognostic potential of HYAL-1 has been explored in prostate cancer. Standard clinical and pathological parameters provide very limited information to clinicians regarding which prostate cancers will progress, and/or have a poor prognosis. As a result, it is difficult to identify which patients need aggressive treatment among those for whom watchful waiting would be sufficient. By performing immunohistochemistry on radical prostatectomy specimens, on whom there was a minimum 5-year follow-up, Posey et al and Ekici et al found that HYAL-1 is highly expressed in specimens from patients who later had a biochemical recurrence (11, 12). HYAL-1 staining in radical prostatectomy specimens appears to be an independent predictor of biochemical recurrence. Furthermore, HYAL-1 staining when combined with HA staining has an 87% accuracy in predicting disease progression (11). It is noteworthy that in early stage prostate cancer specimens while HYAL-1 is exclusively expressed by tumor cells, HA is mostly expressed by the tumor-associated stroma (12), though its cellular origin becomes uncertain as compartmental boundaries erode in progression. These results show that consistent with the function of HYAL-1 in tumor growth, infiltration and angiogenesis, it is most likely a prognostic indicator for disease progression.

6.7. Hyaluronidase and cancer therapeutics

Testicular HAase has been added in cancer chemotherapy regimens to improve drug penetration. Tumor cells growing in 3-dimensional multicellular masses, either *in vitro* as spheroids or as solid tumors *in vivo*, acquire a common resistance to chemotherapeutic drugs (185). The resistance of multicellular spheroids of EMT-6 to 4-hydroperoxycyclophosphamide (4-HC) can be abolished by treatment of the spheroids with HAase (186-188). Consistent with the findings that HAase is necessary for cell cycle progression (122, 123, 169), HAase treatment increases recruitment of disaggregated cells into the cycling pool, and thus renders them more sensitive to a cell cycle dependent drug (186-188). In limited clinical studies, HAase has been used to enhance the efficacy of vinblastine in the treatment of malignant melanoma and Kaposi's sarcoma (189, 190), boron neutron therapy of glioma (191, 192), intravesical mitomycin treatment for bladder cancer (193, 194) and chemotherapy involving cisplatin and vindesine in the treatment of head and neck squamous cell carcinoma (195, 196).

In summary, HAase is an endoglycosidase that functions in tumor growth, infiltration and angiogenesis. At concentrations that are present in tumor tissues, HAase acts as a tumor promoter. However, artificially increasing these concentrations results in HAase functioning as a tumor suppressor. HYAL-1 type HAase regulates cell cycle progression and apoptosis, and therefore, may regulate tumor growth and angiogenesis. The regulation of HAase in cancer appears to be controlled at the transcription level. HAases either alone, or together with HA are potentially accurate diagnostic and prognostic indicators for cancer detection and tumor metastasis. We are only beginning to understand the complex role that this enzyme plays in cancer. In the future because of its role in tumor growth and progression, this enzyme may be targeted for developing novel cancer therapeutics and diagnostics.

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Abbreviations: HA: hyaluronic acid; HAase: hyaluronidase; HAS: hyaluronan synthase; BPH: benign prostatic hyperplasia

Key Words: Hyaluronidase, Hyaluronic Acid, Hyaluronan, Tumor Growth, Infiltration, Angiogenesis, Cancer Diagnosis, Cancer Prognosis, Tumor Promoter, Tumor Suppressor, Review

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