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Targeting Hyaluronic Acid Family for Cancer Chemoprevention and Therapy

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Abstract

Hyaluronic acid or hyaluronan (HA) is perhaps one of the most uncomplicated large polymers that regulates several normal physiological processes and, at the same time, contributes to the manifestation of a variety of chronic and acute diseases, including cancer. Members of the HA signaling pathway (HA synthases, HA receptors, and HYAL-1 hyaluronidase) have been experimentally shown to promote tumor growth, metastasis, and angiogenesis, and hence each of them is a potential target for cancer therapy. Furthermore, as these members are also overexpressed in a variety of carcinomas, targeting of the HA family is clinically relevant. A variety of targeted approaches have been developed to target various HA family members, including small-molecule inhibitors and antibody and vaccine therapies. These treatment approaches inhibit HA-mediated intracellular signaling that promotes tumor cell proliferation, motility, and invasion, as well as induction of endothelial cell functions. Being nontoxic, nonimmunogenic, and versatile for modifications, HA has been used in nanoparticle preparations for the targeted delivery of chemotherapy drugs and other anticancer compounds to tumor cells through interaction with cell-surface HA receptors. This review discusses basic and clinical translational aspects of targeting each HA family member and respective treatment approaches that have been described in the literature.

1. INTRODUCTION

Several members of the hyaluronic acid (HA) family of molecules, HA synthases (i.e., HAS1, HAS2, HAS3), HA receptors (i.e., CD44 and RHAMM), and hyaluronidases (mainly HYAL-1), are critical determinants of tumor growth and progression (Adamia, Pilarski, Belch, & Pilarski, 2013; Ghosh, Kuppusamy, & Pilarski, 2009; Golshani et al., 2007; Karbownik & Nowak, 2013; Orian-Rousseau, 2010; Simpson & Lokeshwar, 2008; Sironen et al., 2011). HA family members promote malignant behavior of tumor cells *in vitro*, and

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tumor growth, metastasis, and angiogenesis in xenograft models (Bharadwaj et al., 2009; Chao, Muthukumar, & Herzberg, 2007; Gurski et al., 2012; Li, Li, Brown, & Heldin, 2007; Lokeshwar, Cerwinka, & Lokeshwar, 2005; Lokeshwar et al., 2006; Siiskonen, Poukka, Tyynela-Korhonen, Sironen, & Pasonen-Seppanen, 2013; Tan et al., 2011). HA family of molecules are also potential diagnostic and prognostic markers for a variety of carcinomas including breast, bladder, endometrial, ovarian, and prostate (Auvinen et al., 2014; Bouga et al., 2010; Chi et al., 2012; Franzmann et al., 2003; Golshani et al., 2007; Gomez et al., 2009; Kramer et al., 2011; Lokeshwar et al., 2000, 2002; Paiva et al., 2005; Yoshida, Matsuda, Naito, & Ishiwata, 2012; Zhang, Chang, & Liu, 2013). In tumor tissues, HA is contributed by both tumor stroma and tumor cells and induces intracellular signaling by binding to HA receptors. HYAL-1 is the major tumor-derived hyaluronidase (HAase) that is almost exclusively expressed by tumor cells. By degrading HA, HAase/HYAL-1 generates small HA fragments, some of which (~10–25 disaccharide units) are angiogenic (Lokeshwar et al., 2001, 1999; West, Hampson, Arnold, & Kumar, 1985). In experimental model systems such as breast, bladder, prostate, and colon, studies have been mainly focused on modulating the expression of individual HA family molecules and assessing their effects on tumor cell phenotypes both *in vitro* and *in vivo*. Each HA synthase or HYAL-1, either alone or coexpressed, contributes to tumor cell proliferation, motility, and invasion, and to enhanced tumor growth, metastasis, and angiogenesis in xenografts; in contrast, knockdown of these genes inhibits tumor cell functions (Adamia et al., 2013; Bharadwaj et al., 2009; Chao et al., 2007; Ghosh et al., 2009; Golshani et al., 2007; Li et al., 2007). In the case of HYAL-1, promotion of tumor cell function is dose dependent. At levels detected in clinical specimens, HYAL-1 promotes tumor growth, invasion/metastasis, and angiogenesis; however, overexpression of HYAL-1 at levels exceeding those expressed in tumor tissues induces apoptosis and inhibits tumor formation (Lokeshwar, Cerwinka, Isoyama, & Lokeshwar, 2005; Lokeshwar & Selzer, 2008). Therefore, while a limited degradation of the pericellular HA matrix generates angiogenic HA fragments which induce intracellular signaling, complete degradation of the HA matrix, as a result of experimental overexpression of HYAL-1, will inhibit tumor growth and progression. Studies on HA-mediated signaling usually do not distinguish between HA and HA fragments present in the pericellular matrix. However, tumor-associated HA consists of both large HA polymers (mol. wt $\geq 0.5\times10^6$ Da) and smaller angiogenic oligosaccharides, the latter correlating with HAase activity in tumor tissues (Franzmann et al., 2003; Lokeshwar et al., 2001). Angiogenic HA fragments have been detected in the urine of patients with bladder cancer and Wilm's tumor, in the saliva of patients with head and neck cancer, and in bladder and prostate tumor tissues (Franzmann et al., 2003; Kumar, West, Ponting, & Gattamaneni, 1989; Lokeshwar, Obek, Soloway, & Block, 1997; Lokeshwar et al., 2001). In contrast to the angiogenic HA fragments, HA oligosaccharides consisting of 2–3 disaccharide units have been shown to have antitumor activity, presumably because they inhibit HA-induced signaling (Ghatak, Misra, & Toole, 2002; Hosono et al., 2007; Toole, Ghatak, & Misra, 2008; Urakawa et al., 2012).

Interaction between pericellular HA/angiogenic fragments and HA receptors induces multiple intracellular pathways. For example, CD44-HA and/or RHAMM-HA signaling promotes cell survival, cancer stemness, motility, and invasion by activating growth factor receptor signaling (e.g., ErbB2, c-Met), PI3/Akt and Erk pathways, small GTPase proteins

(i.e., RhoA and Rac1), Ras, NFkB and src signaling, cytoskeleton reorganization, etc., and some of these pathways, in turn, may induce HA synthase and HA receptor expression (Benitez et al., 2011; Benitez, Yates, Shamaldevi, Bowen, & Lokeshwar, 2013; Bernert, Porsch, & Heldin, 2011; Bharadwaj et al., 2011; Bourguignon, Wong, Earle, Krueger, & Spevak, 2010; Dortet, Veiga, Bonazzi, & Cossart, 2010; Hatano et al., 2011; Kim, Park, Lee, & Jeoung, 2008; Lokeshwar et al., 2010; Misra, Toole, & Ghatak, 2006). CD44 and RHAMM also have compensatory roles and therefore, if HA receptors are to be targeted for cancer therapy, silencing of both receptors may be necessary to completely abrogate HA signaling (Benitez et al., 2011; Lokeshwar et al., 2010; Turley & Naor, 2012). HA-mediated signaling events also induce expression of a variety of cytokines and chemokines, COX2, and matrix metalloproteinases, which promote tumor angiogenesis and invasion/metastasis (Chow, Tauler, & Mulshine, 2010; Dunn et al., 2009; Lokeshwar et al., 2010; Misra et al., 2008, 2006; Porsch et al., 2013; Vincent, Jourdan, Sy, Klein, & Mechti, 2001; Voelcker et al., 2008). HA signaling studies reveal that targeting HA and other HA family members by small-molecule inhibitors, genetic manipulation, and vaccination could be exploited for cancer therapy. In this review, we discuss some of these approaches.

2. TARGETING HA PRODUCTION

2.1. Targeting HA synthases

Although targeting of HA synthases has not been exploited for therapeutic purposes, genetic knockdown studies shed light on the crucial roles that HA synthases play in different types of cancers. For example, HAS1 knockdown in bladder cancer cells induces cell cycle arrest in G2-M phase, followed by apoptosis induction via the extrinsic pathway. HAS1 knockdown also cripples the chemotactic ability and invasion of bladder cancer cells and inhibits tumor growth and angiogenesis over fivefold (Golshani et al., 2008). HAS1 downregulation studies also revealed that there is a feedback loop between HA synthesis and the expression of HA receptors. Knockdown of HAS1 causes transcriptional downregulation of CD44 (Golshani et al., 2008). In the clinical scenario, HAS1 expression is elevated in bladder, prostate, and renal cell carcinomas, correlates with HA presence in tumor tissues, and is an independent predictor of unfavorable outcome (Chi et al., 2012; Golshani et al., 2007). HAS1 mRNA is extensively alternatively spliced, and although the functions of the splice variants are unknown, these splice variants associate with poor survival in multiple myeloma patients. These clinical findings thus corroborate the experimental findings on the functional involvement of HAS1 in tumor growth and progression (Adamia et al., 2005; Kriangkum, Warkentin, Belch, & Pilarski, 2013). Similar to HAS1, HAS2 and HAS3 knockdown inhibits tumor growth and metastasis in breast and osteosarcoma models and recently, HAS2 knockdown has been shown to potentiate radiation-induced DNA damage and apoptosis in cancer cells (Li et al., 2007; Shen et al., 2014; Udabage et al., 2005). HAS2 and HAS3 expression also correlates with clinical outcome, including resistance to chemotherapy and increased risk for anthracycline-related cardiomyopathy (Auvinen et al., 2014; Paiva et al., 2005; Ricciardelli et al., 2013; Wang et al., 2014). However, since cancer cells express more than one HA synthase, targeting HA synthesis using small-molecule inhibitors would be a better approach than inhibiting the expression or function of each individual HA synthase.

2.2. Chemical inhibitors of HA synthesis

2.2.1 4-Methylumbelliferone—4-Methylumbelliferone (4-MU) or 7-hydroxy-4-methylcoumarin is a well-studied inhibitor of HA synthesis (Clarkin, Allen, Wheeler-Jones, Bastow, & Pitsillides, 2011; Kakizaki et al., 2004, 2002; Morohashi et al., 2006; Nakamura et al., 1997; Saito et al., 2013). Mammalian cells synthesize HA, using two building blocks —UDP-glucuronic acid (UGA) and UDP-*N*-acetyl-D-glucosamine. UGA is synthesized by oxidation of UDP-glucose via the enzyme UDP-glucose dehydrogenase. UGA is also a substrate for cellular detoxification enzymes known as UDP-glucuronosyltransferases. In cells treated with 4-MU, UDP-glucuronosyltransferase transfers glucuronic acid onto 4-MU. This depletes the intracellular pool of UGA and HA synthesis is halted. Depending upon the specific isozyme, the K_m values of UDP-glucuronosyltransferase and HAS range from 100 to 900 μM. In several studies using tumor cell lines, the IC_{50} of 4-MU for inhibiting HA synthesis is in the range of 400 μM (Kakizaki et al., 2004, 2002; Lokeshwar et al., 2010; Nakamura et al., 1997). Recently, it has also been shown that 4-MU downregulates HAS2 and HAS3 expression (Saito et al., 2013). The study showed that 4-MU indeed inhibits both HA and sulfated glycosaminoglycan synthesis in the chick limb bud micromass culture (Clarkin et al., 2011). In the cancer arena, however, 4-MU has been extensively studied as a HA synthesis inhibitor and, in addition to its effect on enzymatic inhibition of HA synthesis, has been shown to downregulate HAS2 and HAS3 expression by 60–80% in some cancer cells (Kultti et al., 2009).

Although 4-MU is known as an HA synthesis inhibitor, it is also widely used in assays to measure enzyme activity or quality control, due to its HA-dependent fluorescent properties. In fact, 4-MU (also known as hymecromone) is a popular term in PubMed, with over 1000 citations. The majority of these articles use 4-MU as a fluorescent indicator of food, water, soil, or liver function (including clinical trials of 4-MU) but also include articles that are completely unrelated to 4-MU (over 300 citations). Fewer than 10% of the articles cited in PubMed on 4-MU are related to its use as an HA synthesis inhibitor, less than 5% are on its use in cancer cells either as a fluorescent indicator or as a HA synthesis inhibitor, and even a smaller number of studies have examined the anticancer potential of 4-MU in preclinical models. Over several decades, 4-MU has been investigated in small clinical trials as a choleretic (bile-inducing) and antispasmodic agent (Abate et al., 2001; Camarri & Marchettini, 1988; Garrett, Venitz, Eberst, & Cerda, 1993; Hoffmann, Schwarz, Pohl, Ziegenhagen, & Kruis, 2005; Quaranta, Rossetti, & Camarri, 1984). In fact, 4-MU is sold in Europe and Asia as a dietary supplement to improve liver health. Although it is a coumarin-derivative, 4-MU lacks the antisperminogenic and antiaromatase activities of coumarin and anticoagulant activity of coumadin or warfarin (Chen, Cho, Karlsberg, Zhou, & Yuan, 2004; Crooke, Fitzpatrick, O'Kennedy, & McCormack, 1997; Keating, 1997; Omarbasha, Fair, & Heston, 1989). According to the NIOSH registry, the LD_{50} for 4-MU ranges from 2.8 to 7.3 g/kg (RTECS #: GN7000000) and at doses where 4-MU shows biological efficacy in controlling tumor growth (200–450 mg/kg/day oral dose), it has no serum or organ toxicity (Lokeshwar et al., 2010).

At concentrations of 0.2–1 mM (i.e., ~35–180 μg/ml), 4-MU inhibits proliferation, motility, and invasion, and causes loss of filopodia and focal adhesions, in a variety of cultured tumor

cells (Arai et al., 2011; Hiraga, Ito, & Nakamura, 2013; Lokeshwar et al., 2010; Okuda et al., 2012; Piccioni et al., 2012; Twarock et al., 2011; Uchakina, Ban, & McKallip, 2013; Urakawa et al., 2012). 4-MU inhibits tumor spheroids and osteoclast-like cell formation (Hiraga et al., 2013) and downregulates the expression of both CD44 and RHAMM, suggesting a feedback loop between HA synthesis and HA receptor expression (Lokeshwar et al., 2010). In addition, 4-MU treatment inhibits a variety of HA signaling events, including downregulation of phospho-ErbB2, phospho-Akt, and downstream effectors MMP-2/MMP-9 and IL-8 expression (Fig. 2.1; Lokeshwar et al., 2010; Okuda et al., 2012; Twarock et al., 2011; Urakawa, Nishida, Wasa, et al., 2012). The efficacy of 4-MU as a glucuronate scavenger could contribute to its antitumor effects. For example, it has been shown that 4-MU (1 μM) inhibits glucuronidation and prevents inactivation of androgen in androgen-dependent prostate cancer cells. As a result, 4-MU promotes the androgen-dependent growth of these prostate cancer cells (Wei, Galbenus, Raza, Cerny, & Simpson, 2009). Inhibition of the glucuronidation of androgen by 4-MU at concentrations as low as 1 μM is intriguing. Based solely on the K_m values of UDP-glucuronyltransferases (as described above), at such a low concentration, 4-MU would not be expected to act as a competitive inhibitor of HA synthesis or of glucuronidation. Therefore, its inhibitory potential may be dependent on cell type and expression level of specific UDP-glucuronosyltransferase isozymes, or 4-MU may have other effects than competitive inhibition of glucuronidation or of HA synthesis. Nevertheless, at the concentrations used in cancer cells and tumor models and also the doses at which it is consumed as a dietary supplement, 4-MU is demonstrated to have antitumor activities. For example, in xenograft studies, oral administration of 4-MU has been shown to inhibit tumor growth and metastasis in prostate, B16 melanoma, skin, liver, osteosarcoma, breast, and esophageal cancer model systems (Bhattacharyya et al., 2009; Kudo et al., 2004; Lokeshwar et al., 2010; Nakazawa et al., 2006; Piccioni et al., 2012; Twarock et al., 2011; Urakawa, Nishida, Wasa, et al., 2012; Yoshihara et al., 2005). Recently, 4-MU was shown to inhibit bone metastasis in a breast cancer model (Hiraga et al., 2013). As in the case of *in vitro* studies, some mouse xenograft studies have used 4-MU orally at doses as high as 1–3 g/kg; however, in other studies, 4-MU has shown remarkable efficacy at 200–400 mg/kg doses (Arai et al., 2011; Bhattacharyya et al., 2009; Hiraga et al., 2013; Kudo et al., 2004; Nakazawa et al., 2006; Okuda et al., 2012; Piccioni et al., 2012; Twarock et al., 2011; Urakawa, Nishida, Wasa, et al., 2012; Yoshihara et al., 2005). Based on the FDA's formula of mouse-to-human dose conversion, 200–400 mg/kg doses in mice equates to 1.1–2.2 g/day doses in humans; these are doses at which 4-MU is consumed for improving liver health (Abate et al., 2001; Camarri & Marchettini, 1988; Garrett et al., 1993; Hoffmann et al., 2005; Quaranta et al., 1984; U.S. Department of Health and Human Services, 2005). Considering 4-MU is consumed as a dietary supplement at similar doses, conducting clinical trials to test the toxicity and efficacy profile of 4-MU as an anticancer agent should be possible.

Although the potential of 4-MU as a single agent has been examined in xenograft studies, only two studies have reported its combination with other agents. 4-MU has been shown to enhance the efficacy of gemcitabine in one pancreatic cancer model at 1 g/kg dose (Nakazawa et al., 2006). More recently, 4-MU has been shown to synergize with Sorafenib, a tyrosine kinase inhibitor, approved by the FDA for the treatment of metastatic renal cell

carcinoma (Benitez et al., 2013). In that study, 4-MU synergized with Sorafenib at concentrations at which 4-MU alone did not inhibit HA synthesis and neither agent alone had any inhibitory effects on renal cell carcinoma cells *in vitro* or *in vivo*. However, the combination inhibited HA synthesis, proliferation, motility, and invasion *in vitro* and completely abrogated tumor growth in a Sorafenib-resistant xenograft model without toxicity (Benitez et al., 2013).

Taken together, 4-MU is an orally bioavailable dietary supplement that inhibits HA synthesis and has shown significant promise as an antitumor and antimetastatic agent. With a favorable toxicity profile and high efficacy, this HA synthesis inhibitor has potential for clinical translation.

2.2.2 Other HA synthesis inhibitors—Although not as effective as 4-MU, D-mannose has been shown to inhibit HA synthesis in a dose-dependent manner. Mannose at ~20 mM concentration inhibits HA synthesis by causing a reduction in the cellular concentration of UDP-*N*-acetylhexosamines (i.e., UDP-*N*-acetyl-D-glucosamine and UDP-*N*-acety-D-galactosamine). Mannose treatment was shown to inhibit dermal fibroblast invasion and to prevent enhanced leukocyte binding to HA (Jokela et al., 2008, 2013). However, antitumor effects of mannose in the context of HA synthesis inhibition have not yet been investigated. A curcumin analogue, 1,5-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one (hylin), has been shown to inhibit multidrug resistance protein 5-mediated export of HA in fibroblasts (IC_{50} ~5 μM; Prehm, 2013). However, given that curcumin has poor bioavailability *in vivo*, any of its analogues will need to be tested for their bioavailability before examining their efficacy on HA signaling and on cancer models.

3. TARGETING HA SIGNALING

3.1. HA oligosaccharides

In contrast to the effects of large HA polymers and angiogenic HA fragments, HA oligosaccharides (oHA) of <10 disaccharide units have been shown to possess antitumor activity in cancer cell lines of different tissue origins (Alaniz et al., 2006; Cordo Russo et al., 2008; Fuchs et al., 2013; Slomiany, Dai, Bomar, et al., 2009; Slomiany, Dai, Tolliver, et al., 2009; Slomiany, Grass, Robertson, et al., 2009; Ween, Hummitzsch, Rodgers, Oehler, & Ricciardelli, 2011; Yang et al., 2012). These oHA inhibit anchorage-independent growth, motility, and invasion, and induce apoptosis. These phenotypic effects are a direct consequence of the abrogation of HA signaling, especially the PI3/Akt pathway, and also of the association between CD44 and receptor tyrosine kinases (Ghatak et al., 2002; Slomiany, Dai, Tolliver, et al., 2009; Urakawa, Nishida, Knudson, et al., 2012). oHA induce PTEN expression (Ghatak et al., 2002), inhibit CD44 clustering on the plasma membrane, and block the interaction of CD44 with Emmprin, which is elevated in a variety of carcinomas (see Chapter 12). oHA inhibit plasma membrane localization of monocarboxylate transporters (MCT1 and MCT4) with CD44 and Emmprin, resulting in the inhibition of lactate efflux (Slomiany, Grass, Robertson, et al., 2009). In addition, oHA inhibit association of CD44 with drug transporters BCRP (ABCG2) and P-glycoprotein (ABCB1) in the plasma membrane and increase sensitivity of tumor cells to chemotherapeutic agents such as doxorubicin (DOX) (Slomiany, Dai, Bomar, et al., 2009; Slomiany, Grass, Robertson, et al.,

2009). In fact, in vincristine-resistant lymphoma cell lines, oHA enhance cytotoxicity of vincristine by downregulating PI3-kinase/Akt signaling and also the activity of Pgp-1 (Cordo Russo et al., 2008). oHA also appear to inhibit versican-induced pericellular matrix formation, which is dependent on HA and CD44 interaction; subsequently, oHA inhibit motility and invasion in ovarian cancer cells (Ween et al., 2011). Recently, large polymeric HA was demonstrated to enhance CXCR4 and CXCL12 signaling and, consequently, cause an increase in Erk phosphorylation and phenotypic readouts, including tumor cell motility, capillary sprouting, and angiogenesis. This HA-increased signaling was due to the association of CXCR4 with CD44, and oHA inhibited this association and related signaling (Fuchs et al., 2013).

Therapeutic efficacy of oHA as an antitumor agent has been examined in a few studies. Initially, systemic administration of oHA was shown to inhibit tumor growth in the subcutaneous B16-F10 murine melanoma model (Ghatak et al., 2002). Similarly, systemic administration of oHA inhibits growth of an experimental malignant peripheral nerve sheath tumor (MPNST) model (Slomiany, Dai, Bomar, et al., 2009). In an intratibial growth model of breast cancer, injection of oHA (i.e., decasaccharide) in the tibia inhibited osteolysis and HA accumulation in the bone metastatic lesions (Urakawa, Nishida, Knudson, et al., 2012). In osteosarcoma models, intratumoral injection of HA octasaccharides reduced HA accumulation in local tumors, which consequently caused a significant inhibition of primary tumor growth and distant lung metastasis (Hosono et al., 2007). One of the advantages of oHA is that they are nonimmunogenic, since they are simply repeating disaccharide units, and inhibit tumor cell functions *in vitro* along with tumor growth and metastasis by abrogating CD44 and HA interaction. As discussed above, oHA can improve response of tumor cells to chemotherapeutic agents. For example, paclitaxel conjugated to oHA is internalized by CD44-overexpressing tumor cells and is 50 times more cytotoxic than when administered alone (Journo-Gershfeld, Kapp, Shamay, Kopecek, & David, 2012). Similarly, at concentrations at which oHA alone are not effective, they synergize with DOX and reduce the growth of malignant peripheral nerve sheath tumors (MPNST; Slomiany, Dai, Bomar, et al., 2009).

Taken together, as a single agent, oHA have shown promise in inhibiting CD44-mediated HA signaling in tumor cells and tumor growth at primary and secondary metastatic sites. Interestingly, little work has been done to demonstrate whether or not oHA have similarly inhibitory activity on abrogating HA/RHAMM interaction. Since in recent literature, RHAMM expression has shown promise as a potential biomarker for cancer diagnosis and prognosis, examination of oHA effects on RHAMM-mediated HA signaling will be helpful for the clinical translation of oHA as a cancer treatment. Based on the published studies discussed above, oHA may have better clinical utility in enhancing the efficacy of chemotherapeutic drugs by decreasing pericellular HA matrix. However, before oHA can advance into clinical trials as an anticancer drug or as an adjuvant to chemotherapeutic drugs, pharmacokinetic and pharmacodynamic studies will need to be conducted in preclinical models to determine the therapeutic window. Since the differences in the length of HA oligosaccharides that have angiogenic/tumor-promoting activity and oHA that have antitumor activity are only a few disaccharide units, reliable methods to synthesize and

separate oHA of defined length (6–10 disaccharides) on a large scale will need to be developed for clinical translation.

4. HA AS A CARRIER FOR DRUG DELIVERY

HA has been used for targeted anticancer drug deliveries in the form of HA-coated nanoparticles. Being a hydrophilic large polymer of repeating disaccharide units, HA can either be directly conjugated to drugs or can form self-assembling micelles to generate amphilic nanoparticles. This allows efficient delivery of hydrophobic drugs to target sites, thus improving their bioavailability and half-life. Additional advantages of HA nanosystems include low to no immunogenicity, noninflammatory properties, and good biocompatibility, bioavailability, and biodegradability (Choi, Saravanakumar, Park, & Park, 2012). HA also shows great versatility, as it bears many functional groups that can be used for various modifications and conjugations. All of these properties make HA a central constituent of multifunctional nanoparticles to deliver combination and synergistic therapies. Most importantly, since HA has a strong binding affinity for RHAMM and CD44—two receptors that are commonly overexpressed in various solid tumors and cancer stem cells—the HA nanoparticles can be used for the targeted delivery of chemotherapeutic drugs and other novel treatments (Jaracz, Chen, Kuznetsova, & Ojima, 2005). Many studies have shown successful use of HA in nanoparticle formations, both *in vitro* and *in vivo*.

Direct conjugation of HA to anticancer drugs is a simple yet effective nanoparticle formulation used to improve treatment efficacy. This method involves covalently linking HA to a drug, often using a bond that can readily be cleaved after reaching the target site. The anticancer drug then gains improvement in parameters such as stability, circulation time, solubility, and tumor-targeting specificity (Choi et al., 2012; Jaracz et al., 2005). Small organic or inorganic ions, when conjugated with HA, show increased stability and can be targeted to tumors. For example, the HA-selenium nanoparticle system shows increased stability in circulation and antitumor efficacy (Ren et al., 2013). The common chemotherapy drugs DOX and paclitaxel have also demonstrated increased efficacy and reduced dose-limiting toxicity when incorporated in HA nanoparticles. In the DMBA-induced rat mammary model, HA-paclitaxel significantly increased the antitumor efficacy of paclitaxel than when it was administered alone (Al-Ghananeem et al., 2009).

HA has also been used to generate amphiphilic polymeric nanoparticles that can self-assemble. In these nanoparticles, amphiphilic nanoparticles' hydrophobic ends encapsulate the hydrophobic anticancer drug, and the hydrophilic ends form a protective coating in the form of a spherical micelle. Such coating protects the drug from degradation or absorption until the micelle's contents are released within the target cell (Choi et al., 2010, 2012). To improve the amphilic nature of the HA nanoparticles, HA has been successfully conjugated to various hydrophobic compounds. For example, HA has been conjugated to hydrophobic groups such as 5β-cholanic acid and polyethylene glycol to encapsulate Chlorin e6, a hydrophobic photosensitizer used in photodynamic therapy and imaging. Chlorin e6 was successfully loaded onto HA-based micelles with a high loading capacity and exhibited both improved cancer imaging capabilities and tumor inhibition under laser irradiation (Yoon et al., 2012). Similarly, intravenously delivered DOX conjugated to HA-coated super-

paramagnetic iron oxide nanoparticles was found to accumulate at much higher levels and distribute more widely in the tumor tissue than DOX delivered alone. This resulted in decreased tumor growth of human SKOV-3 xenografts in mice and increased their lifespan (El-Dakdouki et al., 2014).

HA has also been conjugated to other groups that provide more than the ability to self-assemble. For example, HA has been conjugated with poly(L-histidine) for selective delivery of chemotherapeutic drugs such as DOX. Since poly(L-histidine) is a pH-responsive polymer that destabilizes through acidic protonation, the HA-poly(L-histidine) nanoparticles allow sustained drug release specifically in tumor tissues, which naturally have a lower pH (Qiu et al., 2013). Yet another option for the HA nanosystem modification is the addition of other tumor-targeting moieties. As folate receptors are overexpressed in certain tumor types, one study conjugated folate to the outer shell of a HA-octadecyl(C18)-based nanosystem. While both the folate receptor-conjugated and nonconjugated HA-octadecyl (C18) nanoparticles were internalized by MCF-7 breast cancer cells via CD44-mediated endocytosis, folate receptors further enhanced the endocytosis of folate-HA-C18 nanoparticles. However, in this study the *in vivo* efficacy of both nanoparticles was not evaluated (Liu et al., 2011).

As HA holds many functional groups that can be substituted, there is potential for the conjugation of more than one treatment to HA nanosystems, allowing for synergistic combination therapies. In one example, HA was conjugated with all-*trans* retinoid acid, a hydrophobic anticancer drug, to form micelles around paclitaxel. This HA nanosystem was found to have low nonspecific toxicity and high antitumor efficacy in the B16 melanoma model (Yao, Zhang, Zhou, Liu, & Zhang, 2013). Another example of synergistic therapy via HA nanoparticles involves adsorption of a photodynamic therapy agent, hematoporphyrin monomethyl ether, onto HA-derivatized carbon nanotubes. Carbon nanotubes alone have been used previously for photothermal therapy and drug delivery. When conjugated to HA, the carbon nanotubes offer a surface for aromatic hematoporphyrin monomethyl ether to associate with the core of the nanosystem. This combined nanosystem has increased solubility, neutral pH, and targeted delivery. The HA-CNT-HMME nanosystem exhibited effective tumor control in the B16 melanoma model using both photothermal and photodynamic therapies with synergistic effects and reduced nontumor toxicity (Shi et al., 2013).

Finally, hydrophilic treatments can also benefit from HA-based nanocarriers through the formation of ionic nanocomplexes in place of covalent bonds (Choi et al., 2012). The positively charged protein TRAIL can associate with HA through simple mixing, and the HA-TRAIL complexes display increased stability in circulation and higher bioavailability to improve the anticancer effects of TRAIL (Na et al., 2008). Even RNA interference therapy can benefit from forming nanocomplexes with HA, despite sharing a negative charge. Compared to the naked siRNAs that are easily degraded or excreted, and with viral delivery vectors having potential toxicity and immunogenicity drawbacks, HA nanoparticles appear to be an appealing alternative carrier for siRNAs. One study conjugated HA to various polyamines, mainly polyethyleneimine (PEI). This conjugation, when mixed with siRNAs, formed a self-assembling system with a negatively charged surface and a positively charged

core. Through associations with HA-polyethylene glycol nanosystems (HA-PEG), the study found a successful balance of charges in the nanosystem of HA-PEG/HA-PEI/siRNA. This combination allowed for successful tumor-specific uptake via CD44 receptor-mediated endocytosis, along with facilitated endosome escape to release siRNAs into the tumor cell cytoplasm (Ganesh, Iyer, Morrissey, & Amiji, 2013). This system showed the highest target gene knockdown, attesting once more to the versatility, biocompatibility, and importance of HA in nanosystems.

In summary, HA nanoparticles are an attractive delivery system for effective deliveries of antitumor agents, as HA serves both as a tumor-targeting moiety and a drug carrier that combats side effects. For these reasons, HA can potentially be exploited for cancer therapy.

5. TARGETING HA RECEPTORS

5.1. CD44

Since the discovery that CD44 is a stem cell marker in addition to being a HA receptor, targeting CD44 for anticancer therapy has been attempted using DNA vaccines, anti-CD44 monoclonal antibody, and nanoparticle-mediated delivery of CD44siRNA approaches.

5.1.1 CD44 vaccines—CD44 cDNA vaccine has been delivered by implanting virtual lymph nodes in immunocompetent animals to generate anti-CD44 antibodies. These virtual lymph nodes are generated by insertion of CD44-standard or CD44-variant cDNA into a silicone tube filled with 2.5 cm long segment of hydroxylated-polyvinyl acetate wound dressing sponge followed by subcutaneous injection in mice. Using this model, CD44-standard form vaccination was shown to reduce autoimmune encephalomyelitis by induction of apoptosis (Garin et al., 2007). In a similar approach with virtual lymph nodes, effects of CD44 vaccination on tumor growth and lung metastasis were evaluated in a mouse mammary adenocarcinoma model (DA3 cells). The mouse models were injected with virtual lymph nodes containing human CD44 variants (v3–v10) or CD44-standard cDNAs. Immunization resulted in the expression of antibodies against human CD44-variant and CD44-standard forms. In 75% of the animals, mice expressing CD44-variant (v3–v10) antibodies showed a greater inhibition of tumor growth than mice expressing CD44-standard antibodies. The remaining 25% of animals showed reduced tumor growth, and metastasis was eliminated in all animals. In this study, human CD44 cDNA was used to avoid interference from mouse serum proteins; however, this raises a question of whether the injection of human CD44 cDNA in a clinical setting will generate enough antibody response. It is also intriguing that CD44-standard form did not generate the same immune response against tumors (Wallach-Dayan, Rubinstein, Hand, Breuer, & Naor, 2008). Vaccination of animals with dendritic cells and B16 cells coated with anti-CD44 antibodies inhibited lung metastasis (50% reduction) and delayed tumor growth of B16 melanoma cells; 60% of mice remained tumor-free for 8 months. In these models, vaccination induced CD8 T cell activation (Pilon-Thomas, Verhaegen, Kuhn, Riker, & Mule, 2006).

5.1.2 CD44 siRNA delivery—CD44 siRNAs are usually delivered to tumor cells using nanoparticles. For example, biodegradable poly(D,L-lactide-*co*-glycolide) acid nanoparticles have been used to simultaneously deliver CD44 and FAK siRNAs to ovarian cancer

xenografts. Knockdown of both genes reduced tumor growth by inhibiting angiogenesis and proliferation in tumors (Zou et al., 2013). More recently, a nanoscale-based drug delivery system was tested in an ovarian cancer xenograft model. The nanoscale delivery system contained a modified polypropylenimine dendrimer as a carrier, paclitaxel, a synthetic analog of luteinizing hormone-releasing hormone peptide for targeting tumor cells, and siRNA targeted to CD44 mRNA. This dendrimer was able to downregulate CD44 mRNA and protein expression and inhibit tumor growth without toxicity, suggesting that the targeted delivery of CD44 siRNA along with chemotherapeutic agents may be explored for cancer therapy (Shah et al., 2013).

5.1.3 Targeting CD44 for delivering antitumor therapies—Overexpression of CD44 in tumor cells has also been exploited to deliver cytotoxic drugs and siRNAs using HA-coated self-assembling nanoparticles or liposomes. For example, DOX-loaded, HA-coated silica nanoparticles containing a highly fluorescent core to target CD44 have been recently developed for targeting tumor cells. However, their targeted delivery, uptake, and efficacy in experimental tumor models have not yet been evaluated (El-Dakdouki, Pure, & Huang, 2013). Similarly, ONCOFID™-S, a new bioconjugate HA that targets CD44-overexpressing tumor cells, can deliver cytotoxic agents and has shown high antitumor efficacy both *in vitro* and *in vivo* (Serafino et al., 2011). In the ONCOFID™-S bioconjugate, antitumor drugs are covalently bound to HA. These HA-encapsulated siRNA nanoparticles, chemotherapeutic agent-encapsulated liposomes, or covalently bound HA-bioconjugates are selectively taken up by tumors in systemic delivery, and reduce specific gene expression in several xenograft models. The uptake of these nanoparticles or liposomes by tumor cells occurs through HA-receptor-mediated internalization, since incubation of tumor cells with soluble HA inhibits uptake of these materials. This suggests that the delivery of these nanoparticles is occurring through HA-receptor-mediated internalization of nanoparticles and liposomes (Dalla Pozza et al., 2013; Ganesh, Iyer, Gattacceca, Morrissey, & Amiji, 2013).

5.1.4 Targeting of CD44 protein—Anti-CD44 monoclonal antibodies have been used to inhibit tumor growth in experimental model systems. Targeting of CD44 by specific antibodies induces apoptosis in T lymphoma cells by the intrinsic pathway, which, in turn, is dependent on the activation of CD44-associated protein phosphatase 2A (Rajasagi et al., 2010). In chronic lymphocytic leukemia cells, that express high levels of CD44, a humanized monoclonal antibody specific for CD44 (RG7356) was found to be cytotoxic to leukemia B cells without affecting the viability of normal B cells. Systemic administration of this antibody caused complete clearance of leukemia xenografts. Interestingly, the effects of the antibody were not neutralized in the presence of HA, suggesting that CD44 may have functions other than binding to HA that also play a role in leukemia cell growth (Zhang et al., 2013).

As in the case of HA-coated nanoparticles or liposomes, anti-CD44 antibodies have also been used to either image tumors or for delivering chemotherapeutic agents to experimental tumor models. For example, a chimeric monoclonal antibody U36 (cMAb U36) and its F(ab′)2 and Fab′ fragments that recognize the CD44v6 isoform have shown potential for radioimmunotherapy and radioimmunotargeting of experimental tumors (Sandstrom et al.,

2012, 2008). In clinical studies, anti-CD44 antibodies have been used to deliver radioisotopes or mertansine for treatment of CD44-expressing tumors. In these studies, disease stabilization was observed in breast or head and neck tumor patients; however, dose-limiting toxicity was observed with distribution of antibody in the skin, where high levels of CD44 are expressed (Platt & Szoka, 2008). In phase I studies, maximum tolerated dose, safety, and efficacy of the immunoconjugate BIWI 1 (bivatuzumab mertansine), which consists of a highly potent antimicrotubule agent coupled to an anti-CD44v6 monoclonal antibody, was evaluated in head and neck cancer patients. In this study, while three patients showed a partial response, binding of BIWI to CD44v6 on skin keratinocytes mediated serious skin toxicity with a fatal outcome, leading to early termination of this trial (Riechelmann et al., 2008). The HA binding domain is highly conserved in all CD44 isoforms, and therefore, attempts have been made to target this domain using specific monothiophosphate-modified aptamers. These aptamers bind specifically to CD44-expressing tumor cells with high efficacy. However, the *in vivo* bioavailability of these aptamers has not been examined in detail (Somasunderam et al., 2010).

Taken together, CD44 has been targeted for cancer therapy and cancer imaging through vaccination, specific antibodies, siRNAs, and aptamers. However, since targeting of CD44 using CD44v6 monoclonal antibodies in clinical trials caused serious adverse reactions, risk assessment of CD44 targeting by any one of the approaches discussed above needs to be carefully evaluated before using that approach for cancer therapy.

5.2. RHAMM

Since RHAMM is overexpressed in a variety of carcinomas, including acute leukemias, RHAMM vaccines have been tested in phase I and II clinical trials for chronic lymphocytic leukemia and acute myeloid leukemia (AML). Initially, a vaccine was constructed by injecting dendritic cells transfected with *in vitro* transcribed RHAMM mRNA into mice implanted with glioma cells. The vaccine improved the survival of tumor-bearing mice by threefold and histological analysis showed activation of CD4+, CD8+, and CD25+ T lymphocytes (Amano et al., 2007). RHAMM is known to elicit both humoral and cellular immune responses. Efficacy of a peptide-based RHAMM vaccine has been examined in phase I/II clinical trials. In a phase I clinical trial, 10 patients with AML, myelodysplastic syndrome, or multiple myeloma, expressing HLA-A2 and RHAMM expression on tumor cells, were vaccinated with a RHAMM peptide R3 (aa 165–173; ILSLELMKL). Subcutaneous administration of this vaccine increased R3-specific CD8+ T cells and three patients showed partial response, including a significant reduction of blasts in the bone marrow (Schmitt et al., 2008). High dose of the same R3-peptide vaccine again showed immunological responses, including an increase in R3-specific CD8+ cells and regulatory T cells. About 35% of patients also showed a partial clinical response. However, both the clinical and immunological responses were lower than when the vaccine was administered at a lower dose (1 mg vs. 0.3 mg; Greiner et al., 2010). The vaccine had similar immunological (i.e., increase in R3-specific CD8+ cells) and clinical response in patients with chronic lymphocytic leukemia (Giannopoulos et al., 2010). Interestingly, although patients with CML are characterized as having immunosuppression, the vaccine caused profound changes (mostly an increase) in different T cell subsets and in cytokines that are involved in immune

response (i.e., TGF-β, IL-10, IL-2, and TNF) during the vaccination period (Giannopoulos, Wlasiuk, Dmoszynska, Rolinski, & Schmitt, 2011). However, despite the early success with the R3-peptide vaccine, it was recently shown that only baseline RHAMM expression was present in both leukemic stem cells (CD34+CD38−) and hematopoietic stem cells (CD34+CD38−) from healthy control cells. In addition, activation of T cells also increased RHAMM expression. Therefore, the study concluded that RHAMM is not a suitable target for immunotherapy (Snauwaert et al., 2012). Considering CD44 vaccine trials were terminated due to toxicity and RHAMM is ubiquitously expressed and essential for normal cellular functions, RHAMM vaccination for cancer therapy may need more work before it can be translated into clinic.

In summary, although targeting of CD44 and RHAMM appears to be a conceptually attractive strategy, considering its effects on normal cells, and the immune system, the risk versus benefit must be carefully evaluated before clinical usage.

6. TARGETING HAase

As discussed above and in Chapter 1, HAase has been shown to be a critical determinant of cancer growth, metastasis, and angiogenesis. HAase activity and HYAL-1 expression are potentially independent predictors of clinical outcome in several carcinomas. Therefore, targeting HAase activity and/or HYAL-1 expression could potentially be an effective strategy for cancer therapy. Many synthetic and naturally occurring compounds have been tested as HAase inhibitors. These include high molecular mass poly(styrene-4-sulfonate), gossypol, sodium aurothiomalate, fenoprofen, glycerrhizic acid, fatty acids, plant-derived compounds, heparin, and O-sulfated HA (sHA). The activities of most of these inhibitors have been tested against testicular HAase. For example, more than 60 years ago, O-sHA was reported as an inhibitor of testicular HAase (Balazs, Hogberg, & Laurent, 1951; Mio & Stern, 2002). As a result, HAase inhibitors were tried as contraceptives (Balazs et al., 1951; Joyce, Mack, Anderson, & Zaneveld, 1986; Mio & Stern, 2002). In a study of 21 inhibitors, sHA polymers with varying degrees of sulfation were found to be the most effective inhibitors of HYAL-1 (IC_{50} 0.0083–0.019 μM) (Isoyama et al., 2006). The presence of sulfate, and not the degree of sulfation, was found to be the determinant of HAase inhibitory activity displayed by sHA. sHA derivatives inhibited HYAL-1 by a mixed inhibition mechanism (i.e., competitive+uncompetitive) and were 5- to 17-fold better as uncompetitive inhibitors than as competitive inhibitors (Isoyama et al., 2006). Since sHA is a natural compound and is derived from HA, its antitumor activity has been examined in prostate cancer models. sHA with 2.75 degrees of sulfation was found to inhibit prostate cancer cell growth through induction of apoptosis. sHA inhibited HA signaling, with inhibition of PI3-kinase/Akt pathway as the major HA signaling target. The study also showed that there is a feedback loop between Akt and HA receptors. As a result of downregulated Akt activation following sHA treatment, HA receptors were transcriptionally downregulated. Moreover, sHA inhibited the growth of an androgen-independent prostate cancer model, by inhibition of angiogenesis and increased apoptosis in tumor tissues. The tumor inhibitory effects of sHA were accompanied by lack of any serum or organ toxicity (Benitez et al., 2011). The model of sHA action on tumor cells is shown in Fig. 2.2.

Taken together, targeting of tumor-derived HAase may be an attractive strategy for controlling tumor growth and progression.

7. CONCLUSION

The tumor-associated HA-HAase system is an attractive target for cancer therapy, since it regulates major intracellular signaling pathways such as PI3-kinase/Akt, Erk, ras, src, and processes such as epithelial–mesenchymal transition, that impact tumor cell proliferation, invasive phenotype, and angiogenic potential. Among the targeted therapies described in this review, targeting of HA synthesis by a small molecule, 4-MU, seems most promising. Several studies have demonstrated that 4-MU effectively inhibits tumor growth, metastasis, and angiogenesis in experimental tumor models and inhibits a variety of HA signaling pathways. Since 4-MU is already consumed as a dietary supplement at doses comparable to those at which it is effective as an antitumor agent in experimental models, it can be rapidly translated into clinic for cancer therapy. oHA of <10 disaccharides have also shown efficacy in controlling tumor growth and metastasis; however, the challenge for their use in cancer therapy will be to carefully control the length of oHA, since slightly longer HA oligosaccharides can promote tumor growth and metastasis by stimulating HA signaling. The use of HA nanosystems also holds promise for the targeted and safe delivery of chemotherapeutic drugs and other anticancer compounds to tumor cells. Since HA nanoparticles can improve the half-life of anticancer agents and focus the delivery to cells overexpressing HA receptors, many anticancer therapies can potentially be more effective at smaller doses and, thus, benefit from reduced drug-related toxicities. Targeting of HA receptors by antibodies or vaccines has been tested in clinical trials. Although this is potentially an effective approach, serious toxicities have been associated with targeting of HA receptors. Tumor-derived HAase, HYAL-1, has shown potential to be an independent prognostic indicator for disease progression, recurrence, metastasis, and survival and, therefore, a natural target for cancer therapy. However, only the HAase inhibitor, sHA, has been tested and found to have antitumor activity without toxicity in one cancer model. The growing body of literature is demonstrating the involvement of HA family of molecules in tumor growth and progression, and their role as biomarkers in virtually all types of cancers. This will be a great impetus for testing the experimental therapeutic strategies discussed in this review, as well as furthering many new HA-targeted therapies to enter clinical trials for specific tumor types.

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ABBREVIATIONS

4-MU	4-methylumbelliferone
HA	hyaluronic acid
HAase	hyaluronidase

HAS	HA synthase
oHA	HA oligosaccharides
sHA	sulfated hyaluronic acid
UGA	UDP-glucuronic acid

References

Abate A, Dimartino V, Spina P, Costa PL, Lombardo C, Santini A, et al. Hymecromone in the treatment of motor disorders of the bile ducts: A multicenter, double-blind, placebo-controlled clinical study. Drugs Under Experimental and Clinical Research. 2001; 27(5–6):223–231. [PubMed: 11951580]

Adamia S, Pilarski PM, Belch AR, Pilarski LM. Aberrant splicing, hyaluronan synthases and intracellular hyaluronan as drivers of oncogenesis and potential drug targets. Current Cancer Drug Targets. 2013; 13(4):347–361. [PubMed: 23517594]

Adamia S, Reiman T, Crainie M, Mant MJ, Belch AR, Pilarski LM. Intronic splicing of hyaluronan synthase 1 (HAS1): A biologically relevant indicator of poor outcome in multiple myeloma. Blood. 2005; 105(12):4836–4844. http://dx.doi.org/10.1182/blood-2004-10-3825. [PubMed: 15731173]

Alaniz L, Garcia MG, Gallo-Rodriguez C, Agusti R, Sterin-Speziale N, Hajos SE, et al. Hyaluronan oligosaccharides induce cell death through PI3-K/Akt pathway independently of NF-kappaB transcription factor. Glycobiology. 2006; 16(5):359–367. http://dx.doi.org/10.1093/glycob/cwj085. [PubMed: 16461453]

Al-Ghananeem AM, Malkawi AH, Muammer YM, Balko JM, Black EP, Mourad W, et al. Intratumoral delivery of Paclitaxel in solid tumor from biodegradable hyaluronan nanoparticle formulations. AAPS PharmSciTech. 2009; 10(2):410–417. http://dx.doi.org/10.1208/s12249-009-9222-5. [PubMed: 19381833]

Amano T, Kajiwara K, Yoshikawa K, Morioka J, Nomura S, Fujisawa H, et al. Antitumor effects of vaccination with dendritic cells transfected with modified receptor for hyaluronan-mediated motility mRNA in a mouse glioma model. Journal of Neurosurgery. 2007; 106(4):638–645. http://dx.doi.org/10.3171/jns.2007.106.4.638. [PubMed: 17432716]

Arai E, Nishida Y, Wasa J, Urakawa H, Zhuo L, Kimata K, et al. Inhibition of hyaluronan retention by 4-methylumbelliferone suppresses osteosarcoma cells in vitro and lung metastasis in vivo. British Journal of Cancer. 2011; 105(12):1839–1849. http://dx.doi.org/10.1038/bjc.2011.459. [PubMed: 22045192]

Auvinen P, Rilla K, Tumelius R, Tammi M, Sironen R, Soini Y, et al. Hyaluronan synthases (HAS1-3) in stromal and malignant cells correlate with breast cancer grade and predict patient survival. Breast Cancer Research and Treatment. 2014; 143(2):277–286. http://dx.doi.org/10.1007/s10549-013-2804-7. [PubMed: 24337597]

Balazs EA, Hogberg B, Laurent TC. The biological activity of hyaluron sulfuric acid. Acta Physiologica Scandinavica. 1951; 23(2–3):168–178. http://dx.doi.org/10.1111/j.1748-1716.1951.tb00806.x. [PubMed: 14868516]

Benitez A, Yates TJ, Lopez LE, Cerwinka WH, Bakkar A, Lokeshwar VB. Targeting hyaluronidase for cancer therapy: Antitumor activity of sulfated hyaluronic acid in prostate cancer cells. Cancer Research. 2011; 71(12):4085–4095. http://dx.doi.org/10.1158/0008-5472.CAN-10-4610. [PubMed: 21555367]

Benitez A, Yates TJ, Shamaldevi N, Bowen T, Lokeshwar VB. Dietary supplement hymecromone and sorafenib: A novel combination for the control of renal cell carcinoma. Journal of Urology. 2013; 190(1):285–290. http://dx.doi.org/10.1016/j.juro.2012.12.011. [PubMed: 23228386]

Bernert B, Porsch H, Heldin P. Hyaluronan synthase 2 (HAS2) promotes breast cancer cell invasion by suppression of tissue metalloproteinase inhibitor 1 (TIMP-1). Journal of Biological Chemistry. 2011; 286(49):42349–42359. http://dx.doi.org/10.1074/jbc.M111.278598. [PubMed: 22016393]

Bharadwaj AG, Goodrich NP, McAtee CO, Haferbier K, Oakley GG, Wahl JK 3rd, et al. Hyaluronan suppresses prostate tumor cell proliferation through diminished expression of N-cadherin and aberrant growth factor receptor signaling. Experimental Cell Research. 2011; 317(8):1214–1225. http://dx.doi.org/10.1016/j.yexcr.2011.01.026. [PubMed: 21315068]

Bharadwaj AG, Kovar JL, Loughman E, Elowsky C, Oakley GG, Simpson MA. Spontaneous metastasis of prostate cancer is promoted by excess hyaluronan synthesis and processing. American Journal of Pathology. 2009; 174(3):1027–1036. http://dx.doi.org/10.2353/ajpath.2009.080501. [PubMed: 19218337]

Bhattacharyya SS, Paul S, Mandal SK, Banerjee A, Boujedaini N, Khuda-Bukhsh AR. A synthetic coumarin (4-methyl-7 hydroxy coumarin) has anti-cancer potentials against DMBA-induced skin cancer in mice. European Journal of Pharmacology. 2009; 614(1–3):128–136. http://dx.doi.org/10.1016/j.ejphar.2009.04.015. [PubMed: 19393233]

Bouga H, Tsouros I, Bounias D, Kyriakopoulou D, Stavropoulos MS, Papageorgakopoulou N, et al. Involvement of hyaluronidases in colorectal cancer. BMC Cancer. 2010; 10:499. http://dx.doi.org/10.1186/1471-2407-10-499. [PubMed: 20849597]

Bourguignon LY, Wong G, Earle C, Krueger K, Spevak CC. Hyaluronan-CD44 interaction promotes c-Src-mediated twist signaling, microRNA-10b expression, and RhoA/RhoC up-regulation, leading to Rho-kinase-associated cytoskeleton activation and breast tumor cell invasion. Journal of Biological Chemistry. 2010; 285(47):36721–36735. http://dx.doi.org/10.1074/jbc.M110.162305. [PubMed: 20843787]

Camarri E, Marchettini G. Hymecromone in the treatment of symptoms following surgery of the bile ducts. Recenti Progressi in Medicina. 1988; 79(5):198–202. [PubMed: 3051197]

Chao KL, Muthukumar L, Herzberg O. Structure of human hyaluronidase-1, a hyaluronan hydrolyzing enzyme involved in tumor growth and angiogenesis. Biochemistry. 2007; 46(23):6911–6920. http://dx.doi.org/10.1021/bi700382g. [PubMed: 17503783]

Chen S, Cho M, Karlsberg K, Zhou D, Yuan YC. Biochemical and biological characterization of a novel anti-aromatase coumarin derivative. Journal of Biological Chemistry. 2004; 279(46):48071–48078. http://dx.doi.org/10.1074/jbc.M406847200. [PubMed: 15358790]

Chi A, Shirodkar SP, Escudero DO, Ekwenna OO, Yates TJ, Ayyathurai R, et al. Molecular characterization of kidney cancer: Association of hyaluronic acid family with histological subtypes and metastasis. Cancer. 2012; 118(9):2394–2402. http://dx.doi.org/10.1002/cncr.26520. [PubMed: 21887686]

Choi KY, Chung H, Min KH, Yoon HY, Kim K, Park JH, et al. Self-assembled hyaluronic acid nanoparticles for active tumor targeting. Biomaterials. 2010; 31(1):106–114. http://dx.doi.org/10.1016/j.biomaterials.2009.09.030. [PubMed: 19783037]

Choi KY, Saravanakumar G, Park JH, Park K. Hyaluronic acid-based nanocarriers for intracellular targeting: Interfacial interactions with proteins in cancer. Colloids and Surfaces B: Biointerfaces. 2012; 99:82–94. http://dx.doi.org/10.1016/j.colsurfb.2011.10.029. [PubMed: 22079699]

Chow G, Tauler J, Mulshine JL. Cytokines and growth factors stimulate hyaluronan production: Role of hyaluronan in epithelial to mesenchymal-like transition in non-small cell lung cancer. Journal of Biomedicine and Biotechnology. 2010; 2010:485468. http://dx.doi.org/10.1155/2010/485468. [PubMed: 20671927]

Clarkin CE, Allen S, Wheeler-Jones CP, Bastow ER, Pitsillides AA. Reduced chondrogenic matrix accumulation by 4-methylumbelliferone reveals the potential for selective targeting of UDP-glucose dehydrogenase. Matrix Biology. 2011; 30(3):163–168. http://dx.doi.org/10.1016/j.matbio.2011.01.002. [PubMed: 21292001]

Cordo Russo RI, Garcia MG, Alaniz L, Blanco G, Alvarez E, Hajos SE. Hyaluronan oligosaccharides sensitize lymphoma resistant cell lines to vincristine by modulating P-glycoprotein activity and PI3K/Akt pathway. International Journal of Cancer. 2008; 122(5):1012–1018. http://dx.doi.org/10.1002/ijc.23122. [PubMed: 17985348]

Crooke, D.; Fitzpatrick, B.; O'Kennedy, R.; McCormack, T. Coumarins-multifaceted molecules with many analytical and other applications. In: O'Kennedy, R.; Thome, RD., editors. Coumarins biology, applications and mode of action. Chichester: Wiley; 1997. p. 303-331.

Dalla Pozza E, Lerda C, Costanzo C, Donadelli M, Dando I, Zoratti E, et al. Targeting gemcitabine containing liposomes to CD44 expressing pancreatic adenocarcinoma cells causes an increase in

the antitumoral activity. Biochimica et Biophysica Acta. 2013; 1828(5):1396–1404. http://dx.doi.org/10.1016/j.bbamem.2013.01.020. [PubMed: 23384419]

Dortet L, Veiga E, Bonazzi M, Cossart P. CD44-independent activation of the Met signaling pathway by HGF and InlB. Microbes and Infection. 2010; 12(12–13):919–927. http://dx.doi.org/10.1016/j.micinf.2010.07.009. [PubMed: 20670691]

Dunn KM, Lee PK, Wilson CM, Iida J, Wasiluk KR, Hugger M, et al. Inhibition of hyaluronan synthases decreases matrix metalloproteinase-7 (MMP-7) expression and activity. Surgery. 2009; 145(3):322–329. http://dx.doi.org/10.1016/j.surg.2008.11.008. [PubMed: 19231585]

El-Dakdouki MH, Pure E, Huang X. Development of drug loaded nanoparticles for tumor targeting. Part 1: Synthesis, characterization, and biological evaluation in 2D cell cultures. Nanoscale. 2013; 5(9):3895–3903. http://dx.doi.org/10.1039/c3nr33777d. [PubMed: 23529646]

El-Dakdouki MH, Xia J, Zhu DC, Kavunja H, Grieshaber J, O'Reilly S, et al. Assessing the in vivo efficacy of doxorubicin loaded hyaluronan nanoparticles. ACS Applied Materials & Interfaces. 2014; 6(1):697–705. http://dx.doi.org/10.1021/am404946v. [PubMed: 24308364]

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. International Journal of Cancer. 2003; 106(3):438–445. http://dx.doi.org/10.1002/ijc.11252. [PubMed: 12845686]

Fuchs K, Hippe A, Schmaus A, Homey B, Sleeman JP, Orian-Rousseau V. Opposing effects of high- and low-molecular weight hyaluronan on CXCL12-induced CXCR4 signaling depend on CD44. Cell Death and Disease. 2013; 4:e819. http://dx.doi.org/10.1038/cddis.2013.364. [PubMed: 24091662]

Ganesh S, Iyer AK, Gattacceca F, Morrissey DV, Amiji MM. In vivo biodistribution of siRNA and cisplatin administered using CD44-targeted hyaluronic acid nanoparticles. Journal of Controlled Release. 2013; 172(3):699–706. http://dx.doi.org/10.1016/j.jconrel.2013.10.016. [PubMed: 24161254]

Ganesh S, Iyer AK, Morrissey DV, Amiji MM. Hyaluronic acid based self-assembling nanosystems for CD44 target mediated siRNA delivery to solid tumors. Biomaterials. 2013; 34(13):3489–3502. http://dx.doi.org/10.1016/j.biomaterials.2013.01.077. [PubMed: 23410679]

Garin T, Rubinstein A, Grigoriadis N, Nedvetzki S, Abramsky O, Mizrachi-Koll R, et al. CD44 variant DNA vaccination with virtual lymph node ameliorates experimental autoimmune encephalomyelitis through the induction of apoptosis. Journal of Neurological Sciences. 2007; 258(1–2):17–26. http://dx.doi.org/10.1016/j.jns.2007.01.079.

Garrett ER, Venitz J, Eberst K, Cerda JJ. Pharmacokinetics and bioavailabilities of hymecromone in human volunteers. Biopharmaceutics and Drug Disposition. 1993; 14(1):13–39. [PubMed: 8427942]

Ghatak S, Misra S, Toole BP. Hyaluronan oligosaccharides inhibit anchorage-independent growth of tumor cells by suppressing the phosphoinositide 3-kinase/Akt cell survival pathway. Journal of Biological Chemistry. 2002; 277(41):38013–38020. http://dx.doi.org/10.1074/jbc.M202404200. [PubMed: 12145277]

Ghosh A, Kuppusamy H, Pilarski LM. Aberrant splice variants of HAS1 (Hyaluronan Synthase 1) multimerize with and modulate normally spliced HAS1 protein: A potential mechanism promoting human cancer. Journal of Biological Chemistry. 2009; 284(28):18840–18850. http://dx.doi.org/10.1074/jbc.M109.013813. [PubMed: 19451652]

Giannopoulos K, Dmoszynska A, Kowal M, Rolinski J, Gostick E, Price DA, et al. Peptide vaccination elicits leukemia-associated antigen-specific cytotoxic CD8+ T-cell responses in patients with chronic lymphocytic leukemia. Leukemia. 2010; 24(4):798–805. http://dx.doi.org/10.1038/leu.2010.29. [PubMed: 20220777]

Giannopoulos K, Wlasiuk P, Dmoszynska A, Rolinski J, Schmitt M. Peptide vaccination induces profound changes in the immune system in patients with B-cell chronic lymphocytic leukemia. Folia Histochemica et Cytobiologica. 2011; 49(1):161–167. [PubMed: 21526504]

Golshani R, Hautmann SH, Estrella V, Cohen BL, Kyle CC, Manoharan M, et al. HAS1 expression in bladder cancer and its relation to urinary HA test. International Journal of Cancer. 2007; 120(8):1712–1720. http://dx.doi.org/10.1002/ijc.22222. [PubMed: 17230515]

Golshani R, Lopez L, Estrella V, Kramer M, Iida N, Lokeshwar VB. Hyaluronic acid synthase-1 expression regulates bladder cancer growth, invasion, and angiogenesis through CD44. Cancer Research. 2008; 68(2):483–491. http://dx.doi.org/10.1158/0008-5472.CAN-07-2140. [PubMed: 18199543]

Gomez CS, Gomez P, Knapp J, Jorda M, Soloway MS, Lokeshwar VB. Hyaluronic acid and HYAL-1 in prostate biopsy specimens: Predictors of biochemical recurrence. Journal of Urology. 2009; 182(4):1350–1356. http://dx.doi.org/10.1016/j.juro.2009.06.070. [PubMed: 19683287]

Greiner J, Schmitt A, Giannopoulos K, Rojewski MT, Gotz M, Funk I, et al. High-dose RHAMM-R3 peptide vaccination for patients with acute myeloid leukemia, myelodysplastic syndrome and multiple myeloma. Haematologica. 2010; 95(7):1191–1197. http://dx.doi.org/10.3324/haematol.2009.014704. [PubMed: 20081055]

Gurski LA, Xu X, Labrada LN, Nguyen NT, Xiao L, van Golen KL, et al. Hyaluronan (HA) interacting proteins RHAMM and hyaluronidase impact prostate cancer cell behavior and invadopodia formation in 3D HA-based hydrogels. PLoS One. 2012; 7(11):e50075. http://dx.doi.org/10.1371/journal.pone.0050075. [PubMed: 23166824]

Hatano H, Shigeishi H, Kudo Y, Higashikawa K, Tobiume K, Takata T, et al. RHAMM/ERK interaction induces proliferative activities of cementifying fibroma cells through a mechanism based on the CD44-EGFR. Laboratory Investigation. 2011; 91(3):379–391. http://dx.doi.org/10.1038/labinvest.2010.176. [PubMed: 20956971]

Hiraga T, Ito S, Nakamura H. Cancer stem-like cell marker CD44 promotes bone metastases by enhancing tumorigenicity, cell motility, and hyaluronan production. Cancer Research. 2013; 73(13):4112–4122. http://dx.doi.org/10.1158/0008-5472.CAN-12-3801. [PubMed: 23633482]

Hoffmann RM, Schwarz G, Pohl C, Ziegenhagen DJ, Kruis W. Bile acid-independent effect of hymecromone on bile secretion and common bile duct motility. Deutsche Medizinische Wochenschrift. 2005; 130(34–35):1938–1943. http://dx.doi.org/10.1055/s-2005-872606. [PubMed: 16123896]

Hosono K, Nishida Y, Knudson W, Knudson CB, Naruse T, Suzuki Y, et al. Hyaluronan oligosaccharides inhibit tumorigenicity of osteosarcoma cell lines MG-63 and LM-8 in vitro and in vivo via perturbation of hyaluronan-rich pericellular matrix of the cells. American Journal of Pathology. 2007; 171(1):274–286. http://dx.doi.org/10.2353/ajpath.2007.060828. [PubMed: 17591972]

Isoyama T, Thwaites D, Selzer MG, Carey RI, Barbucci R, Lokeshwar VB. Differential selectivity of hyaluronidase inhibitors toward acidic and basic hyaluronidases. Glycobiology. 2006; 16(1):11–21. http://dx.doi.org/10.1093/glycob/cwj036. [PubMed: 16166602]

Jaracz S, Chen J, Kuznetsova LV, Ojima I. Recent advances in tumor-targeting anticancer drug conjugates. Bioorganic and Medicinal Chemistry. 2005; 13(17):5043–5054. http://dx.doi.org/10.1016/j.bmc.2005.04.084. [PubMed: 15955702]

Jokela TA, Jauhiainen M, Auriola S, Kauhanen M, Tiihonen R, Tammi MI, et al. Mannose inhibits hyaluronan synthesis by down-regulation of the cellular pool of UDP-N-acetylhexosamines. Journal of Biological Chemistry. 2008; 283(12):7666–7673. http://dx.doi.org/10.1074/jbc.M706001200. [PubMed: 18201970]

Jokela TA, Kuokkanen J, Karna R, Pasonen-Seppanen S, Rilla K, Kossi J, et al. Mannose reduces hyaluronan and leukocytes in wound granulation tissue and inhibits migration and hyaluronan-dependent monocyte binding. Wound Repair and Regeneration. 2013; 21(2):247–255. http://dx.doi.org/10.1111/wrr.12022. [PubMed: 23464634]

Journo-Gershfeld G, Kapp D, Shamay Y, Kopecek J, David A. Hyaluronan oligomers-HPMA copolymer conjugates for targeting paclitaxel to CD44-overexpressing ovarian carcinoma. Pharmaceutical Research. 2012; 29(4):1121–1133. http://dx.doi.org/10.1007/s11095-012-0672-1. [PubMed: 22350800]

Joyce CL, Mack SR, Anderson RA, Zaneveld LJ. Effect of hyaluronidase, beta-glucuronidase and beta-N-acetylglucosaminidase inhibitors on sperm penetration of the mouse oocyte. Biology of Reproduction. 1986; 35(2):336–346. [PubMed: 3768457]

Kakizaki I, Kojima K, Takagaki K, Endo M, Kannagi R, Ito M, et al. A novel mechanism for the inhibition of hyaluronan biosynthesis by 4-methylumbelliferone. Journal of Biological Chemistry. 2004; 279(32):33281–33289. http://dx.doi.org/10.1074/jbc.M405918200. [PubMed: 15190064]

Kakizaki I, Takagaki K, Endo Y, Kudo D, Ikeya H, Miyoshi T, et al. Inhibition of hyaluronan synthesis in Streptococcus equi FM100 by 4-methylumbelliferone. European Journal of Biochemistry. 2002; 269(20):5066–5075. [PubMed: 12383266]

Karbownik MS, Nowak JZ. Hyaluronan: Towards novel anti-cancer therapeutics. Pharmacological Reports. 2013; 65(5):1056–1074. [PubMed: 24399703]

Keating, GJ. The chemistry and occurrence of coumarins. In: O'Kennedy, R.; O'Kennedy, R.; Thome, RD., editors. Coumarins biology, applications and mode of action. Chichester: Wiley; 1997. p. 23-65.

Kim Y, Park YW, Lee YS, Jeoung D. Hyaluronic acid induces transglutaminase II to enhance cell motility; role of Rac1 and FAK in the induction of transglutaminase II. Biotechnological Letters. 2008; 30(1):31–39. http://dx.doi.org/10.1007/s10529-007-9496-1.

Kramer MW, Escudero DO, Lokeshwar SD, Golshani R, Ekwenna OO, Acosta K, et al. Association of hyaluronic acid family members (HAS1, HAS2, and HYAL-1) with bladder cancer diagnosis and prognosis. Cancer. 2011; 117(6):1197–1209. http://dx.doi.org/10.1002/cncr.25565. [PubMed: 20960509]

Kriangkum J, Warkentin A, Belch AR, Pilarski LM. Alteration of introns in a hyaluronan synthase 1 (HAS1) minigene convert Pre-mRNA [corrected] splicing to the aberrant pattern in multiple myeloma (MM): MM patients harbor similar changes. PLoS One. 2013; 8(1):e53469. http://dx.doi.org/10.1371/journal.pone.0053469. [PubMed: 23301075]

Kudo D, Kon A, Yoshihara S, Kakizaki I, Sasaki M, Endo M, et al. Effect of a hyaluronan synthase suppressor, 4-methylumbelliferone, on B16F-10 melanoma cell adhesion and locomotion. Biochemical and Biophysical Research Communications. 2004; 321(4):783–787. http://dx.doi.org/10.1016/j.bbrc.2004.07.041. [PubMed: 15358095]

Kultti A, Pasonen-Seppanen S, Jauhiainen M, Rilla KJ, Karna R, Pyoria E, et al. 4-Methylumbelliferone inhibits hyaluronan synthesis by depletion of cellular UDP-glucuronic acid and downregulation of hyaluronan synthase 2 and 3. Experimental Cell Research. 2009; 315(11): 1914–1923. http://dx.doi.org/10.1016/j.yexcr.2009.03.002. [PubMed: 19285976]

Kumar S, West DC, Ponting JM, Gattamaneni HR. Sera of children with renal tumours contain low-molecular-mass hyaluronic acid. International Journal of Cancer. 1989; 44(3):445–448. [PubMed: 2550372]

Li Y, Li L, Brown TJ, Heldin P. Silencing of hyaluronan synthase 2 suppresses the malignant phenotype of invasive breast cancer cells. International Journal of Cancer. 2007; 120(12):2557–2567. http://dx.doi.org/10.1002/ijc.22550. [PubMed: 17315194]

Liu Y, Sun J, Cao W, Yang J, Lian H, Li X, et al. Dual targeting folate-conjugated hyaluronic acid polymeric micelles for paclitaxel delivery. International Journal of Pharmaceutics. 2011; 421(1): 160–169. http://dx.doi.org/10.1016/j.ijpharm.2011.09.006. [PubMed: 21945183]

Lokeshwar VB, Cerwinka WH, Isoyama T, Lokeshwar BL. HYAL1 hyaluronidase in prostate cancer: A tumor promoter and suppressor. Cancer Research. 2005; 65(17):7782–7789. http://dx.doi.org/10.1158/0008-5472.CAN-05-1022. [PubMed: 16140946]

Lokeshwar VB, Cerwinka WH, Lokeshwar BL. HYAL1 hyaluronidase: A molecular determinant of bladder tumor growth and invasion. Cancer Research. 2005; 65(6):2243–2250. http://dx.doi.org/10.1158/0008-5472.CAN-04-2805. [PubMed: 15781637]

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 Research. 2006; 66(23):11219–11227. http://dx.doi.org/10.1158/0008-5472.CAN-06-1121. [PubMed: 17145867]

Lokeshwar VB, Lopez LE, Munoz D, Chi A, Shirodkar SP, Lokeshwar SD, et al. Antitumor activity of hyaluronic acid synthesis inhibitor 4-methylumbelliferone in prostate cancer cells. Cancer Research. 2010; 70(7):2613–2623. http://dx.doi.org/10.1158/0008-5472.CAN-09-3185. [PubMed: 20332231]

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. Journal of Urology. 2000; 163(1):348–356. [PubMed: 10604388]

Lokeshwar VB, Obek C, Soloway MS, Block NL. Tumor-associated hyaluronic acid: A new sensitive and specific urine marker for bladder cancer. Cancer Research. 1997; 57(4):773–777. [PubMed: 9044859]

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. Journal of Biological Chemistry. 2001; 276(15):11922–11932. http://dx.doi.org/10.1074/jbc.M008432200. [PubMed: 11278412]

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(1):61–72. http://dx.doi.org/10.1002/cncr.10652. [PubMed: 12115318]

Lokeshwar VB, Selzer MG. Hyalurondiase: Both a tumor promoter and suppressor. Seminars in Cancer Biology. 2008; 18(4):281–287. http://dx.doi.org/10.1016/j.semcancer.2008.03.008. [PubMed: 18448355]

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 Research. 1999; 59(17):4464–4470. [PubMed: 10485499]

Mio K, Stern R. Inhibitors of the hyaluronidases. Matrix Biology. 2002; 21(1):31–37. [PubMed: 11827790]

Misra S, Obeid LM, Hannun YA, Minamisawa S, Berger FG, Markwald RR, et al. Hyaluronan constitutively regulates activation of COX-2-mediated cell survival activity in intestinal epithelial and colon carcinoma cells. Journal of Biological Chemistry. 2008; 283(21):14335–14344. http://dx.doi.org/10.1074/jbc.M703811200. [PubMed: 18326857]

Misra S, Toole BP, Ghatak S. Hyaluronan constitutively regulates activation of multiple receptor tyrosine kinases in epithelial and carcinoma cells. Journal of Biological Chemistry. 2006; 281(46):34936–34941. http://dx.doi.org/10.1074/jbc.C600138200. [PubMed: 16959784]

Morohashi H, Kon A, Nakai M, Yamaguchi M, Kakizaki I, Yoshihara S, et al. Study of hyaluronan synthase inhibitor, 4-methylumbelliferone derivatives on human pancreatic cancer cell (KP1-NL). Biochemical and Biophysical Research Communications. 2006; 345(4):1454–1459. http://dx.doi.org/10.1016/j.bbrc.2006.05.037. [PubMed: 16730656]

Na SJ, Chae SY, Lee S, Park K, Kim K, Park JH, et al. Stability and bioactivity of nanocomplex of TNF-related apoptosis-inducing ligand. International Journal of Pharmaceutics. 2008; 363(1–2):149–154. http://dx.doi.org/10.1016/j.ijpharm.2008.07.013. [PubMed: 18694811]

Nakamura T, Funahashi M, Takagaki K, Munakata H, Tanaka K, Saito Y, et al. Effect of 4-methylumbelliferone on cell-free synthesis of hyaluronic acid. Biochemistry and Molecular Biology International. 1997; 43(2):263–268. [PubMed: 9350333]

Nakazawa H, Yoshihara S, Kudo D, Morohashi H, Kakizaki I, Kon A, et al. 4-Methylumbelliferone, a hyaluronan synthase suppressor, enhances the anticancer activity of gemcitabine in human pancreatic cancer cells. Cancer Chemotherapy and Pharmacology. 2006; 57(2):165–170. http://dx.doi.org/10.1007/s00280-005-0016-5. [PubMed: 16341905]

Okuda H, Kobayashi A, Xia B, Watabe M, Pai SK, Hirota S, et al. Hyaluronan synthase HAS2 promotes tumor progression in bone by stimulating the interaction of breast cancer stem-like cells with macrophages and stromal cells. Cancer Research. 2012; 72(2):537–547. http://dx.doi.org/10.1158/0008-5472.CAN-11-1678. [PubMed: 22113945]

Omarbasha B, Fair WR, Heston WD. Effect of coumarin on the normal rat prostate and on the R-3327H prostatic adenocarcinoma. Cancer Research. 1989; 49(11):3045–3049. [PubMed: 2720665]

Orian-Rousseau V. CD44, a therapeutic target for metastasising tumours. European Journal of Cancer. 2010; 46(7):1271–1277. http://dx.doi.org/10.1016/j.ejca.2010.02.024. [PubMed: 20303742]

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. Gynecologic Oncology. 2005; 98(2):193–202. http://dx.doi.org/10.1016/j.ygyno.2005.02.031. [PubMed: 15936804]

Piccioni F, Malvicini M, Garcia MG, Rodriguez A, Atorrasagasti C, Kippes N, et al. Antitumor effects of hyaluronic acid inhibitor 4-methylumbelliferone in an orthotopic hepatocellular carcinoma model in mice. Glycobiology. 2012; 22(3):400–410. http://dx.doi.org/10.1093/glycob/cwr158. [PubMed: 22038477]

Pilon-Thomas S, Verhaegen M, Kuhn L, Riker A, Mule JJ. Induction of anti-tumor immunity by vaccination with dendritic cells pulsed with anti-CD44 IgG opsonized tumor cells. Cancer Immunology, Immunotherapy. 2006; 55(10):1238–1246. http://dx.doi.org/10.1007/s00262-005-0104-8. [PubMed: 16315029]

Platt VM, Szoka FC Jr. Anticancer therapeutics: Targeting macromolecules and nanocarriers to hyaluronan or CD44, a hyaluronan receptor. Molecular Pharmaceutics. 2008; 5(4):474–486. http://dx.doi.org/10.1021/mp800024g. [PubMed: 18547053]

Porsch H, Bernert B, Mehic M, Theocharis AD, Heldin CH, Heldin P. Efficient TGFbeta-induced epithelial-mesenchymal transition depends on hyaluronan synthase HAS2. Oncogene. 2013; 32(37):4355–4365. http://dx.doi.org/10.1038/onc.2012.475. [PubMed: 23108409]

Prehm P. Curcumin analogue identified as hyaluronan export inhibitor by virtual docking to the ABC transporter MRP5. Food and Chemical Toxicology. 2013; 62:76–81. http://dx.doi.org/10.1016/j.fct.2013.08.028. [PubMed: 23978416]

Qiu L, Li Z, Qiao M, Long M, Wang M, Zhang X, et al. Self-assembled pH-responsive hyaluronic acid-g-poly(L-histidine) copolymer micelles for targeted intracellular delivery of doxorubicin. Acta Biomaterialia. 2013; 10(5):2024–2035. http://dx.doi.org/10.1016/j.actbio.2013.12.025. [PubMed: 24365705]

Quaranta S, Rossetti S, Camarri E. Double-blind clinical study on hymecromone and placebo in motor disorders of the bile ducts after cholecystectomy. Clinica Terapeutica. 1984; 108(6):513–517. [PubMed: 6233070]

Rajasagi M, von Au A, Singh R, Hartmann N, Zoller M, Marhaba R. Anti-CD44 induces apoptosis in T lymphoma via mitochondrial depolarization. Journal of Cellular and Molecular Medicine. 2010; 14(6B):1453–1467. http://dx.doi.org/10.1111/j.1582-4934.2009.00909.x. [PubMed: 19765170]

Ren Y, Zhao T, Mao G, Zhang M, Li F, Zou Y, et al. Antitumor activity of hyaluronic acid-selenium nanoparticles in Heps tumor mice models. International Journal of Biological Macromolecules. 2013; 57:57–62. http://dx.doi.org/10.1016/j.ijbiomac.2013.03.014. [PubMed: 23500433]

Ricciardelli C, Ween MP, Lokman NA, Tan IA, Pyragius CE, Oehler MK. Chemotherapy-induced hyaluronan production: A novel chemoresistance mechanism in ovarian cancer. BMC Cancer. 2013; 13:476. http://dx.doi.org/10.1186/1471-2407-13-476. [PubMed: 24124770]

Riechelmann H, Sauter A, Golze W, Hanft G, Schroen C, Hoermann K, et al. Phase I trial with the CD44v6-targeting immunoconjugate bivatuzumab mertansine in head and neck squamous cell carcinoma. Oral Oncology. 2008; 44(9):823–829. http://dx.doi.org/10.1016/j.oraloncology.2007.10.009. [PubMed: 18203652]

Saito T, Tamura D, Nakamura T, Makita Y, Ariyama H, Komiyama K, et al. 4-Methylumbelliferone leads to growth arrest and apoptosis in canine mammary tumor cells. Oncology Reports. 2013; 29(1):335–342. http://dx.doi.org/10.3892/or.2012.2100. [PubMed: 23124556]

Sandstrom K, Haylock AK, Spiegelberg D, Qvarnstrom F, Wester K, Nestor M. A novel CD44v6 targeting antibody fragment with improved tumor-to-blood ratio. International Journal of Oncology. 2012; 40(5):1525–1532. http://dx.doi.org/10.3892/ijo.2012.1352. [PubMed: 22307465]

Sandstrom K, Nestor M, Ekberg T, Engstrom M, Anniko M, Lundqvist H. Targeting CD44v6 expressed in head and neck squamous cell carcinoma: Preclinical characterization of an 111In-labeled monoclonal antibody. Tumour Biology. 2008; 29(3):137–144. http://dx.doi.org/10.1159/000143399. [PubMed: 18612218]

Schmitt M, Schmitt A, Rojewski MT, Chen J, Giannopoulos K, Fei F, et al. RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses. Blood. 2008; 111(3):1357–1365. http://dx.doi.org/10.1182/blood-2007-07-099366. [PubMed: 17978170]

Serafino A, Zonfrillo M, Andreola F, Psaila R, Mercuri L, Moroni N, et al. CD44-targeting for antitumor drug delivery: A new SN-38-hyaluronan bioconjugate for locoregional treatment of

peritoneal carcinomatosis. Current Cancer Drug Targets. 2011; 11(5):572–585. [PubMed: 21486216]

Shah V, Taratula O, Garbuzenko OB, Taratula OR, Rodriguez-Rodriguez L, Minko T. Targeted nanomedicine for suppression of CD44 and simultaneous cell death induction in ovarian cancer: An optimal delivery of siRNA and anticancer drug. Clinical Cancer Research. 2013; 19(22): 6193–6204. http://dx.doi.org/10.1158/1078-0432.CCR-13-1536. [PubMed: 24036854]

Shen YN, Shin HJ, Joo HY, Park ER, Kim SH, Hwang SG, et al. Inhibition of HAS2 induction enhances the radiosensitivity of cancer cells via persistent DNA damage. Biochemical and Biophysical Research Communications. 2014; 443(3):796–801. http://dx.doi.org/10.1016/j.bbrc.2013.12.026. [PubMed: 24333416]

Shi J, Ma R, Wang L, Zhang J, Liu R, Li L, et al. The application of hyaluronic acid-derivatized carbon nanotubes in hematoporphyrin monomethyl ether-based photodynamic therapy for in vivo and in vitro cancer treatment. International Journal of Nanomedicine. 2013; 8:2361–2373. http://dx.doi.org/10.2147/IJN.S45407. [PubMed: 23843694]

Siiskonen H, Poukka M, Tyynela-Korhonen K, Sironen R, Pasonen-Seppanen S. Inverse expression of hyaluronidase 2 and hyaluronan synthases 1–3 is associated with reduced hyaluronan content in malignant cutaneous melanoma. BMC Cancer. 2013; 13:181. http://dx.doi.org/10.1186/1471-2407-13-181. [PubMed: 23560496]

Simpson MA, Lokeshwar VB. Hyaluronan and hyaluronidase in genitourinary tumors. Frontiers in Bioscience. 2008; 13:5664–5680. [PubMed: 18508614]

Sironen RK, Tammi M, Tammi R, Auvinen PK, Anttila M, Kosma VM. Hyaluronan in human malignancies. Experimental Cell Research. 2011; 317(4):383–391. http://dx.doi.org/10.1016/j.yexcr.2010.11.017. [PubMed: 21134368]

Slomiany MG, Dai L, Bomar PA, Knackstedt TJ, Kranc DA, Tolliver L, et al. Abrogating drug resistance in malignant peripheral nerve sheath tumors by disrupting hyaluronan-CD44 interactions with small hyaluronan oligosaccharides. Cancer Research. 2009; 69(12):4992–4998. http://dx.doi.org/10.1158/0008-5472.CAN-09-0143. [PubMed: 19470767]

Slomiany MG, Dai L, Tolliver LB, Grass GD, Zeng Y, Toole BP. Inhibition of functional hyaluronan-CD44 interactions in CD133-positive primary human ovarian carcinoma cells by small hyaluronan oligosaccharides. Clinical Cancer Research. 2009; 15(24):7593–7601. http://dx.doi.org/10.1158/1078-0432.CCR-09-2317. [PubMed: 19996211]

Slomiany MG, Grass GD, Robertson AD, Yang XY, Maria BL, Beeson C, et al. Hyaluronan, CD44, and emmprin regulate lactate efflux and membrane localization of monocarboxylate transporters in human breast carcinoma cells. Cancer Research. 2009; 69(4):1293–1301. http://dx.doi.org/10.1158/0008-5472.CAN-08-2491. [PubMed: 19176383]

Snauwaert S, Vanhee S, Goetgeluk G, Verstichel G, Van Caeneghem Y, Velghe I, et al. RHAMM/HMMR (CD168) is not an ideal target antigen for immunotherapy of acute myeloid leukemia. Haematologica. 2012; 97(10):1539–1547. http://dx.doi.org/10.3324/haematol.2012.065581. [PubMed: 22532518]

Somasunderam A, Thiviyanathan V, Tanaka T, Li X, Neerathilingam M, Lokesh GL, et al. Combinatorial selection of DNA thioaptamers targeted to the HA binding domain of human CD44. Biochemistry. 2010; 49(42):9106–9112. http://dx.doi.org/10.1021/bi1009503. [PubMed: 20843027]

Tan JX, Wang XY, Li HY, Su XL, Wang L, Ran L, et al. HYAL1 over-expression is correlated with the malignant behavior of human breast cancer. International Journal of Cancer. 2011; 128(6): 1303–1315. http://dx.doi.org/10.1002/ijc.25460. [PubMed: 20473947]

Toole BP, Ghatak S, Misra S. Hyaluronan oligosaccharides as a potential anticancer therapeutic. Current Pharmaceutical Biotechnology. 2008; 9(4):249–252. [PubMed: 18691085]

Turley EA, Naor D. RHAMM and CD44 peptides-analytic tools and potential drugs. Frontiers in Bioscience. 2012; 17:1775–1794.

Twarock S, Freudenberger T, Poscher E, Dai G, Jannasch K, Dullin C, et al. Inhibition of oesophageal squamous cell carcinoma progression by in vivo targeting of hyaluronan synthesis. Molecular Cancer. 2011; 10:30. http://dx.doi.org/10.1186/1476-4598-10-30. [PubMed: 21429221]

U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. 2005. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078932.pdf

Uchakina ON, Ban H, McKallip RJ. Targeting hyaluronic acid production for the treatment of leukemia: Treatment with 4-methylumbelliferone leads to induction of MAPK-mediated apoptosis in K562 leukemia. Leukemia Research. 2013; 37(10):1294–1301. http://dx.doi.org/10.1016/j.leukres.2013.07.009. [PubMed: 23876826]

Udabage L, Brownlee GR, Waltham M, Blick T, Walker EC, Heldin P, et al. Antisense-mediated suppression of hyaluronan synthase 2 inhibits the tumorigenesis and progression of breast cancer. Cancer Research. 2005; 65(14):6139–6150. http://dx.doi.org/10.1158/0008-5472.CAN-04-1622. [PubMed: 16024615]

Urakawa H, Nishida Y, Knudson W, Knudson CB, Arai E, Kozawa E, et al. Therapeutic potential of hyaluronan oligosaccharides for bone metastasis of breast cancer. Journal of Orthopaedic Research. 2012; 30(4):662–672. http://dx.doi.org/10.1002/jor.21557. [PubMed: 21913222]

Urakawa H, Nishida Y, Wasa J, Arai E, Zhuo L, Kimata K, et al. Inhibition of hyaluronan synthesis in breast cancer cells by 4-methylumbelliferone suppresses tumorigenicity in vitro and metastatic lesions of bone in vivo. International Journal of Cancer. 2012; 130(2):454–466. http://dx.doi.org/10.1002/ijc.26014. [PubMed: 21387290]

Vincent T, Jourdan M, Sy MS, Klein B, Mechti N. Hyaluronic acid induces survival and proliferation of human myeloma cells through an interleukin-6-mediated pathway involving the phosphorylation of retinoblastoma protein. Journal of Biological Chemistry. 2001; 276(18): 14728–14736. http://dx.doi.org/10.1074/jbc.M003965200. [PubMed: 11278272]

Voelcker V, Gebhardt C, Averbeck M, Saalbach A, Wolf V, Weih F, et al. Hyaluronan fragments induce cytokine and metalloprotease upregulation in human melanoma cells in part by signalling via TLR4. Experimental Dermatology. 2008; 17(2):100–107. http://dx.doi.org/10.1111/j.1600-0625.2007.00638.x. [PubMed: 18031543]

Wallach-Dayan SB, Rubinstein AM, Hand C, Breuer R, Naor D. DNA vaccination with CD44 variant isoform reduces mammary tumor local growth and lung metastasis. Molecular Cancer Therapeutics. 2008; 7(6):1615–1623. http://dx.doi.org/10.1158/1535-7163.MCT-07-2383. [PubMed: 18566232]

Wang X, Liu W, Sun CL, Armenian SH, Hakonarson H, Hageman L, et al. Hyaluronan synthase 3 variant and anthracycline-related cardiomyopathy: A report from the children's oncology group. Journal of Clinical Oncology. 2014; 32(7):647–653. http://dx.doi.org/10.1200/JCO.2013.50.3557. [PubMed: 24470002]

Ween MP, Hummitzsch K, Rodgers RJ, Oehler MK, Ricciardelli C. Versican induces a pro-metastatic ovarian cancer cell behavior which can be inhibited by small hyaluronan oligosaccharides. Clinical and Experimental Metastasis. 2011; 28(2):113–125. http://dx.doi.org/10.1007/s10585-010-9363-7. [PubMed: 21153687]

Wei Q, Galbenus R, Raza A, Cerny RL, Simpson MA. Androgen-stimulated UDP-glucose dehydrogenase expression limits prostate androgen availability without impacting hyaluronan levels. Cancer Research. 2009; 69(6):2332–2339. http://dx.doi.org/10.1158/0008-5472.CAN-08-3083. [PubMed: 19244115]

West DC, Hampson IN, Arnold F, Kumar S. Angiogenesis induced by degradation products of hyaluronic acid. Science. 1985; 228(4705):1324–1326. [PubMed: 2408340]

Yang C, Cao M, Liu H, He Y, Xu J, Du Y, et al. The high and low molecular weight forms of hyaluronan have distinct effects on CD44 clustering. Journal of Biological Chemistry. 2012; 287(51):43094–43107. http://dx.doi.org/10.1074/jbc.M112.349209. [PubMed: 23118219]

Yao J, Zhang L, Zhou J, Liu H, Zhang Q. Efficient simultaneous tumor targeting delivery of all-trans retinoid acid and paclitaxel based on hyaluronic acid-based multifunctional nanocarrier. Molecular Pharmaceutics. 2013; 10(3):1080–1091. http://dx.doi.org/10.1021/mp3005808. [PubMed: 23320642]

Yoon HY, Koo H, Choi KY, Lee SJ, Kim K, Kwon IC, et al. Tumor-targeting hyaluronic acid nanoparticles for photodynamic imaging and therapy. Biomaterials. 2012; 33(15):3980–3989. http://dx.doi.org/10.1016/j.biomaterials.2012.02.016. [PubMed: 22364699]

Yoshida T, Matsuda Y, Naito Z, Ishiwata T. CD44 in human glioma correlates with histopathological grade and cell migration. Pathology International. 2012; 62(7):463–470. http://dx.doi.org/10.1111/j.1440-1827.2012.02823.x. [PubMed: 22726066]

Yoshihara S, Kon A, Kudo D, Nakazawa H, Kakizaki I, Sasaki M, et al. A hyaluronan synthase suppressor, 4-methylumbelliferone, inhibits liver metastasis of melanoma cells. FEBS Letters. 2005; 579(12):2722–2726. http://dx.doi.org/10.1016/j.febslet.2005.03.079. [PubMed: 15862315]

Zhang J, Chang B, Liu J. CD44 standard form expression is correlated with high-grade and advanced-stage ovarian carcinoma but not prognosis. Human Pathology. 2013; 44(9):1882–1889. http://dx.doi.org/10.1016/j.humpath.2013.02.016. [PubMed: 23664487]

Zhang S, Wu CC, Fecteau JF, Cui B, Chen L, Zhang L, et al. Targeting chronic lymphocytic leukemia cells with a humanized monoclonal antibody specific for CD44. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(15):6127–6132. http://dx.doi.org/10.1073/pnas.1221841110. [PubMed: 23530247]

Zou L, Song X, Yi T, Li S, Deng H, Chen X, et al. Administration of PLGA nanoparticles carrying shRNA against focal adhesion kinase and CD44 results in enhanced antitumor effects against ovarian cancer. Cancer Gene Therapy. 2013; 20(4):242–250. http://dx.doi.org/10.1038/cgt.2013.12. [PubMed: 23492823]

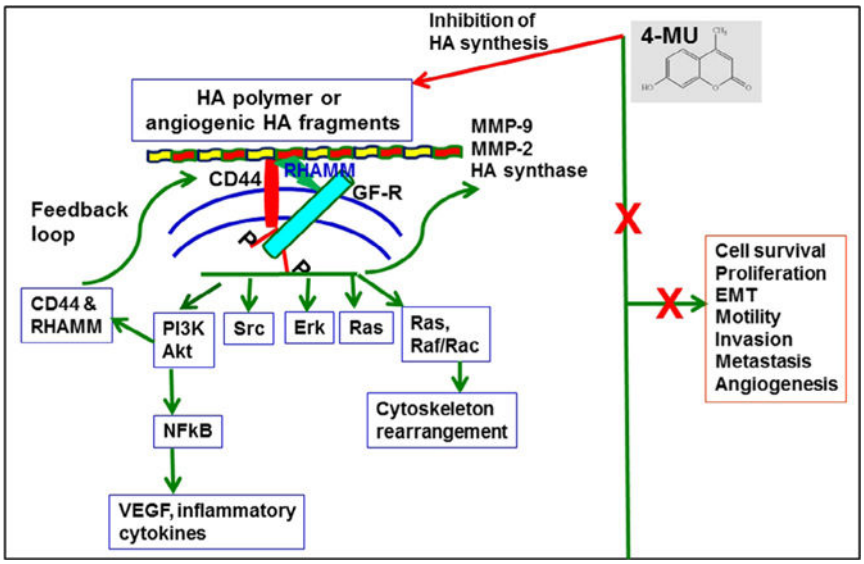

Figure 2.1.

Molecular basis for the antitumor activity of 4-MU. Binding of HA receptors to cell surface HA receptors, CD44 and RHAMM, triggers a variety of signaling events, including complex formation between HA receptors and growth factor receptor protein tyrosine kinases, and activation of downstream effectors such as Akt, NFkB, src, Erk, Ras/Raf/Rac-1. These signaling events culminate in the expression of a variety of inflammatory cytokines, VEGF, matrix metalloproteinases (MMP-2, MMP-9), as well as HA synthase and CD44/RHAMM. By inducing these signaling events and downstream effectors, HA drives cell survival, proliferation, epithelial–mesenchymal interaction, invasion, and motility which lead to tumor growth and progression. Since 4-MU inhibits HA synthesis, it blocks the first event in this signaling cascade and hence shows potent antitumor and antimetastatic efficacy.

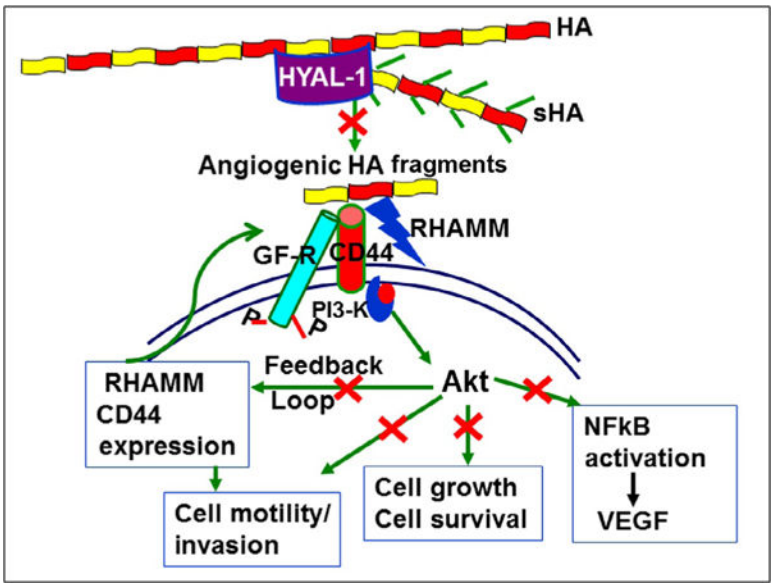

Figure 2.2.

Molecular basis for the antitumor activity of sHA. Tumor-derived HAase, HYAL-1, degrades tumor-associated pericellular HA into angiogenic fragments. These fragments induce HA signaling events described in Fig. 2.1. By inhibiting HYAL-1 activity, sHA blocks the formation of angiogenic HA fragments and hence inhibits HA signaling. The antitumor activity of sHA has been shown to be mediated by inhibition of PI3-kinase/Akt activation and downstream effectors, leading to inhibition of cell survival, proliferation, motility/invasion, and angiogenesis. Adapted from Benitez et al. (2011).