Published in final edited form as:
Semin Cancer Biol. 2008 August ; 18(4): 281–287. doi:10.1016/j.semcancer.2008.03.008.

HYALURONDIASE: BOTH A TUMOR PROMOTER AND SUPPRESSOR

Vinata B. Lokeshwar[1,2] and **Marie G. Selzer**[1]

1*Department of Urology, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, Florida*

2*Department Cell Biology and Anatomy, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, Florida*

Keywords

Hyaluronidase; hyaluronic acid; tumor growth; infiltration; angiogenesis; cancer diagnosis; cancer prognosis; tumor promoter; tumor suppressor

Originally termed as the “spreading factor”, hyaluronidases (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 (1–5). In mammals, testicular HAase present in the sperm acrosome is necessary for the fertilization of the ovum (6). 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 (7–11). In this part of the review, we will focus on the recent advances in our understanding of the role of HAases in cancer.

Hyaluronidases

HAases are a class of enzymes that predominantly degrade hyaluronic acid (HA). However, HAases can also degrade chondroitin sulfate and chondroitin, albeit at a slower rate (12). HAases are endoglycosidases, as they degrade the β-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 (13). 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 (12). 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 (14). On the contrary, PH20 is a neutral active HAase as it is active at pH 7.0 (pH activity profile 3.0 – 9.0) (15).

*Vinata B. Lokeshwar, Ph.D., Department of Urology (M-800), Miller School of Medicine, University of Miami, P.O. Box 016960, Miami, Florida, 33101, Phone: (305) 243-6321, Fax: (305) 243-6893, e-mail: vlokeshw@med.miami.edu.

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 (16–19). PH20, as well as, 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 (12,20–22). HYAL-1 has also been purified from human urine, where it is expressed as two molecular forms (23). Although, HYAL-1 has high specific activity for degrading HA, its concentration in human serum is low (60 ng/ml) (12).

Site directed mutagenesis of PH20, identification of naturally occurring mutations in HYAL-1 and alternatively spliced variants of HYAL1 and HYAL3, crystal structure of bee venom HAase and 3-D x-ray structure of bovine PH20 have revealed the catalytic site of HAases involved in HA degradation (4,24–27). The crystal structure of the bee HAase and x-ray structure of bovine PH20 show that HAases have a classical $(\beta/\alpha)_8$ TIM barrel structure. The dominant feature of the HAase structure is a large grove that extends perpendicular to the barrel axis. In bee HAase, the loops following the β strands 2, 3, and 4 form one wall of the groove, and those of 1, 5, 7 and 7 form the other wall. This groove is large enough to accommodate a hexasaccharide. In bee HAase, the catalytic site that cleaves the glycosaminidic bond between N-acetyl-D-glucosamine and D-glucuronic acid lies in amino acid residues Asp^{111} and Glu^{113} (12). In a susbtrate-assisted acid-base catalytic mechanism Glu^{113} acts as the proton donor, and the N-acetyl group of the substrate acts as the nucleophile. In all 6 mammalian HAases, this Glu residue is conserved along with the Asp and is believed to be responsible for the substrate cleavage. For example, site directed mutagenesis has identified Glu^{148} and Asp^{146} in human PH20 as the important residues involved in the actual catalysis of the glucosminidic linkage. In HYAL-1 the equivalent resideues are Glu^{131} and Asp^{129}. 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 (26). In HYAL-1, this sequence appears in amino acid 301 to 330. Based on the bee HAase crystal structure, the 30 amino acid sequence (amino acid 313 o 342 in the bee HAase sequence), forms β sheets 6 and 7, α-helix 8 and the loops in between (4). Thus, this 30 amino acid sequence is an integral part of one of the walls of the substrate binding groove. In addition, in this 30 amino acid sequence, a Trp residue (Trp^{333}, bee HAase, Trp321 HYAL-1) is conserved in all mammalian and bee HAases and in chitinolytic enzymes and is involved in hydrophobic interaction with the N-acetyl side chain (4). 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 (26).

Among the 6 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 (7,14,15).

HAase expression in tumor cells

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 (28). A modified version of this assay was used by Lokeshwar et al to measure HAase levels in prostate and bladder carcinoma tissues, cells and in the urine of bladder cancer patients (7, 14,15,26,29–33). 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 a HAase assay buffer. Following incubation at 37° 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 benign prostatic hyperplasia tissues (31). This study also linked for the first time, HAase levels to tumor progression. In that study, HAase leveles were 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 (14,31). 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 (29,30,32–34). 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 (35). In addition to genito-urinary tumors, HAase levels are elevated in head and neck squamous cell carcinoma, breast tumors, metastatic tumors and glioma cells (15, 36–47).

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 (7,14). 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 (8,9).

Patients with head and neck squamous cell carcinomas have been shown to have elevated HAase levels in their saliva and HYAL-1 is the major HAase that is expressed in these tumor tissues (15). However, in addition to HYAL1, RT-PCR analysis has revealed PH20 expression in head and neck carcinoma, especially laryngeal carcinoma (36–38). Interestingly, the pH activity profile of the HAase activity expressed in tumor tissues is similar to that of HYAL-1, and not, PH20 (15,36). HAase levels are also shown to be elevated in breast tumors and RT-PCR analysis has detected the expression of PH20, HYAL-2 and HYAL-3 in breast cancer tissues (42,43). As in the case of prostate and bladder carcinomas, HAase levels in metastatic breast tumors are found to be 4-fold higher than those expressed in primary tumors (45). Similarly, HAase levels were higher in brain metastatic lesions of carcinomas other than primary glioblastomas (46). Furthermore, there is some evidence that while less invasive breast cancer cells express HAS3 and HYAL-3, highly invasive cells express HAS2 and HYAL-2 (42). However, how and why the HA production by HAS2 and HA degradation by HYAL-2 promote tumor cell invasion, but HA production by HAS3 and HA degradation by HYAL-3 associates with low-invasive phenotype is unclear. It is noteworthy that in these studies, the expression of HAS and HYAL isoforms was studied only at the transcript level by real time RT-PCR. Given that functionally inactive splice variants of HYAL-1 and HYAL-3 are previously reported (as discussed below), the expression of HYAL genes at the transcript level does not necessarily translate into HAase activity produced by breast cancer or any other cell type. Similar observations regarding HYAL-2 and HYAL-3 expression were reported for endometrial carcinoma. In a relatively small number of endometrial carcinoma specimens (n = 13), HYAL-2 and HYAL-3 mRNA expression, determined by real time RT-PCR was found to be > 1000- and > 30-fold more than HYAL-1, respectively (47).

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 RASSF1 and not a HAase gene (43,48,49). Nonetheless, it was previously believed that HYAL-1 is a tumor suppressor gene (48,50,51). 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 (41).

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.

HAase functions in genitor-urinary tumors (HAase functions in cancer: Stern article)

HAase a tumor promoter

Extensive digestion of HA by HAase generates tetrasaccharides, whereas, limited digestion generates HA fragments, some of which are angiogenic (3 – 25 disaccharide untis). HA fragments of 10 – 15 disaccharide units have been shown to stimulate endothelial cell proliferation, adhesion and capillary formation (52,53). 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 (14,15,54).

Recent evidence based on cDNA transfection studies shows that HYAL1 is involved in tumor growth, muscle infiltration by tumor and tumor angiogenesis (8–10). 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 (8,9). 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 (55). 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 2-years and 2/3rds will die within 5-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 (8,9,14). 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 (10). Expression of HYAL-1 in a human prostate cancer cell line also causes a slight increase in its ability to form lung metastasis in xenograft (56).

HAase a tumor suppressor

Contrary to the tumor promoting effects of HYAL-1, a prevalent concept has been that, in general, HAases are tumor suppressors (48,50,51). 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 (12,43,48,51). 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 (11). Furthermore, Shuster et al showed that administration of super high concentrations of bovine testicular HAase (300 units) caused a ~ 50% regression in breast tumor xenografts (57). The controversy whether HAase is a tumor promoter or a suppressor was recently resolved, when Lokeshwar et al showed that while HYAL-1 levels that are expressed in tumor tissues and cells promote tumor growth, invasion and angiogenesis, HAase levels exceeding 100 milliunits/ 10^6 cells), i.e., at levels that are not naturally expressed by tumor cells, significantly reduce tumor incidence and growth due to induction of apoptosis (8). Therefore, the function of HAase as a tumor promoter or a suppressor is a concentration-dependent phenomenon, but in tumor tissues, the tumor cell-derived HAase acts mainly as a tumor promoter.

Regulation of HAase activity

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 (58–60). 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 (43,50). 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, Frost et al and Junker et al concluded that the HYAL-1 transcript containing the 5' untranslated region is not translated (43,50). However, it is unclear how and why the 5'-untranslated region in the HYAL-1 mRNA prevents translation. 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 that lack HAase activity (26). For example, 5 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 3001 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, that are present in 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 (26).

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 (61). This suggests

that a critical balance between the levels of HYAL-1 and HYAL-1 variants may regulate HYAL-1 function in cancer.

HAase and signaling

HAase 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 anti-sense transfectants show down-regulation of cdc25c, cyclin B1 and cdk1 levels, as well as, cdk1 kinase activity (8,9). In HSC3 oral carcinoma cells, HYAL1 expression caused a 145% increase in the S-phase fraction, with a concomitant decrease in the G0-G1 phase (62).

The mechanism by which HYAL1-induces cell cycle transition and up-regulates 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 p44/42 ERK in murine fibroblasts cells L929 (63). ERK is required for G2-M and G1-S transitions (64). Lokeshwar et al have previously shown that cell surface interaction between HA oligosaccharides and RHAMM stimulates phosphorylation of p42/p44 ERK (activated p42/44ERK) and focal adhesion kinase in human endothelial cells (52). RHAMM co-immunoprecipitates with src and ERK and contains recognition sequences for these kinases, suggesting a direct interaction (65,66). Activated FAK also activates ERK through Grb2 and Shc and PI3 kinase through a direct interaction (67,68). 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 (14,15,54). In addition to ERK activity, transient activation of JNKs is required for G2-M transition. For example, activated JNK may phosphorylate cdc25c and modulate its activity (69). Furthermore, activated JNKs phosphorylate c-jun, which then increases cdc2 expression (70). However, at the present time it is unknown whether hyaluronidase-mediated regulation of the cell cycle involves JNK and/or ERK pathways.

HAase and apoptosis

As discussed above, the super 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 dehydorgenase domain (71). Chang has shown that transient transfection of the murine fibroblast line L929, by HYAL1 or HYAL2 cDNA or ectopic addition of bovine testicular HAase (100 U/ml) enhances TNF-induced cytotoxicity, which is mediated by increased WOX1 expression and prolonged NK_{KB} activation (6372,73). WOX1 is known to induce apoptosis in a p53 independent manner, which involves WOX1 activation (i.e., WOX1-^{P}Tyr33), its translocation to mitochondria and down-regulation of anti-apoptotic proteins bcl2 and $bclx_L$ (72). Although the kinase, which phosphorylates WOX1, is unknown, JNK1 directly interacts with WOX1 (72). JNK is also associated with the mitochondria-mediated apoptotic pathway, as it phosphorylates bcl-2 and bcl_{XL}, and suppresses their anti-apoptotic activity (74,75).

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. HYAL1 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) up-regulation and BID activation. Moreover, inhibiton of Fas expression by Fas siRNA inhibits HYAL1-v1

induced apoptosis (61). These reports suggest that HYAL-1 and its variants are capable of inducing apoptotic pathways, the understanding of which has only recently begun.

HAase 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 (30). 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 (29). 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 (34). 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 (32,33). Hautmann et al have shown a correlation between increased tumor-associated HYAL-1 and HA in tumor tissues and a positive HA-HAase test (76). 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. For example, Eissa et al found that HYAL-1 mRNA expression determined by RT-PCR has > 90% accuracy in detecting bladder cancer (77). Furthermore, HYAL-1 mRNA levels measured in exfoliated cells are elevated in patients with invasive and poorly differentiated carcinoma (55). 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, and as a result, it is difficult to predict which patients need aggressive treatment, from 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 (78,79). Biochemical recurrence is defined as increasing serum prostate specific antigen (PSA) levels following radical prostatectomy and is an indicator of disease progression (i.e., either local recurrence or metastasis to distant sites). 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 (79). It is noteworthy that in prostate cancer specimens while HYAL-1 is exclusively expressed by tumor cells, HA is mostly expressed by the tumor-associated stroma (78). 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.

In a limited number of studies, hyaluronidase expression has also been studied in other carcinomas. For example, there is some evidence that HYAL-1 may be an accurate marker for head and neck squamous cell carcinomas and that salivary HAase levels are elevated in head

and neck cancer patients (15). In addition to HYAL-1, PH20 mRNA levels have been shown to be elevated in primary and lymph node metastatic lesions of laryngeal carcinoma when compared to normal laryngeal tissues (36–38). In contrast to the observations in many other carcinomas, increased HYAL-2 expression inversely correlates with invasion in B-cell lymphomas and may serve as a prognostic indicator (41).

HAase and cancer therapeutics

Testicular HAase has been added in cancer chemotherapy regimens to improve drug penetration. Tumor cells growing in 3-dimensional multicellular masses, such as spheroids *in vitro* and solid tumors acquire resistance to chemotherapeutic drugs (i.e., multicellular resistance) (80). The resistance of multicellular spheroids of EMT-6 to 4-hydroperoxycyclophosphamide (4-HC) can be abolished by treatment of these spheroids by HAase (81–83). Consistent with the findings that HAase is necessary for cell cycle progression (8,9,62), HAase treatment increases recruitment of disaggregated cells into the cycling pool, and thus renders them more sensitive to a cell cycle dependent drug (81–83). In limited clinical studies, HAase has been used to enhance the efficacy of vinblastin in the treatment of malignant melanoma and Kaposi's sarcoma (84,85), boron neutron therapy of glioma (86,87), intravesical mitomycin treatment for bladder cancer (88,89) and chemotherapy involving cisplatin and vindesine in the treatment of head and neck squamous cell carcinoma (90,91). It is noteworthy that the HAase concentrations ($1 \times 10^5 – 2 \times 10^5$ IU) used in these clinical studies far exceed the amount of HAase present in tumor tissues, and therefore, it is unlikely that at these concentrations the infused HAase will act as a tumor promoter.

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.

Acknowledgements

Grant support: NIH/NCI RO1 072821-06 (VBL), NIH R01 DK66100 (VBL)

REFERENCES

1. Nagaraju S, Mahadeswaraswamy YH, Girish KS, Kemparaju K. Venom from spiders of the genus Hippasa: Biochemical and pharmacological studies. Comp Biochem Physiol C Toxicol Pharmacol. 2006(In Press)
2. Morey SS, Kiran KM, Gadag JR. Purification and properties of hyaluronidase from Palamneus gravimanus (Indian black scorpion) venom. Toxicon 2006;47:188–195. [PubMed: 16359718]
3. Girish KS, Kemparaju K. Inhibition of Naja naja venom hyaluronidase: role in the management of poisonous bite. Life Sci 2006;78:1433–1440. [PubMed: 16253285]
4. Markovic-Housley Z, Miglierini G, Soldatova L, Rizkallah PJ, Muller U, Schirmer T. Crystal structure of hyaluronidase, a major allergen of bee venom. Structure 2000;8:1025–1035. [PubMed: 11080624]
5. Skov LK, Seppala U, Coen JJ, Crickmore N, King TP, Monsalve R, et al. Structure of recombinant Ves v 2 at 2.0 Angstrom resolution: structural analysis of an allergenic hyaluronidase from wasp venom. Acta Crystallogr D Biol Crystallogr 2006;62:595–604. [PubMed: 16699186]

6. Gould SF, Bernstein MH. The localisation of bovine sperm hyaluronidase. Differentiation 1975;3:123–132. [PubMed: 1183757]
7. Lokeshwar VB, Young MJ, Goudarzi G, Iida N, Yudin AI, Cherr GN, et al. Identification of bladder tumor-derived hyaluronidase: its similarity to HYAL1. Cancer Res 1999;59:4464–4470. [PubMed: 10485499]
8. Lokeshwar VB, Cerwinka WH, Isoyama T, Lokeshwar BL. HYAL1 hyaluronidase in prostate cancer: a tumor promoter and suppressor. Cancer Res 2005;65:7782–7789. [PubMed: 16140946]
9. Lokeshwar VB, Cerwinka WH, Lokeshwar BL. HYAL1 hyaluronidase: a molecular determinant of bladder tumor growth and invasion. Cancer Res 2005;65:2243–2250. [PubMed: 15781637]
10. Simpson MA. Concurrent expression of hyaluronan biosynthetic and processing enzymes promotes growth and vascularization of prostate tumors in mice. Am J Pathol 2006;169:247–257. [PubMed: 16816377]
11. Jacobson A, Rahmanian M, Rubin K, Heldin P. Expression of hyaluronan synthase 2 or hyaluronidase 1 differentially affect the growth rate of transplantable colon carcinoma cell tumors. Int J Cancer 2002;102:212–219. [PubMed: 12397638]
12. Stern R, Jedrzejas MJ. Hyaluronidases: their genomics, structures, and mechanisms of action. Chem Rev 2006;106:818–839. [PubMed: 16522010]
13. Csoka AB, Frost GI, Stern R. The six hyaluronidase-like genes in the human and mouse genomes. Matrix Biol 2001;20:499–508. [PubMed: 11731267]
14. Lokeshwar VB, Rubinowicz D, Schroeder GL, Forgacs E, Minna JD, Block NL, et al. Stromal and epithelial expression of tumor markers hyaluronic acid and HYAL1 hyaluronidase in prostate cancer. J Biol Chem 2001;276:11922–11932. [PubMed: 11278412]
15. Franzmann EJ, Schroeder GL, Goodwin WJ, Weed DT, Fisher P, Lokeshwar VB. Expression of tumor markers hyaluronic acid and hyaluronidase (HYAL1) in head and neck tumors. Int J Cancer 2003;106:438–445. [PubMed: 12845686]
16. Garg A, Anderson RA, Zaneveld LJ, Garg S. Biological activity assessment of a novel contraceptive antimicrobial agent. J Androl 2005;26:414–421. [PubMed: 15867010]
17. Garg S, Vermani K, Garg A, Anderson RA, Rencher WB, Zaneveld LJ. Development and characterization of bioadhesive vaginal films of sodium polystyrene sulfonate (PSS), a novel contraceptive antimicrobial agent. Pharm Res 2005;22:584–595. [PubMed: 15846466]
18. Hardy CM, Clydesdale G, Mobbs KJ, Pekin J, Lloyd ML, Sweet C, et al. Assessment of contraceptive vaccines based on recombinant mouse sperm protein PH20. Reproduction 2004;127:325–334. [PubMed: 15016952]
19. Suri A. Sperm specific proteins-potential candidate molecules for fertility control. Reprod Biol Endocrinol 2004 Mar 10;2:10. [PubMed: 15012833]
20. Chow G, Knudson W. Characterization of promoter elements of the human HYAL-2 gene. J Biol Chem 2005;280:26904–26912. [PubMed: 15923194]
21. Miller AD. Identification of Hyal2 as the cell-surface receptor for jaagsiekte sheep retrovirus and ovine nasal adenocarcinoma virus. Curr Top Microbiol Immunol 2003;275:179–199. [PubMed: 12596899]
22. Lepperdinger G, Mullegger J, Kreil G. Hyal2--less active, but more versatile? Matrix Biol 2001;20:509–514. [PubMed: 11731268]
23. Csoka AB, Frost GI, Wong T, Stern R. Purification and microsequencing of hyaluronidase isozymes from human urine. FEBS Lett 1997;417:307–310. [PubMed: 9409739]
24. Triggs-Raine B, Salo TJ, Zhang H, Wicklow BA, Natowicz MR. Mutations in HYAL1, a member of a tandemly distributed multigene family encoding disparate hyaluronidase activities, cause a newly described lysosomal disorder, mucopolysaccharidosis IX. Proc Natl Acad Sci U S A 1999;96:6296–6300. [PubMed: 10339581]
25. Arming S, Strobl B, Wechselberger C, Kreil G. In vitro mutagenesis of PH-20 hyaluronidase from human sperm. Eur J Biochem 1997;247:810–814. [PubMed: 9288901]
26. Lokeshwar VB, Schroeder GL, Carey RI, Soloway MS, Iida N. Regulation of hyaluronidase activity by alternative mRNA splicing. J Biol Chem 2002;277:33654–33663. [PubMed: 12084718]

27. Botzki A, Rigden DJ, Braun S, Nukui M, Salmen S, Hoechstetter J, et al. L-Ascorbic acid 6-hexadecanoate, a potent hyaluronidase inhibitor. X-ray structure and molecular modeling of enzyme-inhibitor complexes. J Biol Chem 2004;279:45990–45997. [PubMed: 15322107]
28. Stern M, Stern R. An ELISA-like assay for hyaluronidase and hyaluronidase inhibitors. Matrix 1992;12:397–403. [PubMed: 1283003]
29. Lokeshwar VB, Obek C, Pham HT, Wei D, Young MJ, Duncan RC, et al. Urinary hyaluronic acid and hyaluronidase: markers for bladder cancer detection and evaluation of grade. J Urol 2000;163:348–356. [PubMed: 10604388]
30. Pham HT, Block NL, Lokeshwar VB. Tumor-derived hyaluronidase: a diagnostic urine marker for high-grade bladder cancer. Cancer Res 1997;57:778–783. [PubMed: 9044860]Erratum in: Cancer Res 1997; 57: 1622.
31. Lokeshwar VB, Lokeshwar BL, Pham HT, Block NL. Association of elevated levels of hyaluronidase, a matrix-degrading enzyme, with prostate cancer progression. Cancer Res 1996;56:651–657. [PubMed: 8564986]
32. Hautmann S, Toma M, Lorenzo-Gomez MF, Friedrich MG, Jaekel T, Michl U, et al. Immunocyt and the HA-HAase urine tests for the detection of bladder cancer: a side-by-side comparison. Eur Urol 2004;46:466–471. [PubMed: 15363562]
33. Schroeder GL, Lorenzo-Gomez MF, Hautmann SH, Friedrich MG, Ekici S, Huland, et al. A side by side comparison of cytology and biomarkers for bladder cancer detection. J Urol 2004;172:1123–1126. [PubMed: 15311054]
34. Lokeshwar VB, Schroeder GL, Selzer MG, Hautmann SH, Posey JT, Duncan RC, et al. Bladder tumor markers for monitoring recurrence and screening comparison of hyaluronic acid-hyaluronidase and BTA-Stat tests. Cancer 2002;95:61–72. [PubMed: 12115318]
35. Stern M, Longaker MT, Adzick NS, Harrison MR, Stern R. Hyaluronidase levels in urine from Wilms' tumor patients. J Natl Cancer Inst 1991;83:1569–1574. [PubMed: 1660075]
36. Christopoulos TA, Papageorgakopoulou N, Theocharis DA, Mastronikolis NS, Papadas TA, Vynios DH. Hyaluronidase and CD44 hyaluronan receptor expression in squamous cell laryngeal carcinoma. Biochim Biophys Acta 2006;1760:1039–1045. [PubMed: 16713680]
37. Godin DA, Fitzpatrick PC, Scandurro AB, Belafsky PC, Woodworth BA, Amedee RG, et al. PH20: a novel tumor marker for laryngeal cancer. Arch Otolaryngol Head Neck Surg 2000;126:402–404. [PubMed: 10722016]
38. Victor, Chauzy RC, Girard N, Gioanni J, d'Anjou J, Stora De Novion H, Delpech B. Human breast-cancer metastasis formation in a nude-mouse model: studies of hyaluronidase, hyaluronan and hyaluronan-binding sites in metastatic cells. Int J Cancer 1999;82:77–83. [PubMed: 10360824]
39. Beech DJ, Madan AK, Deng N. Expression of PH-20 in normal and neoplastic breast tissue. J Surg Res 2002;103:203–207. [PubMed: 11922735]
40. Madan AK, Yu K, Dhurandhar N, Cullinane C, Pang Y, Beech DJ. Association of hyaluronidase and breast adenocarcinoma invasiveness. Oncol Rep 1999;6:607–609. [PubMed: 10203600]
41. Bertrand P, Courel MN, Maingonnat C, Jardin F, Tilly H, Bastard C. Expression of HYAL2 mRNA, hyaluronan and hyaluronidase in B-cell non-Hodgkin lymphoma: relationship with tumor aggressiveness. Int J Cancer 2005;113:207–212. [PubMed: 15386412]
42. Udabage L, Brownlee GR, Nilsson SK, Brown TJ. The over-expression of HAS2, Hyal-2 and CD44 is implicated in the invasiveness of breast cancer. Exp Cell Res 2005;310:205–217. [PubMed: 16125700]
43. Junker N, Latini S, Petersen LN, Kristjansen PE. Expression and regulation patterns of hyaluronidases in small cell lung cancer and glioma lines. Oncol Rep 2003;10:609–644. [PubMed: 12684632]Enegd B, King JA, Stylli S, Paradiso L, Kaye AH, Novak U. Overexpression of hyaluronan synthase-2 reduces the tumorigenic potential of glioma cells lacking hyaluronidase activity. Neurosurgery 2002;50:1311–1318. [PubMed: 12015850]
45. Bertrand P, Girard N, Duval C, d'Anjou J, Chauzy C, Menard JF, et al. Increased hyaluronidase levels in breast tumor metastases. Int J Cancer 1997;73:327–346. [PubMed: 9359477]
46. Delpech B, Laquerriere A, Maingonnat C, Bertrand P, Freger P. Hyaluronidase is more elevated in human brain metastases than in primary brain tumours. Anticancer Res 2002;22:2423–2427. [PubMed: 12174938]

47. Paiva P, Van Damme MP, Tellbach M, Jones RL, Jobling T, Salamonsen LA. Expression patterns of hyaluronan, hyaluronan synthases and hyaluronidases indicate a role for hyaluronan in the progression of endometrial cancer. Gynecol Oncol 2005;98:193–202. [PubMed: 15936804]
48. Csoka AB, Frost GI, Heng HH, Scherer SW, Mohapatra G, Stern R. The hyaluronidase gene HYAL1 maps to chromosome 3p21.2-p21.3 in human and 9F1-F2 in mouse, a conserved candidate tumor suppressor locus. Genomics 1998;48:63–70. [PubMed: 9503017]
49. Ji L, Nishizaki M, Gao B, Burbee D, Kondo M, Kamibayashi C, et al. Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities in vitro and in vivo. Cancer Res 2002;62:2715–2720. [PubMed: 11980673]
50. Frost GI, Mohapatra G, Wong TM, Csoka AB, Gray JW, Stern R. HYAL1LUCA-1, a candidate tumor suppressor gene on chromosome 3p21.3, is inactivated in head and neck squamous cell carcinomas by aberrant splicing of pre-mRNA. Oncogene 2000;19:870–877. [PubMed: 10702795]
51. Stern R. Hyaluronan metabolism: a major paradox in cancer biology. Pathol Biol (Paris) 2005;53:372–382. [PubMed: 16085113]
52. Lokeshwar VB, Selzer MG. Differences in hyaluronic acid-mediated functions and signaling in arterial, microvessel, and vein-derived human endothelial cells. J Biol Chem 2000;275:27641–27649. [PubMed: 10882722]
53. Takahashi Y, Li L, Kamiryo M, Asteriou T, Moustakas A, Yamashita H, et al. Hyaluronan fragments induce endothelial cell differentiation in a CD44- and CXCL1/ GRO1-dependent manner. J Biol Chem 2005;280:24195–24204. [PubMed: 15843382]
54. Lokeshwar VB, Obek C, Soloway MS, Block NL. Tumor-associated hyaluronic acid: a new sensitive and specific urine marker for bladder cancer. Cancer Res 1997;57:773–777. [PubMed: 9044859] Erratum in: Cancer Res 1998; 58: 3191.
55. Aboughalia AH. Elevation of hyaluronidase-1 and soluble intercellular adhesion molecule-1 helps select bladder cancer patients at risk of invasion. Arch Med Res 2006;37:109–116. [PubMed: 16314195]
56. Patel S, Turner PR, Stubberfield C, Barry E, Rohlff CR, Stamps A, et al. Hyaluronidase gene profiling and role of hyal-1 overexpression in an orthotopic model of prostate cancer. Int J Cancer 2002;97:416–424. [PubMed: 11802201]Erratum in: Int J Cancer 2002; 98: 957.
57. Shuster S, Frost GI, Csoka AB, Formby B, Stern R. Hyaluronidase reduces human breast cancer xenografts in SCID mice. Int J Cancer 2002;102:192–197. [PubMed: 12385018]
58. Marsit CJ, Hasegawa M, Hirao T, Kim DH, Aldape K, Hinds PW, et al. Loss of heterozygosity of chromosome 3p21 is associated with mutant TP53 and better patient survival in non-small-cell lung cancer. Cancer Res 2004;64:8702–8707. [PubMed: 15574780]
59. Hilbe W, Auberger J, Dirnhofer S, Schmid T, Erdel M, Duba HC. High rate of molecular alteration in histologically tumour-free bronchial epithelium of NSCLC patients detected by multicolour fluorescence in situ hybridisation. Oncol Rep 2006;15:1233–1240. [PubMed: 16596192]
60. Pizzi S, Azzoni C, Bottarelli L, Campanini N, D'Adda T, Pasquali C, et al. RASSF1A promoter methylation and 3p21.3 loss of heterozygosity are features of foregut, but not midgut and hindgut, malignant endocrine tumours. J Pathol 2005;206:409–416. [PubMed: 15887288]
61. Lokeshwar VB, Estrella V, Lopez L, Kramer M, Gomez P, Soloway MS, et al. HYAL1-v1, an alternatively spliced variant of hyal1 hyaluronidase: a negative regulator of bladder cancer. Cancer Res. (2006; revised manuscript submitted).
62. Lin G, Stern R. Plasma hyaluronidase (Hyal-1) promotes tumor cell cycling. Cancer Lett 2001;163:95–101. [PubMed: 11163112]
63. Chang NS. Hyaluronidase activation of c-Jun N-terminal kinase is necessary for protection of L929 fibrosarcoma cells from staurosporine-mediated cell death. Biochem Biophys Res Commun 2001;283:278–286. [PubMed: 11327694]
64. Liu X, Yan S, Zhou T, Terada Y, Erikson RL. The MAP kinase pathway is required for entry into mitosis and cell survival. Oncogene 2004;23:763–776. [PubMed: 14737111]
65. Zhang S, Chang MC, Zylka D, Turley S, Harrison R, Turley EA. The hyaluronan receptor RHAMM regulates extracellular-regulated kinase. J Biol Chem 1998;273:11342–11348. [PubMed: 9556628]

66. Hall CL, Lange LA, Prober DA, Zhang S, Turley EA. pp60(c-src) is required for cell locomotion regulated by the hyaluronanreceptor RHAMM. Oncogene 1996;13:2213–2224. [PubMed: 8950989]

67. McLean GW, Carragher NO, Avizienyte E, Evans J, Brunton VG, Frame MC. The role of focal-adhesion kinase in cancer - a new therapeutic opportunity. Nat Rev Cancer 2005;5:505–515. [PubMed: 16069815]

68. Mitra SK, Hanson DA, Schlaepfer DD. Focal adhesion kinase: in command and control of cell motility. Nat Rev Mol Cell Biol 2005;6:56–68. [PubMed: 15688067]

69. Mingo-Sion AM, Marietta PM, Koller E, Wolf DM, Van Den Berg CL. Inhibition of JNK reduces G2/M transit independent of p53, leading to endoreduplication, decreased proliferation, and apoptosis in breast cancer cells. Oncogene 2004;23:596–604. [PubMed: 14724588]

70. Goss VL, Cross JV, Ma K, Qian Y, Mola PW, Templeton DJ. SAPK/JNK regulates cdc2/cyclin B kinase through phosphorylation and inhibition of cdc25c. Cell Signal 2003;15:709–718. [PubMed: 12742231]

71. Chang NS. Transforming growth factor-beta1 blocks the enhancement of tumor necrosis factor cytotoxicity by hyaluronidase Hyal-2 in L929 fibroblasts. BMC Cell Biol 2002;3:8. [PubMed: 11960552]

72. Chang NS, Doherty J, Ensign A. JNK1 physically interacts with WW domain-containing oxidoreductase (WOX1) and inhibits WOX1-mediated apoptosis. J Biol Chem 2003;278:9195–9202. [PubMed: 12514174]

73. Chang NS, Pratt N, Heath J, Schultz L, Sleve D, Carey GB, et al. Hyaluronidase induction of a WW domain-containing oxidoreductase that enhances tumor necrosis factor cytotoxicity. J Biol Chem 2001;276:3361–3370. [PubMed: 11058590]

74. Basu A, Haldar S. Identification of a novel Bcl-xL phosphorylation site regulating the sensitivity of taxol- or 2-methoxyestradiol-induced apoptosis. FEBS Lett 2003;538:41–47. [PubMed: 12633850]

75. Deng X, Xiao L, Lang W, Gao F, Ruvolo P, May WS Jr. Novel role for JNK as a stress-activated Bcl2 kinase. J Biol Chem 2001;276:23681–23688. [PubMed: 11323415]

76. Hautmann SH, Lokeshwar VB, Schroeder GL, Civantos F, Duncan RC, Gnann R, et al. Elevated tissue expression of hyaluronic acid and hyaluronidase validates the HA-HAase urine test for bladder cancer. J Urol 2001;165:2068–2074. [PubMed: 11371930]

77. Eissa S, Kassim SK, Labib RA, El-Khouly IM, Ghaffer TM, Sadek M, et al. Detection of bladder carcinoma by combined testing of urine for hyaluronidase and cytokeratin 20 RNAs. Cancer 2005;103:1356–1362. [PubMed: 15717321]

78. Posey JT, Soloway MS, Ekici S, Sofer M, Civantos F, Duncan RC, et al. Evaluation of the prognostic potential of hyaluronic acid and hyaluronidase (HYAL1) for prostate cancer. Cancer Res 2003;63:2638–2644. [PubMed: 12750291]

79. Ekici S, Cerwinka WH, Duncan R, Gomez P, Civantos F, Soloway MS, et al. Comparison of the prognostic potential of hyaluronic acid, hyaluronidase (HYAL-1), CD44v6 and microvessel density for prostate cancer. Int J Cancer 2004;112:121–129. [PubMed: 15305383]

80. Green SK, Francia G, Isidoro C, Kerbel RS. Antiadhesive antibodies targeting E-cadherin sensitize multicellular tumor spheroids to chemotherapy in vitro. Mol Cancer Ther 2004;3:149–159. [PubMed: 14985455]

81. St Croix B, Man S, Kerbel RS. Reversal of intrinsic and acquired forms of drug resistance by hyaluronidase treatment of solid tumors. Cancer Lett 1998;131:35–44. [PubMed: 9839618]

82. Kerbel RS, St Croix B, Florenes VA, Rak J. Induction and reversal of cell adhesion-dependent multicellular drug resistance in solid breast tumors. Hum Cell 1996;9:257–264. [PubMed: 9183656]

83. Croix BS, Rak JW, Kapitain S, Sheehan C, Graham CH, Kerbel RS. Reversal by hyaluronidase of adhesion-dependent multicellular drug resistance in mammary carcinoma cells. J Natl Cancer Inst 1996;88:1285–1296. [PubMed: 8797768]

84. Spruss T, Bernhardt G, Schonenberger H, Schiess W. Hyaluronidase significantly enhances the efficacy of regional vinblastine chemotherapy of malignant melanoma. J Cancer Res Clin Oncol 1995;121:193–202. [PubMed: 7751317]

85. Smith KJ, Skelton HG, Turiansky G, Wagner KF. Hyaluronidase enhances the therapeutic effect of vinblastine in intralesional treatment of Kaposi's sarcoma. Military Medical Consortium for the

Advancement of Retroviral Research (MMCARR). J Am Acad Dermatol 1997 Feb;36(2 Pt 1):239–242. [PubMed: 9039176]

86. Haselsbeger K, Radner H, Pendl G. Boron neutron capture therapy for glioblastoma: improvement of boron biodistribution by hyaluronidase. Cancer Lett 1998;131:109–111. [PubMed: 9839625]
87. Pillwein K, Fuiko R, Slavc I, Czech T, Hawliczek G, Bernhardt G, et al. Hyaluronidase additional to standard chemotherapy improves outcome for children with malignant brain tumors. Cancer Lett 1998;131:101–108. [PubMed: 9839624]
88. Hobarth K, Maier U, Marberger M. Topical chemoprophylaxis of superficial bladder cancer with mitomycin C and adjuvant hyaluronidase. Eur Urol 1992;21:206–210. [PubMed: 1499626]
89. Maier U, Baumgartner G. Metaphylactic effect of mitomycin C with and without hyaluronidase after transurethral resection of bladder cancer: randomized trial. J Urol 1989;141:529–530. [PubMed: 2493098]
90. Klocker J, Sabitzer H, Raunik W, Wieser S, Schumer J. Hyaluronidase as additive to induction chemotherapy in advanced squamous cell carcinoma of the head and neck. Cancer Lett 1998;131:113–115. [PubMed: 9839626]
91. Klocker J, Sabitzer H, Raunik W, Wieser S, Schumer J. Combined application of cisplatin, vindesine, hyaluronidase and radiation for treatment of advanced squamous cell carcinoma of the head and neck. Am J Clin Oncol 1995;18:425–428. [PubMed: 7572761]

Abbreviations used

HA, hyaluronic acid; HAase, hyaluronidase.