

## IN FOCUS

## Targeting the Tumor Microenvironment in Cancer: Why Hyaluronidase Deserves a Second Look

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**Summary:** Increased extracellular matrix (ECM) deposition is a characteristic observed in many solid tumors. Increased levels of one ECM component—namely, hyaluronan (HA)—leads to reduced elasticity of tumor tissue and increased interstitial fluid pressure. Multiple initial reports showed that the addition of hyaluronidase (HYAL) to chemotherapeutic regimens could greatly improve efficacy. Unfortunately, the bovine HYAL used in those studies was limited therapeutically by immunologic responses to treatment. Newly developed recombinant human HYAL has recently been introduced into clinical trials. In this article, we describe the role of HA in cancer, methods of targeting HA, and clinical studies performed to date, and we propose that targeting HA could now be an effective treatment option for patients with many different types of solid tumors. *Cancer Discovery*; 1(4): 291–96. ©2011 AACR.

### INTRODUCTION

Many solid tumors develop extensive fibroses, a result of what is termed the desmoplastic reaction (reviewed in ref. 1). Desmoplasia leads to a significant increase in the production of extracellular matrix (ECM) proteins, as well as extensive proliferation of myofibroblast-like cells. The result is the formation of a dense and fibrous connective tissue that is composed of multiple ECM components, including collagen types I, III, and IV; fibronectin; laminin; hyaluronan (HA); and the glycoprotein osteonectin [also known as secreted protein, acidic and rich in cysteine (SPARC); Fig. 1]. This fibroinflammatory component of the tumor (sometimes called stroma) contributes to an increase in tumor interstitial fluid pressure, blocking perfusion of anticancer therapies to the tumor

cells, and contributes generally to chemoresistance (ref. 2; see accompanying box for individual components and their proposed mechanism of chemoresistance). Consequently, targeting the components of the stromal compartment, in conjunction with cytotoxic agents directed against tumor cells, is gaining traction as a potential approach to treating patients and overcoming chemoresistance.

The concept of directing therapies toward the stromal compartment as a means to enhance drug perfusion is supported by a recent report revealing that stromal depletion by nab-paclitaxel (which accumulates in tumor tissues high in SPARC) resulted in improved gemcitabine delivery in a primary human xenograft model for pancreatic cancer (3). In addition, using a genetically engineered mouse model of pancreatic cancer, Olive and colleagues (4) showed that stromal depletion by Hedgehog pathway inhibitors enhanced the intratumor concentration of gemcitabine and resulted in significantly increased survival of tumor-bearing mice.

Enzymes that degrade the ECM have also been proposed as stroma-targeting agents. However, the immunoreactivity and pH sensitivity of ECM-targeting agents such as the collagenases have been problems that have limited their study *in vivo*. Another ECM component that may be targeted by a degrading enzyme is HA. HA, a linear polysaccharide

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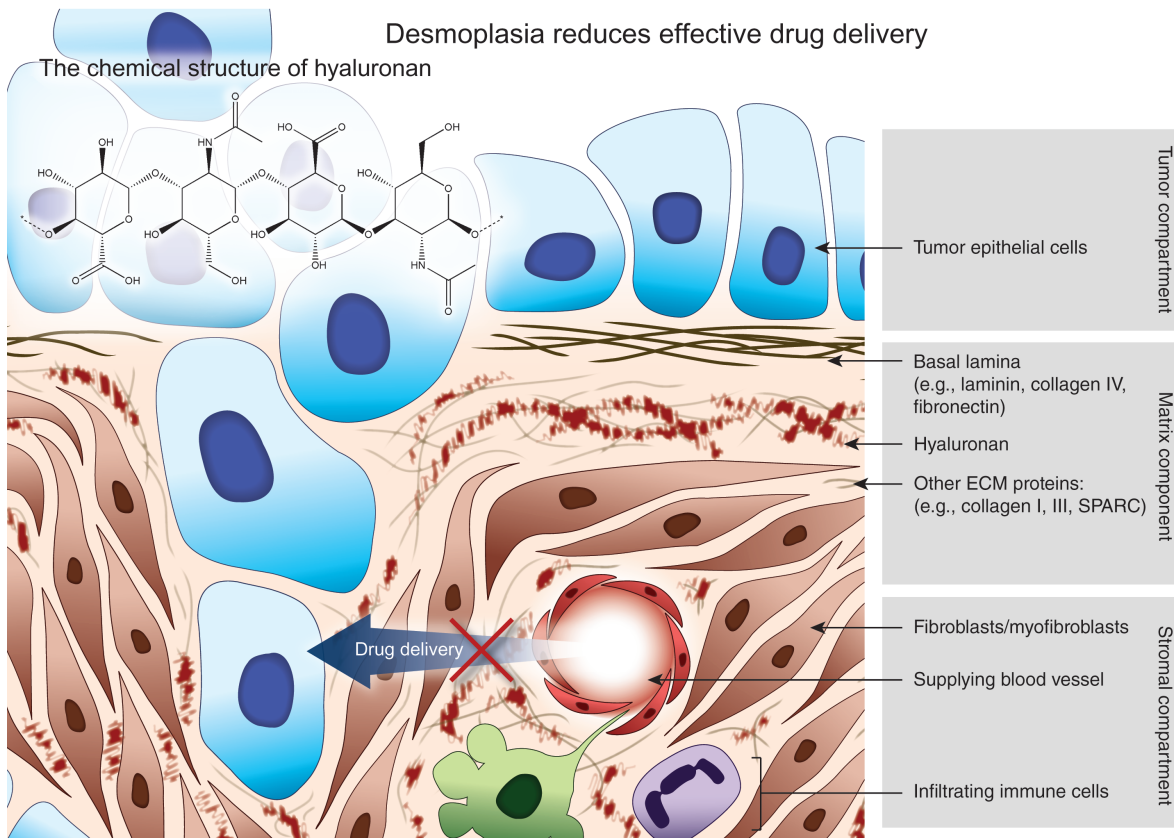
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### ECM COMPONENTS THAT MAY CONTRIBUTE TO CHEMORESISTANCE

ECM component	Functional role in chemoresistance
Collagen I, III, IV	Enhances tumor cell proliferation, structural support of ECM
Decorin	Binds TGF- $\beta$ , tightens collagen fibrils
HA	Synergizes with collagen network, increases interstitial fluid pressure
Versican	Enhances tumor cell proliferation, confers resistance to apoptosis
Fibronectin	Confers resistance to apoptosis
Laminin	Confers resistance to apoptosis
Osteonectin/SPARC	Enhances tumor cell proliferation and metastasis



**Figure 1.** Invading epithelial tumor cells and the associated tumor microenvironment. Dissolution of the basement membrane is accompanied by the production and secretion of numerous ECM components, including the collagens, fibronectin, laminin, and HA, as part of the myofibroblast-mediated desmoplastic reaction. Infiltrating immune cells also contribute to the signaling involved in the desmoplastic reaction. Expansion of the stromal compartment and production of the ECM proteins are thought to result in greater intratumoral pressure and contribute to a reduction in effective drug delivery. Inset, chemical structure of HA. HA is a linear polysaccharide composed of repeating units of glucuronic acid and *N*-acetylglucosamine. HA is a nonsulfated GAG and participates as an integral component of the ECM.

composed of glucuronic acid and *N*-acetylglucosamine, plays an important role in a diverse range of cellular processes (Fig. 1, inset). Elements of HA and HA metabolism are thought to be involved in biological functions related to cell proliferation, tissue hydration, cell motility, inflammation, angiogenesis, and malignancy. HA is distributed universally in the extracellular spaces of most tissues, with especially high concentrations found within connective tissues. Unlike the other protein-bound and sulfated glycosaminoglycans (GAG), HA is a unique polyanionic and protein-free polysaccharide that has an exceptional ability to increase viscosity, expand volume, and provide structural support in various locations and contexts within the body. Although steady-state levels of HA are generally quite low in most normal tissues, HA levels dramatically increase with many disease states, such as vascular disease (e.g., atherosclerosis) and cancer. Furthermore, high HA levels have been correlated with poor prognosis in many different cancer types, including gastric, colorectal, breast, ovarian, and bladder cancer. Because HA functions in ion exchange and may also act as a molecular sieve that

prevents the penetration of drugs, it is thought that treatment with agents that degrade HA, such as a hyaluronidase (HYAL), may have the potential to increase penetration of drugs through the stromal compartment and ultimately into tumor cells. Given the important role of HA and the ECM in solid tumors, it is possible that targeting the ECM with agents such as HYAL could be effective in improving therapeutic outcomes in patients with solid tumors. This possibility has led us to consider the following question: Why are we currently not targeting the tumor stroma with HYAL in our treatment of cancer?

## HYALURONAN AND ITS ROLE IN CANCER

HA is a protein-free, acetone-insoluble polysaccharide first isolated from hyaloid (or vitreous) matter and reported to contain uronic acid. It is ubiquitously distributed throughout the human body, with particularly high concentrations in the skin, eyes, cartilage, and synovial fluid. The structure of HA gives it great capacity to interact with water molecules, resulting in a vastly increased volume, as well as

increased viscosity of HA-containing solutions. These properties have also led many to infer that HA is largely an inert molecule functioning to maintain the physical volume and rigidity of connective tissue. However, with the discovery of the HA-binding proteins, or hyaladherins, it became clear that their functional reach was much greater. The discovery of the proteoglycans, the aggrecan and link protein, and the HA-binding surface receptors, CD44 and RHAMM, revealed that HA is involved in the direct signaling of many biological processes, including cell proliferation, migration, adhesion, and even the recruitment of leukocytes such as the neutrophils.

Studies of many cancer types, including pancreatic ductal adenocarcinoma (PDAC), indicate that an accumulation of HA occurs in neoplastic tissues. Indeed, it appears that most epithelial tumors exhibit high levels of HA localizing to their peritumoral, or stromal, compartments. Of interest, high HA levels have also been detected at the invasive front of growing tumors, suggesting that HA may be involved not only in cell proliferation but possibly in invasion as well. Indeed, Bertrand and colleagues (5) observed a 4.4-fold ( $\pm 0.4$ ) increase in HA staining relative to adjacent normal tissue at the invasive edges in breast tumors, whereas only a 3.3-fold ( $\pm 0.4$ ) increase was seen in central locations within the tumor ( $P < 0.05$ ). HA interaction with CD44 facilitates colon tumor cell migration, as well as migration in other tumor models, including breast and brain cancer cells. The level of HA itself correlates with overall tumor aggressiveness and increased cell migration and proliferation in breast and ovarian cancer. Tumor cells that overexpress the HA synthase, HAS1, to varying degrees experience increased proliferation rates. Although it remains to be seen whether HAS1 may serve as a definitive tumor biomarker, urine HA and HYAL levels may be used as suitable markers for bladder cancer, including assessment of tumor grade. In addition, we now know that HA levels correlate with malignancy in mesothelioma and may function as a potential diagnostic marker. It seems clear that a balance of the activity of the HA synthases (HAS) with HYAL activity is necessary for normal tissue function.

## TARGETING HYALURONAN

In normal tissues, HA levels are maintained through a balance of synthesis by HAS and degradation by the enzyme HYAL. HA is synthesized in mammals via the expression of 3 related HAS: HAS1, HAS2, and HAS3. Corticosteroids can inhibit the synthesis of HA by HAS. Indeed, the addition of cortisol to cultured aortic smooth muscle cells can reduce the production of HA by as much as 50%. This effect was also observed in a recent study by Gebhardt and colleagues (6), wherein they report a rapid decrease of approximately 50% of HA levels, as well as a reduction in HAS2 expression, in the skin following topical treatment with dexamethasone. Except in the treatment of patients with hematologic malignancies, clinical research on the addition of corticosteroids to anticancer therapies for patients with solid tumors has been limited (aside from preventing nausea and vomiting).

In addition to corticosteroids, the HAS inhibitor 4-methylumbelliferone (MU) has been developed and proposed as an alternative approach to lowering HA levels. Intriguingly, MU has been shown to increase the efficacy of gemcitabine by inhibiting the growth of cancer cell lines over gemcitabine alone, without significant growth inhibition itself. Furthermore, Yoshihara and colleagues (7) have shown that MU decreases liver metastases in a mouse model for melanoma. In addition, MU also reduces tumorigenicity, including reduced proliferation and motility, in esophageal squamous cell carcinoma or prostate cancer cells. MU is currently being investigated in clinical trials for the treatment of patients with chronic hepatitis infection ([www.clinicaltrials.gov](http://www.clinicaltrials.gov), NCT00225537), although no studies to determine if the effects of MU will enhance anticancer therapies in patients have yet been reported. With several very recent reports on the efficacy of MU in breast or prostate cancer xenograft mouse models showing significant reduction in tumor growth, studies of MU's efficacy in human trials are likely to be forthcoming (8).

Altering the breakdown of HA has also been proposed as a means to target HA accumulation. Catabolism of HA and balance of HA levels are mainly mediated by the HYALs. Six genes that encode for the different HYALs have been identified, including *HYAL1*, *-2*, *-3*, *-4*, *PHYAL1*, and *PH20*. The HYALs catalyze the hydrolysis of HA and function as endo- $\beta$ -acetyl-hexosaminidases. *HYAL1* and *-2* maintain the highest enzymatic activity in mammals, turning over as much as a third of the total HA each day. The addition of HYAL to chemotherapeutics enhances the catabolism of HA, as well as significantly increases the efficacy of chemotherapeutics, even in tumors previously deemed chemoresistant. The effectiveness of HYAL in improving chemotherapies has been explored in multiple tumor types, including breast, brain, melanoma, and sarcoma (Table 1). The synergistic effect of adding HYAL to chemotherapeutics is thought to aid in cancer treatment by reducing intratumoral pressure or by breaking down the ability of HA to function as a molecular sieve (2). Alternatively, the synergistic benefit may instead occur by means of a chemosensitizing effect.

In cell-culture models, the addition of HYAL decreases intrinsic chemoresistance in spheroid models of cancer, resulting in greatly disaggregated spheroids, increased drug penetration, and increased cell death (9). Using a breast cancer xenograft model, however, Beckenlehner and colleagues (10) in 1992 showed an increased susceptibility to doxorubicin when animals were pretreated with HYAL prior to doxorubicin. Other investigators have shown that HYAL pretreatment can result in increased intratumor drug concentrations. Indeed, Muckenschabel and colleagues (11) in 1996 observed increases as high as 16- to 32-fold in tumor-specific melphalan concentrations in a melanoma study. Mounting evidence suggests that drugs may fail owing to an inability to attain significant intratumor concentrations (2). Thus, the finding that HYAL pretreatment increased intratumor drug concentration is particularly exciting, as it may enhance the efficacy of current therapies in patients.

**Table 1. Early clinical studies investigating the coadministration of bovine hyaluronidase with chemotherapy**

Study	Trial type	Tumor type	Chemotherapy	Number of patients	Endpoint	Results
Klocker et al. (16)	Phase II	Adv. SCC-HN	Cisplatin/ vindesine	48	Response	CR in 84%, 47% survival >5 y
Baumgartner et al. (12)	Phase III	Bladder cancer	Mitomycin C	56	Recurrence	27% vs. 59% recurrence in HYAL-treated vs. untreated
Pillwein et al. (14)	Phase II	Malignant brain	Carboplatin/ etoposide	40	Survival	3-y survival, 84% vs. 50% in HYAL-treated vs. untreated
Smith et al. (13)	Phase I	Kaposi's sarcoma	Vinblastine	6	Toxicity/ recurrence	0% vs. 50% recurrence in HYAL-treated lesions, no added toxicity
Baumgartner et al. (20)	Phase I	Gastrointestinal and others	Adriamycin and others	12	Toxicity/ recurrence	PR/MR in 5 of 12 resistant, no added toxicity

Abbreviations: Adv. SCC-HN, advanced squamous cell carcinoma of the head and neck; CR, complete response; HYAL, hyaluronidase; MR, minimal response; PR, partial response

## CLINICAL STUDIES INCORPORATING HYALURONIDASE

Multiple preliminary clinical studies have demonstrated increased efficacy with bovine HYAL pretreatment in cancer patients (Table 1). Baumgartner and colleagues (12) in 1998 reviewed the early pilot clinical trials involving bovine HYAL. In a small study of 6 patients, the addition of intralesional bovine HYAL to intralesional vinblastine treatment was shown to be more effective at treating cutaneous lesions of Kaposi's sarcoma than vinblastine alone and resulted in reduced recurrence (13). This reduced recurrence was also seen in a study reporting on the addition of bovine HYAL to standard carboplatin and etoposide treatment for malignant brain tumors (14). In this study, which included 40 pediatric brain cancer patients, both event-free survival and overall survival at 36 months were significantly improved with the addition of a 30-minute infusion of bovine HYAL prior to chemotherapy treatment (Table 1). Similarly, in a study focusing on the effects of mitomycin C in combination with bovine HYAL administered intravesically, recurrence in bladder cancer patients was reduced from 32% in mitomycin C only-treated patients to 7% with the addition of HYAL ( $P < 0.05$ ; ref. 15). In 2 studies, which enrolled a total of 80 patients, bovine HYAL delivered intravenously was added to chemotherapy or chemotherapy plus radiation therapy for advanced squamous cell carcinoma of the head and neck. In these studies, a complete response was achieved in 84% of those treated, with a 47% survival observed over 5 years (16). In a study of 43 patients with high-grade astrocytoma, it was reported that the addition of HYAL did not produce a significant improvement in tumor regression (12). However, the authors suggest that any synergy of HYAL may have been obscured after being compared with a different, more effective agent later in the study. More specifically, the drug used in the first segment of the astrocytoma study,

lomustine [also called CCNU, or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea], is only slightly effective against astrocytoma as a single agent; thus HYAL did not produce a significant improvement relative to the more effective agent used in segment 2, carmustine [also called BCNU, or 1,3-bis(2-chloroethyl)-1-nitrosourea; ref. 12].

Considering these results, one might ask why HYAL has not been studied more fully or gained broader acceptance. One particular limitation of HYAL as a therapeutic agent in prior studies has been the development of allergic reactions by patients to this enzyme because of its bovine origin. Indeed, multiple studies report that as many as 32% of patients harbor reactive IgE antibodies to the bovine HYAL preparation, prior to therapy, inducing various reactions ranging from urticaria to tachycardia to shock (12). Bovine HYAL treatment has resulted in allergic reactions, even anaphylaxis. Furthermore, the development of anti-HYAL antibodies following treatment limits its usefulness in any subsequent treatment, with elevated antibodies present for 6 weeks or more following intravenous or intramuscular treatment.

Recombinant human HYAL has been developed in recent years. The recombinant human material eliminates the risk of disease transmission via contaminants found in animal-derived HYAL. The recombinant human material did not induce allergic reactions in a cohort of 100 volunteer subjects who were injected intradermally (17). The recombinant human molecule is now used subcutaneously, consistent with the FDA-approved label, to help with dispersion and absorption of various injected drugs (17). Recombinant HYAL is currently being investigated under different formulations for both superficial bladder cancer (phase I/II, NCT00318643), for which it is being used as purified recombinant material, and for solid tumors (phase I, NCT00834704), for which it is being used in the pegylated form.

Considering the biological role of HA, and the many locations in which it is found, side effects of repeated HYAL treatment as part of an anticancer regimen might induce

inflammation or pain in the joints. It appears that some side effects of enhanced HYAL activity in normal tissue observed in earlier studies, however, were controlled by the administration of corticosteroids (12). The ongoing phase I trial used pegylated material, PEGPH20, because of the improved half-life of the recombinant enzyme. In this study, 50 µg/kg induced grade 3 muscle/joint pain, whereas doses of 0.5 µg/kg and 0.75 µg/kg of HYAL were generally well tolerated (18). Additional work in canine models suggesting amelioration of musculoskeletal events by use of dexamethasone is also being examined in the ongoing phase I trial.

Given the role of the stromal compartment in PDAC and other cancers, it is likely that targeting HA in pancreatic cancer has potential for improving current therapies (1). With the clinical availability of recombinant HYAL, prospects for targeting HA in the treatment of cancer are improved, particularly in cancer types known to be fibrotic. In a recent conference report, Thompson and colleagues (19) observed a 50% increase in median survival time in mice bearing pancreatic cancer xenograft tumors treated with gemcitabine plus HYAL. Taken together, these results warrant further clinical investigation of targeting HA in a variety of tumors, including PDAC, in which the stroma is thought to play a key role in limiting drug delivery.

## CONCLUDING REMARKS

With such promising preliminary clinical results following the addition of early forms (i.e., bovine) of HYAL to chemotherapeutic treatments, we should again reconsider the power in targeting components of the tumor microenvironment, especially HA. On the basis of current and past clinical studies, future therapeutic regimens for patients with cancer may significantly benefit from agents targeting critical pathways in the development, progression, and perpetuation of the tumor stroma, particularly HA. Certainly, one must remain cognizant of the function of HA in many other parts of the body. Its role in synovial fluid or the vitreous humor could become problematic following long-term treatment with HYAL. However, with the development of a recombinant HYAL, some of the significant limitations (i.e., immune reactions) to targeting HA with bovine HYAL have been addressed. These developments will allow for greater utility in studying HYAL as part of an anticancer therapy regimen by yielding greater flexibility in route of administration as well as treatment schedule in clinical trials. Even using bovine HYAL, targeting HA as part of a combination regimen has shown promise in the clinic. Using recombinant human HYAL as a component of an anticancer regimen is now possible. It also has a key advantage over other ECM-targeting alternatives in that it is available now. Pegylated recombinant HYAL is in ongoing phase I trials. Although the enzyme will likely cause some musculoskeletal events, and may also present challenges in wound healing, inhibiting a key stromal component such as HA with recombinant human HYAL could improve the clinical outcomes in individuals with the most deadly types of cancer, such as PDAC. In such a disease, any potential improvement in the effective delivery of therapeutics should be cause for serious consideration.

## Disclosure of Potential Conflicts of Interest

D.D. Von Hoff maintains a consulting relationship with Halozyme Therapeutics, maker of a human recombinant hyaluronidase. No potential conflicts of interest were disclosed by the other authors.

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