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Roles and Targeting of the HAS/Hyaluronan/CD44 Molecular System in Cancer

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Abstract

Synthesis, deposition, and interactions of hyaluronan (HA) with its cellular receptor CD44 are crucial events that regulate the onset and progression of tumors. The intracellular signaling pathways initiated by HA interactions with CD44 leading to tumorigenic responses are complex. HA molecules may perform dual functions depending on their concentration and size. Overexpression of variant isoforms of CD44 (CD44v) is most commonly linked to cancer progression, whereas their loss is associated with inhibition of tumor growth. In this review, we highlight that regulation of HA synthases (HASes) by post-translational modifications such as O-GlcNAcylation and ubiquitination, or by environmental factors or the action of microRNAs is important for HA synthesis and secretion in the tumor microenvironment. Moreover, we focus on the roles and interactions of CD44 with various proteins that reside extra- and intracellularly, as well as on cellular membranes with particular reference to the CD44-HA axis in cancer stem cell functions, and the importance of CD44/CD44v6 targeting to inhibit tumorigenesis in colon cancer cell models.

Keywords: Hyaluronan, hyaluronan synthase, CD44, Tissue-specific-targeting, Cancer, Cancer Stem Cells

Hyaluronan: Structure and Metabolism

Hyaluronan (HA) is one of the most prominent macromolecules of the extracellular matrix (ECM) that belongs to the family of glycosaminoglycans (GAGs). The repeating disaccharidic unit of HA is composed of N-acetyl-D-glucosamine and D-glucuronic acid which are linked by beta bonds (-β1,3-*N*-acetyl-D-glucosamine- β1,4-D-glucuronic acid-)$_n$. It can form oligomers or polymers that may reach a size of 6 to 8 MDa with a length of about 1 nm. Even though HA is found predominantly in the ECM, it may also be found in traces intracellularly or on the cell surface, usually non-covalently linked to glycoproteins. A unique characteristic of HA is that among the GAG family members it is the only one that is not covalently bound onto a core protein and is not sulfated. HA is ubiquitously distributed in all connective tissues and ECMs in mammals, it has gel properties and is associated with high hydration [1] and, thus, is more abundant in the rapidly growing fetal tissues, especially in the first weeks of gestation [2], than in mature adult tissues. The role of an HA-rich environment is to promote cell proliferation and migration, which is important during embryogenesis for the quick movement of stem cells to the location of organ development [3, 4]. Moreover, HA is a necessary regulatory element in the bone marrow hematopoiesis [5]. Because of the similarity of development and tumorigenesis in the activation of cell functions, the HA-rich environment was studied by various research groups, demonstrating the importance of this polysaccharide for several tumor cell functions, including proliferation and metastasis. The size of HA is critical for tumor progression, since high-molecular-weight HA (HMWHA) is functionally linked to the proliferation of cells and tissue development, whereas the low-molecular-weight HA (LMWHA) modulates angiogenesis and has pro-inflammatory effects [6, 7]. Both HMWHA and LMWHA can act as signaling molecules through the interaction with cell surface receptors, such as CD44, RHAMM, LYVE, and ECM molecules, such as the proteoglycan versican. Recent studies on the naked-mole rat showed that the longevity and resistance to cancer that present these animals are

correlated to HMWHA [8]. Specifically, it was found that HMWHA in naked-mole rats is five times or even much larger than HA in mouse or human and that the abundant accumulation of HA in the tissues of this animal is due to a unique amino acid sequence of the HA synthesizing enzyme, HAS2, and a decrement of the activity of hyaluronidases (HYALs).

The size of HA depends on the enzymes related to its metabolism. Although simple in structure it is synthesized and degraded by multiple enzymes. The family of the enzymes that synthesize HA are called hyaluronan synthases (HASes). In mammals, they exist as three HAS isoforms (HAS1, 2 and 3) that share 55-71% sequence identity [9] and differ in their enzymatic ability to form HA matrices and to establish the product size [10]. On the other hand, degradation occurs by the HYALs, which are endoglucosidases that degrade HA to LMWHA fragments that are internalized by endocytosis and are further degraded by other enzymes in endosomes or lysosomes [11]. There are six different HYALs (HYAL-1, -2, -3, -4, -P1, PH-20) that, with the exception of HYAL-P1, share ~40% of amino acid identity. The enzymatic activity of HYALs depends on the pH of the environment. For instance, HYAL-1, -2 and -3 are active at acidic pH, whereas PH-20 is active at neutral pH [12].

Hyaluronan Synthases: Regulation of the Enzymatic Activity

Structurally, HAS isoenzymes have molecular masses from 42 to 64 kDa. They are localized within the plasma membrane and consist of both multiple membrane-spanning regions and large cytosolic loops. The catalytic activity of HASes resides to the inner face of membrane with active sites for the two precursors, UDP-glucosamine and UDP-glucuronic acid, and the product is secreted or translocated to the ECM through the HAS proteins [13]. Recent studies demonstrated that a pool of HASes is localized in the ER-Golgi, but with no enzymatic activity [14]. However, when mesangial cells were grown in hyperglycaemic conditions, it was likely that HA synthesis was initiated within the cell through an activated protein kinase C (PKC)-dependent pathway [15].

All HASes have been shown to form homo- and heteromeric dimers complexes with each other [16, 17], when cells were transiently transfected with plasmids containing the three HASes, whereas complexes of HAS1-HAS2, HAS2-HAS2 and HAS2-HAS3 were observed among endogenously expressed HASes. The interaction occurred mainly via the uncharacterized N-terminal 86-amino acid domain(s). Complexes were detected in both plasma membrane and Golgi apparatus. Interestingly, among all homomeric complexes, HAS1 has the lowest activity whereas HAS3 has the highest activity. Moreover, HAS1 transfection causes a reduction of the synthesis of HA mediated by HAS2 and HAS3, suggesting a function cooperation between the isoenzymes [17].

HASes activity is also regulated by the traffic of these enzymes to/from the plasma membrane. Specifically, it was demonstrated that HA synthesis from HAS3 and an enlarged cell surface HA coat were increased when this enzyme was localized in the plasma membrane [18]. The endocytosis of HAS3 is dependent on the Rab10 GTPase, which means that the blocking or the stimulation of this protein controls the trafficking of HAS3 and consequently the HA secretion. Reduction of HA synthesis by enhancement of HAS3 endocytosis may result to a reduced amount of HAS3 in microvesicles which, as demonstrated before, mediate extracellular communication during tumor progression [19].

Post-translational modifications and environmental factors, such as hypoxia or glucose availability, may affect HASes enzymatic activity and HA synthesis [20]. Indeed, HAS2 was found to be active when it is monoubiquitinated at lysine 190 [16]. Under conditions of low cellular energy status, where the ratio ATP/AMP is low, it was shown that HAS2 is phosphorylated by the adenosine monophosphate activated protein kinase (AMPK) at threonine 110, which resides to a cytoplasmic loop, resulting in a decreased HA synthesis [21]. Moreover, in aortic smooth muscle cells, HAS2 can be regulated by O-*N*-acetylglucosamination (O-GlcNAcylation), which increases its activity and stability. This modification has an important impact in altered glucose metabolism, such as in

cancer cells where the Warburg effect is observed and hyperglycaemic conditions lead to an increase of UDP-*N*-acetylglucosamine (UDP-GlcNAc) that, in turn, directs to an increase of O-GlcNAcylation [22]. Recently, it was found that O-GlcNAcylation of histone and the opening of chromatin in proximity of HAS2 promoter are enhanced by the natural antisense transcript for HAS2 (HAS2-AS1) [23]. In addition, hyaluronan metabolism can be a rheostat for controlling an acceptable normal range of cytosolic UDP-GlcNAc concentrations in order to maintain normal cell functions [24]. The role of the UDP-sugars was intensively studied in COS-1 cells, transiently transfected with different plasmids encoding the three HASes [25]. As shown by this study, HAS2 and HAS3 but not HAS1 are able to synthesize HA and form a HA cell coat. Increasing the amount of glucosamine in the growth medium, which in turn increases the UDP-GlcNAc, showed a significant increase of HA synthesis by HAS1. Moreover, glucose-free medium resulted to a depletion of UDP-sugars and a decrease of HA synthesis by all HASes, even though this phenomenon was more evident in HAS1 activity, less in HAS2 and almost unaffected in HAS3 [25]. Thus, hyper- or hypoglycaemic conditions drastically affect the activity of HASes by both the post-translational modification of O-GlcNAcylation and by the disposition of UDP-sugars.

In the last years, many studies are focused on the regulation of signaling pathways through epigenetic regulation of the synthesis of specific proteins, by the action of microRNAs (miRNAs). One of these miRNAs, miR-23a-3p, was shown to target HAS2, acting as an inhibitor of HAS2 gene expression which results in a decrease of HA synthesis [26]. Similarly, miR-7 targets and decreases the expression of the epidermal growth factor receptor (EGFR), negatively affecting the HA-mediated CD44-EGFR pathway and decreasing indirectly the expression of HAS2 [27]. The regulation of HASes and HA synthesis by stimuli deriving from the cross-talk between cancer and stromal cells are summarized in figure 1.

Hyaluronan and Hyaluronan Metabolic Enzymes in Cancers

In the last decades, studies regarding the role of HA in tumors have been focused on the enzymes of HA metabolism and on the binding of HA to its cellular receptors. The role of HA in the tumor microenvironment has been widely studied and it is now clear that it accumulates in the stroma of a variety of tumors, activating signaling pathways that modulate cell motility, proliferation, differentiation and invasiveness [28, 29]. The increased concentration of HA in many cancers is associated with malignant progression and poor survival as in the case of colorectal, breast, prostate, lung, ovarian, and gastric tumors [30]. Moreover, alterations in HA size are closely related to cancer progression, since LMWHA promotes angiogenesis that is required for growth and metastasis of tumor, whereas HMWHA has the opposite effect [7, 31-33]. Notably, HA through its interaction with its major cellular receptor CD44 is able to induce and maintain epithelial–mesenchymal transition (EMT), a critical event in the initiation of metastasis [33, 34]. EMT is a process that is mainly induced by transforming growth factor beta (TGFβ), which, as demonstrated recently, requires the expression of HAS2 [35]. Moreover, TGFβ that can be found within the cancer exosomes can cause differentiation of fibroblasts to myofibroblasts, a phenomenon that is accompanied by an increase of the pericellular HA coat [36]. Thus, cellular processes that are enhanced by secreted factors and favor cancer progression are correlated to an increase of HA in cancer microenvironment.

Induction of HASes gene expression is closely related to cancer progression and aggressiveness, even though different types of tumor cells and cancer stages show a preference of HAS induction. For instance, high amounts of HA and a preferential HAS2 mRNA overexpression is observed in highly invasive breast cancer cells, whereas low amounts of HA synthesis and a gene expression of HAS1 and HYAL1 are observed in less invasive breast cancer cells [37]. In fact, silencing of HAS2 in the highly invasive breast cancer cells showed a decreased aggressiveness and migration and a

concomitant up-regulation of HAS1, HAS3 and HYAL1, ensuing to a 50% decrease of HA [37]. Clinical studies regarding the HAS2 expression on breast carcinomas of various types showed that HAS2 was expressed in 30.6% of invasive ductal carcinomas and 72.7% of metaplastic carcinomas [38]. Interestingly, this latter carcinoma is a subtype related to EMT [38] that in turn, as showed recently, is strictly related to the estrogen receptors alpha (ERα) [39] and beta (ERβ) both of which regulate the synthesis and turnover of tumor ECM [40]. Porsch and colleagues have demonstrated that in NMuMG mammary epithelial cells the TGFβ-induced EMT was dependent on HAS2 expression, but not on extracellular HA [41].

In breast cancer diagnosis, HA has been used as a biomarker. In a recent study that included the analysis of LMWHA in serum obtained from patients of different stages of breast cancer, it was demonstrated that the serum LMWHA level (but not the total HA) was significantly correlated with lymph node metastasis [42]. Similarly, *in vitro* studies showed that the high-invasive cells produce more LMWHA, suggesting that smaller size of HA promotes migration and invasion of breast cancer cells [42].

In leiomyosarcoma, HA is found also in high levels. This increment of HA and gene expression of HAS2 and HAS3 seems to be correlated to the amount of versican, as depletion of versican showed a decreased production and accumulation of HA and down-regulation of both synthases [43]. Interestingly, it was demonstrated that when leiomyosarcoma cells stably expressing versican siRNA were injected into nude mice, the resulting tumors displayed significantly less versican and HA staining, had lower volumes, and had reduced levels of mitosis as compared with controls [43]. Moreover, versican and HA increase ovarian cancer cell migration and invasion in a CD44-dependent manner [43]. On the other hand, tumor cells also express elevated levels of versican which interact with myeloid cells to promote an inflammatory response, through stimulating cytokine release, and metastasis. Versican, by binding to hyaluronan, influences T lymphocyte

phenotypes and in part controls the ability of these cells to synthesize and secrete cytokines that influence the immune response [44-46]. Thus, ECM proteoglycans, such as versican, play an important role in the induction of HASes and, subsequently, of HA synthesis in inflammation and tumors.

In endometrial cancer it was found that the expression of HAS1, but not HAS2 or HAS3, was related to tumor characteristics, such as the lymph-vascular space involvement, the depth of myometrial invasion and the histological grade [47]. Moreover, HAS1-positive patients showed increased serum HA [47]. Increased amounts of HA in specimens of histological grade 2 of endometrial cancer are associated with myometrial invasion, whereas the expression of HASes and HYAL2 and 3 did not vary among the tumor grades [48].

HAS3 induction and accumulation of HA in the tumor microenvironment are correlated to an increased tumor growth and aggressiveness in prostate and pancreatic cancer. Specifically, aggressive prostate tumor cells express 20-fold higher levels of HAS3, but it was demonstrated that the HA produced by HAS3 reduced tumor growth and adhesion [49]. This phenomenon is inversed by the action of HYAL1, showing that the role of HA in tumor growth is associated with its molecular size. HMWHA produced by HAS3 may suppress prostate tumor growth but induce cell migration [50], whereas LMWHA associated with the high expression of HYAL1 favours angiogenesis which is important for the first stages of tumor. Contrarily to prostate cancer, elevated levels of HAS3 promote pancreatic tumor growth by modulating the cancer microenvironment, including the increase of HA, collagens and proteoglycans [51]. In particular, *in vivo* experiments showed that overexpression of HAS3 resulted to faster growing xenograft tumors, accompanied with an abundant accumulation of extracellular HA and a decreased adhesion of epithelial cells. The removal of the extracellular HA by treatment with pegylated human recombinant HYAL (PEGPH20) showed a significant decrease of the growth rate of pancreatic

cancer cells-HAS3 tumors compared to parental tumors. Similar treatments of HAS2-overexpressing pancreatic tumors with PEGPH20 showed a weaker effect on growth rate, which still grew more slowly [51].

Lung cancer is among the most frequent cancers in males. Histologically, adenocarcinomas (AD) and squamous cell carcinomas (SCC) are the two most common carcinoma subtypes, accounting for ~90% of all lung cancer in most countries. Similarly to prostate cancer, HAS3 and HYAL1 were significantly increased also in lung cancer, specifically in AD and SCC [52]. Additionally, elevated HAS3 expression was correlated to patients with smoking history, whereas HYAL1 was strongly related to the lung prognosis [52].

Hyaluronan as Soluble Biomarker in Malignant Pleural Mesothelioma

Soluble biomarkers are crucial for early detection of various diseases, particularly cancers with deleterious prognosis. Malignant mesothelioma is a mesenchymal tumor originating from the mesothelial cells covering the body cavities**.** Accumulation of a pleural effusion is one of the first signs of the disease, providing also the first biological material for diagnosis. Biochemical-, ultra-structural-, molecular- and immunocytochemical characterization of the effusion can be used as adjuvant methods to the morphological diagnosis of a malignant mesothelioma [53] allowing differentiation from the main differential diagnostic alternatives comprising metastatic AD or reactive mesothelial hyperplasia. A high HA concentration was recognized early as a specific and reliable biomarker of malignant mesothelioma in malignant effusions [54-60] and it still constitutes one of the most established soluble biomarkers, also holding promise for future multi-parameter biomarker approaches [61].

The level of HA exceeding 75 μg HA-derived uronic acid (230 μg HA) per mL in effusion is strongly indicating a mesothelioma, whereas benign effusions often contain less than 10 μg HA-derived

uronic acid (30 μg HA) per mL. Moderately elevated values are however sometimes seen in effusions caused by other conditions such as bacterial infections, but the HA level measured in these conditions never riches the above mentioned cutoff levels.

The high HA levels seen in mesothelioma effusions have been regarded as an indication of the mesenchymal origin of this tumor. Both epithelioid- and sarcomatoid mesothelioma cells produce this polysaccharide, with a higher rate in the epithelioid phenotype [59, 62]. HA is the most specific among established mesothelioma biomarkers, but a conclusive diagnosis can be achieved more often if it is combined with mesothelin. Several attempts have been made to combine biomarkers in diagnostic batteries [63, 64]. Such combined biomarker panels have greater diagnostic accuracy than individual biomarkers and they also carry significant prognostic information provided by the addition of HA [65]. The largest of these studies consists of a two-step model combining HA and N-ERC/mesothelin. This model predicts the presence of mesothelioma with high specificity [64].

Biomarkers are also useful for monitoring the results of therapeutic interventions. Serum or plasma HA concentration reflects the tumor burden [66]. The diagnostic value of serum analysis however, seems to be low, depending on the rapid turnover of this polysaccharide in the blood stream and the fact that other conditions such as rheumatoid arthritis and liver disease also will cause elevated serum HA levels [67-69].

CD44: Structure, Diversity and Interactions

CD44 proteins are single-span transmembrane glycoproteins that are encoded by one gene composed of 20 exons that are alternatively spliced. The presence of GAG chains in some CD44 isoforms classifies CD44 molecules as part-time proteoglycans [70, 71]. Structurally, all CD44 proteins consist of three major domains: an extracellular portion or ectodomain, a transmembrane domain, and a cytoplasmic or intracellular domain (ICD). CD44 ectodomain is composed of a

common N-terminal globular domain and a stem membrane-proximal region, which accounts for the heterogeneity of this protein family, while the transmembrane and intracellular domains are common in all CD44 proteins. Among the 20 exons of CD44 gene, the ten central exons (exons 6-15) known as “variant” exons (v1-v10) are excised or included in various combinations by alternate splicing in the membrane-proximal stem region [72]. The complexity of the CD44 protein family is further enhanced by post-translational modifications, such as N- and O-glycosylations, and chondroitin or heparan sulfate additions in the extracellular portion [73, 74]. Standard CD44 (CD44s) does not contain any variant exon in its ectodomain and is widely expressed. In contrast, the expression of CD44 variant isoforms (CD44v) takes place only under specific developmental conditions [75]. Interestingly, CD44v are expressed in a variety of different cancers, particularly in advanced stages [76]. Specifically, the variant 6 of CD44 (CD44v6, in which exon 6 is expressed) is of particular interest because it is over expressed in various cancers and plays a significant role in disease onset and progression [29, 74, 77-79].

CD44 proteins participate in the reception of a variety of microenvironmental signals, thereby regulating cell-cell and cell-matrix adhesion, and controlling cell proliferation, differentiation, migration, and survival. The binding ability of CD44 to ECM components, particularly to HA but also to growth factors, cytokines, and metalloproteinases, as well as the co-receptor functions of CD44 molecules and the multiple binding motifs residing in their ICD are features that might explain the nodal contribution of CD44 to tumorigenesis.

CD44 is the main cellular receptor for HA. The binding of HA to CD44 regulates numerous cellular processes including inflammation, wound healing, tumor growth, and metastasis [80, 81]. HA binding is mediated in part by the link domain, consisting of 90 amino acids (residues 32-123), as well as other sequences flanking the link domain that form a lobular extension, creating a larger HA-binding domain [82]. The minimal size of HA fragments binding to CD44 corresponds to three to

five disaccharide units. This is important since newly synthesized HMWHA is degraded by HYALs in smaller fragments (LMWHA) that still can bind to CD44 but often exert opposite effects compared to HMWHA molecules giving rise to the known size-dependent functions of HA in physiological and pathological processes [73].

Post-translational modification of CD44 proteins regulate their ability to bind to a number of ECM components, including HA, collagens, fibronectin, laminin, fibrin, osteopontin (OPN), and serglycin. In particular, chondroitin and heparan sulfate on amino acid sequences in the CD44 stem region encoded by specific variant exons allow CD44 to interact with collagen, fibronectin, and laminin [83-85]. The degree of sulfation of CD44 sugar side chains also regulates its ability to bind to fibrin, while sialylation inhibits the interaction of CD44 with HA [86, 87]. OPN, a highly negatively charged matricellular protein, binds cells via integrins and CD44, and these interactions are involved in adhesion, migration, homing, survival, and proliferation of tumor, and stromal cells [88]. Compared to HA, OPN binding to CD44 has different effects on chemotaxis and aggregation [89]. Serglycin, an intracellular proteoglycan secreted by various cell types, interacts with and regulates the activity and availability of numerous inflammatory mediators to their extracellular target molecules, and plays a role in inflammation and tumor progression and metastasis [90]. Importantly, CD44-serglycin interactions have been implicated in the growth of malignant melanomas, in part through the regulation of angiogenesis [91].

At cellular membranes, numerous studies show that CD44 acts as a signaling platform controlling cell surface receptors of diverse structure and function. Heparan sulfate-modified CD44 isoforms (mainly CD44v3) can bind to growth factors, such as HB-EGF (heparin-binding epidermal growth factor), FGF-2 (fibroblast growth factor-2), VEGF (vascular endothelial growth factor), HGF (hepatocyte growth factor) [70, 92] thereby modulating their activities and interactions with their cognate receptors. Subsequently, various CD44 isoforms have been demonstrated to regulate the

signaling of RTKs (Receptor Tyrosine Kinases), such as Met, VEGFR-2 (vascular endothelial growth factor receptor-2), PDGFR (platelet-derived growth factor receptor), and EGFR in both HS-dependent and independent manners. Moreover, CD44v regulate the activity of matrix metalloproteinases (MMPs), such as MMP-7, MMP-9, and MT1-MMP, either involved in the maturation of growth factors and subsequent activation of their cognate receptors, or the degradation of ECM for tumor cell invasion and metastasis [93-97]. Furthermore, CD44 isoforms, like CD44v6, act as co-receptors for RTKs such as Met exerting a dual function: on one hand, CD44 ectodomain controls Met activation in a ligand-independent manner, and on the other hand, the CD44ICD binds to the ezrin-radixin-moesin (ERM) family of proteins and to the cytoskeleton, providing an intracellular platform for Met signaling [98]. Moreover, Met internalization and its intracellular signaling were shown to depend on CD44v6 [99]. In a similar mode, CD44v6 regulates VEGFR-2 activation [100]. CD44v are also involved in the regulation of PDGFRβ and FGFR activation [101-103]. Importantly, the specific requirements of a given RTK for particular CD44 isoforms in its signaling (for example, CD44v6 for Met, VEGFR-2, EGFR, and CD44v3 for FGFR, ErbB4) as well as the unique ligand-binding properties of specific CD44 isoforms to specifically support ligand-induced RTK activation, allows the design and production of specific diagnostic and therapeutic tools [100, 104, 105].

It is worth noting that CD44 proteins collaborate not only with RTKs, but also with the serine/threonine kinase receptors TGFβ-RI and TGFβ-RII, G protein-coupled receptors (CXCL12-CXCR4 axis), as well as the chondroitin sulfated form of CD74, which acts as a receptor for macrophage-migration inhibitory factor (MIF) (reviewed in [73]). More recently, CD44, which constitutes a Wnt-target gene, was shown to regulate Wnt signaling [106]. The role of CD44 in Wnt signaling was highly dependent to the binding of ERM proteins to CD44ICD, but independen to HA binding.

Although CD44ICD is only 72 aa residues long and devoid of any enzymatic activity, it contains motifs implicated in interactions with a plethora of binding partners, including proteins involved in cytoskeletal reorganization, transcription, apoptosis, survival, endocytosis, and intracellular transport [74, 107, 108]. Additionally, the CD44ICD undergoes regulatory phosphorylation at specific serine residues (Ser291, Ser316, Ser325) that determines the specific interactions between CD44 molecules and effector proteins [108-110]. Interestingly, CD44 (either full length or cleaved CD44ICD) can translocate to the nucleus acting, at least in part, as a transcriptional regulator [111, 112].

Several extracellular cues are dependent on the interaction of ankyrin and ERM proteins with the CD44ICD. Numerous studies have suggested that CD44 transduces both contact inhibition cues and HA-mediated signaling through its differential interaction with ERM proteins and merlin (a tumor suppressor ERM-related protein), and these interactions can regulate CD44-HA binding [113-116]. Furthermore, HA/CD44-mediated tumor cell-specific phenotypes and functions are closely linked to the activation of small GTP-binding proteins, such as RhoA, Rac1 and Cdc42 (RhoGTPases) [117]. IQ motif containing GTPase activating protein (IQGAP1), an essential scaffolding protein, was shown to have important roles in HA-induced actin cytoskeleton remodeling, and functional properties through its interaction with CD44ICD by inhibiting the intrinsic GTPase activities of Rac1 and RhoA thereby stabilizing their GTP-bound forms [107, 118, 119].

In the context of carcinogenesis, the contribution of CD44 to the inhibition of apoptosis is very important. Among the underlying mechanisms, the HA-dependent activation of TGFβ1, HB-EGF and OPN by CD44v6 isoforms as well as the inhibition of Fas signaling by CD44 are the most well characterized [74, 120, 121]. Jung et al. suggested that CD44v6 isoforms account for the formation of a pre-metastatic niche that promotes survival of tumor cells and induces chemoresistance [122, 123]. Another possible mechanism may involve iASPP, a novel CD44 interacting protein that was

revealed by the proteomic approach described in Skandalis et al. [107]. iASPP is known to inhibit p53-mediated apoptosis in mammalian cells [124]. Given that CD44 has crucial tumor-promoting functions in tumor cells lacking p53 function [125], and that CD44 confers growth advantage on breast Cancer Stem Cells (CSCs) [126], this finding may shed light on the mechanisms whereby CD44 is involved in cancer cell survival and evolution.

The CD44-Hyaluronan Axis in Cancer Stem Cell Function

Given the multitude of functions exerted by CD44 and its ligand at the cell biological level, it is not surprising that they have been linked to stem-cell like properties. According to the CSC concept, a small proportion of cells within a given tumor has the properties of self-renewal, remarkably high proliferative potential, expresses high levels of multidrug-resistance proteins, shows apoptosis resistance, a highly efficient DNA repair system, and a considerable developmental plasticity [127, 128]. These functional properties distinguish these so-called tumor-initiating cells from the majority of cells within the tumor, and have been linked to increased resistance to chemotherapy, radiotherapy, and even targeted therapies [129-133]. Depending on signals from a specialized cellular and extracellular matrix environment, the so-called stem cell niche, CSCs are thought to be responsible for reconstituting after therapy, which has been initially successful in targeting the bulk of tumor cells [134, 135]. Notably, in combination with other markers, CD44 is a CSC marker for a large number of tumor entities, including breast, colon, gall bladder, gastric, liver, ovarian, pancreatic, prostate and head and neck cancer (reviewed in [128]).

Given the prominent role of CD44 in signaling pathways that modulate cancer cell behavior, its use as a CSC marker suggests a functional link. Indeed, several studies have shed new light on some of the mechanisms by which CD44 modulates the functional properties of CSCs, thus rendering them attractive targets for antitumoral therapies that may eradicate a tumor cell population particularly

relevant for recurrence and therapeutic resistance. In this context, several studies indicate that the interaction between HA and its receptor CD44 is of functional importance even for non-malignant stem cells. For example, human bone marrow-derived mesenchymal stem cells were shown to express high levels of HAS1–3 [136]. Consequently, these cells secrete huge amounts of HA resulting in the formation of a substantial HA coat functionally interacting with CD44. These findings underscore the importance of autocrine HA secretion for the maintenance of a proper stem cell niche. However, HA is also relevant in a CSC context. For example, porous chitosan-HA scaffolds have been used as a mimic of the microenvironment [137]. Regarding the role of CD44 as marker, in several instances, specific isoforms of CD44 could be linked to the malignant CSC phenotype. For example, Lau et al. recently demonstrated that CD44v8-10 is the quantitatively most important CD44 variant expressed in gastric cancer cells, verifying its role by limiting dilution and serial transplantation assays [138]. Only exogenous CD44v8-10, but not standard CD44, was able to increase the frequency of tumor initiation in a mouse xenograft model. The tumor-initiating potential of the malignant cells could be markedly reduced by silencing of CD44, and rescued by CD44v8-10 expression, demonstrating the particular relevance of this isoform for gastric cancer CSCs. An important mechanistic study on the role of another CD44 variant and the impact of the microenvironment on its expression has recently been performed by Todaro et al. [139]. The authors showed that clonogenic colorectal CSCs express CD44v6, and revealed that this CD44 variant played a major role for their ability to migrate and to generate metastatic tumors. Notably, cells in the tumor microenvironment were able to induce CD44v6 expression by secreting HGF, OPN and SDF-1. The resulting activation of the Wnt/beta-catenin pathway promoted migration and metastasis in a xenograft model. A clinicopathological study revealed that expression of CD44v6 correlated to a poor patient survival, whereas the finding that phosphatidylinositol 3-kinase

inhibition selectively killed the CD44v6 CSC population raises hopes for future therapeutic targeting approaches.

In another landmark study, Bourguignon and colleagues demonstrated the presence of a subpopulation of CSCs characterized by high levels of CD44v3 expression in a human head and neck SCC cell line, HSC-3 [140]. The cell population showed the self-renewal and was capable of generating heterogeneous cell populations. At a functional level, these properties mark these cells as a CSC population. Notably, it did not only express high levels of the surrogate stem cell marker ALDH1 [128], but also the transcription factors Oct4, Sox2, and Nanog, which are part of the so-called 'Yamanaka-factor'-signature needed to reprogram differentiated cells into an embryonic stem cell-like state [141]. The authors were able to link the interaction of HS with CD44v3 to the formation of an Oct4-Sox2-Nanog complex, and to its nuclear translocation. Further mechanistic investigations revealed a complex regulatory circuit, demonstrating that microRNA miR-302 expression is controlled by Oct4-Sox2-Nanog binding sites in its promoter, and that the CD44-HA interaction resulted in miR-302 upregulation. In turn, the epigenetic regulators AOF1/AOF2 and DNMT1 were suppressed, whereas the survival proteins cIAP-1, cIAP-2 and XIAP were upregulated, leading to increased self-renewal, clonality, and resistance to the chemotherapeutic drug cisplatin. Application of an anti-miR-302 inhibitor could revert this phenotype, suggesting a possible therapeutic approach for the CSC phenotype imposed by the CD44v3-HA interaction. Another link between the interaction of CD44 with HA and a miRNA-dependent mechanism of CSC regulation was recently revealed in a study on colon cancer [142]. Using the cell lines HCT-15 and HCT-116 as an in vitro model, the authors demonstrated that CD44 expressing cells formed more colonies in soft agar and displayed increased tumorigenicity in vivo compared to cells devoid of CD44 expression. Importantly, the interaction of CD44 and HA stimulated c-Src kinase activity, which resulted in a reduced expression of miR-203 in HCT-15 cells, a miRNA known as an inhibitor of

‘stemness’ [143]. Interestingly, a part of the mechanism of miR-203 regulation included an interaction of the EMT-related factor snail with its promoter [142]. This is noteworthy, as it was recently shown that massive HA production promotes acquisition of CSC expression signatures in breast cancer through the coordinated regulation of Twist and Snail [144], providing independent proof of concept in a different experimental system.

A link between CD44 and the Nanog, Sox2 and Oct4 transcription factor network was recently also demonstrated for breast cancer CSCs: Cho et al. overexpressed the CD44ICD in breast cancer cells, and observed an increase in mammosphere formation as a readout of CSC activity [145]. Downregulation of CD44ICD decreased the expression levels and nuclear localization of the stemness factors, whereas overexpression of CD44ICD reversed these effects. Moreover, an impact of CD44 on transcriptional activation of Oct4 and Sox 2 was demonstrated at the promoter level using luciferase reporter assays. As gamma secretase inhibitor treatment (which blocks the cleavage of CD44ICD) interfered with mammosphere formation, this approach may represent a possible therapeutic strategy targeting the CD44-dependent CSC compartment in breast cancer. Supporting this concept, the relevance of ADAM17-mediated cleavage of CD44 had previously also been demonstrated in HNSCC cancer stem cells [146]. Overall, these studies clearly demonstrate the relevance of CD44 isoform expression, and of functional CD44-HA interactions for the acquisition and maintenance of a CSC phenotype. Consequently, an interference with this signaling pathway or the use of CD44/HA as targeting strategies for cytotoxic drugs have been the subject of numerous therapeutic targeting approaches.

Strategies include the use of HA-functional amphipathic and redox-responsive polymer particles for the simultaneous delivery of chemotherapeutics and hedgehog pathway inhibitors to breast CSCs [147], the use of CD44-targeted docetaxel conjugates [148], the functionalisation of liposomes with anti-CD44 aptamers [149], intracellular targeting of CD44-positive cells with self-assembling,

protein-based nanoparticles [150], the use of T cells targeting the CD44v6 to mediate antitumoral effects against multiple myeloma and acute myeloid leukemia [151], Cdk2 kinase inhibition [152], and even nutritional approaches such as vitamin D-analog-mediated notch inhibition as a strategy to diminish the CD44-positive CSC population in breast cancer [153]. The diversity of these approaches and the current intense research in this area generate hope for a successful translation of CD44-HA-mediated CSC targeting approaches into clinical applications in the near future. Further therapeutic strategies which do not exclusively target the stemness-related functions of CD44 are explored in the following section.

Targeting CD44 in Cancer: Approaches and Perspectives

Cancer cells need constant supply of nutrients through microvasculature networks present in the stroma where HA serves as one of the critical components [122, 154-156]. Many pathways in cancer are over activated downstream of CD44 that support tumor progression [78, 102, 154, 157-161]. In animal models, increases in HA production and expression of splice variant CD44 have been observed. In human tumors, overexpression of CD44 variants and the presence of high HA levels have also been found [76, 78, 161, 162].

Why is CD44 a more convenient target in cancer therapy instead of HA? Methods to eliminate HA production in a tissue-specific manner are not available as of today, whereas reagents (antibodies or HA) are now at hand for targeting CD44 variants. HA is used by several investigators as a vehicle to transport anticancer drugs for at least three reasons. First, HA binds CD44 variants. Second, HA-drug conjugate is concentrated on the cell surface and internalized. Third, toxicity due to conjugated drug is low compared to the free drug. HMWHA binds to a number of CD44 receptors on the cell surface [3]. A simple calculation shows how many CD44 molecules would bind to HA having a molecular mass of 500,000. Minimal molecular mass of HA that binds to one CD44

molecule on the cell surface is 2500. A molecular mass of 500,000 would bind 200 CD44 receptors. Thus, upon binding HA-CD44 will be internalized and rapidly eliminated from circulation by the liver hepatocytes [163]. Therefore, targeting CD44 is the best choice.

As already mentioned, serine residues of the CD44 cytoplasmic region are important for signal transduction. A peptide from CD44 cytoplasmic region containing phosphoserine at residue 325 has become of interest to block CD44 signaling and cancer cell functions. Conjugated to cell penetrating protein penetratin, the delivered peptide (pSer325) reduced movement of melanoma cells *in vitro* [109]. In prostate cancer cells, peptides containing either pSer325 or pSer323 and pSer325 interrupted the activation of CD44/MMP-9 complex [156]. An octapeptide (peptide A6) that binds specifically to CD44 [164] and selected from human urokinase plasminogen activator inhibited migration, invasion and metastasis of cancer cells. One possible explanation is by intervention of the uPA-dependent signaling pathway [165].

It is clear that the peptides that bind to HA have drawn attention because of their similarity to CD44 binding domain. Such peptides are Pep-1 (Dodecamer) and BH-P (a 42 amino acid peptide having three BX7B HA-binding motifs present in CD44). Both peptides demonstrated antitumor activities in animal models [166, 167].

It is now established that CD44v6 is a co-receptor for VEGF/VEGFR2 and HGF/c-Met and has been the focus of attention because: (i) transfection of CD44v6 induced metastatic properties on cells which were otherwise non-metastatic [77], (ii) a five amino acid peptide from v6 exon inhibited the co-receptor function of CD44v6 and as a result stopped the vascularization in tumors, prevented growth, migration and invasion [100, 168], (iii) the availability and use of monoclonal antibodies (e.g., 2F10, VFF4, VFF7, VFF18, U36, BIWA) against specific domains containing 42 amino acids encoded by exon6 to screen almost 10,000 tumors and metastatic tissues of sections by immunohistochemical analysis showed presence of CD44v6 in >80-90% cases, (iv) CD44v6 is also

present in non-epithelial tumors [169]. These findings suggested a tumorigenic role of CD44v6 and, thus, became a high value target for antibody-based cancer therapy. Antibodies against the CD44v6 can selectively deliver a radioisotope (chimeric ^{186}Re-U36 antibody, humanized monoclonal antibody ^{186}Re-bivatuzumab (BIWA 4)), toxins and cytotoxic drugs to cancer cells. The phase I clinical trial performed with radiolabeled or toxin conjugated CD44v6 antibodies in HNSCC patients showed promising results. Encouraged by the initial response, Phase I dose escalation trial with anti-CD44v6 antibody-mertansine was conducted on seven patients who had developed untreatable HNSCC or esophagus. One out of seven developed severe side-effects on skin - apoptosis of keratinocytes and epidermal necrolysis, and the patient died. This could be due to expression of CD44v6 in the skin tissue [170]. Therefore, tissue-specific expression of CD44shRNA would be a safe alternative to drug conjugate.

When complexed with polyetheleneimine (PEI), non-viral plasmid vectors mediate unspecific interactions with non-target cells and blood components, which results in a rapid clearance from the circulation. These unfavorable effects can be minimized by “shielding” with polyethylene glycol (PEG). PEGylation of PEI-plasmid polyplexes can protect the plasmid vector from systemic nuclease degradation, increased circulating life-span and reduce the toxicity of polyplexes [171]. To increase the transfection efficiency in target tumor cells having high levels of transferrin receptors (Tf-R) on the surface, transferrin (Tf), which is an iron-transporting protein, is conjugated to PEI (Tf-PEI) before PEGylation. Therefore, the shielded particles (i.e. plasmid DNA/PEG-Tf-PEI) target more specifically tumor cells than the surrounding normal cells, as Tf-receptor is 100-fold over expressed in tumor cells than normal colon cells. Internalization of plasmid DNA/Tf-PEG-PEI is promoted by receptor-mediated endocytosis [172, 173].

To target CD44v6 one must know: (a) what to deliver – a mixture of conditionally silenced plasmid pSico-CD44v6shRNA and plasmid containing colon-specific Fabpl-promoter controlling Cre

recombinase (pFabpl-cre), (b) how to deliver - both plasmids can be delivered in nanoparticles by coating with Tf-PEG-PEI, and (c) where to deliver – systemically by in mice injecting into peritoneum to target tumor and non-tumor cells.

The conditional expression of the plasmid and the mechanism of selective action on CD44v6 expression are as follows: Fabpl promoter will produce Cre recombinase in the colon and intestine cells since Fabpl promoter is leaky. Activation of conditional plasmid pSicoCD44v6shRNA takes place by the recombinase which removes the floxed region and the U6 promoter works on the CD44v6 sequence. In the case of colon/intestinal colon cancer and since CD44v6 is only present in colon tumor cells, shRNA will form a double stranded mRNA which will be degraded by dicer enzyme. In the normal colon cells, CD44v6shRNA will be also produced. Since no CD44v6 mRNA is formed in these cells the shRNA will do nothing and decayed with time [174, 175]. When these nanoparticles, containing pSicoCD44v6shRNA and pFabpl-cre, are injected systemically into the peritoneum of the Apc Min/+ mice, they seek out Tf-receptor-positive cells, undergo endocytosis and then synthesize CD44v6shRNA, knockdown CD44v6 expression, and inhibit CD44v6 signaling. As a result, it prevents tumor cell growth and induces apoptosis [78, 176].

Testing ground of the nanoparticle plasmid delivery approach was the colon cancer cell models where transfection of pSV-β-gal/Tf-PEG-PEI-nanoparticles and a mixture of Tf-PEG-PEI-nanoparticles containing pSicoCD44v6shRNA and pFabpl-Cre were first verified [29, 78, 176]. Following this experiment, Misra et al. successfully demonstrated *in vivo* that the CD44v6shRNA is localized into the colon tumor cells by an end point assay of CD44v6 expression, and by perturbation of HA-CD44v6 interaction, as reflected in the reduction in the number of tumors [78]. The possible application of tissue-specific approach by Misra et al [78] is to detect disseminated tumor growth. Recent unpublished animal studies in the C57Bl/6 mice demonstrated that systemic delivery of a mixture of two plasmids containing prostate specific Probasin-Cre/Tf-PEG-PEI-

nanoparticles and floxed pSTOP-beta galactosidase/Tf-PEG-PEI-nanoparticles, can target prostate epithelial cells in mice. This novel approach opens up novel ways to combat cancer, and to understand tumorigenesis *in vivo.*

Conclusions

Activated HA-CD44 signaling regulates a variety of cellular processes, such as cell survival, migration, invasion, adhesion, and differentiation in a cell type and context-dependent manner. This signaling can be modulated by a large number of other cellular receptors including VEGFR, EGFR, c-Met, and LRP6, which in fact determine the functional outcome of HA-CD44 signaling, as illustrated extensively in figure 2. As described in this review, targeting the interaction of HA-CD44 can inhibit the malignant processes at multiple stages. This can be accomplished by attenuation of HA synthesis by blocking the HASes activity, or providing a sustained source of drug at the tumor site, by targeting CD44 with a CD44 blocking antibody, or by a peptide approach, or more specifically by tissue-specific targeting of specific variants of CD44v that are overexpressed in certain tumors like CD44v6 in colon tumor cells described in the present review.

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Legends to Figures:

Figure 1: Overview of HASes regulation and HA levels in paracrine interactions between cancer and stromal cells.

(1) O-GlcNAcylation by increased amounts of GlcNAc and ubiquitination induce HAS2 activity, increasing the synthesized HA amounts. (2) Dimerization of HAS2 with each of the three HASes stimulates HA synthesis. (3) Contrarily, low levels of energy in terms of ATP/AMP ratio increase AMPK levels resulting to HAS2 phosphorylation which blocks its synthesizing capacity. (4) HAS2 transcription is positively regulated by HAS2-AS1, whereas inhibition of HAS2 mRNA is targeted directly by miR-23a-3p which decreases drastically its synthesis and activity. (5) Among various mechanistic roles as membrane receptor, (a) CD44 regulates the secretion of growth and soluble factors and the activity of MMPs, (b) which act as paracrine factors on stromal cells by inducing HASes and increasing HA synthesis. (6) TGF-beta also induces HA synthesis in a similar manner, whereas it may be implicated in stromal cell differentiation and EMT that enhance tumor progression. (7a) HMW-HA synthesized by activated by tumor cell-derived stromal cells is linked to CD44, formulates complexes with RTKs receptors and versican, regulating by this cancer cell proliferation and (7b) tumor cell migration and invasion. (8) On the other hand, degradation of HA to LMW-HA by HYALs activity, which contribute to inflammation conditions and high tumor aggressiveness, and binding to CD44 leads to a neoangiogenesis which is essential for tumor cell metastasis.

Figure 2. CD44v, receptor tyrosine kinases and apoptosis resistance. This figure shows the complex interplay between CD44, HA, and various receptor tyrosine kinases (RTKs) leading to anti-apoptotic signaling, multidrug resistance and contribution to feed-back information on HA production. CD44-HA binding (Green color), is accompanied by activation of Src→Ras→Erk

pathway for anti-apoptotic signaling. Also, the binding activates (via phosphorylation) the cytoplasmic tail, which leads to the attachment of Ezrin, Moesin and Radixin (ERM) as well as the actin cytoskeleton. As a co-receptor, CD44v6 binds either to c-MET or VEGFR or EGF and form complexes. The co-receptor and receptor kinase function depends on CD44v6 link through its v6 region. Each pair thus sends a powerful combined signal. For example, CD44v6 binds to hepatocyte growth factor (HGF, a ligand for c-MET) and presents it to c-MET. HGF also binds to c-MET. The ligand activates MET and the downstream signaling cascades require sustained activation of CD44-associated phosphorylated ERM and SRC (Src) signaling via the Ras-MAPK and the PI3K-Akt pathway**.** CD44v6-VEGFR activates Erk, sending an anti-apoptotic signal to endothelial cells forming blood vessels**.** β-catenin is one of the key players in cancer progression and is present in normal cells as a complex (consisting of GSK3, axin, active LRP6, Dishelved, and β-catenin) that is meant for its degradation. When Wnt protein binds to frizzle receptor, the complex releases β-catenin, which activates gene expression of cyclooxygenase 2 (COX2), resulting in the formation of PGE2. HA-CD44v6 interaction also activates COX2, and subsequent production of PGE2. Particularly in colorectal cancer the CD44–ERBB2 complex provides a strong apoptotic resistance through stimulation of COX2 transcription via PI3K and β-catenin. In turn, COX2 induced PGE2 stimulates HA synthesis. Plasma membrane contains lipid rafts where reside CD44v6, PI3 kinase, multidrug resistance protein1 (MDR1), and several RTKs, including (ERBB2, ErbB3, IGF1R-β, PDGFR-β), VEGFR, EGFR, and c-MET. PI3-kinase promotes phosphorylation and activation of the RTKs. PI3K also activates Akt and downstream anti-apoptotic events contributing which contribute to drug resistance of cancer cells. ERBB2 most likely forms complex with CD44 and PI3K via Gab-1 protein. CD44-HA crosstalk with RTKs, and non-RTKs (Src) regulates the Ras and ERK pathways. However, HA and PI3K stimulate MDR1 expression, and the stimulatory effects of PI3K would be due mainly to its

feedback stimulation of HA production by a positive feedback loop. In the lipid raft, MDR1 is associated with CD44.

Figure 1.

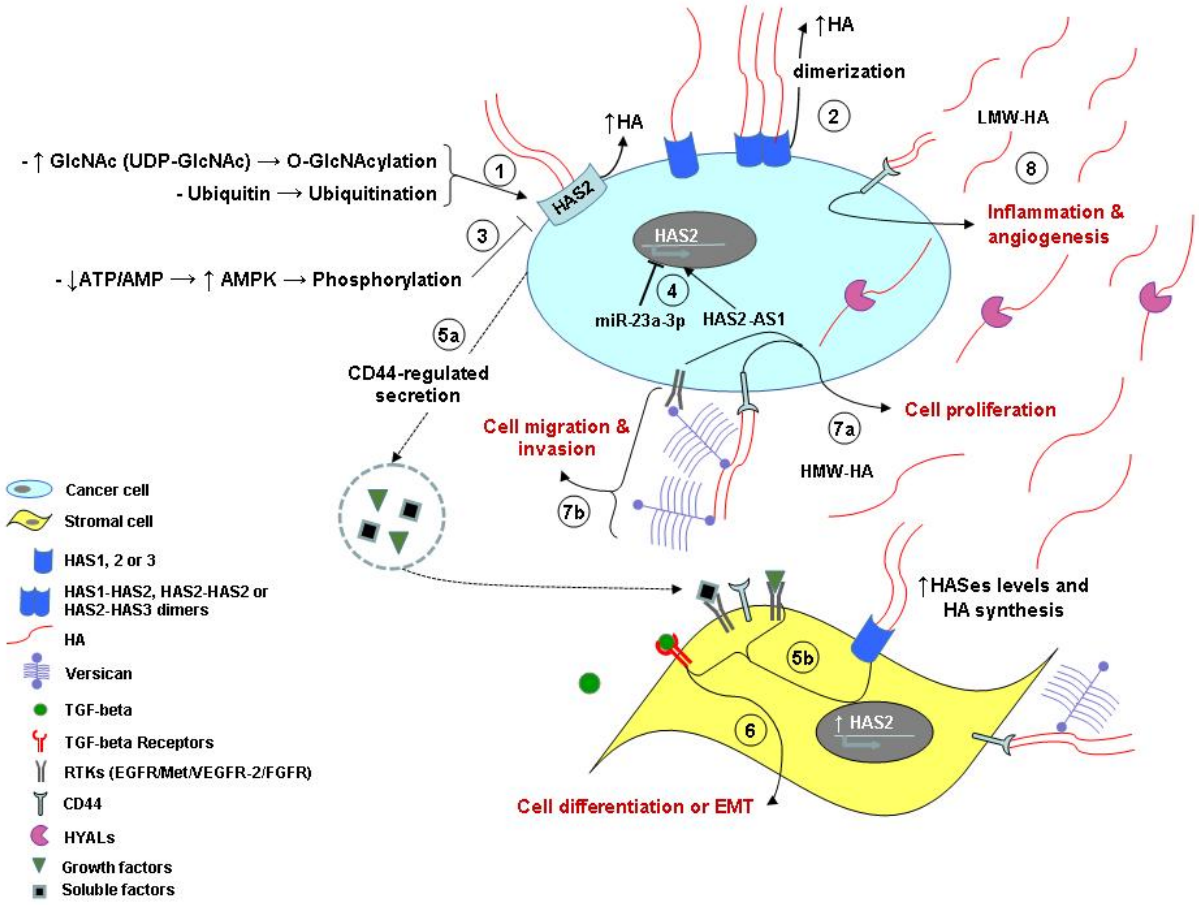
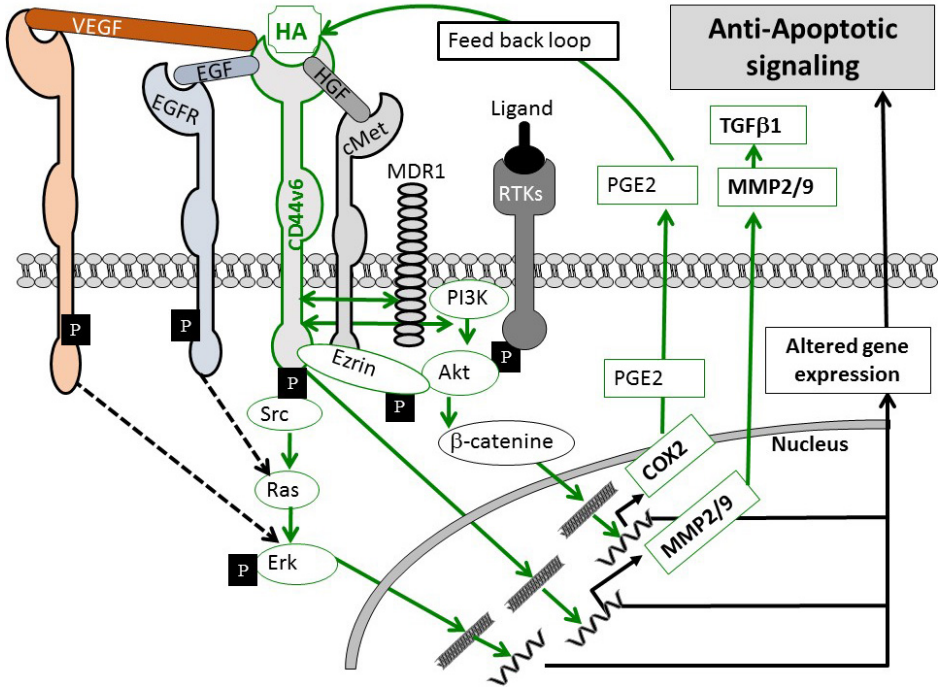

↑HA
dimerization
↑HA
LMW-HA
- ↑ GlcNAc (UDP-GlcNAc) ⟶ O-GlcNAcylation
- Ubiquitin ⟶ Ubiquitination
1
2
8
HAS2
Inflammation & angiogenesis
3
HAS2
- ↓ATP/AMP ⟶ ↑ AMPK ⟶ Phosphorylation
4
miR-23a-3p
HAS2-AS1
5a
CD44-regulated secretion
Cell migration & invasion
Cell proliferation
7a
HMW-HA
7b
↑HASes levels and HA synthesis
5b
↑ HAS2
6
Cell differentiation or EMT
Cancer cell
Stromal cell
HAS1, 2 or 3
HAS1-HAS2, HAS2-HAS2 or HAS2-HAS3 dimers
HA
Versican
TGF-beta
TGF-beta Receptors
RTKs (EGFR/Met/VEGFR-2/FGFR)
CD44
HYALs
Growth factors
Soluble factors

Figure 2


VEGF
HA
EGF
HGF
EGFR
cMet
CD44v6
MDR1
Ligand
RTKs
Feed back loop
Anti-Apoptotic signaling
TGFβ1
PGE2
MMP2/9
PI3K
Ezrin
Akt
Src
Ras
Erk
β-catenine
PGE2
Altered gene expression
Nucleus
COX2
MMP2/9
P

References

[1] L. Rugheimer, J. Olerud, C. Johnsson, T. Takahashi, K. Shimizu, P. Hansell, Hyaluronan synthases and hyaluronidases in the kidney during changes in hydration status, Matrix Biol 28(7) (2009) 390-5.

[2] L. Raio, A. Cromi, F. Ghezzi, A. Passi, E. Karousou, M. Viola, D. Vigetti, G. De Luca, P. Bolis, Hyaluronan content of Wharton's jelly in healthy and Down syndrome fetuses, Matrix biology : journal of the International Society for Matrix Biology 24(2) (2005) 166-74.

[3] B.P. Toole, Hyaluronan: from extracellular glue to pericellular cue, Nature reviews. Cancer 4(7) (2004) 528-39.

[4] D. Vigetti, M. Viola, R. Gornati, M. Ori, I. Nardi, A. Passi, G. De Luca, G. Bernardini, Molecular cloning, genomic organization and developmental expression of the Xenopus laevis hyaluronan synthase 3, Matrix Biol 22(6) (2003) 511-7.

[5] V. Goncharova, N. Serobyan, S. Iizuka, I. Schraufstatter, A. de Ridder, T. Povaliy, V. Wacker, N. Itano, K. Kimata, I.A. Orlovskaja, Y. Yamaguchi, S. Khaldoyanidi, Hyaluronan expressed by the hematopoietic microenvironment is required for bone marrow hematopoiesis, The Journal of biological chemistry 287(30) (2012) 25419-33.

[6] G.M. Campo, A. Avenoso, A. D'Ascola, V. Prestipino, M. Scuruchi, G. Nastasi, A. Calatroni, S. Campo, The stimulation of adenosine 2A receptor reduces inflammatory response in mouse articular chondrocytes treated with hyaluronan oligosaccharides, Matrix Biol 31(6) 338-51.

[7] F. Gao, Y. Liu, Y. He, C. Yang, Y. Wang, X. Shi, G. Wei, Hyaluronan oligosaccharides promote excisional wound healing through enhanced angiogenesis, Matrix Biol 29(2) 107-16.

[8] X. Tian, J. Azpurua, C. Hine, A. Vaidya, M. Myakishev-Rempel, J. Ablaeva, Z. Mao, E. Nevo, V. Gorbunova, A. Seluanov, High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat, Nature 499(7458) (2013) 346-9.

[9] A. Jacobson, J. Brinck, M.J. Briskin, A.P. Spicer, P. Heldin, Expression of human hyaluronan synthases in response to external stimuli, The Biochemical journal 348 Pt 1 (2000) 29-35.

[10] N. Itano, T. Sawai, M. Yoshida, P. Lenas, Y. Yamada, M. Imagawa, T. Shinomura, M. Hamaguchi, Y. Yoshida, Y. Ohnuki, S. Miyauchi, A.P. Spicer, J.A. McDonald, K. Kimata, Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties, The Journal of biological chemistry 274(35) (1999) 25085-92.

[11] H. Yoshida, A. Nagaoka, A. Kusaka-Kikushima, M. Tobiishi, K. Kawabata, T. Sayo, S. Sakai, Y. Sugiyama, H. Enomoto, Y. Okada, S. Inoue, KIAA1199, a deafness gene of unknown function, is a new hyaluronan binding protein involved in hyaluronan depolymerization, Proceedings of the National Academy of Sciences of the United States of America 110(14) (2013) 5612-7.

[12] E.J. Franzmann, G.L. Schroeder, W.J. Goodwin, D.T. Weed, P. Fisher, V.B. Lokeshwar, Expression of tumor markers hyaluronic acid and hyaluronidase (HYAL1) in head and neck tumors, International journal of cancer. Journal international du cancer 106(3) (2003) 438-45.

[13] N. Itano, K. Kimata, Mammalian hyaluronan synthases, IUBMB life 54(4) (2002) 195-9.

[14] K. Rilla, H. Siiskonen, A.P. Spicer, J.M. Hyttinen, M.I. Tammi, R.H. Tammi, Plasma membrane residence of hyaluronan synthase is coupled to its enzymatic activity, The Journal of biological chemistry 280(36) (2005) 31890-7.

[15] J. Ren, V.C. Hascall, A. Wang, Cyclin D3 mediates synthesis of a hyaluronan matrix that is adhesive for monocytes in mesangial cells stimulated to divide in hyperglycemic medium, J Biol Chem 284(24) (2009) 16621-32.

[16] E. Karousou, M. Kamiryo, S.S. Skandalis, A. Ruusala, T. Asteriou, A. Passi, H. Yamashita, U. Hellman, C.H. Heldin, P. Heldin, The activity of hyaluronan synthase 2 is regulated by dimerization and ubiquitination, The Journal of biological chemistry 285(31) (2010) 23647-54.

[17] G. Bart, N.O. Vico, A. Hassinen, F.M. Pujol, A.J. Deen, A. Ruusala, R.H. Tammi, A. Squire, P. Heldin, S. Kellokumpu, M.I. Tammi, Fluorescence Resonance Energy Transfer (FRET) and

Proximity Ligation Assays Reveal Functionally Relevant Homo- and Heteromeric Complexes among Hyaluronan Synthases HAS1, HAS2, and HAS3, The Journal of biological chemistry 290(18) (2015) 11479-90.
[18] A.J. Deen, K. Rilla, S. Oikari, R. Karna, G. Bart, J. Hayrinen, A.R. Bathina, A. Ropponen, K. Makkonen, R.H. Tammi, M.I. Tammi, Rab10-mediated endocytosis of the hyaluronan synthase HAS3 regulates hyaluronan synthesis and cell adhesion to collagen, The Journal of biological chemistry 289(12) (2014) 8375-89.
[19] V. Muralidharan-Chari, J.W. Clancy, A. Sedgwick, C. D'Souza-Schorey, Microvesicles: mediators of extracellular communication during cancer progression, Journal of cell science 123(Pt 10) (2010) 1603-11.
[20] D. Vigetti, A. Passi, Hyaluronan synthases posttranslational regulation in cancer, Advances in cancer research 123 (2014) 95-119.
[21] D. Vigetti, M. Clerici, S. Deleonibus, E. Karousou, M. Viola, P. Moretto, P. Heldin, V.C. Hascall, G. De Luca, A. Passi, Hyaluronan synthesis is inhibited by adenosine monophosphate-activated protein kinase through the regulation of HAS2 activity in human aortic smooth muscle cells, The Journal of biological chemistry 286(10) (2011) 7917-24.
[22] D. Vigetti, M. Viola, E. Karousou, G. De Luca, A. Passi, Metabolic control of hyaluronan synthases, Matrix biology : journal of the International Society for Matrix Biology (2013).
[23] D. Vigetti, S. Deleonibus, P. Moretto, T. Bowen, J.W. Fischer, M. Grandoch, A. Oberhuber, D.C. Love, J.A. Hanover, R. Cinquetti, E. Karousou, M. Viola, M.L. D'Angelo, V.C. Hascall, G. De Luca, A. Passi, Natural antisense transcript for hyaluronan synthase 2 (HAS2-AS1) induces transcription of HAS2 via protein O-GlcNAcylation, J Biol Chem 289(42) 28816-26.
[24] V.C. Hascall, A. Wang, M. Tammi, S. Oikari, R. Tammi, A. Passi, D. Vigetti, R.W. Hanson, G.W. Hart, The dynamic metabolism of hyaluronan regulates the cytosolic concentration of UDP-GlcNAc, Matrix Biol 35 14-7.
[25] K. Rilla, S. Oikari, T.A. Jokela, J.M. Hyttinen, R. Karna, R.H. Tammi, M.I. Tammi, Hyaluronan synthase 1 (HAS1) requires higher cellular UDP-GlcNAc concentration than HAS2 and HAS3, The Journal of biological chemistry 288(8) (2013) 5973-83.
[26] K. Rock, J. Tigges, S. Sass, A. Schutze, A.M. Florea, A.C. Fender, F.J. Theis, J. Krutmann, F. Boege, E. Fritsche, G. Reifenberger, J.W. Fischer, miR-23a-3p causes cellular senescence by targeting hyaluronan synthase 2: possible implication for skin aging, The Journal of investigative dermatology 135(2) (2015) 369-77.
[27] A.C. Midgley, T. Bowen, A.O. Phillips, R. Steadman, MicroRNA-7 inhibition rescues age-associated loss of epidermal growth factor receptor and hyaluronan-dependent differentiation in fibroblasts, Aging cell 13(2) (2014) 235-44.
[28] J. Iijima, K. Konno, N. Itano, Inflammatory alterations of the extracellular matrix in the tumor microenvironment, Cancers 3(3) (2011) 3189-205.
[29] S. Misra, P. Heldin, V.C. Hascall, N.K. Karamanos, S.S. Skandalis, R.R. Markwald, S. Ghatak, Hyaluronan-CD44 interactions as potential targets for cancer therapy, The FEBS journal 278(9) (2011) 1429-43.
[30] R.H. Tammi, A. Kultti, V.M. Kosma, R. Pirinen, P. Auvinen, M.I. Tammi, Hyaluronan in human tumors: pathobiological and prognostic messages from cell-associated and stromal hyaluronan, Seminars in cancer biology 18(4) (2008) 288-95.
[31] M. Slevin, J. Krupinski, J. Gaffney, S. Matou, D. West, H. Delisser, R.C. Savani, S. Kumar, Hyaluronan-mediated angiogenesis in vascular disease: uncovering RHAMM and CD44 receptor signaling pathways, Matrix Biol 26(1) (2007) 58-68.
[32] R.K. Sironen, M. Tammi, R. Tammi, P.K. Auvinen, M. Anttila, V.M. Kosma, Hyaluronan in human malignancies, Experimental cell research 317(4) (2011) 383-91.
[33] B. Cieply, C. Koontz, S.M. Frisch, CD44S-hyaluronan interactions protect cells resulting from EMT against anoikis, Matrix Biol 48 55-65.

[34] N. Itano, K. Kimata, Altered hyaluronan biosynthesis in cancer progression, Seminars in cancer biology 18(4) (2008) 268-74.
[35] A. Moustakas, P. Heldin, TGFbeta and matrix-regulated epithelial to mesenchymal transition, Biochimica et biophysica acta 1840(8) (2014) 2621-34.
[36] J. Webber, R. Steadman, M.D. Mason, Z. Tabi, A. Clayton, Cancer exosomes trigger fibroblast to myofibroblast differentiation, Cancer research 70(23) (2010) 9621-30.
[37] Y. Li, L. Li, T.J. Brown, P. Heldin, Silencing of hyaluronan synthase 2 suppresses the malignant phenotype of invasive breast cancer cells, International journal of cancer. Journal international du cancer 120(12) (2007) 2557-67.
[38] H.C. Lien, Y.H. Lee, Y.M. Jeng, C.H. Lin, Y.S. Lu, Y.T. Yao, Differential expression of hyaluronan synthase 2 in breast carcinoma and its biological significance, Histopathology 65(3) (2014) 328-39.
[39] Z. Piperigkou, P. Bouris, M. Onisto, M. Franchi, D. Kletsas, A.D. Theocharis, N.K. Karamanos, Estrogen receptor beta modulates breast cancer cells functional properties, signaling and expression of matrix molecules, Matrix Biol 43 (2016) 42-60.
[40] P. Bouris, S.S. Skandalis, Z. Piperigkou, N. Afratis, K. Karamanou, A.J. Aletras, A. Moustakas, A.D. Theocharis, N.K. Karamanos, Estrogen receptor alpha mediates epithelial to mesenchymal transition, expression of specific matrix effectors and functional properties of breast cancer cells, Matrix Biol 43 (2015) 42-60.
[41] H. Porsch, B. Bernert, M. Mehic, A.D. Theocharis, C.H. Heldin, P. Heldin, Efficient TGFbeta-induced epithelial-mesenchymal transition depends on hyaluronan synthase HAS2, Oncogene 32(37) (2013) 4355-65.
[42] M. Wu, M. Cao, Y. He, Y. Liu, C. Yang, Y. Du, W. Wang, F. Gao, A novel role of low molecular weight hyaluronan in breast cancer metastasis, FASEB journal : official publication of the Federation of American Societies for Experimental Biology 29(4) (2015) 1290-8.
[43] P.A. Keire, S.L. Bressler, J.M. Lemire, B. Edris, B.P. Rubin, M. Rahmani, B.M. McManus, M. van de Rijn, T.N. Wight, A role for versican in the development of leiomyosarcoma, The Journal of biological chemistry 289(49) (2014) 34089-103.
[44] S.P. Evanko, S. Potter-Perigo, L.J. Petty, G.A. Workman, T.N. Wight, Hyaluronan Controls the Deposition of Fibronectin and Collagen and Modulates TGF-beta1 Induction of Lung Myofibroblasts, Matrix Biol 42 74-92.
[45] M.Y. Chang, Y. Tanino, V. Vidova, M.G. Kinsella, C.K. Chan, P.Y. Johnson, T.N. Wight, C.W. Frevert, A rapid increase in macrophage-derived versican and hyaluronan in infectious lung disease, Matrix Biol 34 1-12.
[46] S.P. Evanko, S. Potter-Perigo, P.L. Bollyky, G.T. Nepom, T.N. Wight, Hyaluronan and versican in the control of human T-lymphocyte adhesion and migration, Matrix Biol 31(2) 90-100.
[47] H. Yabushita, T. Kishida, K. Fusano, K. Kanyama, L. Zhuo, N. Itano, K. Kimata, M. Noguchi, Role of hyaluronan and hyaluronan synthase in endometrial cancer, Oncology reports 13(6) (2005) 1101-5.
[48] P. Paiva, M.P. Van Damme, M. Tellbach, R.L. Jones, T. Jobling, L.A. Salamonsen, Expression patterns of hyaluronan, hyaluronan synthases and hyaluronidases indicate a role for hyaluronan in the progression of endometrial cancer, Gynecologic oncology 98(2) (2005) 193-202.
[49] A.G. Bharadwaj, K. Rector, M.A. Simpson, Inducible hyaluronan production reveals differential effects on prostate tumor cell growth and tumor angiogenesis, The Journal of biological chemistry 282(28) (2007) 20561-72.
[50] A.G. Bharadwaj, J.L. Kovar, E. Loughman, C. Elowsky, G.G. Oakley, M.A. Simpson, Spontaneous metastasis of prostate cancer is promoted by excess hyaluronan synthesis and processing, The American journal of pathology 174(3) (2009) 1027-36.
[51] A. Kultti, C. Zhao, N.C. Singha, S. Zimmerman, R.J. Osgood, R. Symons, P. Jiang, X. Li, C.B. Thompson, J.R. Infante, M.A. Jacobetz, D.A. Tuveson, G.I. Frost, H.M. Shepard, Z. Huang,

Accumulation of extracellular hyaluronan by hyaluronan synthase 3 promotes tumor growth and modulates the pancreatic cancer microenvironment, BioMed research international 2014 (2014) 817613.
[52] V.K. de Sa, L. Carvalho, A. Gomes, A. Alarcao, M.R. Silva, P. Couceiro, V. Sousa, F.A. Soares, V.L. Capelozzi, Role of the extracellular matrix in variations of invasive pathways in lung cancers, Braz J Med Biol Res 46(1) 21-31.
[53] A. Hjerpe, V. Ascoli, C.W. Bedrossian, M.E. Boon, J. Creaney, B. Davidson, A. Dejmek, K. Dobra, A. Fassina, A. Field, P. Firat, T. Kamei, T. Kobayashi, C.W. Michael, S. Onder, A. Segal, P. Vielh, Guidelines for the cytopathologic diagnosis of epithelioid and mixed-type malignant mesothelioma. Complementary statement from the international mesothelioma interest group, also endorsed by the international academy of cytology and the papanicolaou society of cytopathology, Acta cytologica 59(1) (2015) 2-16.
[54] G. Blix, Hyaluronic acid in the pleural and peritoneal fluids from a case of mesothelioma, Acta Societatis Medicorum Upsaliensis 56(1-2) (1951) 47-50.
[55] A. Hjerpe, Liquid-chromatographic determination of hyaluronic acid in pleural and ascitic fluids, Clinical chemistry 32(6) (1986) 952-6.
[56] C. Friman, P.E. Hellstrom, M. Juvani, H. Riska, Acid glycosaminoglycans (mucopolysaccharides) in the differential diagnosis of pleural effusion, Clinica chimica acta; international journal of clinical chemistry 76(3) (1977) 357-61.
[57] J.S. Harington, J.C. Wagner, M. Smith, The detection of hyaluronic acid in pleural fluids of cases with diffuse pleural mesotheliomas, British journal of experimental pathology 44 (1963) 81-3.
[58] R. Havez, P. Degand, A. Boersma, C. Richet, [Detection and estimation of hyaluronic acid in the pleural fluid of mesothelioma], Clinica chimica acta; international journal of clinical chemistry 33(2) (1971) 443-54.
[59] M. Nurminen, A. Dejmek, G. Martensson, A. Thylen, A. Hjerpe, Clinical utility of liquid-chromatographic analysis of effusions for hyaluronate content, Clinical chemistry 40(5) (1994) 777-80.
[60] G. Martensson, A. Thylen, U. Lindquist, A. Hjerpe, The sensitivity of hyaluronan analysis of pleural fluid from patients with malignant mesothelioma and a comparison of different methods, Cancer 73(5) (1994) 1406-10.
[61] V. Panou, M. Vyberg, U.M. Weinreich, C. Meristoudis, U.G. Falkmer, O.D. Roe, The established and future biomarkers of malignant pleural mesothelioma, Cancer treatment reviews 41(6) (2015) 486-495.
[62] L. Welker, M. Muller, O. Holz, E. Vollmer, H. Magnussen, R.A. Jorres, Cytological diagnosis of malignant mesothelioma--improvement by additional analysis of hyaluronic acid in pleural effusions, Virchows Archiv : an international journal of pathology 450(4) (2007) 455-61.
[63] B. Grigoriu, B. Chahine, F. Zerimech, M. Gregoire, M. Balduyck, M.C. Copin, P. Devos, P. Lassalle, A. Scherpereel, Serum mesothelin has a higher diagnostic utility than hyaluronic acid in malignant mesothelioma, Clinical biochemistry 42(10-11) (2009) 1046-50.
[64] F. Mundt, G. Nilsonne, S. Arslan, K. Csuros, G. Hillerdal, H. Yildirim, M. Metintas, K. Dobra, A. Hjerpe, Hyaluronan and N-ERC/mesothelin as key biomarkers in a specific two-step model to predict pleural malignant mesothelioma, PloS one 8(8) (2013) e72030.
[65] J. Creaney, I.M. Dick, A. Segal, A.W. Musk, B.W. Robinson, Pleural effusion hyaluronic acid as a prognostic marker in pleural malignant mesothelioma, Lung cancer 82(3) (2013) 491-8.
[66] A. Thylen, J. Wallin, G. Martensson, Hyaluronan in serum as an indicator of progressive disease in hyaluronan-producing malignant mesothelioma, Cancer 86(10) (1999) 2000-5.
[67] J.R. Fraser, T.C. Laurent, A. Engstrom-Laurent, U.G. Laurent, Elimination of hyaluronic acid from the blood stream in the human, Clinical and experimental pharmacology & physiology 11(1) (1984) 17-25.

[68] A. Engstrom-Laurent, L. Loof, A. Nyberg, T. Schroder, Increased serum levels of hyaluronate in liver disease, Hepatology 5(4) (1985) 638-42.
[69] A. Engstrom-Laurent, R. Hallgren, Circulating hyaluronate in rheumatoid arthritis: relationship to inflammatory activity and the effect of corticosteroid therapy, Annals of the rheumatic diseases 44(2) (1985) 83-8.
[70] K.L. Bennett, D.G. Jackson, J.C. Simon, E. Tanczos, R. Peach, B. Modrell, I. Stamenkovic, G. Plowman, A. Aruffo, CD44 isoforms containing exon V3 are responsible for the presentation of heparin-binding growth factor, J Cell Biol 128(4) (1995) 687-98.
[71] R.V. Iozzo, L. Schaefer, Proteoglycan form and function: A comprehensive nomenclature of proteoglycans, Matrix Biol 42 11-55.
[72] C. Tolg, M. Hofmann, P. Herrlich, H. Ponta, Splicing choice from ten variant exons establishes CD44 variability, Nucleic Acids Res 21(5) (1993) 1225-9.
[73] V. Orian-Rousseau, J. Sleeman, CD44 is a multidomain signaling platform that integrates extracellular matrix cues with growth factor and cytokine signals, Advances in cancer research 123 (2014) 231-54.
[74] H. Ponta, L. Sherman, P.A. Herrlich, CD44: from adhesion molecules to signalling regulators, Nature reviews. Molecular cell biology 4(1) (2003) 33-45.
[75] S. Goodison, V. Urquidi, D. Tarin, CD44 cell adhesion molecules, Molecular pathology : MP 52(4) (1999) 189-96.
[76] D. Naor, S. Nedvetzki, I. Golan, L. Melnik, Y. Faitelson, CD44 in cancer, Critical reviews in clinical laboratory sciences 39(6) (2002) 527-79.
[77] U. Gunthert, M. Hofmann, W. Rudy, S. Reber, M. Zoller, I. Haussmann, S. Matzku, A. Wenzel, H. Ponta, P. Herrlich, A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells, Cell 65(1) (1991) 13-24.
[78] S. Misra, V.C. Hascall, C. De Giovanni, R.R. Markwald, S. Ghatak, Delivery of CD44 shRNA/nanoparticles within cancer cells: perturbation of hyaluronan/CD44v6 interactions and reduction in adenoma growth in Apc Min/+ MICE, The Journal of biological chemistry 284(18) (2009) 12432-46.
[79] T. Ishida, Immunohistochemical expression of the CD44 variant 6 in colorectal adenocarcinoma, Surgery today 30(1) (2000) 28-32.
[80] P.W. Noble, D. Jiang, Matrix regulation of lung injury, inflammation, and repair: the role of innate immunity, Proceedings of the American Thoracic Society 3(5) (2006) 401-4.
[81] P. Heldin, E. Karousou, B. Bernert, H. Porsch, K. Nishitsuka, S.S. Skandalis, Importance of hyaluronan-CD44 interactions in inflammation and tumorigenesis, Connective tissue research 49(3) (2008) 215-8.
[82] P. Teriete, S. Banerji, M. Noble, C.D. Blundell, A.J. Wright, A.R. Pickford, E. Lowe, D.J. Mahoney, M.I. Tammi, J.D. Kahmann, I.D. Campbell, A.J. Day, D.G. Jackson, Structure of the regulatory hyaluronan binding domain in the inflammatory leukocyte homing receptor CD44, Molecular cell 13(4) (2004) 483-96.
[83] A.E. Faassen, J.A. Schrager, D.J. Klein, T.R. Oegema, J.R. Couchman, J.B. McCarthy, A cell surface chondroitin sulfate proteoglycan, immunologically related to CD44, is involved in type I collagen-mediated melanoma cell motility and invasion, The Journal of cell biology 116(2) (1992) 521-31.
[84] S. Jalkanen, M. Jalkanen, Lymphocyte CD44 binds the COOH-terminal heparin-binding domain of fibronectin, The Journal of cell biology 116(3) (1992) 817-25.
[85] D. Naor, R.V. Sionov, D. Ish-Shalom, CD44: structure, function, and association with the malignant process, Advances in cancer research 71 (1997) 241-319.
[86] C.S. Alves, K. Konstantopoulos, PDGF suppresses the sulfation of CD44v and potentiates CD44v-mediated binding of colon carcinoma cells to fibrin under flow, PloS one 7(10) (2012) e41472.

[87] D.G. Jackson, Immunological functions of hyaluronan and its receptors in the lymphatics, Immunological reviews 230(1) (2009) 216-31.
[88] P.Y. Wai, P.C. Kuo, Osteopontin: regulation in tumor metastasis, Cancer metastasis reviews 27(1) (2008) 103-18.
[89] G.F. Weber, S. Ashkar, M.J. Glimcher, H. Cantor, Receptor-ligand interaction between CD44 and osteopontin (Eta-1), Science 271(5248) (1996) 509-12.
[90] A. Korpetinou, S.S. Skandalis, V.T. Labropoulou, G. Smirlaki, A. Noulas, N.K. Karamanos, A.D. Theocharis, Serglycin: at the crossroad of inflammation and malignancy, Frontiers in oncology 3 (2014) 327.
[91] A. Purushothaman, B.P. Toole, Serglycin proteoglycan is required for multiple myeloma cell adhesion, in vivo growth, and vascularization, The Journal of biological chemistry 289(9) (2014) 5499-509.
[92] M. Jones, L. Tussey, N. Athanasou, D.G. Jackson, Heparan sulfate proteoglycan isoforms of the CD44 hyaluronan receptor induced in human inflammatory macrophages can function as paracrine regulators of fibroblast growth factor action, The Journal of biological chemistry 275(11) (2000) 7964-74.
[93] W.H. Yu, J.F. Woessner, Jr., J.D. McNeish, I. Stamenkovic, CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling, Genes & development 16(3) (2002) 307-23.
[94] L.Y. Bourguignon, Z. Gunja-Smith, N. Iida, H.B. Zhu, L.J. Young, W.J. Muller, R.D. Cardiff, CD44v(3,8-10) is involved in cytoskeleton-mediated tumor cell migration and matrix metalloproteinase (MMP-9) association in metastatic breast cancer cells, Journal of cellular physiology 176(1) (1998) 206-15.
[95] L.Y. Bourguignon, D. Zhu, H. Zhu, CD44 isoform-cytoskeleton interaction in oncogenic signaling and tumor progression, Frontiers in bioscience : a journal and virtual library 3 (1998) d637-49.
[96] H. Mori, T. Tomari, N. Koshikawa, M. Kajita, Y. Itoh, H. Sato, H. Tojo, I. Yana, M. Seiki, CD44 directs membrane-type 1 matrix metalloproteinase to lamellipodia by associating with its hemopexin-like domain, The EMBO journal 21(15) (2002) 3949-59.
[97] C.I. Kung, C.Y. Chen, C.C. Yang, C.Y. Lin, T.H. Chen, H.S. Wang, Enhanced membrane-type 1 matrix metalloproteinase expression by hyaluronan oligosaccharides in breast cancer cells facilitates CD44 cleavage and tumor cell migration, Oncology reports 28(5) (2012) 1808-14.
[98] V. Orian-Rousseau, L. Chen, J.P. Sleeman, P. Herrlich, H. Ponta, CD44 is required for two consecutive steps in HGF/c-Met signaling, Genes & development 16(23) (2002) 3074-86.
[99] S. Hasenauer, D. Malinger, D. Koschut, G. Pace, A. Matzke, A. von Au, V. Orian-Rousseau, Internalization of Met requires the co-receptor CD44v6 and its link to ERM proteins, PloS one 8(4) (2013) e62357.
[100] M. Tremmel, A. Matzke, I. Albrecht, A.M. Laib, V. Olaku, K. Ballmer-Hofer, G. Christofori, M. Heroult, H.G. Augustin, H. Ponta, V. Orian-Rousseau, A CD44v6 peptide reveals a role of CD44 in VEGFR-2 signaling and angiogenesis, Blood 114(25) (2009) 5236-44.
[101] L. Li, C.H. Heldin, P. Heldin, Inhibition of platelet-derived growth factor-BB-induced receptor activation and fibroblast migration by hyaluronan activation of CD44, The Journal of biological chemistry 281(36) (2006) 26512-9.
[102] S. Misra, B.P. Toole, S. Ghatak, Hyaluronan constitutively regulates activation of multiple receptor tyrosine kinases in epithelial and carcinoma cells, The Journal of biological chemistry 281(46) (2006) 34936-41.
[103] L. Sherman, D. Wainwright, H. Ponta, P. Herrlich, A splice variant of CD44 expressed in the apical ectodermal ridge presents fibroblast growth factors to limb mesenchyme and is required for limb outgrowth, Genes & development 12(7) (1998) 1058-71.

[104] V. Orian-Rousseau, H. Morrison, A. Matzke, T. Kastilan, G. Pace, P. Herrlich, H. Ponta, Hepatocyte growth factor-induced Ras activation requires ERM proteins linked to both CD44v6 and F-actin, Molecular biology of the cell 18(1) (2007) 76-83.
[105] C. Cheng, M.B. Yaffe, P.A. Sharp, A positive feedback loop couples Ras activation and CD44 alternative splicing, Genes & development 20(13) (2006) 1715-20.
[106] M. Schmitt, M. Metzger, D. Gradl, G. Davidson, V. Orian-Rousseau, CD44 functions in Wnt signaling by regulating LRP6 localization and activation, Cell death and differentiation 22(4) (2015) 677-89.
[107] S.S. Skandalis, I. Kozlova, U. Engstrom, U. Hellman, P. Heldin, Proteomic identification of CD44 interacting proteins, IUBMB life 62(11) (2010) 833-40.
[108] R.F. Thorne, J.W. Legg, C.M. Isacke, The role of the CD44 transmembrane and cytoplasmic domains in co-ordinating adhesive and signalling events, Journal of cell science 117(Pt 3) (2004) 373-80.
[109] D. Peck, C.M. Isacke, Hyaluronan-dependent cell migration can be blocked by a CD44 cytoplasmic domain peptide containing a phosphoserine at position 325, Journal of cell science 111 (Pt 11) (1998) 1595-601.
[110] G. Tzircotis, R.F. Thorne, C.M. Isacke, Directional sensing of a phorbol ester gradient requires CD44 and is regulated by CD44 phosphorylation, Oncogene 25(56) (2006) 7401-10.
[111] J.L. Lee, M.J. Wang, J.Y. Chen, Acetylation and activation of STAT3 mediated by nuclear translocation of CD44, The Journal of cell biology 185(6) (2009) 949-57.
[112] M. Kajita, Y. Itoh, T. Chiba, H. Mori, A. Okada, H. Kinoh, M. Seiki, Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration, The Journal of cell biology 153(5) (2001) 893-904.
[113] L.Y. Bourguignon, K. Peyrollier, E. Gilad, A. Brightman, 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, The Journal of biological chemistry 282(2) (2007) 1265-80.
[114] I. Stamenkovic, Q. Yu, Merlin, a "magic" linker between extracellular cues and intracellular signaling pathways that regulate cell motility, proliferation, and survival, Current protein & peptide science 11(6) (2010) 471-84.
[115] Y. Bai, Y.J. Liu, H. Wang, Y. Xu, I. Stamenkovic, Q. Yu, Inhibition of the hyaluronan-CD44 interaction by merlin contributes to the tumor-suppressor activity of merlin, Oncogene 26(6) (2007) 836-50.
[116] H. Morrison, L.S. Sherman, J. Legg, F. Banine, C. Isacke, C.A. Haipek, D.H. Gutmann, H. Ponta, P. Herrlich, The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44, Genes & development 15(8) (2001) 968-80.
[117] L.Y. Bourguignon, Hyaluronan-mediated CD44 activation of RhoGTPase signaling and cytoskeleton function promotes tumor progression, Seminars in cancer biology 18(4) (2008) 251-9.
[118] L.Y. Bourguignon, E. Gilad, K. Rothman, K. Peyrollier, Hyaluronan-CD44 interaction with IQGAP1 promotes Cdc42 and ERK signaling, leading to actin binding, Elk-1/estrogen receptor transcriptional activation, and ovarian cancer progression, The Journal of biological chemistry 280(12) (2005) 11961-72.
[119] I. Kozlova, A. Ruusala, O. Voytyuk, S.S. Skandalis, P. Heldin, IQGAP1 regulates hyaluronan-mediated fibroblast motility and proliferation, Cellular signalling 24(9) (2012) 1856-62.
[120] Q. Yu, I. Stamenkovic, Transforming growth factor-beta facilitates breast carcinoma metastasis by promoting tumor cell survival, Clinical & experimental metastasis 21(3) (2004) 235-42.
[121] A. Mielgo, M. van Driel, A. Bloem, L. Landmann, U. Gunthert, A novel antiapoptotic mechanism based on interference of Fas signaling by CD44 variant isoforms, Cell death and differentiation 13(3) (2006) 465-77.

[122] T. Jung, D. Castellana, P. Klingbeil, I. Cuesta Hernandez, M. Vitacolonna, D.J. Orlicky, S.R. Roffler, P. Brodt, M. Zoller, CD44v6 dependence of premetastatic niche preparation by exosomes, Neoplasia 11(10) (2009) 1093-105.
[123] T. Jung, W. Gross, M. Zoller, CD44v6 coordinates tumor matrix-triggered motility and apoptosis resistance, The Journal of biological chemistry 286(18) (2011) 15862-74.
[124] E.A. Slee, S. Gillotin, D. Bergamaschi, C. Royer, S. Llanos, S. Ali, B. Jin, G. Trigiante, X. Lu, The N-terminus of a novel isoform of human iASPP is required for its cytoplasmic localization, Oncogene 23(56) (2004) 9007-16.
[125] S. Godar, T.A. Ince, G.W. Bell, D. Feldser, J.L. Donaher, J. Bergh, A. Liu, K. Miu, R.S. Watnick, F. Reinhardt, S.S. McAllister, T. Jacks, R.A. Weinberg, Growth-inhibitory and tumor-suppressive functions of p53 depend on its repression of CD44 expression, Cell 134(1) (2008) 62-73.
[126] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison, M.F. Clarke, Prospective identification of tumorigenic breast cancer cells, Proceedings of the National Academy of Sciences of the United States of America 100(7) (2003) 3983-8.
[127] R. Pardal, M.F. Clarke, S.J. Morrison, Applying the principles of stem-cell biology to cancer, Nature reviews. Cancer 3(12) (2003) 895-902.
[128] B. Greve, R. Kelsch, K. Spaniol, H.T. Eich, M. Gotte, Flow cytometry in cancer stem cell analysis and separation, Cytometry. Part A : the journal of the International Society for Analytical Cytology 81(4) (2012) 284-93.
[129] H.E. Lee, J.H. Kim, Y.J. Kim, S.Y. Choi, S.W. Kim, E. Kang, I.Y. Chung, I.A. Kim, E.J. Kim, Y. Choi, H.S. Ryu, S.Y. Park, An increase in cancer stem cell population after primary systemic therapy is a poor prognostic factor in breast cancer, British journal of cancer 104(11) (2011) 1730-8.
[130] J. Chen, Y. Li, T.S. Yu, R.M. McKay, D.K. Burns, S.G. Kernie, L.F. Parada, A restricted cell population propagates glioblastoma growth after chemotherapy, Nature 488(7412) (2012) 522-6.
[131] D.R. Boulbes, G.B. Chauhan, Q. Jin, C. Bartholomeusz, F.J. Esteva, CD44 expression contributes to trastuzumab resistance in HER2-positive breast cancer cells, Breast cancer research and treatment 151(3) (2015) 501-13.
[132] T. Aso, M. Matsuo, H. Kiyohara, K. Taguchi, F. Rikimaru, M. Shimokawa, Y. Segawa, Y. Higaki, H. Umeno, T. Nakashima, M. Masuda, Induction of CD44 variant 9-expressing cancer stem cells might attenuate the efficacy of chemoradioselection and Worsens the prognosis of patients with advanced head and neck cancer, PloS one 10(3) (2015) e0116596.
[133] S.H. Sahlberg, D. Spiegelberg, B. Glimelius, B. Stenerlow, M. Nestor, Evaluation of cancer stem cell markers CD133, CD44, CD24: association with AKT isoforms and radiation resistance in colon cancer cells, PloS one 9(4) (2014) e94621.
[134] M.A. LaBarge, The difficulty of targeting cancer stem cell niches, Clinical cancer research : an official journal of the American Association for Cancer Research 16(12) (2010) 3121-9.
[135] S. Hosonuma, Y. Kobayashi, S. Kojo, H. Wada, K. Seino, K. Kiguchi, B. Ishizuka, Clinical significance of side population in ovarian cancer cells, Human cell 24(1) (2011) 9-12.
[136] C. Qu, K. Rilla, R. Tammi, M. Tammi, H. Kroger, M.J. Lammi, Extensive CD44-dependent hyaluronan coats on human bone marrow-derived mesenchymal stem cells produced by hyaluronan synthases HAS1, HAS2 and HAS3, The international journal of biochemistry & cell biology 48 (2014) 45-54.
[137] S.J. Florczyk, K. Wang, S. Jana, D.L. Wood, S.K. Sytsma, J.G. Sham, F.M. Kievit, M. Zhang, Porous chitosan-hyaluronic acid scaffolds as a mimic of glioblastoma microenvironment ECM, Biomaterials 34(38) (2013) 10143-50.
[138] W.M. Lau, E. Teng, H.S. Chong, K.A. Lopez, A.Y. Tay, M. Salto-Tellez, A. Shabbir, J.B. So, S.L. Chan, CD44v8-10 is a cancer-specific marker for gastric cancer stem cells, Cancer research 74(9) (2014) 2630-41.
[139] M. Todaro, M. Gaggianesi, V. Catalano, A. Benfante, F. Iovino, M. Biffoni, T. Apuzzo, I. Sperduti, S. Volpe, G. Cocorullo, G. Gulotta, F. Dieli, R. De Maria, G. Stassi, CD44v6 is a marker

of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis, Cell stem cell 14(3) (2014) 342-56.
[140] L.Y. Bourguignon, G. Wong, C. Earle, L. Chen, Hyaluronan-CD44v3 interaction with Oct4-Sox2-Nanog promotes miR-302 expression leading to self-renewal, clonal formation, and cisplatin resistance in cancer stem cells from head and neck squamous cell carcinoma, The Journal of biological chemistry 287(39) (2012) 32800-24.
[141] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126(4) (2006) 663-76.
[142] S.Y. Ju, S.H. Chiou, Y. Su, Maintenance of the stemness in CD44(+) HCT-15 and HCT-116 human colon cancer cells requires miR-203 suppression, Stem cell research 12(1) (2014) 86-100.
[143] R. Yi, M.N. Poy, M. Stoffel, E. Fuchs, A skin microRNA promotes differentiation by repressing 'stemness', Nature 452(7184) (2008) 225-9.
[144] T. Chanmee, P. Ontong, N. Mochizuki, P. Kongtawelert, K. Konno, N. Itano, Excessive hyaluronan production promotes acquisition of cancer stem cell signatures through the coordinated regulation of Twist and the transforming growth factor beta (TGF-beta)-Snail signaling axis, The Journal of biological chemistry 289(38) (2014) 26038-56.
[145] Y. Cho, H.W. Lee, H.G. Kang, H.Y. Kim, S.J. Kim, K.H. Chun, Cleaved CD44 intracellular domain supports activation of stemness factors and promotes tumorigenesis of breast cancer, Oncotarget 6(11) (2015) 8709-21.
[146] P. Kamarajan, J.M. Shin, X. Qian, B. Matte, J.Y. Zhu, Y.L. Kapila, ADAM17-mediated CD44 cleavage promotes orasphere formation or stemness and tumorigenesis in HNSCC, Cancer medicine 2(6) (2013) 793-802.
[147] K. Hu, H. Zhou, Y. Liu, Z. Liu, J. Liu, J. Tang, J. Li, J. Zhang, W. Sheng, Y. Zhao, Y. Wu, C. Chen, Hyaluronic acid functional amphipathic and redox-responsive polymer particles for the co-delivery of doxorubicin and cyclopamine to eradicate breast cancer cells and cancer stem cells, Nanoscale 7(18) (2015) 8607-18.
[148] N. Goodarzi, M.H. Ghahremani, M. Amini, F. Atyabi, S.N. Ostad, N. Shabani Ravari, N. Nateghian, R. Dinarvand, CD44-targeted docetaxel conjugate for cancer cells and cancer stem-like cells: a novel hyaluronic acid-based drug delivery system, Chemical biology & drug design 83(6) (2014) 741-52.
[149] W. Alshaer, H. Hillaireau, J. Vergnaud, S. Ismail, E. Fattal, Functionalizing Liposomes with anti-CD44 Aptamer for Selective Targeting of Cancer Cells, Bioconjugate chemistry (2014).
[150] M. Pesarrodona, N. Ferrer-Miralles, U. Unzueta, P. Gener, W. Tatkiewicz, I. Abasolo, I. Ratera, J. Veciana, S. Schwartz, Jr., A. Villaverde, E. Vazquez, Intracellular targeting of CD44+ cells with self-assembling, protein only nanoparticles, International journal of pharmaceutics 473(1-2) (2014) 286-95.
[151] M. Casucci, B. Nicolis di Robilant, L. Falcone, B. Camisa, M. Norelli, P. Genovese, B. Gentner, F. Gullotta, M. Ponzoni, M. Bernardi, M. Marcatti, A. Saudemont, C. Bordignon, B. Savoldo, F. Ciceri, L. Naldini, G. Dotti, C. Bonini, A. Bondanza, CD44v6-targeted T cells mediate potent antitumor effects against acute myeloid leukemia and multiple myeloma, Blood 122(20) (2013) 3461-72.
[152] M. Opyrchal, J.L. Salisbury, I. Iankov, M.P. Goetz, J. McCubrey, M.W. Gambino, L. Malatino, G. Puccia, J.N. Ingle, E. Galanis, A.B. D'Assoro, Inhibition of Cdk2 kinase activity selectively targets the CD44(+)/CD24(-)/Low stem-like subpopulation and restores chemosensitivity of SUM149PT triple-negative breast cancer cells, International journal of oncology 45(3) (2014) 1193-9.
[153] J.Y. So, J. Wahler, S. Das Gupta, D.M. Salerno, H. Maehr, M. Uskokovic, N. Suh, HES1-mediated inhibition of Notch1 signaling by a Gemini vitamin D analog leads to decreased CD44(+)/CD24(-/low) tumor-initiating subpopulation in basal-like breast cancer, The Journal of steroid biochemistry and molecular biology 148 (2015) 111-21.

[154] S. Misra, S. Ghatak, B.P. Toole, Regulation of MDR1 expression and drug resistance by a positive feedback loop involving hyaluronan, phosphoinositide 3-kinase, and ErbB2, The Journal of biological chemistry 280(21) (2005) 20310-5.
[155] Q. Yu, I. Stamenkovic, Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion, Genes & development 13(1) (1999) 35-48.
[156] B. Desai, T. Ma, J. Zhu, M.A. Chellaiah, Characterization of the expression of variant and standard CD44 in prostate cancer cells: identification of the possible molecular mechanism of CD44/MMP9 complex formation on the cell surface, Journal of cellular biochemistry 108(1) (2009) 272-84.
[157] S. Ghatak, V.C. Hascall, R.R. Markwald, S. Misra, Stromal hyaluronan interaction with epithelial CD44 variants promotes prostate cancer invasiveness by augmenting expression and function of hepatocyte growth factor and androgen receptor, The Journal of biological chemistry 285(26) (2010) 19821-32.
[158] S. Misra, L.M. Obeid, Y.A. Hannun, S. Minamisawa, F.G. Berger, R.R. Markwald, B.P. Toole, S. Ghatak, Hyaluronan constitutively regulates activation of COX-2-mediated cell survival activity in intestinal epithelial and colon carcinoma cells, The Journal of biological chemistry 283(21) (2008) 14335-44.
[159] L.Y. Bourguignon, E. Gilad, K. Peyrollier, Heregulin-mediated ErbB2-ERK signaling activates hyaluronan synthases leading to CD44-dependent ovarian tumor cell growth and migration, The Journal of biological chemistry 282(27) (2007) 19426-41.
[160] S. Ghatak, S. Misra, B.P. Toole, Hyaluronan constitutively regulates ErbB2 phosphorylation and signaling complex formation in carcinoma cells, The Journal of biological chemistry 280(10) (2005) 8875-83.
[161] S. Ghatak, S. Misra, B.P. Toole, Hyaluronan oligosaccharides inhibit anchorage-independent growth of tumor cells by suppressing the phosphoinositide 3-kinase/Akt cell survival pathway, The Journal of biological chemistry 277(41) (2002) 38013-20.
[162] Y. Yamada, N. Itano, H. Narimatsu, T. Kudo, K. Morozumi, S. Hirohashi, A. Ochiai, M. Ueda, K. Kimata, Elevated transcript level of hyaluronan synthase1 gene correlates with poor prognosis of human colon cancer, Clinical & experimental metastasis 21(1) (2004) 57-63.
[163] E.N. Harris, S.V. Kyosseva, J.A. Weigel, P.H. Weigel, Expression, processing, and glycosaminoglycan binding activity of the recombinant human 315-kDa hyaluronic acid receptor for endocytosis (HARE), The Journal of biological chemistry 282(5) (2007) 2785-97.
[164] R.S. Piotrowicz, B.B. Damaj, M. Hachicha, F. Incardona, S.B. Howell, M. Finlayson, A6 peptide activates CD44 adhesive activity, induces FAK and MEK phosphorylation, and inhibits the migration and metastasis of CD44-expressing cells, Molecular cancer therapeutics 10(11) (2011) 2072-82.
[165] D.D. Boyd, S.J. Kim, H. Wang, T.R. Jones, G.E. Gallick, A urokinase-derived peptide (A6) increases survival of mice bearing orthotopically grown prostate cancer and reduces lymph node metastasis, The American journal of pathology 162(2) (2003) 619-26.
[166] M.E. Mummert, D.I. Mummert, L. Ellinger, A. Takashima, Functional roles of hyaluronan in B16-F10 melanoma growth and experimental metastasis in mice, Molecular cancer therapeutics 2(3) (2003) 295-300.
[167] X.M. Xu, Y. Chen, J. Chen, S. Yang, F. Gao, C.B. Underhill, K. Creswell, L. Zhang, A peptide with three hyaluronan binding motifs inhibits tumor growth and induces apoptosis, Cancer research 63(18) (2003) 5685-90.
[168] A. Matzke, P. Herrlich, H. Ponta, V. Orian-Rousseau, A five-amino-acid peptide blocks Met- and Ron-dependent cell migration, Cancer research 65(14) (2005) 6105-10.
[169] K.H. Heider, H. Kuthan, G. Stehle, G. Munzert, CD44v6: a target for antibody-based cancer therapy, Cancer immunology, immunotherapy : CII 53(7) (2004) 567-79.

[170] B.M. Tijink, J. Buter, R. de Bree, G. Giaccone, M.S. Lang, A. Staab, C.R. Leemans, G.A. van Dongen, 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, Clinical cancer research : an official journal of the American Association for Cancer Research 12(20 Pt 1) (2006) 6064-72.
[171] M. Kursa, G.F. Walker, V. Roessler, M. Ogris, W. Roedl, R. Kircheis, E. Wagner, Novel shielded transferrin-polyethylene glycol-polyethylenimine/DNA complexes for systemic tumor-targeted gene transfer, Bioconjugate chemistry 14(1) (2003) 222-31.
[172] N.C. Bellocq, S.H. Pun, G.S. Jensen, M.E. Davis, Transferrin-containing, cyclodextrin polymer-based particles for tumor-targeted gene delivery, Bioconjugate chemistry 14(6) (2003) 1122-32.
[173] Z.M. Qian, H. Li, H. Sun, K. Ho, Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway, Pharmacological reviews 54(4) (2002) 561-87.
[174] A.C. Prost, F. Menegaux, P. Langlois, J.M. Vidal, M. Koulibaly, J.L. Jost, J.J. Duron, J.P. Chigot, P. Vayre, A. Aurengo, J.C. Legrand, G. Rosselin, C. Gespach, Differential transferrin receptor density in human colorectal cancer: A potential probe for diagnosis and therapy, International journal of oncology 13(4) (1998) 871-5.
[175] H. Shinohara, D. Fan, S. Ozawa, S. Yano, M. Van Arsdell, J.L. Viner, R. Beers, I. Pastan, I.J. Fidler, Site-specific expression of transferrin receptor by human colon cancer cells directly correlates with eradication by antitransferrin recombinant immunotoxin, International journal of oncology 17(4) (2000) 643-51.
[176] S. Ghatak, V.C. Hascall, F.G. Berger, M.M. Penas, C. Davis, E. Jabari, X. He, J.S. Norris, Y. Dang, R.R. Markwald, S. Misra, Tissue-specific shRNA delivery: a novel approach for gene therapy in cancer, Connective tissue research 49(3) (2008) 265-9.