Published in final edited form as:
Mol Pharm. 2008 ; 5(4): 474–486. doi:10.1021/mp800024g.

Anticancer Therapeutics: Targeting Macromolecules and Nanocarriers to Hyaluronan or CD44, a Hyaluronan Receptor

Virginia M. Platt[†] and **Francis C. Szoka Jr**[†,‡]

[†]Joint Graduate Group in Bioengineering, University of California, Berkeley and San Francisco

[‡]Department of Biopharmaceutical Sciences and Pharmaceutical Chemistry, University of California San Francisco

Abstract

The complex system involved in the synthesis, degradation, and binding of the high molecular weight glycosaminoglycan hyaluronic acid (hyaluronan or HA) provides a variety of structures that can be exploited for targeted cancer therapy. In many cancers of epithelial origin there is an up-regulation of CD44, a receptor that binds HA. In other cancers, HA in the tumor matrix is over-expressed. Both CD44 on cancer cells and HA in the matrix have been targets for anti-cancer therapy. Even though CD44 is expressed in normal epithelial cells and HA is part of the matrix of normal tissues, selective targeting to cancer is possible. This is because macromolecular carriers predominantly extravasate into the tumor and not normal tissue; thus CD44-HA targeted carriers administered intravenously localize preferentially into tumors. Anti-CD44 antibodies have been used in patients to deliver radioisotopes or mertansine for treatment of CD44 expressing tumors. In early phase clinical trials, patients with breast or head and neck tumors treated with anti-CD44 conjugates experienced stabilized disease. A dose-limiting toxicity was associated with distribution of the antibody-drug conjugate to the skin, a site in the body with a high level of CD44. HA has been used as a drug carrier and a ligand on liposomes or nanoparticles to target drugs to CD44 over-expressing cells. Drugs can be attached to HA via the carboxylate on the glucuronic acid residue, the hydroxyl on the *N*-acetylglucosamine, or the reducing end which are located on a repeating disaccharide. Drugs delivered in HA-modified liposomes exhibited excellent anti-tumor activity both *in vitro* and in murine tumor models. The HA matrix is also a potential target for anti-cancer therapies. By manipulating the interaction of HA with cell surface receptors, either by degrading it with hyaluronidase or by interfering with CD44-HA interactions using soluble CD44 proteins, tumor progression was blocked. Finally, cytotoxic drugs or pro-drug converting enzymes can be attached to the HA matrix to generate a cytotoxic fence around the tumor. This review describes how the complex interplay among cancer biology, the CD44-HA interaction, drug carriers and drug targeting has been used to improve anti-cancer therapies. As these approaches evolve, they hold forth the prospect of significantly improved targeted anti-cancer treatments.

Keywords

Antibody; Biodistribution; Cancer; Chemotherapy; Drug-conjugate; EPR effect; Liposome; Polymer; Prodrug; Recombinant Protein

1. The Biology of the CD44-Hyaluronan Interaction

Hyaluronan (HA) (Figure 1) is a high molecular weight glycosaminoglycan, extracellular matrix component essential for proper cell growth, organ structural stability, and tissue organization. The amount of HA in a tissue depends upon on a complex interplay among HA synthesis by HA synthases,[1] HA internalization by cell surface receptors,[2] and extracellular

degradation by hyaluronidases.[3] HA turnover is due to local cellular catabolism, removal by an HA endocytosing receptor (LYVE-1) on cells located in the lymphatics,[4] and systemic clearance from the blood by the HARE receptor on liver sinusoidal endothelial cells.[5] In skin, HA has a half-life of over a day.[6] In contrast, circulating high molecular weight HA (HMW-HA) has a half-life of two to five minutes.[7] The net outcome of the various HA clearance processes results in a total turnover of about five grams of HA per day in humans.[8]

HA acts through CD44, its principal receptor, and RHAMM (receptor for HA mediated motility),[9] to regulate cell proliferation and movement. Many of the downstream pathways following CD44 activation become deregulated in cancer, leading to tumor growth, progression and metastasis.[10 - 12] Changes in both CD44 and HA expression have been widely observed in tumors from cancer patients and occur in animal models of tumor growth.[13] During carcinogenesis, expression of the standard form of CD44 is upregulated in certain cancers. Non-native CD44 variants may also arise from alternate splicing of cytoplasmic regions of CD44 during translation.[13]

Interference with the CD44-HA interaction by either targeting drugs to CD44,[14] targeting drugs to the HA matrix[15] or interfering with HA matrix-CD44 interactions[16] are viable strategies for cancer treatments. The CD44-HA system is illustrated in Figure 2. In this figure we indicate options for anti-cancer therapeutics that specifically interfere with or exploit aspects of the CD44-HA pathway. This review discusses these options.

2. Targeting to CD44 with Anti-CD44 Antibodies or Hyaluronan

Although the ideal anticancer drug would have high specificity and activity against cancers, most drugs distribute throughout the body and are toxic to healthy as well as neoplastic cells. To minimize adverse effects, drugs can be formulated to increase the concentration at the target site and decrease drug concentrations elsewhere in the body. This goal has been achieved to some extent with anti-CD44 antibody-drug conjugates, which target drugs to CD44 that is overexpressed on various tumors.[13] Anti-CD44 antibody-drug conjugates have shown promise during clinical development (Table 2). However, CD44 is endogenously expressed at low levels in healthy tissues;[17] because of this expression, side effects still occur.[18, 19]

Antibody targeted therapies are effective in cancer treatments for three principal reasons: (1) The epitope recognized by the antibody is over-expressed on the tumor cell. (2) The antibody has better access to the tumor than to normal tissues that also express the epitope. (3) The antibody circulates for a long enough period that a high fraction of the injected dose passes through the tumor. The therapeutic advantages of mechanisms one and three are self-evident. Mechanism two arises because tumors often exhibit a phenomenon known as the enhanced permeability and retention effect (EPR).[20] The properties of the EPR effect are summarized in Table 1. This phenomenon is thought to occur because solid tumors have a much leakier blood supply than healthy capillary beds; particles of up to approximately 0.5 μm can extravasate into the tumor from the blood.[20] Tumors also lack a well-developed lymphatic system. This combination allows macromolecules or nanoparticles to passively accumulate within tumors due to leakage from the improperly formed tumor vasculature and remain within the tumor due to limited lymphatic clearance.[20]

Administering a drug in a carrier alters the drug's distribution profile by directing the drug away from sites of toxicity and, by exploiting the EPR effect, into the tumor. Successfully targeted anti-cancer therapies utilize a specific drug target and also exploit the altered biology of cancerous tissue to achieve a therapeutic effect. Drug carriers may also improve drug circulation time, stability, or solubility, depending on the carrier's properties.[21]

HA exhibits a number of properties of a successful drug carrier. This water soluble, non-immunogenic polysaccharide has multiple functional groups available for chemical conjugation[22 - 24] (Figure 1). Furthermore, since it is the major CD44 ligand, HA can be used to target cells on which CD44 is expressed[24, 25] (Table 3). Several reviews have previously discussed the advantages of HA as a drug carrier and a targeting ligand for cancer, as well as other pathologies.[22 - 27]

HA targeting increases drug accumulation on CD44 expressing cells and HA-attached drug can enter the cell via endocytosis.[28, 29] Interestingly, CD44 binding and endocytotic activity, and therefore the cellular localization of HA targeted drugs, are greatly affected by CD44 post-translational modifications. Such modifications are not constant across all CD44 expressing cells. Glycosylation is required for CD44 to bind HA on certain cell types, while glycosylations rich in sialic acid decrease HA binding.[30 - 32] Circulating lymphocytes express CD44 but do not bind HA until the CD44 is deglycosylated upon lymphocyte activation.[30, 33, 34] For CD44 to internalize via endocytosis it must be acylated.[35] Thus posttranslational modifications of CD44 greatly influence its ability to bind and internalize HA and must be considered during development of an HA-ligand targeted carrier.

Carriers, such as HMW-HA, can crosslink multiple receptors, potentially inducing binding and endocytosis. Drug potency is improved by increased cellular uptake of the drug-carrier complex and, for certain drugs, by circumvention of multidrug resistance efflux pumps.[36] Potency may also be improved by altering the drug's location inside the cell so that the intrinsic activity of the drug in the carrier is higher than the intrinsic activity of the free drug.[37, 38] A confounding difficulty for HMW-HA as a targeting carrier is that HA is cleared from circulation by the liver.[39] For HMW-HA to be a useful intravenous targeting carrier, strategies must be devised to reduce HA clearance from the blood.

Drug carrier-target cell interactions can be tuned to be effective only at sites of high receptor density by taking advantage of multiple ligands with moderate affinity but high specificity.[40] In the case of CD44, this can be achieved by employing short HA oligosaccharides as targeting moieties on a larger carrier.[37, 41] CD44 is reported to interact with a minimum HA length of 6 to 8 saccharides.[42] The extracellular portion of CD44 with an octasaccharide HA positioned in the binding groove is illustrated in Figure 3.[43] Selecting HA oligosaccharides long enough to bind to CD44 but too short to bind to the HARE receptor may permit an HA-targeted carrier to avoid elimination by the liver while maintaining targeting to cells that over-express CD44. This is because short oligosaccharides maintain a high enough affinity with individual CD44 that binding to multiple CD44 by different HA on the same carrier creates an avidity strong enough for effective targeting (Table 4).

2A. Anti-CD44 Antibody Conjugates

The most effective strategy for CD44 targeted anti-cancer therapeutics currently under investigation is anti-CD44 antibodies that "actively" target drugs to CD44, disrupt CD44 matrix interactions and, by occupying CD44, induce CD44 signaling, which can cause apoptosis.[44] Anti-CD44 antibodies as targeting ligands for either radio-labels or anti-cancer chemotherapeutics in squamous cell carcinoma (SCC) of the head and neck (Table 2) stabilized disease progression in several patients.[18, 19]

A number of monoclonal antibodies capable of targeting SCC were identified.[45] The most specific antibody was developed as a clinical radioisotope carrier by converting it to a human/mouse chimera that had a slightly lower half-life in circulation but a much higher capacity for cell lysis in a mouse xenograft tumor model.[46] Later improvements led to antibodies specific for CD44v6, a variant over-expressed in SCC. The antibody VFF18 was shown to target SCC in spontaneous epidermoid carcinoma bearing nude mice.[47] This mouse monoclonal antibody

(renamed BIWA 1) and a similar chimeric antibody, U36, were evaluated for targeting, pharmacokinetic parameters, and safety in humans.[48] The monoclonal antibody BIWA 1 targeted SCC cells *in vivo*, was safe and localized to tumors; however, anti-mouse antibodies were found in patients.[48] The chimeric U36 antibody was also well-tolerated and resulted in the highest *in vivo* tumor targeting compared to several other monoclonal or humanized antibodies.[49, 50] Treatment of refractory head and neck SCC patients with U36 conjugated to the radioisotope rhenium-186 (^{186}Re) resulted in partially stabilized disease at the maximum tolerated dose.[51] To reduce immunogenicity, a humanized monoclonal antibody, bivatuzumab (BIWA 4), was developed. Bivatuzumab radio-labeled with either technetium-99m (^{99m}TC) or ^{186}Re was safely administered to patients. There was minimal toxicity and a minimal anti-antibody response.[52, 53] Treatment of patients with ^{186}Re-BIWA 4 resulted in stable disease in three of the six patients at the maximum tolerated dose, but the tumor did not completely regress in any patient.[52] The ^{186}Re-BIWA 4 antibody conjugate was also well tolerated in patients with early stage breast cancer but did not target the tumor as effectively as in the patients with head and neck tumors. In the breast cancer patients, tumor localization appeared unrelated to CD44v6 expression and patients experienced unfavorable accumulation of the conjugate in non-target organs.[54]

Conjugation of BIWA 4 to a cytotoxic drug, mertansine, resulted in dose-limiting toxicities associated with skin-related disorders, including one fatal event of toxic epidermal necrolysis, which manifested by detachment of the epidermis from the dermis. This toxicity was most likely due to the presence of CD44v6 in the skin.[17] BIWA 4 antibodies (with or without conjugated mertansine) had half-lives of over 3 days, with low interpatient pharmacokinetic variability.[18, 19] This slow elimination allowed the antibody to reach the skin as well as the targeted tumor. There was limited anti-antibody response in the patients which did not seem to influence the pharmacokinetics of the BIWA 4-mertansine. Stable disease was achieved in 50% of patients with CD44v6 positive metastatic breast cancer, who previously received anthracycline and taxanes, regardless which of eight dose levels they received.[55]

Although the clinical trials for BIWA 4 were stopped due to skin-related toxicities, the antibody-drug conjugate showed some clinical success (disease stabilization or tumor regression) in both SCC and metastatic breast cancer. Using this antibody as a targeting moiety on a drug carrier (which would have less access to the skin), thus altering its distribution via physical mechanisms, may allow for an alternative use of these antibodies as targeting ligands in a clinical setting.

2B. Hyaluronan Conjugates as Drug Carriers

HA has several chemical groups to which drugs can be conjugated.[22] The structure of HA, with possible chemical modification sites, is shown in Figure 1. The carboxylate on the glucuronic acid, the *N*-acetylglucosamine hydroxyl, and the reducing end have all been successfully utilized in conjugation reactions with drugs. The acetyl group may possibly be enzymatically removed from the *N*-acetylglucosamine and so is also a potential site for drug conjugation.[56] Drug conjugation creates a macromolecular prodrug; the conjugated drug becomes active upon release from the HA. A summary of various HA conjugates with specific conjugation methods used, as well as the cell lines and details of the conjugated drugs or drug carriers, is listed in Table 3.

In many of the studies described below, uptake of HA conjugates was measured using radio-labeled conjugates in which binding at 4 °C was compared to uptake at 37 °C,[57, 58] fluorescent HA conjugates (such as FITC or BODIPY),[14, 28, 29, 59] or the drug as a visualizing agent itself (doxorubicin).[60] These measurements provided direct evidence that HA conjugates were specifically and efficiently internalized into cells that expressed CD44.

The earliest reports of an HA-drug conjugate designed to specifically target over-expressed CD44 were performed by Akima *et al.*, who showed uptake of a fluorescent HA conjugate in Lewis lung carcinoma cells.[14] More importantly, this HA-drug conjugate decreased cancer progression in several models of cancer. HA-conjugated mitomycin c reduced systemic toxicity and allowed a higher relative dose to be administered in mice bearing ascites tumors in the foot pad, although it did not decrease metastasis more than unconjugated mitomycin c. When treating primary ascites tumors implanted into the back of mice, HA-mitomycin c decreased tumor burden more than free mitomycin. An epirubicin conjugate showed no improvement. In a metastatic lung carcinoma model, HA-mitomycin c decreased the number of metastatic lung nodules at a much lower dose that free mitomycin c.[14]

Drug-HA conjugates have also been used to deliver drugs with physical properties that limit dosing. For instance, the antimitotic chemotherapeutic agent paclitaxel has low aqueous solubility. Conjugation of paclitaxel to HA increases drug solubility and, when incubated with cells that express CD44, a LMW-HA-paclitaxel conjugate increased cellular uptake and cytotoxicity *in vitro*.[28] CD44 negative control fibroblasts tolerated a much higher dose of conjugate drug than CD44 positive cells.[28] Uptake of HA-conjugated paclitaxel was dose-dependent and CD44-specific, as it could be blocked by both excess HA and anti-CD44 antibodies. Notably, uptake could not be blocked by chondroitin sulfate, a sulfated polysaccharide of glucuronic acid and N-acetylglucosamine similar to HA. Luo *et al.* found that HA-drug conjugates are internalized via CD44 and drug is released mainly by intracellular enzymatic hydrolysis.[28, 29] Others have devised improved synthetic methods for HA-paclitaxel conjugates.[61]

Auzenne *et al.* observed CD44-specific paclitaxel cytotoxicity *in vitro* in two human ovarian carcinoma cell lines, NMP-1 and SK-OV-3. *In vivo*, the HA-paclitaxel conjugate decreased tumor burden in mice bearing abdominal tumors of either cell type. HA-paclitaxel conjugate delivered intraperitoneally in the NMP-1 tumor model also moderately increased survival time compared to multiple doses of free drug. Preliminary *in vivo* toxicity studies showed that the HA conjugate was well tolerated.[62]

Conjugation of drug to HMW-HA can also serve to physically localize the drug. *In vitro* treatment of transitional cell carcinoma (superficial bladder cancer) cells showed a higher anti-proliferative effect than free paclitaxel. Injection of the conjugate within the bladder did not improve therapeutic efficacy and only slightly decreased the amount of paclitaxel reaching the systemic circulation. However, the HA-paclitaxel conjugate had significantly decreased local toxicity.[63]

To improve the uptake of carboranes for boron neutron capture therapy the carborane was attached to HA. This conjugate was taken up specifically by CD44 positive cells, but the amount of conjugate internalized in each cell line was not directly proportional to the receptor density. Cells that expressed 10-fold fewer CD44 receptors internalized similar amounts of HA conjugate. Meo *et al.* suggested that cell lines with lower expression of CD44 may recycle CD44, increasing the uptake of the drug conjugate.[58] In this case, healthy cells that express low endogenous levels of CD44 would also take up the conjugate; however, activation of the carborane occurs only in regions irradiated by the neutron beam so that therapy can be concentrated in the tumor.

Attaching butyric acid, a histone deacetylase inhibitor, to HMW-HA targets the drug conjugate to CD44 expressing cells, leads to internalization, and improves *in vivo* delivery by altering the release profile of the drug. Coradini *et al.* showed, in several human tumor models (breast, lung, melanoma and hepatocellular), that HA-conjugated butyric acid increased apoptosis, inhibited cell growth *in vitro* and decreased tumor burden *in vivo*. Initial studies of HA-

conjugated butyric acid showed rapid cellular uptake in a human breast carcinoma cell line *in vitro*. This interaction was blocked by an anti-CD44 antibody.[59] Further *in vitro* studies in both primary and metastatic non-small cell lung cancer cell lines also showed increased uptake of the HA-butyric acid conjugate. The most effective HA-butyric acid conjugate resulted in a 10-fold decrease in IC_{50} compared to free butyric acid, suggesting that intracellular delivery of butyric acid, via the HA internalization pathway, increases cytotoxicity. Daily *in vivo* intratumoral injections both significantly decreased primary tumor mass and reduced metastases. Intraperitoneal and subcutaneous injection of butyric acid conjugates in intrasplenic hepatocellular carcinoma or melanoma mouse models resulted in fewer liver metastases.[57] Intratumoral treatment of non-small cell lung carcinoma bearing mice showed a decrease in the number of metastatic colonies in the lung.[64] However, intravenous injection resulted in rapid accumulation in clearance organs.[57]

HA-drug conjugates have effective cytotoxicity at a cellular level, but studies performed *in vivo* often did not employ intravenous dosing, opting for intratumoral, subcutaneous or intraperitoneal injection, and avoided many HA clearance mechanisms. The short half-life of HMW-HA conjugates[57, 65, 66] is most likely due to HA clearance from the blood by the HARE receptor present in the liver and spleen.[5]

Improvement in the distribution of HA conjugated drugs *in vivo* may be achieved by reducing endogenous HA blood clearance mechanisms. Luo *et al.* suggested that systemic HA clearance can be reduced by pre-treatment with a high molecular weight saccharide that competes with HA for binding during clearance but not during target cell drug internalization.[29] HARE binds chondroitin sulfate and HA; CD44 binds only to HA.[39]

In rats with hepatic metastases of colon adenocarcinoma cells, pretreatment by intravenous injection of either chondroitin sulfate or HA resulted in higher tumor accumulation of a subsequent intravenous HA dose.[67] In perfused liver, pre-blocking with an anti-HARE antibody or immediate pre-dosing with free HA resulted in decreased HA clearance of the second dose.[68] These results parallel the *in vitro* observations of Luo *et al.*, strongly suggesting that cancer treatments utilizing HA targeting to CD44 over-expressing tumor types may benefit from pretreatment with a HARE blocking moiety such as free chondroitin sulfate, unconjugated HA, or antibodies.[29, 67, 68]

2C. Hyaluronan in a Targeted Nanocarrier System

When attached to a nanocarrier, HA can act as a protective structural component, a small (oligosaccharide) targeting moiety, and a targeting coating. Pharmacokinetic properties, such as circulation time and biodistribution, are influenced by incorporating the targeting and cell-specific uptake properties of HA onto large carriers. Cargos delivered to CD44 over-expressing cells include anti-cancer drugs: epirubicin,[14] doxorubicin,[37, 38, 41, 69, 70, 71] paclitaxel,[28, 29, 62, 63] and mitomycin c,[14, 70] as well as agents such as siRNA,[72] iron oxide magnetic particles,[73] and DNA.[74] These studies are summarized in Tables 3 and 4.

Several different methods have been used to improve the distribution of doxorubicin, which has significant cardiotoxicity as a free drug. Liposomes (nanosized phospholipid vesicles) bearing oligosaccharide HA conjugated to a phosphatidylethanolamine lipid have been used as a targeted drug carrier for doxorubicin.[37, 38, 41, 69, 70] Two different approaches to attach HA to liposomes were developed.[41, 75] In the earliest report, HMW-HA was coupled via the glucuronic carboxylate to phosphatidylethanolamine in pre-formed liposomes.[75] This coupling method results in multipoint attachment of the HA to a liposome. The number and distribution of the resulting attachment points was difficult to characterize. The HMW-HA liposomes were originally proposed as a bioadhesive formulation[75] and then subsequently used to target cytotoxic drugs to tumors.[69, 70] Peer and co-workers encapsulated doxorubicin or mitomycin

c within the aqueous core of the HMW-HA liposome. Mitomycin c encapsulated in HMW-HA liposomes increased cytotoxicity compared to non-targeted liposomes (but not free drug) in CD44 over-expressing cells.[70]

HMW-HA liposomes were very successful at decreasing tumor burden in several different tumor models. HA coated liposomes carrying either doxorubicin or mitomycin c also showed an increased circulation time over traditional, non-targeted liposomes[69, 70] with a half-life slightly less than that of PEGylated "stealth" liposomes. This method of conjugation, in which a HMW-HA was attached to the liposome surface at multiple sites, avoided immediate clearance by the liver HA receptor. Both drug-loaded HA-coated liposomes increased drug accumulation in the CD44 expressing tumor, decreased systemic toxicity, and increased survival time in multiple cancer models.[69, 70] Disease regression included significantly decreased solid tumor growth and a reduced metastatic lung tumor burden in colon cancer models for mitomycin c[70] and doxorubicin containing targeted liposomes, increased survival in models of intraperitoneal ascites tumors, and reduced tumor burden in a solid pancreatic tumor model.[69]

The second conjugation method attached oligosaccharide HA via the reducing end of a phosphatidylethanolamine by reductive amination.[41] A mixture of oligosaccharides of increasing disaccharide number was coupled to phosphatidylethanolamine.[41] The precise composition of the oligosaccharide mixture was not completely specified. This lipid-HA was incorporated at a defined surface density in the liposome and the binding of radiolabeled and fluorescent liposomes to cells that expressed different levels of CD44 examined. Liposome uptake was dependent on the expression of CD44. The uptake was CD44 specific and could be blocked by both free HA and anti-CD44 antibodies.[41] Liposome uptake was also dependent on the density of the HA oligosaccharide targeting ligand,[37] suggesting that receptor clustering might occur because of multivalent ligand presentation on the liposomal surface. Targeting was observed with as little as 0.1 mol % HA-ligand on the liposome. Encapsulated doxorubicin was significantly more cytotoxic to CD44 overexpressing cell types in culture than was free drug. Uptake of targeted liposomes was modeled and provided a quantitative confirmation of the hypothesis that the increased cytotoxicity of the HA-targeted liposome stemmed specifically from the internalization of the doxorubicin containing liposomes.[37]

Doxorubicin-containing PLGA (polylactide-*co*-glycolide) nanoparticles showed improved tumor accumulation when targeted with HA. In two separate studies, HA was conjugated to PLGA via a polyethylene glycol (PEG) spacer prior to particle formation[71] or directly onto the surface of a pre-formed particle.[76] Both types of coated nanoparticles showed improved sustained release compared to untargeted nanoparticles. Pre-formed particles also showed higher specific uptake and cytotoxicity in CD44 over-expressing cell types than in cells with low CD44 expression.[76] *In vivo* studies using particles with HA conjugated via a PEG spacer showed significantly decreased hemolytic and subacute toxicity profiles and modestly increased doxorubicin levels within the tumor.[71] However, there was no significant difference between the uptake of targeted and non-targeted particles in the liver. Most importantly there was no difference in tumor progression among the non-drug treated control, the targeted or non-targeted PLGA nanoparticle.[71] In this case, incorporating a targeting ligand onto the particle did not improve drug therapy.

Cross-linked HA particles have also been used for the protected delivery of DNA[74] and siRNA.[72] DNA incorporated prior to cross-linking of the HA carboxylate side chains was release from the particle upon exposure to hyaluronidase but not buffer. Release was sustained for several weeks. However, HA mediated CD44 specificity of transfection was not investigated. Rather, targeting was achieved by antibodies to specific selectins.[74] Thiosulfated HA nanogels cross-linked with a glutathione labile disulfide bond were developed for siRNA delivery. These

nanogels had CD44 specific uptake. Release of siRNA by glutathione, an intracellular reductive agent, induced silencing of a fluorescent reporter protein gene in a colon carcinoma cell line *in vitro*.[72]

Results from the above studies using HA targeted nanocarriers are consistent with many of the studies performed on HA-drug conjugates and validate the potential of targeting CD44 to improve anticancer drug delivery.

2D. Alternate CD44 Targeting Ligands

Alternatives to HA for highly specific targeting ligands are also being investigated. Peptide mimetics of both HA itself[77] and a collagen triple helix peptide that interacts specifically with a chondroitin sulfate proteoglycan modification found on CD44 variants over-expressed in metastatic melanoma[78] were designed to interact with the HA receptors RHAMM and CD44 respectively. The CD44 specific peptide amphiphile was incorporated into liposomes via a C_{16} tail and engendered specificity for CD44 positive cells, with liposome uptake correlating with the amount of CD44 on the surface of a human metastatic melanoma line studied *in vitro*.[78]

3. Targeting to the Hyaluronan Matrix

Based on the intimate relationship between tumor survival and the extracellular matrix, regulated in part by the CD44-HA interaction, HA itself is also a viable target for anti-cancer treatments. Manipulating the interaction between cancer cells and HA by disrupting HA binding to cell surface receptors can sometimes lead to disease regression. HA within the matrix, acting as a reservoir for nascent HA binding proteins,[79] can be used as a conjugate-drug target site and provides a further opportunity for improved targeted chemotherapeutic delivery.

Degradation of the HA matrix by exogenously added hyaluronidase decreases tumor growth.[80] Intravenous injection of hyaluronidase into mice bearing human breast carcinoma tumors resulted in substantial tumor regression. Although the tumors were grown initially in the presence of an HA gel, and grew more slowly without an HA matrix,[80] this study illustrated the combinatorial effect of targeted treatment (hyaluronidase disruption of the HA matrix) and utilization of cancer biology (access of the hyaluronidase to the tumor, potentially via the EPR phenomenon). A conceptually simple method to increase the passive targeting of nanocarriers to tumors is to inject hyaluronidase into the tumor. The disruption of the HA matrix permitted increased delivery of intravenously injected untargeted liposomes containing doxorubicin in a human osteosarcoma solid tumor model.[81]

Disrupting binding of HA to the cell surface with soluble HA binding proteins can also decrease tumor progression. The soluble portion of HA binding receptors (either as individual molecules or as immunoglobulin fusion constructs) decreased tumor growth in several cell lines and animal tumor models. Administration of CD44-immunoglobulin fusion protein significantly decreased *in vitro* tumor cell growth in models of human melanoma[82] and lymphoma.[16] Soluble CD44-immunoglobin constructs, injected intravenously[16] or delivered by slow release infusion pump implanted subcutaneously near the tumor[82] decreased tumor growth and inhibited invasion.

Mammary carcinoma and malignant melanoma transfected to over-express soluble CD44 had significantly decreased HA binding *in vitro* and slower tumor progression *in vivo*.[83 - 85] Transfected mammary carcinoma cells showed decreased monolayer invasion, HA binding, and HA internalization.[83] When injected intraperitoneally, cells expressing soluble CD44 grew poorly and did not invade or form tumors on the peritoneal wall. When injected intravenously,

the cells could both adhere and invade pulmonary stroma, but underwent apoptosis before forming tumors.[84] Melanoma cells expressing soluble CD44 bound less HA *in vitro* and formed fewer tumors *in vivo*.[85] Decreased proliferation was not observed in all tumor cell lines since over-expression of soluble CD44 in human colon carcinoma cells increased proliferation *in vitro*.[86]

Addition of exogenous soluble RHAMM[87, 88] and a peptide mimetic of RHAMM consisting of three repeats of an HA binding motif,[89] inhibited tumor growth. *In vivo*, pretreatment of fibrosarcoma cells with soluble RHAMM prior to either subcutaneous or intravenous injection decreased primary tumor formation, limited metastasis, and decreased lung nodule formation.[87] *In vitro* studies using the RHAMM peptide mimetic suggested that soluble RHAMM may cause apoptosis.[83]

Soluble CD44 and RHAMM mutated to eliminate HA binding ability did not exhibit any anti-tumor effects, suggesting that interference with HA binding between the cell and the tumor HA matrix may also contribute to decreased tumor growth.[84, 85] The above studies showed that decreased tumor growth by over-expression of soluble HA binding proteins is due to both increased apoptosis and physical mechanisms of CD44-matrix disruption.[83 - 85, 87, 88] These results suggest that either mechanism may be a potential avenue for improved anti-cancer therapeutics.

The tumor matrix can be a target for drug delivery. It is a multivalent ligand accessible from the blood due to the EPR effect. The concept of matrix attachment therapy is to target the tumor matrix with a drug carrier or prodrug converting enzyme. This concept has a number of advantages over targeting the tumor itself: (1) The number of binding sites is very large so that a high concentration of a matrix targeted system can accumulate at the site. (2) The tumor cannot shed the matrix as it can shed a surface antigen. (3) The tumor cannot directly internalize the delivery system, as it could a system targeted to the cell surface. (4) Targeting the matrix may kill matrix-associated fibroblasts that supply growth factors that promote tumor growth. (5) Targeting the matrix may also kill local endothelial cells that provide a blood supply to the growing tumor. The combination of these mechanisms could have a synergistic effect to stop tumor progression.

To implement matrix attachment therapy, Park and co-workers[15] prepared a recombinant fusion protein consisting of a soluble-HA binding domain fused to a yeast cytosine deaminase. The fusion protein could convert the prodrug 5-fluorocytosine into cytotoxic 5-fluorouracil. When the fusion protein was injected intratumorally into a C26 colon carcinoma tumor model, and the animals were supplied with 5-fluorocytosine in the drinking water, tumor progression was slowed and long-term survivors ensued. The presence of the drug, active enzyme and an HA binding component were all required to observe an anti-tumor effect.[15] This strategy of targeting the matrix may also be possible for other carriers, such as liposomes, polymeric micelles, or nanocapsules.

4. Conclusion

The importance of the CD44-HA interaction in tumor growth and progression provides multiple opportunities for intervention: targeting therapeutics to the CD44 receptor, interfering with the CD44-HA signaling pathway, removing the HA matrix with hyaluronidase to enable passive carrier uptake, or targeting the HA tumor matrix to provide a sustained drug source within the tumor. Anti-CD44 antibodies targeting either radioisotopes or cytotoxic drugs have shown promise in human clinical trials. The targeting of drug-loaded liposomes with surface attached HA has shown promise in various animal tumor models. Alternative approaches, such as targeting the tumor matrix to disrupt CD44-HA signaling pathways or targeting matrix HA

to provide a sustained source of drug, are promising research avenues in the quest for effective approaches for the control of tumor progression and metastasis.

Acknowledgments

This work was partially funded by NIH: R01 CA107268 and R01 GM061851

We are indebted to the constructive comments and support of the Szoka Laboratory

References

1. Weigel PH, DeAngelis PL. Hyaluronan synthases: a decade-plus of novel glycosyltransferases. J. Biol. Chem 2007;282:36777–36781. [PubMed: 17981795]
2. Hua Q, Knudson CB, Knudson W. Internalization of hyaluronan by chondrocytes occurs via receptor-mediated endocytosis. J. Cell. Sci 1993;106(Pt 1):365–375. [PubMed: 7505784]
3. Stern R, Jedrzejas MJ. Hyaluronidases: their genomics, structures, and mechanisms of action. Chem. Rev 2006;106:818–839. [PubMed: 16522010]
4. Banerji S, Ni J, Wang SX, Clasper S, Su J, Tammi R, Jones M, Jackson DG. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. J. Cell Biol 1999;144:789–801. [PubMed: 10037799]
5. Zhou B, Weigel JA, Fauss L, Weigel PH. Identification of the hyaluronan receptor for endocytosis (HARE). J. Biol. Chem 2000;275:37733–37741. [PubMed: 10952975]
6. Tammi R, Saamanen AM, Maibach HI, Tammi M. Degradation of newly synthesized high molecular mass hyaluronan in the epidermal and dermal compartments of human skin in organ culture. J. Invest. Dermatol 1991;97:126–130. [PubMed: 2056182]
7. Fraser JR, Laurent TC, Laurent UB. Hyaluronan: its nature, distribution, functions and turnover. J. Intern. Med 1997;242:27–33. [PubMed: 9260563]
8. Laurent, TC.; Fraser, JR. Catabolism of Hyaluronan. In: Henriksen, JH., editor. Degradation of Bioactive Substances: Physiology and Pathophysiology. CRC Press; Boston: 1991. p. 249-288.
9. Pilarski LM, Masellis-Smith A, Belch AR, Yang B, Savani RC, Turley EA. RHAMM, a receptor for hyaluronan-mediated motility, on normal human lymphocytes, thymocytes and malignant B cells: a mediator in B cell malignancy? Leuk. Lymphoma 1994;14:363–374. [PubMed: 7529076]
10. Bourguignon LY, Zhu H, Shao L, Chen YW. CD44 interaction with tiam1 promotes Rac1 signaling and hyaluronic acid-mediated breast tumor cell migration. J. Biol. Chem 2000;275:1829–1838. [PubMed: 10636882]
11. Bourguignon LY, Singleton PA, Zhu H, Diedrich F. Hyaluronan-mediated CD44 interaction with RhoGEF and Rho kinase promotes Grb2-associated binder-1 phosphorylation and phosphatidylinositol 3-kinase signaling leading to cytokine (macrophage-colony stimulating factor) production and breast tumor progression. J. Biol. Chem 2003;278:29420–29434. [PubMed: 12748184]
12. Bourguignon LY, Peyrollier K, Gilad E, Brightman A. Hyaluronan-CD44 interaction with neural Wiskott-Aldrich syndrome protein (N-WASP) promotes actin polymerization and ErbB2 activation leading to beta-catenin nuclear translocation, transcriptional up-regulation, and cell migration in ovarian tumor cells. J. Biol. Chem 2007;282:1265–1280. [PubMed: 17092940]
13. Naor D, Nedvetzki S, Golan I, Melnik L, Faitelson Y. CD44 in cancer. Crit. Rev. Clin. Lab. Sci 2002;39:527–579. [PubMed: 12484499]
14. Akima K, Ito H, Iwata Y, Matsuo K, Watari N, Yanagi M, Hagi H, Oshima K, Yagita A, Atomi Y, Tatekawa I. Evaluation of antitumor activities of hyaluronate binding antitumor drugs: synthesis, characterization and antitumor activity. J. Drug Target 1996;4:1–8. [PubMed: 8798873]
15. Park J, Stull RA, Huang Z, Cao L, Dy EE, Sperinde JJ, Yokoyama JS, Platt VM, Szoka FC Jr. Matrix Attachment Anti-Tumor Therapy Mediated by 5-Fluorocytosine and a Recombinant Fusion Protein Containing TSG-6 Hyaluronan Binding Domain and Yeast Cytosine Deaminase. Mol. Pharm. Submitted

16. Sy MS, Guo YJ, Stamenkovic I. Inhibition of tumor growth in vivo with a soluble CD44-immunoglobulin fusion protein. J. Exp. Med 1992;176:623–627. [PubMed: 1500863]
17. Mackay CR, Terpe HJ, Stauder R, Marston WL, Stark H, Gunthert U. Expression and modulation of CD44 variant isoforms in humans. J. Cell Biol 1994;124:71–82. [PubMed: 7507492]
18. Tijink BM, Buter J, de Bree R, Giaccone G, Lang MS, Staab A, Leemans CR, van Dongen GA. A phase I dose escalation study with anti-CD44v6 bivatuzumab mertansine in patients with incurable squamous cell carcinoma of the head and neck or esophagus. Clin. Cancer Res 2006;12:6064–6072. [PubMed: 17062682]
19. Sauter A, Kloft C, Gronau S, Bogeschdorfer F, Erhardt T, Golze W, Schroen C, Staab A, Riechelmann H, Hoermann K. Pharmacokinetics, immunogenicity and safety of bivatuzumab mertansine, a novel CD44v6-targeting immunoconjugate, in patients with squamous cell carcinoma of the head and neck. Int. J. Oncol 2007;30:927–935. [PubMed: 17332932]
20. Maeda H, Seymour LW, Miyamoto Y. Conjugates of anticancer agents and polymers: advantages of macromolecular therapeutics in vivo. Bioconjug. Chem 1992;3:351–362. [PubMed: 1420435]
21. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science 2004;303:1818–1822. [PubMed: 15031496]
22. Pouyani T, Prestwich GD. Functionalized derivatives of hyaluronic acid oligosaccharides: drug carriers and novel biomaterials. Bioconjug. Chem 1994;5:339–347. [PubMed: 7948100]
23. Prestwich GD, Marecak DM, Marecek JF, Vercruysse KP, Ziebell MR. Controlled chemical modification of hyaluronic acid: synthesis, applications, and biodegradation of hydrazide derivatives. J. Control. Release 1998;53:93–103. [PubMed: 9741917]
24. Vercruysse KP, Prestwich GD. Hyaluronate derivatives in drug delivery. Crit. Rev. Ther. Drug Carrier Syst 1998;15:513–555. [PubMed: 9822869]
25. Jaracz S, Chen J, Kuznetsova LV, Ojima I. Recent advances in tumor-targeting anticancer drug conjugates. Bioorg. Med. Chem 2005;13:5043–5054. [PubMed: 15955702]
26. Liao YH, Jones SA, Forbes B, Martin GP, Brown MB. Hyaluronan: pharmaceutical characterization and drug delivery. Drug Deliv 2005;12:327–342. [PubMed: 16253949]
27. Yadav AK, Mishra P, Agrawal GP. An insight on hyaluronic acid in drug targeting and drug delivery. J. Drug Target 2008;16:91–107. [PubMed: 18274931]
28. Luo Y, Prestwich GD. Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bioconjugate. Bioconjug. Chem 1999;10:755–763. [PubMed: 10502340]
29. Luo Y, Ziebell MR, Prestwich GD. A hyaluronic acid-taxol antitumor bioconjugate targeted to cancer cells. Biomacromolecules 2000;1:208–218. [PubMed: 11710102]
30. Katoh S, Zheng Z, Oritani K, Shimozato T, Kincade PW. Glycosylation of CD44 negatively regulates its recognition of hyaluronan. J. Exp. Med 1995;182:419–429. [PubMed: 7543137]
31. Skelton TP, Zeng C, Nocks A, Stamenkovic I. Glycosylation provides both stimulatory and inhibitory effects on cell surface and soluble CD44 binding to hyaluronan. J. Cell Biol 1998;140:431–446. [PubMed: 9442118]
32. Rochman M, Moll J, Herrlich P, Wallach SB, Nedvetzki S, Sionov RV, Golan I, Ish-Shalom D, Naor D. The CD44 receptor of lymphoma cells: structure-function relationships and mechanism of activation. Cell Adhes. Commun 2000;7:331–347. [PubMed: 10714394]
33. Hathcock KS, Hirano H, Murakami S, Hodes RJ. CD44 expression on activated B cells. Differential capacity for CD44-dependent binding to hyaluronic acid. J. Immunol 1993;151:6712–6722. [PubMed: 7505013]
34. Lesley J, English N, Perschl A, Gregoroff J, Hyman R. Variant cell lines selected for alterations in the function of the hyaluronan receptor CD44 show differences in glycosylation. J. Exp. Med 1995;182:431–437. [PubMed: 7543138]
35. Thankamony SP, Knudson W. Acylation of CD44 and its association with lipid rafts are required for receptor and hyaluronan endocytosis. J. Biol. Chem 2006;281:34601–34609. [PubMed: 16945930]
36. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev 2002;54:631–651. [PubMed: 12204596]
37. Eliaz RE, Nir S, Szoka FC Jr. Interactions of hyaluronan-targeted liposomes with cultured cells: modeling of binding and endocytosis. Methods Enzymol 2004;387:16–33. [PubMed: 15172155]

38. Eliaz RE, Nir S, Marty C, Szoka FC Jr. Determination and modeling of kinetics of cancer cell killing by doxorubicin and doxorubicin encapsulated in targeted liposomes. Cancer Res 2004;64:711–718. [PubMed: 14744789]
39. Harris EN, Kyosseva SV, Weigel JA, Weigel PH. Expression, processing, and glycosaminoglycan binding activity of the recombinant human 315-kDa hyaluronic acid receptor for endocytosis (HARE). J. Biol. Chem 2007;282:2785–2797. [PubMed: 17145755]
40. Mammen M, Choi S, Whitesides GM. Polvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors. Angew. Chem. Int. Ed 1998;37:2754–2794.
41. Eliaz RE, Szoka FC Jr. Liposome-encapsulated doxorubicin targeted to CD44: a strategy to kill CD44-overexpressing tumor cells. Cancer Res 2001;61:2592–2601. [PubMed: 11289136]
42. Lesley J, Hascall VC, Tammi M, Hyman R. Hyaluronan binding by cell surface CD44. J. Biol. Chem 2000;275:26967–26975. [PubMed: 10871609]
43. Banerji S, Wright AJ, Noble M, Mahoney DJ, Campbell ID, Day AJ, Jackson DG. Structures of the Cd44-hyaluronan complex provide insight into a fundamental carbohydrate-protein interaction. Nat. Struct. Mol. Biol 2007;14:234–239. [PubMed: 17293874]
44. Song G, Liao X, Zhou L, Wu L, Feng Y, Han ZC. HI44a, an anti-CD44 monoclonal antibody, induces differentiation and apoptosis of human acute myeloid leukemia cells. Leuk. Res 2004;28:1089–1096. [PubMed: 15289023]
45. Quak JJ, Van Dongen G, Brakkee JG, Hayashida DJ, Balm AJ, Snow GB, Meijer CJ. Production of a monoclonal antibody (K 931) to a squamous cell carcinoma associated antigen identified as the 17-1A antigen. Hybridoma 1990;9:377–387. [PubMed: 2210778]
46. Brakenhoff RH, van Gog FB, Looney JE, van Walsum M, Snow GB, van Dongen GA. Construction and characterization of the chimeric monoclonal antibody E48 for therapy of head and neck cancer. Cancer Immunol. Immunother 1995;40:191–200. [PubMed: 7728778]
47. Heider KH, Sproll M, Susani S, Patzelt E, Beaumier P, Ostermann E, Ahorn H, Adolf GR. Characterization of a high-affinity monoclonal antibody specific for CD44v6 as candidate for immunotherapy of squamous cell carcinomas. Cancer Immunol. Immunother 1996;43:245–253. [PubMed: 9003471]
48. Stroomer JW, Roos JC, Sproll M, Quak JJ, Heider KH, Wilhelm BJ, Castelijns JA, Meyer R, Kwakkelstein MO, Snow GB, Adolf GR, van Dongen GA. Safety and biodistribution of 99mTechnetium-labeled anti-CD44v6 monoclonal antibody BIWA 1 in head and neck cancer patients. Clin. Cancer Res 2000;6:3046–3055. [PubMed: 10955783]
49. Colnot DR, Wilhelm AJ, Cloos J, Roos JC, de Bree R, Quak JJ, Snow GB, van Dongen GA. Evaluation of limited blood sampling in a preceding 99mTc-labeled diagnostic study to predict the pharmacokinetics and myelotoxicity of 186Re-cMAb U36 radioimmunotherapy. J. Nucl. Med 2001;42:1364–1367. [PubMed: 11535726]
50. Verel I, Heider KH, Siegmund M, Ostermann E, Patzelt E, Sproll M, Snow GB, Adolf GR, van Dongen GA. Tumor targeting properties of monoclonal antibodies with different affinity for target antigen CD44V6 in nude mice bearing head-and-neck cancer xenografts. Int. J. Cancer 2002;99:396–402. [PubMed: 11992408]
51. Colnot DR, Ossenkoppele GJ, Roos JC, Quak JJ, de Bree R, Borjesson PK, Huijgens PC, Snow GB, van Dongen GA. Reinfusion of unprocessed, granulocyte colony-stimulating factor-stimulated whole blood allows dose escalation of 186Relabeled chimeric monoclonal antibody U36 radioimmunotherapy in a phase I dose escalation study. Clin. Cancer Res 2002;8:3401–3406. [PubMed: 12429627]
52. Borjesson PK, Postema EJ, Roos JC, Colnot DR, Marres HA, van Schie MH, Stehle G, de Bree R, Snow GB, Oyen WJ, van Dongen GA. Phase I therapy study with (186)Re-labeled humanized monoclonal antibody BIWA 4 (bivatuzumab) in patients with head and neck squamous cell carcinoma. Clin. Cancer Res 2003;9:3961S–72S. [PubMed: 14506195]
53. Colnot DR, Roos JC, de Bree R, Wilhelm AJ, Kummer JA, Hanft G, Heider KH, Stehle G, Snow GB, van Dongen GA. Safety, biodistribution, pharmacokinetics, and immunogenicity of 99mTc-labeled humanized monoclonal antibody BIWA 4 (bivatuzumab) in patients with squamous cell carcinoma of the head and neck. Cancer Immunol. Immunother 2003;52:576–582. [PubMed: 14627130]

54. Koppe M, Schaijk F, Roos J, Leeuwen P, Heider KH, Kuthan H, Bleichrodt R. Safety, pharmacokinetics, immunogenicity, and biodistribution of (186)Re-labeled humanized monoclonal antibody BIWA 4 (Bivatuzumab) in patients with early-stage breast cancer. Cancer Biother. Radiopharm 2004;19:720–729. [PubMed: 15665619]
55. Rupp U, Schoendorf-Holland E, Eichbaum M, Schuetz F, Lauschner I, Schmidt P, Staab A, Hanft G, Huober J, Sinn HP, Sohn C, Schneeweiss A. Safety and pharmacokinetics of bivatuzumab mertansine in patients with CD44v6-positive metastatic breast cancer: final results of a phase I study. Anticancer Drugs 2007;18:477–485. [PubMed: 17351401]
56. Duncan MB, Liu M, Fox C, Liu J. Characterization of the N-deacetylase domain from the heparan sulfate N-deacetylase/N-sulfotransferase 2. Biochem. Biophys. Res. Commun 2006;339:1232–1237. [PubMed: 16343444]
57. Coradini D, Zorzet S, Rossin R, Scarlata I, Pellizzaro C, Turrin C, Bello M, Cantoni S, Speranza A, Sava G, Mazzi U, Perbellini A. Inhibition of hepatocellular carcinomas in vitro and hepatic metastases in vivo in mice by the histone deacetylase inhibitor HA-But. Clin. Cancer Res 2004;10:4822–4830. [PubMed: 15269158]
58. Meo CD, Panza L, Capitani D, Mannina L, Banzato A, Rondina M, Renier D, Rosato A, Crescenzi V. Hyaluronan as carrier of carboranes for tumor targeting in boron neutron capture therapy. Biomacromolecules 2007;8:552–559. [PubMed: 17291079]
59. Coradini D, Pellizzaro C, Miglierini G, Daidone MG, Perbellini A. Hyaluronic acid as drug delivery for sodium butyrate: improvement of the anti-proliferative activity on a breast-cancer cell line. Int. J. Cancer 1999;81:411–416. [PubMed: 10209956]
60. Luo Y, Bernshaw NJ, Lu ZR, Kopecek J, Prestwich GD. Targeted delivery of doxorubicin by HPMA copolymer-hyaluronan bioconjugates. Pharm. Res 2002;19:396–402. [PubMed: 12033370]
61. Leonelli F, La Bella A, Francescangeli A, Joudioux R, Capodilupo A, Quagliariello M, Migneco LM, Bettolo RM, Crescenzi V, De Luca G, Renier D. A New and Simply Available Class of Hydrosoluble Bioconjugates by Coupling Paclitaxel to Hyaluronic Acid through a 4-Hydroxybutanoic Acid Derived Linker. Helv. Chim. Acta 2005;88:154–159.
62. Auzenne E, Ghosh SC, Khodadadian M, Rivera B, Farquhar D, Price RE, Ravoori M, Kundra V, Freedman RS, Klostergaard J. Hyaluronic acid-paclitaxel: antitumor efficacy against CD44(+) human ovarian carcinoma xenografts. Neoplasia 2007;9:479–486. [PubMed: 17603630]
63. Rosato A, Banzato A, De Luca G, Renier D, Bettella F, Pagano C, Esposito G, Zanovello P, Bassi P. HYTAD1-p20: a new paclitaxel-hyaluronic acid hydrosoluble bioconjugate for treatment of superficial bladder cancer. Urol. Oncol 2006;24:207–215. [PubMed: 16678050]
64. Coradini D, Pellizzaro C, Abolafio G, Bosco M, Scarlata I, Cantoni S, Stucchi L, Zorzet S, Turrin C, Sava G, Perbellini A, Daidone MG. Hyaluronic-acid butyric esters as promising antineoplastic agents in human lung carcinoma: a preclinical study. Invest. New Drugs 2004;22:207–217. [PubMed: 15122068]
65. Fraser JR, Dahl LB, Kimpton WG, Cahill RN, Brown TJ, Vakakis N. Elimination and subsequent metabolism of circulating hyaluronic acid in the fetus. J. Dev. Physiol 1989;11:235–242. [PubMed: 2607093]
66. Sugahara S, Okuno S, Yano T, Hamana H, Inoue K. Characteristics of tissue distribution of various polysaccharides as drug carriers: influences of molecular weight and anionic charge on tumor targeting. Biol. Pharm. Bull 2001;24:535–543. [PubMed: 11379776]
67. Mahteme H, Graf W, Larsson BS, Gustafson S. Uptake of hyaluronan in hepatic metastases after blocking of liver endothelial cell receptors. Glycoconj. J 1998;15:935–939. [PubMed: 10052598]
68. Weigel JA, Raymond RC, McGary C, Singh A, Weigel PH. A blocking antibody to the hyaluronan receptor for endocytosis (HARE) inhibits hyaluronan clearance by perfused liver. J. Biol. Chem 2003;278:9808–9812. [PubMed: 12645574]
69. Peer D, Margalit R. Tumor-targeted hyaluronan nanoliposomes increase the antitumor activity of liposomal Doxorubicin in syngeneic and human xenograft mouse tumor models. Neoplasia 2004;6:343–353. [PubMed: 15256056]
70. Peer D, Margalit R. Loading mitomycin C inside long circulating hyaluronan targeted nano-liposomes increases its antitumor activity in three mice tumor models. Int. J. Cancer 2004;108:780–789. [PubMed: 14696107]

71. Yadav AK, Mishra P, Mishra AK, Mishra P, Jain S, Agrawal GP. Development and characterization of hyaluronic acid-anchored PLGA nanoparticulate carriers of doxorubicin. Nanomedicine 2007;3:246–257. [PubMed: 18068091]
72. Lee H, Mok H, Lee S, Oh YK, Park TG. Target-specific intracellular delivery of siRNA using degradable hyaluronic acid nanogels. J. Control. Release 2007;119:245–252. [PubMed: 17408798]
73. Kumar A, Sahoo B, Montpetit A, Behera S, Lockey RF, Mohapatra SS. Development of hyaluronic acid-Fe2O3 hybrid magnetic nanoparticles for targeted delivery of peptides. Nanomedicine 2007;3:132–137. [PubMed: 17572355]
74. Yun YH, Goetz DJ, Yellen P, Chen W. Hyaluronan microspheres for sustained gene delivery and site-specific targeting. Biomaterials 2004;25:147–157. [PubMed: 14580918]
75. Yerushalmi N, Arad A, Margalit R. Molecular and cellular studies of hyaluronic acid-modified liposomes as bioadhesive carriers for topical drug delivery in wound healing. Arch. Biochem. Biophys 1994;313:267–273. [PubMed: 8080272]
76. Hyung W, Ko H, Park J, Lim E, Park SB, Park YJ, Yoon HG, Suh JS, Haam S, Huh YM. Novel hyaluronic acid (HA) coated drug carriers (HCDCs) for human breast cancer treatment. Biotechnol. Bioeng 2008;99:442–454. [PubMed: 17625788]
77. Ziebell MR, Zhao ZG, Luo B, Luo Y, Turley EA, Prestwich GD. Peptides that mimic glycosaminoglycans: high-affinity ligands for a hyaluronan binding domain. Chem. Biol 2001;8:1081–1094. [PubMed: 11731299]
78. Rezler EM, Khan DR, Lauer-Fields J, Cudic M, Baronas-Lowell D, Fields GB. Targeted drug delivery utilizing protein-like molecular architecture. J. Am. Chem. Soc 2007;129:4961–4972. [PubMed: 17397150]
79. Day AJ, Prestwich GD. Hyaluronan-binding proteins: tying up the giant. J. Biol. Chem 2002;277:4585–4588. [PubMed: 11717315]
80. 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]
81. Eikenes L, Tari M, Tufto I, Bruland OS, de Lange Davies C. Hyaluronidase induces a transcapillary pressure gradient and improves the distribution and uptake of liposomal doxorubicin (Caelyx) in human osteosarcoma xenografts. Br. J. Cancer 2005;93:81–88. [PubMed: 15942637]
82. Bartolazzi A, Peach R, Aruffo A, Stamenkovic I. Interaction between CD44 and hyaluronate is directly implicated in the regulation of tumor development. J. Exp. Med 1994;180:53–66. [PubMed: 7516417]
83. Yu Q, Toole BP, Stamenkovic I. Induction of apoptosis of metastatic mammary carcinoma cells in vivo by disruption of tumor cell surface CD44 function. J. Exp. Med 1997;186:1985–1996. [PubMed: 9396767]
84. Peterson RM, Yu Q, Stamenkovic I, Toole BP. Perturbation of hyaluronan interactions by soluble CD44 inhibits growth of murine mammary carcinoma cells in ascites. Am. J. Pathol 2000;156:2159–2167. [PubMed: 10854236]
85. Ahrens T, Sleeman JP, Schempp CM, Howells N, Hofmann M, Ponta H, Herrlich P, Simon JC. Soluble CD44 inhibits melanoma tumor growth by blocking cell surface CD44 binding to hyaluronic acid. Oncogene 2001;20:3399–3408. [PubMed: 11423990]
86. Subramaniam V, Gardner H, Jothy S. Soluble CD44 secretion contributes to the acquisition of aggressive tumor phenotype in human colon cancer cells. Exp. Mol. Pathol 2007;83:341–346. [PubMed: 17945213]
87. Mohapatra S, Yang X, Wright JA, Turley EA, Greenberg AH. Soluble hyaluronan receptor RHAMM induces mitotic arrest by suppressing Cdc2 and cyclin B1 expression. J. Exp. Med 1996;183:1663–1668. [PubMed: 8666924]
88. Akiyama Y, Jung S, Salhia B, Lee S, Hubbard S, Taylor M, Mainprize T, Akaishi K, van Furth W, Rutka JT. Hyaluronate receptors mediating glioma cell migration and proliferation. J. Neurooncol 2001;53:115–127. [PubMed: 11716065]
89. Xu XM, Chen Y, Chen J, Yang S, Gao F, Underhill CB, Creswell K, Zhang L. A peptide with three hyaluronan binding motifs inhibits tumor growth and induces apoptosis. Cancer Res 2003;63:5685–5690. [PubMed: 14522884]

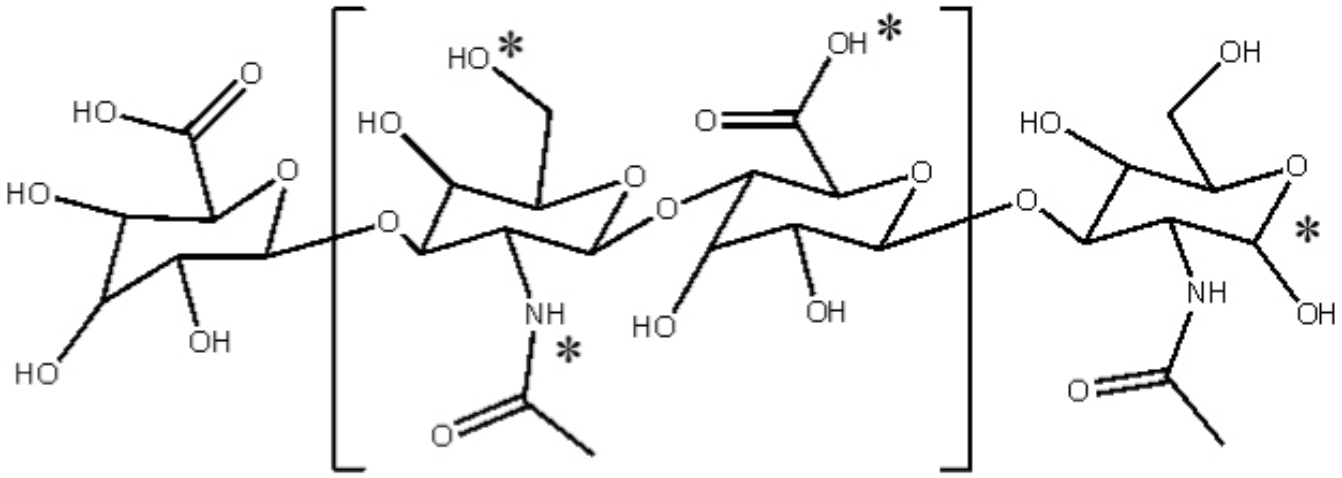

Figure 1.
HA Structure: polymeric repeat of *D*-glucuronic acid and *N*-acetylglucosamine. The asterisk (*) represents potential sites of chemical conjugation

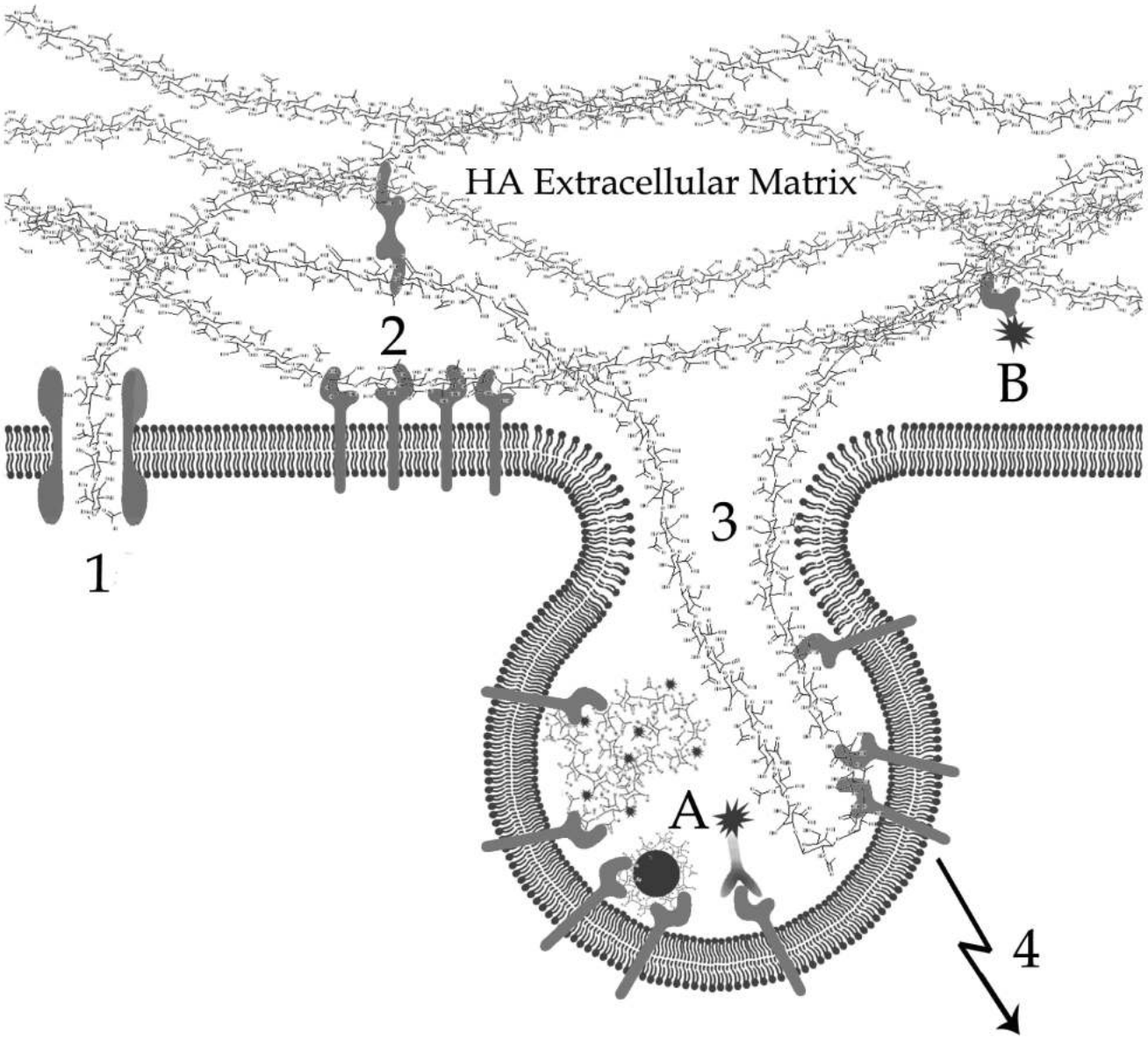

Figure 2.
HA regulation: (1) HA is synthesized by HA synthases on the cell surface, (2) interacts with extracellular binding proteins and cell surface receptors which (3) internalize HA and lead to (4) degradation by hyaluronidases. The CD44-HA system can be utilized for (A) intracellular delivery of antibody- or HA-conjugated drugs and drug carriers or for (B) extracellular localization of HA binding proteins or protein conjugates

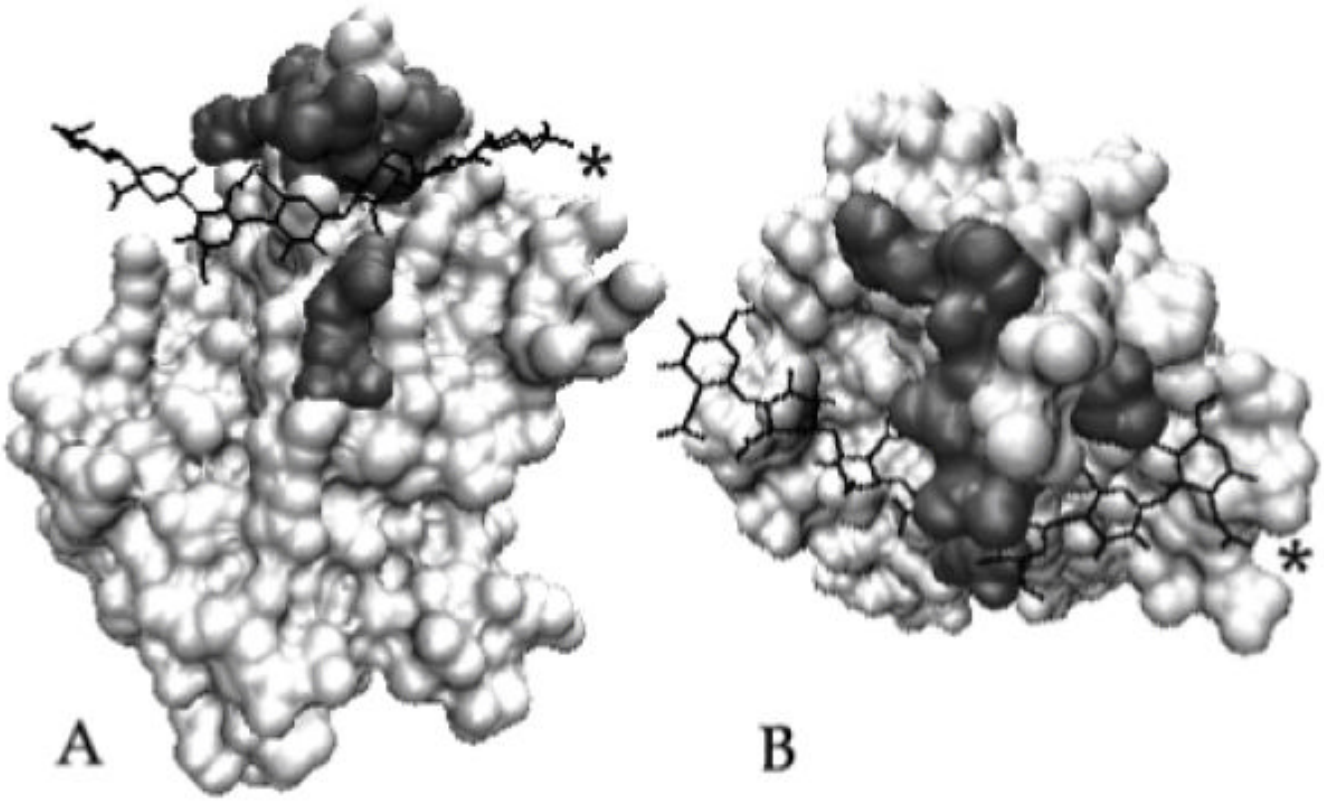

Figure 3.
HA-CD44 binding interaction: The structure of the extracellular portion of CD44 is shown with important binding residues shown in gray (A front view, B top view). Saccharides 2–8 of a co-crystallized HA8 are shown in the link domain binding groove (* reducing end). (Reproduced from Protein Data Bank structure reported in Banerji 2007.[43])

Table 1

Hallmarks of the Enhanced Permeability and Retention (EPR) Effect

Healthy Tissue	Cancerous Tissue
Small molecules diffuse across capilary walls	Small molecules diffuse across capillary walls and enter through large gaps in the capillary walls
Large molecules can not diffuse due to tight capillary cell junctions	Large molecules enter through large gaps in the capillary walls
Small molecules are cleared by a wall-formed lymphatic system	No lymphatic clearance is present

Table 2

Clinical Trials of Antibody Anti-CD44 Conjugates

Antibody	Drug	Injection Method	Cancer Type	Effect	Ref.
U36	Re-186	Single intravenous	Head and Neck Squamous Cell Carcinoma	Stable disease in 5 of 9 patients, mild myelotoxicity	51
BIWA 4	Tc-99m	Single intravenous	Head and Neck Squamous Cell Carcinoma	Tumor targeting	53
BIWA 4	Re-186	Dose escalation	Head and Neck Squamous Cell Carcinoma	Stable disease in 3 of 6 patients, limiting myelotoxicity	52
BIWA 4	Re-186	Single intravenous	Early Stage Breast Cancer	Moderate tumor identification, no correlation with CD44v6 expression	54
BIWA 4	Mertansine	Dose escalation	Head and Neck Squamous Cell Carcinoma	Moderate disease stabilization, skin toxicity	18
BIWA 4	Mertansine	Dose escalation	Head and Neck Squamous Cell Carcinoma	Low interpatient pharmacokinetic variability, skin toxicity	19
BIWA 4	Mertansine	Dose escalation	CD44v6 Positive Metastatic Breast Cancer	Stable disease in 12 of 24 patients, dose limiting toxicity	55

Table 3

Targeted Drugs and Drug Carriers *in Vitro*

Carrier	Drug	Linkage	Cell Lines	Effect	Ref.
LMW-HA	Paclitaxel	Hydrazide to Glucuronic Acid carboxylate	HBL-100 (human mammary carcinoma) HCT-116 (human colon carcinoma) SK-OV-3 (human ovarian carcinoma)	Cytotoxicity, CD44 specific uptake	28, 29
HMW-HA	Butyrate	Ester to N-Acetyl Glucosamine hydroxyl	MCF-7 (human mammary adenocarcinoma) HepB3 (human hepatocellular carcinoma) NCI-H460 (human non-small cell lung carcinoma) NCI-H460M (human non-small cell lung carcinoma)	Inhibited proliferation, CD44 specific uptake	57, 59, 64
HMW-HA	Paclitaxel	Ester to Glucuronic Acid carboxylate	RT-4 (human bladder carcinoma) RT-112/84 (human transitional cell carcinoma)	Cytotoxicity	63
HMW-HA	Paclitaxel	Hydrazide to Glucuronic Acid carboxylate	NMP-1 (human ovarian carcinoma) SK-OV-3ip (human ovarian carcinoma)	Cytotoxicity	62
HMW-HA	Carborane	Ester to Glucuronic Acid carboxylate	HT-29 (human colorectal adenocarcinoma) MCF-7 (human mammary adenocarcinoma) OVCAR-3 (human ovarian adenocarcinoma) RT-112/84 (human transitional cell carcinoma)	CD44 specific uptake	58
LMW-HA HMW-HA	siRNA	Disulfide crosslinked via the Glucuronic Acid carboxylate	HCT-116 (human colon carcinoma)	CD44 specific uptake, gene silencing	72
HMW-HA Fe_2O_3 Particle	Peptide	Hydrazide crosslinked via the Glucuronic Acid carboxylate	A549 (human alveolar squamous carcinoma)	Peptide internalization	73
HPMA Polymer LMW-HA	Doxorubicin	Hydrazide to Glucuronic Acid carboxylate	HBL-100 (human mammary carcinoma) HCT-116 (human colon carcinoma) SK-OV-3 (human ovarian carcinoma	Cytotoxicity, CD44 specific uptake	60
Liposome HA Oligos	Doxorubicin	Reductive amination to terminal end	B16F10 (murine melanoma)	Cytotoxicity, CD44 specific uptake	37, 38, 41
HMW-HA PLGA Particle	Doxorubicin	Amide to Glucuronic Acid carboxylate	MDA-MB-231 (human breast adenocarcinoma)	Cytotoxicity, CD44 specific uptake	76

Table 4

Targeted Drugs and Drug Carriers *in Vivo*

Carrier	Drug	Injection Method	Tumor Model	Effect	Ref.
Anti-CD44 Antibodies	Re-186	Intravenous	HNX-OE (human HNSC carcinoma) in HSD nude mice via subcutaneous implantation	Delayed tumor growth	50
HMW-HA	Epirubicin, Mitomycin C	Intraperitoneal, subcutaneous	MH-134 (murine ascites hepatoma) in C3H/He mice via subcutaneous foot pad or back injection LLC (murine lung carcinoma) in C57BL/6 mice via subcutaneous abdominal injection	Decreased tumor size, inhibited lymph node and lung metastasis, decreased systemic toxicity	14
HMW-HA	Butyrate	Intraperitoneal, subcutaneous	LL3 (murine lung carcinoma) or B16F10 (murine melanoma) in BD2F1 mice via intrasplenic injection	Reduced liver metastasis, increased survival time	57
HMW-HA	Butyrate	Intratumoral	LL3 (murine lung carcinoma) in BD2F1 mice via subcutaneous injection	Inhibited tumor growth, reduced lung metastasis	64
HMW-HA	Paclitaxel	Intraperitoneal	RT-112/84 (human transitional cell carcinoma) in SCID mice via subcutaneous flank injection	Inhibited tumor growth, decrease local toxicity	63
HMW-HA	Paclitaxel	Intraperitoneal	NMP-1 (human ovarian carcinoma) or SK-OV-3ip (human ovarian carcinoma) in HSD mice via intraperitoneal injection	Reduced tumor burden, decreased systemic toxicity, increased survival time	62
HMW-HA	DNA	Intramuscular	Rat Hind Limb	Transfection	74
Liposome HMW-HA	Mitomycin C, Doxorubicn	Intravenous	C26 (murine colon carcinoma) in BALB/c mice via footpad injection B16F10 (murine melanoma) or D122 (murine metastatic lung carcinoma) C57BL/6 mice via intravenous tail vein injection	Prolonged drug circulation time, increased drug accumulation in tumors, decreased metastasis, inhibited tumor growth, prolonged survival	69, 70
Liposome HMW-HA			P388/ADR (murine leukemia) in BDF_1 mice via intraperitoneal injection PANC-1 (human pancreatic carcinoma) in CD1-Nu mice via subcutaneous injection		
PLGA particle HMW-HA	Doxorubicin	Intravenous	EAT(Ehrlich ascites) in BALB/c mice via intraperitoneal injection	Moderately increased tumor accumulation, weak tumor growth inhibition	71