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Understanding Hyaluronan Receptor (CD44) Interaction, HA-CD44 Activated Potential Targets in Cancer Therapeutics

Gul-e-Saba Chaudhry[1]*, Abdah Akim[2], Muhammad Naveed Zafar[3], Naila Safdar[4], Yeong Yik Sung[1], Tengku Sifzizul Tengku Muhammad[1]

[1]*Institute of Marine Biotechnology, University Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia*
[2]*Department of Biomedical Sciences, Universiti Putra Malaysia, Seri Kembangan, Selangor, Malaysia,*
[3]*Department of Chemistry, Quaid-i-Azam University, Islamabad, 45320, Pakistan*
[4]*Department of Environmental Sciences, Fatima Jinnah University, Rawalpindi, Pakistan*

Corresponding author
Name: Gul-e-Saba Chaudhry*
E-mail: sababiochem@gmail.com
        gul.saba@umt.edu.my
Contact: +609-6683810
Fax : +609-6683810

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Abstract

Cancer is a complex mechanism involving a series of cellular events. The glycoproteins such as hyaluronan (HA) are a significant element of extracellular matrix (ECM), involve in the onset of cancer developmental process. The pivotal roles of HA in cancer progression depend on dysregulated expression in various cancer. HA, also gain attention due to consideration as a primary ligand of CD44 receptor. The CD44, complex transmembrane receptor protein, due to alternative splicing in the transcription process, various CD44 isoforms predominantly exist. The overexpression of distinct CD44 isoforms (CD44v) standard (CD44s) depends on the tumour type and stage. The receptor proteins, CD44 engage in a variety of biological processes, including cell growth, apoptosis, migration, and angiogenesis. HA-CD44 interaction trigger survival pathways that result in cell proliferation, invasion ultimately complex metastasis. The interaction and binding of ligand-receptor HA-CD44 regulate the downstream cytoskeleton pathways involve in cell survival or cell death. Thus, targeting HA, CD44 (variant and standard) isoform, and HA-CD44 binding consider as an attractive and useful approach towards cancer therapeutics. The use of various inhibitors of HA, hyaluronidase (HYALs), and utilizing targeted Nano-delivery of anticancer agents and antibodies against CD44, peptides gives promising results *invitro* and *invivo*. However, they are in clinical trials with favourable and unfavourable outcomes, which reflects the need for various modifications in targeting agents and a better understanding of potential targets in tumour progression pathways.

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ECM, a potential source of cancer progression initiators

The extracellular environment is surrounding the cells considered as complex matrices (ECMs) necessary for support and communication between cells (Figure 1). The extracellular matrices composed of various glycol-proteins, which play an essential role in structure as well as functional aspects. The components include; i) proteoglycans, ii) proteins including collagens (fibrillar), iii) elastins proteins, iv) fibronectins proteins and v) laminins and the particular class of glycosaminoglycan (GAGs).[1-2] These ECM components could act as ligand molecules, and due to ligand-receptor binding, the signal transduction pathways triggered that lead to a variety of disease propagation and cytoskeleton and chromatin structures organization aswell[3-7]. Hyaluronan belongs to glycosaminoglycans, an integral hydrophilic part of the matrix. The surrounding proteins interact with integrin protein receptors where CD44 and RHAMM receptors got special attention due to cancer development and growth[8-9]. The components in matrix regulate the fundamental process in the cell, any dysregulation considered as essential hallmarks of cancer.[10-17] Additionally, ECM regulates the expression of stromal cells and indirectly the tumorigenic microenvironment. Any dysregulation leads to the development of inflammation and the generation of a disease microenvironment.[13] However, alteration in ECM is quite often due to variation in the expression level of matrix-degrading enzymes.[19] The integrin-FAK signaling due to modification of the ECM results in reactivating dormant tumour cells.[18] The alterations in ECM promotes the formation of a tumorigenic microenvironment leads to metastasis.[20]

A major component of ECM, Hyaluronan

Hyaluronan is linear repeating units of β glucuronic acid and β -N-acetyl glucosamine. HA, a principal component of the extracellular matrix in cancer cells, as well as normal cells, involved in various developmental process[30] Various enzymes involved in the synthesis of different molecular weight (MW) of Hyaluronan. HA produced by HA synthases (HAS1-3). The disaccharides unit of N-acetyl-D-glucosamine and D-glucuronic acid polymerize via β-(1-3)-glucuronidic bond.[26] Similarly, HA synthases HAS2 produces the larger HA more than 2 MDa; and HAS1 and HAS3 produces smaller HA with a range of less than 2 MDa.[27] Along with synthases, hyaluronidases (HYAL1-3) fragment the higher MW HA to medium MMW (via HYAL2) and lower LMW (via HYAL1) into oHA.[28-29] Alteration in a post-translational modification in the HAS2 produce mutations involve the replacement of one amino acid asparagines by another amino acid serines.[30-31] Also, hyaluronidases (HYALs) mutations have linked to tumour resistance in the naked mole-rat.[31]

HA involve in various developmental processes such as cell growth, proliferation, migration, tissue development, embryogenesis, and wound healing.[42] HA is the primary ligand of CD44 receptors.[30] Various modifications in the splicing mechanism and glycosylation process produce of CD44 variants. These variants are differentiated by structural and functional aspects mainly responsible for a pro-inflammatory activity via cell-ECM component interacts.[22-25] The HA-receptor mediated signal transduction occurs via CD44, the primary receptor for HA-binding and RHAMM (receptor for HA mediated mobility), toll-like receptors (TLR2, TLR4), hyaluronan receptor for endocytosis (HARE), hyaluronan-binding protein 1 (HABP1), and lymphatic vessel endothelial receptor for hyaluronan 1 (LYVE1).[42]

CD44 receptor

CD44 transmembrane protein, glycoprotein, encoded by 19 exons compromised gene positioned on chromosome 11 in humans.[53] The protein is a transmembrane protein with three visible domains includes outermost extracellular domain, ii) middle transmembrane domain, and iii) innermost intracellular domain. The N-terminal region is essential for cellular interaction with ECM components such as ligand HA and due to the presence of area responsible for the formation of the various variant. During post-transcription modifications, alternative splicing occurs in variant exons (nine exons) results in the formation of variant isoforms (CD44v). However, constant exons encode the CD44 standard (CD44s). A-part from hyaluronic acid (HA), CD44 receptor have an affinity towards other ligands including osteopontin, collagen, chondroitin, fibronectin, and sulfated proteoglycan.[54-58] The HA major extracellular matrix component is having a more binding affinity towards all isoforms of the CD44 receptor protein. The HA binds to CD44 (Figure 2) at extracellular domain causes conformational changes, which leads to activation of CD44 and recruitment of various other cytoplasmic and membrane-bound cytoplasmic proteins (adaptor molecules) trigger the downstream cell survival, growth and tumour progression pathways.[59]

HA-CD44 interaction pathways in cancer progression

HA interaction with CD44 receptor leads to cell survival, cell growth, invasion, and metastasis via signaling networks include; RhoGTPases and PI3K/AKT pathway[83, 148] and other chemoresistance pathways via ROK activation (Figure 3). CD44 receptor triggers cell survival promoted the tumour metastasis. The HA ligand upon binding with CD44 triggers signal cascade via activating the cytoplasmic domain of CD44 and which results in the recruitment of various protein a series of cell signaling events. The CD44-cytoplasmic domain activated proteins such as ankyrin, merlin, and ERM involve in actin polymerization mediated by ERM proteins results in cytoskeleton rearrangements for tumour invasion and migration.[84]

CD44 interaction is associated with metastasis in several tumours.[85-86] The role of CD44 in tumour progression quite clear; however, its involvement in trigger apoptosis inhibits tumour invasion and progression. CD44 in inducing apoptosis and reducing cell invasion is HA MW dependable but still contradictory which show the response differ in differ tissue also along with MW.[89-90] The high molecular weight HA blocked invasion and small molecular weight oligomerized HA to initiate apoptosis and via inhibition the activation of cell survival and growth pathway (AKT) in breast, colon and mammary cancer cells.[91-92]

Besides AKT, RhoGTPases triggers a cascade of events from cell surface receptors to downstream signals. RhoGTPases acts as response molecules which act in the response of signal or external stimuli and regulates the various process of cytoskeleton reorganization results in cell invasion and metastasis. RhoGTPases belong to Ras superfamily, includes RhoA, Rac1, and Cdc42. These Rho proteins exist in an inactive form and convert to active form upon binding of GTP molecules. The RhoGTPase involve in normal cell invasion and development. However, the overexpression in various human cancers link them to cancer progression and poor prognosis.[98-101] Additionally, an important kinase as Rho kinase (ROK) regulates the process of tumour invasion and metastasis via myosin light chain phosphatase. The myosin light chain phosphatase promotes the secretion of ECM degrading proteins as matrix metalloproteins (MMPs). The activation of MMPs via CD44 receptor protein help in invasion and also angiogenesis via degradation of collagen.[102-103] The MMPs include MMP-2 (type IV collagenase), MMP-9 (type V collagenase), MMP-3 (stromelysins) and MMP-1 (interstitial fibroblast-

type collagenase) degrade ECM materials, crucial in tumor invasion and metastases.

HA/CD44 interaction mediates cytoskeleton activation via critical RhoA signaling. RhoA regulation cellular functions via alteration of intracellular Ca^2 levels. The intracellular Ca^2 mobilization mediated via essential Phospholipase C (PLC). Upon activated, Phospholipase C (PLCs) converted to inositol trisphosphate (IP3) via PIP2 (hydrolyzed), which results in the release of Ca^2 from deposit storage. This released Ca^2 stimulates cytoskeleton rearrangement and eventually tumour progression.[104-106] In breast tumour cells[107-109], HA activation of CD44 leads to the expression of multidrug resistance gene (P-glycoprotein) and anti-apoptotic gene Bcl expression, which promotes tumour cell proliferation and survival.[107-109] Apoptosis is a highly regulated mechanism of cell death characterized by morphological and biochemical changes, including various enzymes proteins that lead to cell death and maintain cell growth.[110]

However, the recent proposed HA-CD44 induced tumour progression signaling events in cancer by Bourguignon 2019.[39] The interaction and binding of HA to CD44v upregulate Ras/Cdc42/Rac1/RhoA/NFkB expression, and various cytokines production involve in tumour cell survival, growth, invasion, and migration. As we already discussed, HA-AKT activation stimulates PI3K/AKT activation also the production of LncRNA (UCA1). LncRNA triggered tumour cell migration and invasion via ROK. Moreover, HA/CD44 also responsible for chemoresistance via activation of transcriptional factor-induced miR-10b, miRNA-21, and miR-302 expression. The JNK activity causes activation of c-Jun via phosphorylation. The activated c-Jun binds to the miR-21 promoter and induces the up-regulation of miR-21 expression. Similarly, HA-CD44 interaction triggers the complex formation include; Nanog, Stat-3, DROSHA, and p68. This complex bind to the miR-21 promoter region, resulting in the expression of miR-21. The miRNA expression upregulates IAP protein, involved in tumour cell survival, and chemoresistance. Activated miR-21 stimulates upregulation in RhoC and ROK-regulated tumour cell migration and invasion. In contrast, activated miRNA-302 expression involves in chemoresistance also due to HA-CD44 interaction. HA binding to CD44 promotes the recruitment and build association between cytoplasmic domain of CD44v3 and OCT4/SOX2/Nanog. This OCT4/SOX2/Nanog induce IAP (cIAP-1, cIAP-2, and XIAP) expression via the upregulation of miR-302 gene expression. IAPs involve in tumour cell growth and chemoresistance in tumour cells. Moreover, the cytoskeleton activation by miRNA-10b expression trigger via HA-CD44 interaction. HA binding to CD44 promotes c-Src activation via phosphorylation, the activated c-Src causes phosphorylation of Twist. The activated Twist act as TF, activate the mR-10b expression, promotes tumour cell migration, and invasion.

HA and CD44 role in Cancer regulation

The microenvironment outside the cell is a critical regulator of the fundamental growth of the cell. Alteration in cell growth trigger rapid growth and migration causes metastasis.[43] HA is biopolymer and the primary component of ECM, provides a hydrophilic part to the matrix and cellular support but gain special attention when it comes to cell survival, growth, and differentiation.[44] However, HA contributes to abrupt growth and invasion of cancer cells. Due to its hydrophilic nature, HA can form a protective lining around the tumour cell to protect the tumour cells from immune attack.[45-46] Any transcriptional or translational disruption in HA synthases or hyaluronidases can lead to over or fragmented production of HA. In several tumours, HAS and HYAL regulate the production of HA, which linked to tumour development and metastasis. Besides, HYAL fragmented HA some ROS-fragmented

HA also found to be involved in overproduction of HA in tumours growth, invasion and spreading.[47] The over-expression of synthase HAS2 and HAS3 results in enhancing HA overproduction, which triggers the production sarcomas and other melanoma cells.[48-49] HA trigger the formation of new blood vessels and also circulation of tumour cells via growth and invasion seen in some studies. HA induce angiogenesis and lymphatic invasion in tumour transgenic mouse models.[50-51] Apart from the above concern, interstitial fluid pressure cause by HA also highlighted in drug delivery. By using HYALs, it degrades HA, reduces the pressure, and improve the efficacy of therapeutics.[52]

The CD44 exists in various isoforms and gain much importance due to involvement in the pathogenesis of cancer. The variant form of CD44v could use as a biomarker for disease (cancer) prognosis. Several isoforms linked to specific cancer and play a role in cell growth and metastasis. The CD44v found to be involved in tumour progression and invasion. The overexpressed form of CD44v6 linked to invasion in endocrine-resistant breast cancer cells via activation of epidermal growth factor receptor (EGFR) signalling.[60] As cancer is a multistage disease, and the CD44v expression also have an association with various stages which ensure the precise involvement of CD44v in tumour progression. CD44v6v appearance only noticed in advanced stages of pancreatic cancer during the metastatic behaviour of cells and not visible in a benign tumour.[61] Increased expression of CD44v6 in pancreatic cancer link to metastasis.[62-63] The CD44v6 role in metastasis confirmed by transfection study confirms the involvement of CD44v6 in rat pancreatic adenocarcinoma.[64] Various treatment against CD44v6 linked metastasis include bFGF, EGF, TNFα, and TGF-β1(growth factors and cytokines) unable to reduce the surface expression of CD44v6 receptor. Similarly, Interferon (IFN) γ also not wholly inhibit the expression of CD44v6.[65] The studies also confirm the CD44v as a survival marker. The presence of enhancing expression of CD44v6 link to shorter survival time as compared to tumours deprived of CD44v6.[66] Also, the positive expression of CD44v6 and CD44v9 together link to metastasis with low survival rate.[67-68] Also, the standard form of CD44 regulates the metastasis in tumour cells. Studies show that down-regulation of CD44s detected in human metastasis tissue.

Silencing the expression of CD44v9 (via short hairpin RNA) overturned the gathering of various proteins (HSP90, PI3K, and others) into lipid raft-like structures and attenuate the downstream signal cascade of tumour progression.[71] The isoforms CD44v6 and CD44v9 overexpressed in prostate cancer (PC3-epithelial cells).[69] The function of CD44v6 evaluated using small interfering RNA (siRNA) in prostate cancer cells and Knock-down of CD44v6 linked to increasing chemo/radiosensitivity.[70] Similarly, overexpression of CD44v3 isoform triggered a substantial increase in cell migration. In neck squamous cell carcinoma (HNSCC), elevated CD44v3 levels noticed.[72] The inhibitor study using, anti-CD44v3 antibody shows the reverse cisplatin resistance and decrease cell proliferation.[73] In colorectal cancer, CD44v6 and CD44v10 overexpression detected which linked to poor prognosis, cell migration and metastasis.[74-76] Various cytokines regulate CD44v6 expression, which activates Wnt/β-catenin pathway and promotes migration and metastasis.[77] Previous study shows that CD44v operates in a specific and overlapping manner and by studying CD44v help to understand the various variant as a potential target in drug delivery. The variant form CD44v8-10 involve in the protection of gastrointestinal cancer cells against ROS (reactive oxygen species), CD44v4-10 and CD44v8-10 promoted adenoma and gastric tumour initiation.[78-79] Similarly, another variant CD44v9 found to be overexpression gastric adenocarcinoma.[80] Overexpression of CD44v6 activate the phosphorylation of AKT, autophagy flux, and ERKs which result in cell survival and growth. The colon cancer cells treated with chemotherapy drugs with enhancing the expression of CD44v6 reduce cytotoxicity.[82]

Strategies to target HA and CD44 interaction

HA as a therapeutic target

The rich level HA in various tumours and metastatic cancers responsible for cell growth proliferation, invasion, angiogenesis, metastasis along with acquiring resistance in response to anticancer agents. HA abundance in pancreatic ductal adenocarcinoma (PDAC) links to a lower survival rate. Hyaluronan interaction with CD44 results in cell growth, invasion, and metastasis. To prevent the interaction and downstream pathways of cancer propagation, Hyaluronan ligand, CD44 receptor with its variants, and HA-CD44 interaction could consider as important targets in drug development. In the case of HA, the strategies are i) Hyaluronidases along with chemotherapeutics, ii) use of Hyaluronan oligosaccharides (oHA) to induce apoptosis, iii) Inhibitors of Hyaluronan and iv) Hyaluronan-base drugs.

HYALs are well-known enzymes that can fragment HA. Previous studies demonstrate that the expression of wild type HYAL-1 (HYAL-1-v1) induces apoptosis via G2-M arrest in bladder cancer cells.[110] However, due to adverse impact as an anti-adhesive on normal tissue, HYALs have limitations and side effects.[111] Interestingly, commercial bovine testicular or bacterial HYAL act as an anti-adhesive compound on EMT-6 tumour spheroids improve the chemosensitivity by increasing the accessibility.[112] In regards to drug therapeutics, HA also increase the intestinal fluid pressure and hinder the drug penetration in pancreatic ductal adenocarcinomas. The application of PEGPH20 increases vessel size and recover the increased interstitial fluid. The treatment of Gem+PEGPH20 in placebo control trials in KPC improves the survival rate and significant reduction of metastatic tumour burden.[113] Interestingly, hyaluronan oligosaccharides (oHA) trigger the induction of apoptosis in lymphoma cell lines via inhibition of downstream cancer survival pathways. The oligosaccharides (oHA) inhibited PIP3 production, involve in PI3-K activation and results in deactivation of Akt (via reduced phosphorylation) without involving NF-kB activation in lymphoma cells.

Hyaluronan (HA) found to be abundant in most cancers. Previous studies show that 4-methylumbelliferone (MU) inhibitor of HA synthesis, deplete uridine diphosphate glucuronic acid, and the down-regulation of HAS human skin fibroblasts. Similarly, MU inhibits HA production in pancreatic cancer cells (KP1-NL) and decreases liver metastases in mice injected with pancreatic cancer cells.[114] 4-Methylumbelliferone was then later shown to inhibit HA production and to have anticancer activities in mouse models of various cancers. However, the inhibition depends on MW of HA, as HMW-HA revoke the inhibitory and non-metastatic effects of MU on MIA PaCa-2 cells. Studies have shown that MU inhibits HA synthesis in at least two ways. First, MU depletes UDP-GlcUA, which is a precursor of HA.[115] Second, MU reduces the expression of HAS mRNA.[116] However, no studies have described the mechanisms through which MU reduces HAS mRNA expression. However, more studies need to do as the MU in inhibition of overall HAS mRNA and effect on CD44 expression still not clear yet. Some reports have indicated that MU suppresses HAS mRNA expression,[117-119] whereas another report stated that HAS mRNA expression upregulated.[120] However, its right to be said that the effect of MU on expression depends on various cell types with specific up-regulation or down-regulation.

Hyaluronan, a versatile biopolymer, increases the solubility of poorly soluble anticancer drugs. Hyaluronan improve the efficacy of anticancer drugs with improving solubility and biocompatibility via various mode as follows; i) HA-conjugate drugs, ii) HA-encapsulated drugs, iii) gagomers, iv) micelles and v) Nanocarriers. The paclitaxel (PTX), the potent antimitotic agent inhibits cell growth, but due to

poor water solubility limits its application in drug therapeutics. The HA-PTX conjugated drug name as HYTAD1-p20 with improve solubility and biocompatibility in the bladder and other cancers.[121-122] Similarly, HA-conjugated-PTX-N-hydroxysuccinimide ester (PTX-NHS) and HA-SN-38 (CPT11 (irinotecan) metabolite) used against CD44 expressing cell line (colon, breast, gastric and others) reduced tumour cell growth and metastasis.[123-127]

The side effects of potential anticancer also hinder the application of potential compounds in drug delivery. However, *in-vitro* and *in-vivo* HA encapsulated drugs, HA-5-fluorouracil (HyFIVETM) and HA-doxorubicin (5- FU) show reasonable cytotoxic efficacy, undergoing clinical trials.[128-129] Self-assemble Lipid molecule coated with hyaluronan (HMW) as liposomes or lipoplexes loaded drug deliver plasmid DNA and siRNA (small interfering RNA) also used against several cancers including head and cancer, expressed CD44 receptor.[130-135] The HA micelles are self-organized structure, HA polymer (carboxyl group) conjugate to another polymer, i.e., PEG (polyethylene) amino group. HA-micelles with paclitaxel (HA-PTX), doxorubicin (HA-DOX) HA-DOX, and HA-salinomycin produced effective cytotoxicity on DC44 expressing cells.[136-138] Nanoparticles act as Nanocarriers for delivery the therapeutic agent to target site hold promising features include; dots (quantum and nanodots, nanodots graphene,[139,142,141] carbon nanotubes,[140] and nanoparticles gold and iron oxide nanoparticles.[143-144] and silica nanoparticles,[145] and they have found to acquire novel characteristics after their conjugation with HA.[146] Due to improve the efficacy of HA conjugated nanocarriers delivered anticancer drugs such as HA-epirubicin,[147] HA-DOX,[133] HA-PTX,[149, 186] and HA-MMC,[147] as well as HA-siRNA make HA attractive polymer in drug delivery. Similarly, drug-resistant tumor cell treatment improved via combination therapy of HA (HA–CUR/DOX-NPs). The improve efficacy was achieved via down-regulate the expression of P-gp and induce apoptosis (regulating Bax/Bcl-2),[150] thus increasing the therapeutic effect.

CD44 as a therapeutic target

The CD44 essential receptor protein involves in cancer cell growth, survival, and metastasis in the majority of cancer. HA is the primary ligand of CD44 receptor; upon binding, to the receptor, the ligand-receptor interaction activates the down-stream cancer pathways.[151] CD44 do exist in various isoforms of CD44v along with CD44s play a significant role progression of cancer and prognosis.[152-154]

Several studies regarding the role of CD44s and CD44v in the onset of cancer propagation. However, the function of CD44 isoforms differs in various cancers and different outcomes CD44 isoforms. As in one study, the CD44s (standard isoform) aberrant expression or loss is linked to an increased risk of metastasis, as observed in malignant breast cancer.[153] Along with this, CD44s indorsed invasion in breast tumour and liver metastasis.[154] Amongst isoforms of CD44, CD44v (3, 6 and, 7 and 8) linked to the propagation of breast cancer.[52-53] In lymph node metastasis, CD44v6 expression was upregulated, while both CD44v (v3 and v6) were upregulated invasive cribriform breast tumours. However, the downregulation in CD44v4 in the invasive cribriform breast but upregulated in trans-endothelial cell metastasis. More research needs to done further to clarify the specific role of CD44s and CD44v variants.

Antibodies to inhibit the CD44 mediated cancer progression also show remarkable results in inhibition of HA-CD44 downstream pathways. The binding of antibodies (anti-CD44) to CD44 variants that expressed in a variety of cancers interfere with the binding of HA to CD44 receptor cells and disrupt CD44 matrix interactions. This interruption cause alteration in CD44, triggered cancer progression pathways as well as induce apoptosis.[160] The use of several peptides, The Pep-1 and BH-P, inhibit the proliferation of melanoma tumour cells in nude mice xenograft models.[165] The CD44v6 isoforms identified as markers of CSCs in colon cancer. As a receptor CD44v6 involve in matrix assembly and link with metastasis.[167-168] Therefore, targeting CD44v6 in colon cancer is a promising therapeutic approach. The siRNA/shRNA use in the inhibition of CD44 receptor expression successfully blocks the interaction (HA-CD44) and induction of cancer progression pathways. The using cell-specific shRNA delivery approach to inhibit HA-CD44v6 interaction inhibited distant growth in colon tumour and downstream tumour progression pathways.[173, 185]

The double approach in targeting CD44 by using an antibody as well as cytotoxic drugs have studied. The cytotoxic drug (mertansine) conjugated to an antibody against CD44v6 used in early phase clinical trials.[161-162] Similarly, another drug bivatuzumab (anti-CD44v6 monoclonal antibody) along with combinational drug mertansine also undergoing clinical trials.[163-164] However, due to toxicity towards healthy cells (CD44v6 positive expression), the phase I clinical trials did not consider as successful. By studying this limitation of antibody therapy, the quest for alternative therapies increased.

Natural products show the remarkable result in cancer therapeutics. Besides various synthetic inhibitor, different natural potential compounds such as silibinin, zerumbone, gemini, curcumin, epigallocatechin gallate, and apigenin down-regulate the expression of CD44 isoforms.[174] Silibinin bioactive component, isolated from plant *Silybum marianum* used in phase II clinical trials in prostate cancer and. Silibinin inhibits cell growth in a time-dependent manner in BxPC3 and Panc1 pancreatic cancer cell lines. The active compound (Silibinin) act as a transcription regulator in CD44 expression decreased the expression of levels of CD44v7-10 RNA and well as translated protein in prostate cancer cells.[178-180] Another potential regulator of the CD44 receptor cell, Zerumbone, belongs to the terpene class of phytochemicals. The expression level of CD44 in breast cancer cells downregulated by Zerumbone via inhibiting phosphorylation of STAT3 which involve in EGF-induced CD44 expression.[175-177] Similarly, members of vitamin D family Gemini also act as a transcription regulator. As a transcription regulator, it downregulates the expression of CD44 protein level, inhibited the invasion and metastasis in mice model.[181-182] The enhance synergistic effect of curcumin (potential active compound) and epigallocatechin gallate (catechin) inhibit the expression of the CD44 receptor on cancer cells. The combine approaches successfully reduced CD44- positive (CSC) and downregulate the phosphorylation of STAT3 expression.[183] Also, potent phytochemical apigenin belongs to flavonoid group improve the cell survival in a prostate cancer patient by dose-dependent inhibition in expression of CD44 in CSC by upregulating the expression of p21 and p27.[184] Along with previous reports and our research various natural extract and fractions rich of potential phytochemicals and targeted delivery produce effective cytotoxicity via induction of apoptosis (*in-vitro*).[187-195] However, the study of receptor-ligand molecular interactions required to understand the inhibition of anti-apoptosis pathways involves in cancer progression.

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Conflict of interest

Authors have no conflict of interest.

Ethical Issue

Not applicable.

Conclusion

HA versatile biopolymer, the primary ligand of CD44 receptor cells. HA-CD44 interaction and binding can regulate cytoskeleton protein activation in various tumour cells. The cytoskeleton activation triggers different downstream pathways involved in tumour propagation. The HA and CD44, along with signaling proteins in the cytoskeleton could be suitable markers for cancer drug therapeutics. The CD44 exists in various isoforms and regulate the cell survival signaling pathways in a variety of cancers. The alternative splicing triggers the variant isoform of CD44, and the expression level is a critical regulator in various cancers. Here, we discussed the understanding of the HA-CD44 interaction/binding and discussed different activated pathways involve in cancer cell growth, survival, and metastasis via signaling networks include; RhoGTPases and PI3K/AKT pathway. HA-CD44 activation stimulates AKT activation via ROK (kinase) triggered tumour cell migration and invasion. Also, HA/CD44 also responsible for chemoresistance via the up-regulation of miR-10b, miRNA-21, and miR-302 expression. The overexpression of various CD44 isoforms depends on different cell types and stages—the fate of cancer cells regulated by isoforms, CD44v, and standard CD44s. Thus, targeting HA by using HYALs, targeting HA synthase and HA-inhibitors, useful strategy to inhibit HA trigger CD44-signaling pathways. Similarly, targeting CD44(variant) by application of CD44 anti-bodies, peptides HA-Nano carries and natural CD44 negative regulator showing optimal success. Thus, extensive studies show that CD44 has the potential to be an effective drug therapeutics.

References

1. Theocharis AD, Skandalis SS, Tzanakakis GN, Karamanos NK. Proteoglycans in health and disease: novel roles for proteoglycans in malignancy and their pharmacological targeting. *FEBS J* 2010;277(19): 3904–3923.   doi:10.1111/j.1742-4658.2010.07800.x

2. Schaefer L, and Schaefer RM. Proteoglycans: from structural compounds to signaling molecules. *Cell and Tissue Res* 2010; 339(1): 237–246, 2010. doi:10.1007/s00441-009-0821-y

3. Xu R, Boudreau A, Bissell MJ. Tissue architecture and function: dynamic reciprocity via extra- and intra-cellular matrices. *Cancer and Metastasis Rev* 2009; 28(1-2): 167–176

4. Wiseman BS, Sternlicht MD, Lund LR. Alexander CM, Mott J, Bissell MJ, et al. Site-specific inductive and inhibitory activities of MMP-2 and MMP-3 orchestrate mammary gland branching morphogenesis. *J Cell Biol* 2003; 162(6): 1123–1133.   doi:10.1083/jcb.200302090

5. Stickens D, Behonick DJ, Ortegaetal N. Heyer B, Hartenstein, Yu Y, el al. Altered endochondral bone development in matrix metalloproteinase 13-deficient mice. *Development* 2004; 131(23): 5883–5895. doi:10.1242/dev.01461

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

6.   Rebustini IT, Myers C, Lassiter KS. Surmak A, Szabova L, Holmbeck K, et al. MT2-MMP-dependent release of collagen IV NC1 domains regulates sub- mandibular gland branching morphogenesis. *Developmental Cell* 2009;17(4): 482–493. doi:10.1016/j.devcel.2009.07.016

7.   Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb Perspect Biol* 2011; 3(12): a005058. doi:10.1101/cshperspect. a005058

8.   Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;110(6): 673–687. doi:10.1016/s0092-8674(02)00971-6

9.   Maxwell CA, McCarthy J, Turley E. Cell-surface and mitotic-spindle RHAMM: moonlighting or dual oncogenic functions?. *J Cell Sci* 2008;121(7): 925–932. doi:10.1242/jcs.022038

10.   Nikitovic D, Kouvidi K, Karamanos NK, Tzanakakis GN. The roles of hyaluronan/RHAMM/CD44 and their respective interactions along the insidious pathways of fibrosarcoma progression. *Biomed Res Int*. 2013;2013: 929531. doi:10.1155/2013/929531

11.   Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ. Collagen reorganization at the tumor- stromal interface facilitates local invasion. *BMC Medicine* 2006; 4: 38-38. doi:10.1186/1741-7015-4-38

12. Levental KR, Yu H, Kass L. Lakins JN, Egeblad M, Erler JT, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* 2009; 139(5): 891–906. doi:10.1016/j.cell.2009.10.027

13. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. *J Cell Biol* 2012;196(4): 395–406.   doi:10.1083/jcb.201102147

14. Nikitovic D, Chatzinikolaou G, Tsiaoussis J, Tsatsakis A, Karamanos NK, Tzanakakis GN. Insights into targeting colon cancer cell fate at the level of proteoglycans/glycosaminoglycans. *Curr Med Chem* 2012;19(25): 4247– 4258. doi:10.2174/092986712802884268

15. Gialeli C, Theocharis AD, Karamanos NK, Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J* 2011; 278(1): 16–27. doi:10.1111/j.1742-4658.2010.07919.x

16. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature* 2004; 432(7015): 332–337. doi:10.1038/nature03096

17. Cirri P, Chiarugi P. Cancer-associated-fibroblasts and tumour cells: a diabolic liaison driving cancer progression. *Cancer Metastasis Rev* 2012; 31(1-2): 195–208. doi:10.1007/s10555-011-9340-x

18. Barkan D, Green JE, Chambers AF. Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. *European J Cancer* 2010; 46(7): 1181–1188. doi: 10.1016/j.ejca.2010.02.027

19. Ilan N, Elkin M, Vlodavsky I. Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. *Int J Biochem Cell Biol* 2006;38(12): 2018–2039. doi:10.1016/j.biocel.2006.06.004

20. Skandalis SS, Aletras AJ, Gialeli C, Theocharis AD, Afratis N, Tzanakakis GN, et al. Targeting the tumor proteasome as a mechanism to control the synthesis and bioactivity of matrix macromolecules. *Current Medicinal Chemistry* 2012;12(8) :1068–1082.

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

doi:10.2174/156652412802480943

21. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. *Cell* 1990;61(7): 1303–1313. doi:10. 1016/0092- 8674(90)90694- A.

22. Misra S, Heldin P, Hascall VC, Karamanos NK, Skandalis SS, Markwald RR, et al. Hyaluronan-CD44 interactions as potential targets for cancer therapy. *FEBS J* 2011 278(9): 1429–43. doi:10.1111/j.1742-4658.2011. 08071.x

23. Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 2003; 4(1): 33–45. doi:10.1038/nrm1004

24. Naor D, Nedvetzki S, Golan I, Melnik L, Faitelson Y. CD44 in cancer. *Crit Rev Clin Lab Sci* 2008; 39(6): 527–579. doi:10.1080/10408360290795574

25. Naor D, Sionov RV, Ish-Shalom D. CD44: structure, function, and association with the malignant process. *Adv Cancer Res* 1997;71: 241–319. doi:10.1016/ S0065- 230X (08)60101-3

26. Vigetti D, Viola M, Karousou E, De Luca G, Passi A. Metabolic control of hyaluronan synthases. *Matrix Biol* 2014;35: 8–13. doi: 10.1016/j.matbio.2013.10.002

27. Itano N, Sawai T, Yoshida M, Lenas P, Yamada Y, Imagawa M, et al. Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. *J Biol. Chem* 1999; 274(35): 25085–25092. doi:10.1074/jbc.274.35.25085

28. Maharjan AS, Pilling D, Gomer RH. High and low molecular weight hyaluronic acid differentially regulate human fibrocyte differentiation. *PLoS ONE* 2011;6(10): e26078. doi:10.1371/journal.pone.0026078

29. Stern, R. Devising a pathway for hyaluronan catabolism: Are we there yet? *Glycobiology* 2003; 13(12): 105–115. doi:10.1093/glycob/cwg112

30. Bohaumilitzky L, Huber AK, Stork EM, Wengert S, Woelfl F, Boehm H. A trickster in disguise: Hyaluronan's ambivalent roles in the matrix. *Front Oncol* 2017; *7*: 242. doi:10.3389/fonc.2017.00242

31. Tian X, Azpurua J, Hine C, Vaidya A, Myakishev-Rempel M, Ablaeva J, et al. High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature* 2013; 499(7458): 346–349. doi:10.1038/nature12234

32. Underhill CB, Green SJ, Comoglio PM, Tarone G. The hyaluronate receptor is identical to a glycoprotein of Mr 85,000 (gp85) as shown by a monoclonal antibody that interferes with binding activity. *J Biol Chem* 1987; 262(27): 13142–6.

33. Stamenkovic I, Amiot M, Pesando JM, Seed B. A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. *Cell* 1989; 56(6): 1057–1062. doi:10.1016/0092-8674(89)90638-7

34. Lesley J, Schulte R, Hyman R. Binding of hyaluronic acid to lymphoid cell lines is inhibited by monoclonal antibodies against Pgp-1. *Exp Cell Res* 1990;187: 224–33. doi:10.1016/0014-4827(90)90085-O

35. Miyake K, Underhill CB, Lesley J, Kincade PW. Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. *J Exp Med* 1990 172(1): 69–75. doi:10.1084/jem.172.1.69

36. Ghatak S, Hascall VC, Markwald RR, Misra S. Stromal hyaluronan interaction with epithelial CD44 variants promotes prostate cancer invasiveness by augmenting expression and function of hepatocyte growth factor and androgen receptor. *J Biol Chem* 2010; 285(26):19821–19832. doi:10.1074/jbc.M110. 104273

37. Hardwick C, Hoare K, Owens R, Hohn HP, Hook M, Moore D, et al. Molecular cloning of a novel hyaluronan receptor that mediates tumor motility. *J Cell Biol* 1992; 117(6): 1343–1350. doi:10.1083/jcb.117.6.1343

38. Turley EA, Austen L, Vandeligt K, Clary C. Hyaluronan and a cell-associated hyaluronan binding

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

protein regulate the locomotion of ras-transformed cells. *J Cell Biol* 1991;112(5): 1041–7. doi:10.1083/jcb.112.5.1041

39. Bourguignon LYW. Matrix Hyaluronan-CD44 Interaction Activates MicroRNA and LncRNA Signaling Associated with Chemoresistance, Invasion, and Tumor Progression. *Front Oncol*. 2019;9: 492. doi:10.3389/fonc.2019.00492

40. Tammi MI, Day AJ, Turley EA. Hyaluronan and homeostasis: a balancing act. *J Biol Chem* 2002;277: 4581–4. doi:10.1074/jbc.R100037200

41. Jiang D, Liang J, Noble PW. Hyaluronan as an immune regulator in human diseases. *Physiol Rev* 2011;91(1): 221-264. doi:10.1152/physrev.00052.2009

42. Bissell MJ, Radisky DC, Rizki A, Weaver VM, Petersen OW. The organizing principle: micro environmental influences in the normal and malignant breast. Differentiation. 2002 70: 537–46. doi:10.1046/j.1432- 0436.2002.700907.x

43. Jiang WG, Sanders AJ, Katoh M, Ungefroren H, Gieseler F, Prince M. et al. Tissue invasion and metastasis: Molecular, biological and clinical perspectives. *Semin Cancer Biol*. 2015;35: 244-S275. doi:10.1016/j.semcancer.2015.03.008

44. Laurent TC, Fraser JR. Hyaluronan. *FASEB J* 1992; 6(7): 2397–2404.

45. McBride WH, Bard JB. Hyaluronidase-sensitive halos around adherent cells. Their role in blocking lymphocyte-mediated cytolysis. *J Exp Med* 1979;149: 507–15. doi:10.1084/jem.149.2.507.

46. Gately CL, Muul LM, Greenwood MA, Papazoglou S, Dick SJ, Kornblith PL, et al. In vitro studies on the cell-mediated immune response to human brain tumors. II. Leukocyte-induced coats of glycosaminoglycan increase the resistance of glioma cells to cellular immune attack. *J Immunol* 1984; 133(6): 3387–95.

47. Stern R, Jedrzejas MJ. Hyaluronidases: their genomics, structures, and mechanisms of action. *Chem Rev* 2006;106(3): 818–839. doi:10.1021/cr050247k.

48. Kosaki R, Watanabe K, Yamaguchi Y. Overproduction of hyaluronan by expression of the hyaluronan synthase Has2 enhances anchorage-independent growth and tumorigenicity. *Cancer Res* 1999; 59(5): 1141–1145.

49. Liu N, Gao F, Han Z, Xu X, Underhill CB, Zhang L. Hyaluronan synthase 3 overexpression promotes the growth of TSU prostate cancer cells. *Cancer Res* 2001; 61(13): 5207–5214.

50. Itano N, Zhuo L, Kimata K. Impact of the hyaluronan-rich tumor microenvironment on cancer initiation and progression. *Cancer Sci* 2008;99(9): 1720–5. doi:10.1111/j.1349-7006.2008.00885.x

51. West DC, Kumar S. The effect of hyaluronate and its oligosaccharides on endothelial cell proliferation and monolayer integrity. *Exp Cell Res* 1989; 183(1): 179–196. doi:10.1016/0014-4827(89)90428- X

52. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012;21(3): 418–29. doi:10. 1016/j.ccr.2012.01.007

53. Naor D, Nedvetzki S, Golan I, Melnik L, Faitelson Y. CD44 in cancer. *Crit Rev Clin Lab Sci*. 2002; 39(6): 527–79. doi:10.1080/10408360290795574

54. Weber GF, Ashkar S, Glimcher MJ, Cantor H. Receptor-ligand interaction between CD44 and

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

osteopontin (Eta-1). *Science*. 1996; 271(5248): 509–12. doi:10.1126/science.271.5248.509

55. Faassen AE, Schrager JA, Klein DJ, Oegema TR, Couchman JR, McCarthy JB. A cell surface chondroitin sulfate proteoglycan, immunologically related to CD44, is involved in type I collagen-mediated melanoma cell motility and invasion. *J Cell Biol* 1992; 116(2): 521–531. doi:10.1083/jcb.116.2.521

56. Knutson JR, Iida J, Fields GB, McCarthy JB. CD44/chondroitin sulfate proteoglycan and alpha 2 beta 1 integrin mediate human melanoma cell migration on type IV collagen and invasion of basement membranes. *Mol Biol Cell* 1996;7(3): 383–96. doi:10.1091/mbc.7.3.383

57. Jalkanen M, Elenius K, Salmivirta M. Syndecan—a cell surface proteoglycan that selectively binds extracellular effector molecules. *Adv Exp Med Biol*. 1992; 313: 79–85. doi:10.1007/978-1-4899-2444-5-8

58. Toyama-Sorimachi N, Sorimachi H, Tobita Y, Kitamura F, Yagita H, Suzuki K, et al. A novel ligand for CD44 is serglycin, a hematopoietic cell lineage-specific proteoglycan. Possible involvement in lymphoid cell adherence and activation. *J Biol Chem* 1995; 270(13): 7437–44. doi:10.1074/jbc.270.13.7437

59. Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 2003; 4(1): 33–45. doi:10.1038/nrm1004

60. Bellerby R, Smith C, Kyme S, Gee J, Gunthert U, Green A, et al. Overexpression of specific CD44 isoforms is associated with aggressive cell features in acquired endocrine resistance. *Front Oncol* 2016; 6:145. doi:10.3389/fonc.2016.00145

61. Wielenga VJ, Heider KH, Offerhaus GJ, Adolf GR, van den Berg FM, Ponta H, et al. Expression of CD44 variant proteins in human colorectal cancer is related to tumor progression. *Cancer Res* 1993; 53(20): 4754–6.

62. Rall CJ, Rustgi AK. CD44 isoform expression in primary and metastatic pancreatic adenocarcinoma. *Cancer Res* 1995; 55(9): 1831–5.

63. Castella EM, Ariza A, Ojanguren I, Mate JL, Roca X, Fernandez-Vasalo A, et al. Differential expression of CD44v6 in adenocarcinoma of the pancreas: an immunohistochemical study. *Virchows Arch* 1996; 429(4–5): 191–195.

64. Gunthert U, Hofmann M, Rudy W, Reber S, Zoller M, Haussmann I, et al. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell* 1991; 65(1): 13–24. doi:10.1016/0092-8674(91)90403-l

65. Ringel J, Jesnowski R, Schmidt C, Ringel J, Kohler HJ, Rychly J, et al. CD44 in normal human pancreas and pancreatic carcinoma cell lines. *Teratog Carcinog Mutagen* 2001;21(1): 97–106. doi.org/10.1002/1520-6866(2001)21:1<97:AID-TCM9>3.0.CO;2-O

66. Gotoda T, Matsumura Y, Kondo H, Saitoh D, Shimada Y, Kosuge T, et al. Expression of CD44 variants and its association with survival in pancreatic cancer. *Jpn J Cancer Res* 1998; 89(10): 1033–40. doi: 10.1111/j.1349-7006. 1998.tb00493.x

67. Zhou G, Chiu D, Qin D, Niu L, Cai J, He L, et al. Detection and clinical significance of CD44v6 and integrin-beta1 in pancreatic cancer patients using a triplex real-time RT-PCR assay. *Appl Biochem Biotechnol* 2012;167(8): 2257–2268. doi:10.1007/s12010-012-9752-2

68. Li Z, Chen K, Jiang P, Zhang X, Li X, Li Z. CD44v/CD44s expression patterns are associated with the survival of pancreatic carcinoma patients. *Diagn Pathol* 2014; 9: 79. doi: 10.1186/1746-1596-9-79

69. Hernandez JR, Kim JJ, Verdone JE, Liu X, Torga G, Pienta KJ, et al. Alternative CD44 splicing identifies epithelial prostate cancer cells from the mesenchymal counterparts. *Med Oncol*

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

2015; 32(5): 159.   doi:10.1007/s12032-015-0593-z

70. Ni J, Cozzi PJ, Hao JL, Beretov J, Chang L, Duan W, et al. CD44 variant 6 is associated with prostate cancer metastasis and chemo-/radioresistance. *Prostate* 2014; 74(6): 602–617. doi:10.1002/pros.22775

71. Ghatak S, Hascall VC, Markwald RR, Misra S. Stromal hyaluronan interaction with epithelial CD44 variants promotes prostate cancer invasiveness by augmenting expression and function of hepatocyte growth factor and androgen receptor. *J Biol Chem* 2010; 285(26): 19821–19832.   doi:10.1074/jbc.M110.104273

72. Reategui EP, de Mayolo AA, Das PM, Astor FC, Singal R, Hamilton KL, et al. Characterization of CD44v3- containing isoforms in head and neck cancer. *Cancer Biol Ther* 2006; 5(9): 1163–1168.   doi:10.4161/cbt.5.9.3065

73. Wang SJ, Wreesmann VB, Bourguignon LY. Association of CD44 V3- containing isoforms with tumor cell growth, migration, matrix metalloproteinase expression, and lymph node metastasis in head and neck cancer. *Head Neck* 2007; 29(6): 550–558. doi:10.1002/hed.20544

74. Mulder JW, Kruyt PM, Sewnath M, Oosting J, Seldenrijk CA, Weidema WF, et al. Colorectal cancer prognosis and expression of exon- v6-containing CD44 proteins. *Lancet* 1994; 344(8935): 1470–2. doi:10.1016/s0140-6736(94)90290-9

75. Kaufmann M, Heider KH, Sinn HP, von Minckwitz G, Ponta H, Herrlich P. CD44 variant exon epitopes in primary breast cancer and length of survival. *Lancet* 1995; 345(8950): 615–9. doi:10.1016/s0140-6736(95)90521-9

76. Yamao T, Matsumura Y, Shimada Y, Moriya Y, Sugihara K, Akasu T, et al. Abnormal expression of CD44 variants in the exfoliated cells in the feces of patients with colorectal cancer. *Gastroenterology* 1998; 114(6): 1196–1205. doi:10.1016/s0016-5085(98)70425-1

77. Todaro M, Gaggianesi M, Catalano V, Benfante A, Iovino F, Biffoni M, et al. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell* 2014; 14(3): 342–56. doi: 10.1016/j.stem.2014.01.009

78. Ishimoto T, Nagano O, Yae T, Tamada M, Motohara T, Oshima H, et al. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc (−) and thereby promotes tumor growth. *Cancer Cell* 2011; 19(3): 387–400. doi: 10.1016/j.ccr.2011.01.038

79. Zeilstra J, Joosten SP, van Andel H, Tolg C, Berns A, Snoek M, et al. Stem cell CD44v isoforms promote intestinal cancer formation in Apc(min) mice downstream of Wnt signaling. *Oncogene* 2014; 33(5): 665–70. doi:10.1038/onc.2012.611

80. Mayer B, Jauch KW, Gunthert U, Figdor CG, Schildberg FW, Funke I, et al. De-novo expression of CD44 and survival in gastric cancer. *Lancet* 1993; 342(8878): 1019–22.   doi:10.1016/0140-6736(93)92879-x

81. Ishimoto T, Oshima H, Oshima M, Kai K, Torii R, Masuko T, et al. CD44+ slow-cycling tumor cell expansion is triggered by cooperative actions of Wnt and prostaglandin E2 in gastric tumorigenesis. *Cancer Sci* 2010; 101(3): 673–678. doi:10.1111/j.1349-7006.2009.01430.x

82. Lv L, Liu HG, Dong SY, Yang F, Wang QX, Guo GL, et al. Upregulation of CD44v6 contributes to

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

acquired chemoresistance via the modulation of autophagy in colon cancer SW480 cells. *Tumour Biol* 2016;37(7): 8811–24. doi:10.1007/s13277-015-4755-6

83. Okamoto I, Kawano Y, Murakami D, Sasayama T, Araki N, Miki T, et al. Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. *J Cell Biol* 2001; 155(5): 755-762.   doi:10.1083/jcb.200108159

84. Bretscher A, Edwards K, Fehon RG. ERM proteins and merlin: integrators at the cell cortex. *Nat Rev Mol Cell Biol* 2002; 3(8): 586-99. doi:10.1038/nrm882

85. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. *Genes Dev* 2000; 14(2): 163-176.

86. Yu Q, Stamenkovic I. Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev* 1999;13(1): 35-48. doi: 10.1101/gad.13.1.35

87. Toole BP, Wight TN, Tammi MI. Hyaluronan-Cell Interactions in Cancer and Vascular Disease. *J Biol Chem* 2002;277(7): 4593-6.   doi:10.1074/jbc.R100039200

88. Ahrens T, Assmann V, Fieber C, Termeer CC, Herrlich P, Hofmann M, et al. CD44 is the Principal Mediator of Hyaluronic-Acid-Induced Melanoma Cell Proliferation. *J Invest Dermatol* 2001;116(1): 93-101. doi:10.1046/j.1523-1747.2001.00236.x

89. Deed R, Rooney P, Kumar P, Norton JD, Smith J, Freemont AJ, et al. Early-response gene signalling is induced by angiogenic oligosaccharides of hyaluronan in endothelial cells. Inhibition by non-angiogenic, high-molecular-weight hyaluronan. *Int J Cancer* 1997;71(2): 251-6. doi:10.1002/(sici)1097-0215(19970410)71:2<251: aid-ijc21>3.0.co;2-j

90. Naor D, Nedvetzki S, Golan I, Melnik L, Faitelson Y. CD44 in cancer. *Crit Rev Clin Lab Sci* 2002; 39(6): 527-79. doi:10.1080/10408360290795574

91. Lopez JI, Camenisch TD, Stevens MV, Sands BJ, McDonald J, Schroeder JA. CD44 attenuates metastatic invasion during breast cancer progression. *Cancer Res* 2005; 65(15): 6755-6763.

92. Ghatak S, Misra S, Toole BP. Hyaluronan Oligosaccharides Inhibit Anchorage-independent Growth of Tumor Cells by Suppressing the Phosphoinositide 3-Kinase/Akt Cell Survival Pathway. *J Biol Chem* 2002; 277(41): 38013-20.   doi:10.1074/jbc.M202404200

93. Bourguignon LY. CD44-mediated oncogenic signaling and cytoskeleton activation during mammary tumor progression. *J Mammary Gland Biol Neoplasia* 2001;6(3): 287–297.
94. Lambert S, Yu H, Prchal JT, Lawler J, Ruff P, Speicher D, et al. cDNA sequence for human erythrocyte ankyrin. *Proc Natl Acad Sci USA* 1990; 87(5): 1730–1734. doi: 10.1073/pnas.87.5.1730
95. Lux SE, John KM, Bennett V. Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. *Nature* 1990; 344(6261): 36–42. doi:10.1038/344036a0
96. Lokeshwar VB, Fregien N, Bourguignon LY. Ankyrin-binding domain of CD44(GP85) is required for the expression of hyaluronic acid-mediated adhesion function. *J Cell Biol* 1994; 126(4): 1099–1109. doi: 10.1083/jcb.126.4.1099
97. Bourguignon LY, Peyrollier K, Gilad E, Brightman A. Hyaluronan-CD44 interaction with neural Wiskott-Aldrich syndrome protein (N-WASP) promotes actin polymerization and ErbB2

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

activation leading to beta-catenin nuclear translocation, transcriptional up-regulation, and cell migration in ovarian tumor cells. *J Biol Chem* 2007;282(2): 1265–1280. doi:10.1074/jbc.M604672200

98. Hall A. Rho GTPase and the actin cytoskeleton. *Science* 1998; 279: 509–514. doi:10.1126/science.279.5350.509

99. Xiaoyu L, Bing L. RhoGTPases and their role in cancer. *Oncol Res* 2003; 13(6): 323–331. doi: 10.3727/096504003108748528

100. Suwa H, Ohshio G, Imamura T, Watanabe G, Arii S, Imamura M, et al. Overexpression of the rho C gene correlates with progression of ductal adenocarcinoma of the pancreas. *Br J Cancer* 1998;77(1): 147–152. doi:10.1038/bjc.1998.23

101. Bourguignon LY, Peyrollier K, Xia W, Gilad E. Hyaluronan-CD44 inter- action activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. *J Biol Chem* 2008; 283(25): 17635–17651. doi: 10.1074/jbc.M800109200

102. Chen WT. Proteases associated with invadopodia, and their role in degradation of extracellular matrix. *Enzyme Protein* 1996;49(1-3): 59–71. doi:10.1159/000468616

103. Gum R, Lengyel E, Juarez J, Chen JH, Sato H, Seiki M, et al. Stimulation of 92-kDa gelatinase B promoter activity by ras is mitogen-activated protein kinase 1-independent and requires multiple transcription factor binding sites including closely spaced PEA3/ets and AP-1 sequences. *J Biol Chem* 1996;271: 10672–10680. doi:10.1074/jbc.271.18.10672

104. Bourguignon LY, Gilad E, Brightman A, Diedrich F, Singleton P. Hyaluronan-CD44 interaction with leukemia-associated RhoGEF and epidermal growth factor receptor promotes Rho/Ras co- activation, phospholipase C epsilon-Ca^{2+} signaling, and cytoskeleton modification in head and neck squamous cell carcinoma cells. *J Biol Chem* 2006; 281(20): 14026-14040. doi:10.1074/jbc.M507734200

105. Bourguignon LYW, Zhu H, Shao L, Zhu D, Chen YW. Rho-kinase (ROK) promotes CD44v3,8– 10- ankyrin interaction and tumor cell migration in metastatic breast cancer cells. *Cell Motil Cytoskeleton* 1999; 43(4): 269-287. doi:10.1002/(SICI)1097-0169(1999)43:4<269:AID-CM1>3.0.CO;2-5

106. Singleton PA, Bourguignon LYW. CD44v10 interaction with Rho-kinase (ROK) activates inositol 1,4,5-triphosphate (IP3) receptor-mediated Ca^{2+} signaling during hyaluronan (HA)-induced endothelial cell migration. *Cell Motil Cytoskeleton* 2002; 53(4): 293-316. doi:10.1002/cm.10078

107. Lakshman M, Subramaniam V, Rubenthiran U, Jothy S. CD44 promotes resistance to apoptosis in human colon cancer cells. *Exp Mol Pathol* 2004; 77(1): 18–25. doi: 10.1016/j.yexmp.2004.03.002

108. Yasuda M, Tanaka Y, Fujii K, Yasumoto K. CD44 stimulation down-regulates Fas expression and Fas-mediated apoptosis of lung cancer cells. *Int Immunol* 2001; 13(10): 1309–19. doi:10.1093/intimm/13.10.1309

109. Park YS, Huh JW, Lee JH, Kim HR. shRNA against CD44 inhibits cell proliferation, invasion and migration, and promotes apoptosis of colon carcinoma cells. *Oncol Rep* 2012; 27(2): 339–346. doi:10.3892/or.2011.1532

110. Jan R, Chaudhry GE. Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. *Adv Pharm Bull* 2019; 9(2): 205-218. doi: 10.15171/apb.2019.024

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

111. Lokeshwar VB, Estrella V, Lopez L, Kramer M, Gomez P, Soloway MS, et al. HYAL1-v1, an alternatively spliced variant of HYAL1 hyaluronidase: a negative regulator of bladder cancer. *Cancer Res* 2006; 66(23): 11219–27. doi:10.1158/ 0008- 5472.CAN- 06- 1121

112. Croix B, Man S, Kerbel RS. Reversal of intrinsic and acquired forms of drug resistance by hyaluronidase treatment of solid tumors. *Cancer Lett* 1998; 131(1): 35–44. doi:10.1016/S0304- 3835(98)00199- 2

113. Croix B, Rak JW, Kapitain S, Sheehan C, Graham CH, Kerbel RS. Reversal by hyaluronidase of adhesion-dependent multicellular drug resistance in mammary carcinoma cells. *J Natl Cancer Inst* 1996; 88(18): 1285–1296. doi:10.1093/ jnci/88.18.1285.

114. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012;21(3): 418–429. doi:10. 1016/j.ccr.2012.01.007.

115. Nagase H, Kudo D, Suto A. Yoshida E, Suto S, Negishi M, et al. 4-Methylumbelliferone Suppresses Hyaluronan Synthesis and Tumor Progression in SCID Mice Intra-abdominally Inoculated with Pancreatic Cancer Cells. *Pancreas* 2017; 46(2): 190–197. doi:10.1097/MPA.0000000000000741.

116. Kakizaki I, Kojima K, Takagaki K. Endo M, Kannagi R, Ito M, et al. A novel mechanism for the inhibition of hyaluronan biosynthesis by 4-methylumbelliferone. *J Biol Chem* 2004; 279: 33281–33289. doi:10.1074/jbc.M405918200

117. Kultti A, Pasonen-Seppanen S, Jauhiainen M. Rilla KJ, Karna R, Pyyoria E, et al. 4-Methylumbelliferone inhibits hyaluronan synthesis by depletion of cellular UDP–glucuronic acid and downregulation of hyaluronan synthase 2 and 3. *Exp Cell Res* 2009; 315(11): 1914–1923. doi:10.1016/j.yexcr.2009.03.002

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

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

120. Arai E, Nishida Y, Wasa J, Urakawa H, Zhuo L, Kimata K, et al. Inhibition of hyaluronan retention by 4-methylumbelliferone suppresses osteosarcoma cells in vitro and lung metastasis in vivo. *Br J Cancer* 2011;105: 1839–1849. doi:10.1038/bjc.2011.459

121. Luo Y, Ziebell MR, Prestwich GD. A hyaluronic acid-taxol antitumor bio- conjugate targeted to cancer cells. Biomacromolecules 2000; 1(2): 208–18. doi:10. 1021/bm000283n.

122. Rosato A, Banzato A, De Luca G, Renier D, Bettella F, Pagano C, et al. HYTAD1-p20: a new paclitaxel-hyaluronic acid hydrosoluble bioconjugate for treatment of superficial bladder cancer. *Urol Oncol* 2006; 24(3): 207–15. doi:10.1016/j.urolonc.2005.08.020.

123. Luo Y, Prestwich GD. Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bioconjugate. *Bioconjug Chem* 1999; 10(5): 755–63. doi:10.1021/ bc9900338

124. Luo Y, Kirker KR, Prestwich GD. Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery. *J Control Release* 2000; 69(1):169–84. doi:10. 1016/S0168-3659(00)00300- X.

125. Serafino A, Zonfrillo M, Andreola F, Psaila R, Mercuri L, Moroni N, et al. CD44-targeting for antitumor drug delivery: a new SN-38-hyaluronan bioconjugate for locoregional treatment of peritoneal carcinomatosis. *Curr Cancer Drug Targets* 2011; 11(5): 572–85. doi:10.2174/156800911795655976.

126. Bassi PF, Volpe A, D'Agostino D, Palermo G, Renier D, Franchini S, et al. Paclitaxel-

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

hyaluronic acid for intravesical therapy of bacillus Calmette-Guerin refractory carcinoma in situ of the bladder: results of a phase I study. *J Urol* 2011; 185(2): 445–9. doi:10.1016/j.juro.2010.09.073

127.	Montagner IM, Banzato A, Zuccolotto G, Renier D, Campisi M, Bassi P, et al. Paclitaxel-hyaluronan hydrosoluble bioconjugate: mechanism of action in human bladder cancer cell lines. *Urol Oncol* 2013; 31(7): 1261–9. doi:10.1016/ j.urolonc.2012.01.005

128.	Rosenthal MA, Gibbs P, Brown TJ, Wong S, Uren S, Ellis A, et al. Phase I and pharmacokinetic evaluation of intravenous hyaluronic acid in combination with doxorubicin or 5-fluorouracil. *Chemotherapy* 2005;51(2-3): 132–41. doi:10. 1159/000085621

129.	Gibbs P, Brown TJ, Ng R, Jennens R, Cinc E, Pho M, et al. A pilot human evaluation of a formulation of irinotecan and hyaluronic acid in 5- fluorouracil-refractory metastatic colorectal cancer patients. *Chemotherapy* 2009; 55(1): 49–59. doi:10.1159/000180339 .

130.	Peer D, Margalit R. Loading mitomycin C inside long circulating hyaluronan targeted nano-liposomes increases its antitumor activity in three mice tumor models. *Int J Cancer* 2004; 108(5): 780–9. doi:10.1002/ijc.11615.

131.	Peer D, Margalit R. Tumor-targeted hyaluronan nanoliposomes increase the antitumor activity of liposomal doxorubicin in syngeneic and human xenograft mouse tumor models. *Neoplasia* 2004; 6(4): 343–53. doi:10.1593/neo. 03460

132.	Surace C, Arpicco S, Dufay-Wojcicki A, Marsaud V, Bouclier C, Clay D, et al. Lipoplexes targeting the CD44 hyaluronic acid receptor for efficient transfection of breast cancer cells. *Mol Pharm* 2009; 6(4): 1062–73. doi:10.1021/ mp800215d

133.	Eliaz RE, Szoka FC Jr. Liposome-encapsulated doxorubicin targeted to CD44: a strategy to kill CD44-overexpressing tumor cells. *Cancer Res* 2001; 61(6): 2592–601.

134.	Ruhela D, Riviere K, Szoka FC Jr. Efficient synthesis of an aldehyde functional ized hyaluronic acid and its application in the preparation of hyaluronan-lipid conjugates. *Bioconjug Chem* 2006;17(5): 1360–3. doi:10.1021/bc0600721

135.	Dufay Wojcicki A, Hillaireau H, Nascimento TL, Arpicco S, Taverna M, Ribes S, et al. Hyaluronic acid-bearing lipoplexes: physico-chemical characterization and in vitro targeting of the CD44 receptor. *J Control Release* 2012; 162(3): 545–52. doi:10.1016/j.jconrel.2012.07.015

136.	Liu Y, Sun J, Cao W, Yang J, Lian H, Li X, et al. Dual targeting folate-conjugated hyaluronic acid polymeric micelles for paclitaxel delivery. *Int J Pharm* 2011; 421(1): 160–169. doi:10.1016/j.ijpharm.2011.09.006

137.	Qiu L, Li Z, Qiao M, Long M, Wang M, Zhang X, et al. Self-assembled pH- responsive hyaluronic acid-g-poly(l-histidine) copolymer micelles for targeted intracellular delivery of doxorubicin. *Acta Biomater* 2014;10(5): 2024–35. doi:10. 1016/j.actbio.2013.12.025.

138.	Zhang Y, Zhang H, Wang X, Wang J, Zhang X, Zhang Q. The eradication of breast cancer and cancer stem cells using octreotide modified paclitaxel active targeting micelles and salinomycin passive targeting micelles. *Biomaterials* 2012;33(2): 679–91. doi:10.1016/j.biomaterials.2011.09.072.

139.	Pellegrino T, Kudera S, Liedl T, Munoz Javier A, Manna L, Parak WJ. On the

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

development of colloidal nanoparticles towards multifunctional structures and their possible use for biological applications. *Small* 2005; 1(1): 48–63. doi:10. 1002/smll.200400071.

140.   Tenne R. Inorganic nanotubes and fullerene-like nanoparticles. *Nat Nanotechnol* 2006; 1:103–11. doi:10.1038/nnano.2006.62.

141.   Baker SN, Baker GA. Luminescent carbon nanodots: emergent nanolights. *Angew Chem Int Ed Engl* 2010; 49(38): 6726–6644. doi:10.1002/anie.200906623.

142.   Geim AK. Graphene: status and prospects. *Science* 2009; 324(5934): 1530–4. doi:10. 1126/science.1158877.

143.   Lee MY, Yang JA, Jung HS, Beack S, Choi JE, Hur W, et al. Hyaluronic acid- gold nanoparticle/interferon alpha complex for targeted treatment of hepatitis C virus infection. *ACS Nano* 2012; 6(11): 9522–9531. doi:10.1021/nn302538y.

144.   Kumar A, Sahoo B, Montpetit A, Behera S, Lockey RF, Mohapatra SS. Development of hyaluronic acid-Fe2O3 hybrid magnetic nanoparticles for targeted delivery of peptides. *Nanomedicine* 2007; 3(2): 132–137. doi:10.1016/j.nano.2007. 03.001.

145.   Lu J, Liong M, Li Z, Zink JI, Tamanoi F. Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles for cancer therapy in animals. *Small* 2010; 6(16): 1794–805. doi:10.1002/smll.201000538.

146.   Cho HJ, Yoon HY, Koo H, Ko SH, Shim JS, Lee JH, et al. Self-assembled nanoparticles based on hyaluronic acid-ceramide (HA-CE) and Pluronic(R) for tumor-targeted delivery of docetaxel. *Biomaterials* 2011;32(29): 7181–7190. doi:10.1016/j.biomaterials.2011.06.028.

147.   Akima K, Ito H, Iwata Y, Matsuo K, Watari N, Yanagi M, et al. Evaluation of antitumor activities of hyaluronate binding antitumor drugs: synthesis, characterization and antitumor activity. *J Drug Target* 1996; 4(1): 1–8. doi:10. 3109/10611869609046255.

148.   Gul-e-Saba, Abdah A Abdullah MA. Hyaluronan-mediated CD44 Receptor Cancer Cells Progression and the Application of Controlled Drug-delivery System. *Int J Curr Chem* 2010; 1(4): 245-265.

149.   Gul-e-Saba, Abdah A. Abdullah MA. Cytotoxic Effects of Drug-loaded Hyaluronan-Glutaraldehyde Cross-Linked Nanoparticles and the Release Kinetics Modeling. *J Adv Chem Eng* 2014; 1(104): 2.

150.   Zhao MD, Li JQ, Chen FY, Dong W, Wen LJ, Fei WD, et al. Co-Delivery of Curcumin and Paclitaxel by Core-Shell Targeting Amphiphilic Copolymer to Reverse Resistance in the Treatment of Ovarian Cancer. *Int J Nanomedicine* 2019; 14: 9453–9467. doi:10.2147/IJN.S224579.

151.   Hrabarova E, Juranek I, Soltes L. Pro-oxidative effect of peroxynitrite regarding biological systems: a special focus on high-molar-mass hyaluronan degradation. *Gen Physiol Biophys* 2011; 30(3): 223–38. doi:10.4149/gpb201103223.

152.   Ghatak S, Misra S, Norris RA, Moreno-Rodriguez RA, Hoffman S, Levine RA, et al. Periostin induces intracellular cross-talk between kinases and hyaluronan in atrioventricular valvulogenesis. *J Biol Chem* 2014;289(12): 8545–61. doi:10. 1074/jbc.M113.539882.

153.   Hrabarova E, Valachova K, Juranek I, Soltes L. Free-radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions: evaluation of antioxidative effect of cysteine-derived compounds. *Chem Biodivers* 2012; 9(2): 309–17. doi:10.1002/cbdv.201100046.

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

154.   Soltes L, Mendichi R, Kogan G, Schiller J, Stankovska M, Arnhold J. Degradative action of reactive oxygen species on hyaluronan. *Biomacromolecules* 2006; 7(3): 659–668. doi:10.1021/bm050867v.

155.   Knudson W, Chow G, Knudson CB. CD44-mediated uptake and degradation of hyaluronan. *Matrix Biol* 2002; 21(1): 15–23. doi:10.1016/S0945-053X(01) 00186- X.

156.   Jackson DG. Biology of the lymphatic marker LYVE-1 and applications in research into lymphatic trafficking and lymphangiogenesis. *APMIS* 2004; 112(7-8): 526–38. doi:10.1111/j.1600- 0463.2004.apm11207- 0811.x.

157.   Stern R, Jedrzejas MJ. Hyaluronidases: their genomics, structures, and mech anisms of action. *Chem Rev* 2006; 106(3): 818–839. doi:10.1021/cr050247k.

158.   Roden L, Campbell P, Fraser JR, Laurent TC, Pertoft H, Thompson JN. Enzymic pathways of hyaluronan catabolism. *Ciba Found Symp* 1989; 143: 60–76. doi: 10.1002/9780470513774.ch5

159.   Frost GI, Csoka AB, Wong T, Stern R. Purification, cloning, and expression of human plasma hyaluronidase. [published correction appears in Biochem Biophys Res Commun. 2004; 319(2): 705. Csoka, T B [corrected to Csoka, A B]]. Biochem Biophys Res Commun. 1997; 236(1):10-15. doi:10.1006/bbrc.1997.6773

160.   Song G, Liao X, Zhou L, Wu L, Feng Y, Han ZC. HI44a, an anti-CD44 mono- clonal antibody, induces differentiation and apoptosis of human acute myeloid leukemia cells. *Leuk Res* 2004; 28(10):1089–96. doi:10.1016/j.leukres.2004.02.005.

161.   de Bree R, Roos JC, Quak JJ, den Hollander W, Wilhelm AJ, van Lingen A, et al. Biodistribution of radiolabeled monoclonal antibody E48 IgG and F(ab')2 in   patients with head and neck cancer. *Clin Cancer Res* 1995;1(3): 277–86.

162.   Borjesson PK, Postema EJ, Roos JC, Colnot DR, Marres HA, van Schie MH, et al. Phase I therapy study with (186) Re-labeled humanized monoclonal antibody BIWA 4 (bivatuzumab) in patients with head and neck squamous   cell carcinoma. *Clin Cancer Res* 2003; 9(10 Pt 2): 3961–3972.

163.   Heider KH, Sproll M, Susani S, Patzelt E, Beaumier P, Ostermann E, et al. Char acterization of a high-affinity monoclonal antibody specific for CD44v6 as candidate for immunotherapy of squamous cell carcinomas. *Cancer Immunol Immunother* 1996; 43(4): 245– 53. doi:10.1007/s002620050329.

164.   Schrijvers AH, Quak JJ, Uyterlinde AM, van Walsum M, Meijer CJ, Snow GB, et al. MAb U36, a novel monoclonal antibody successful in immunotargeting of squamous cell carcinoma of the head and neck. *Cancer Res* 1993; 53(18): 4383–90.

165.   Mummert ME, Mummert DI, Ellinger L, Takashima A. Functional roles of hyaluronan in B16-F10 melanoma growth and experimental metastasis in mice. *Mol Cancer Ther* 2003; 2(3): 295–300.

166.   Xu XM, Chen Y, Chen J, Yang S, Gao F, Underhill CB, et al. A peptide with three hyaluronan binding motifs inhibits tumor growth and induces apoptosis. *Cancer Res* 2003; 63(18): 5685–90.

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

167.   Klingbeil P, Marhaba R, Jung T, Kirmse R, Ludwig T, Zoller M. D44 variant isoforms promote metastasis formation by a tumor cell-matrix cross-talk that supports adhesion and apoptosis resistance. *Mol Cancer Res* 2009; 7(2): 168–79. doi:10.1158/1541- 7786.MCR- 08- 0207.

168.   Todaro M, Gaggianesi M, Catalano V, Benfante A, Iovino F, Biffoni M, et al. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell* 2014; 14(3): 342–56. doi:10.1016/ j.stem.2014.01.009.

169.   Wielenga VJ, Smits R, Korinek V, Smit L, Kielman M, Fodde R, et al. Expression of CD44 in Apc and Tcf mutant mice implies regulation by the WNT pathway. *Am J Pathol* 1999; 154(2): 515–23. doi:10.1016/S0002- 9440(10)65297- 2.

170.   Misra S, Hascall VC, Berger FG, Markwald RR, Ghatak S. Hyaluronan, CD44, and cyclooxygenase-2 in colon cancer. *Connect Tissue Res* 2008; 49(3): 219–24. doi:10.1080/03008200802143356

171.   Misra S, Hascall VC, De Giovanni C, Markwald RR, Ghatak S. Delivery of CD44 shRNA/nanoparticles within cancer cells: perturbation of hyaluronan/CD44v6 interactions and reduction in adenoma growth in Apc Min/+ MICE. *J Biol Chem* 2009; 284(18): 12432–46. doi:10.1074/jbc.M806772200.

172.   Ghatak S, Hascall VC, Berger FG, Penas MM, Davis C, Jabari E, et al. Tissue- specific shRNA delivery: a novel approach for gene therapy in cancer. *Connect Tissue Res* 2008; 49(3): 265–9. doi:10.1080/03008200802147845.

173.   Paul CP, Good PD, Winer I, Engelke DR. Effective expression of small interfering RNA in human cells. *Nat Biotechnol* 2002; 20(5): 505–8. doi:10.1038/ nbt0502- 505.

174.   Stauder R, Eisterer W, Thaler J, Gunthert U. CD44 variant isoforms in non- Hodgkin's lymphoma: a new independent prognostic factor. *Blood* 1995;85(10): 2885–99.

175.   Kainz C, Kohlberger P, Tempfer C, Sliutz G, Gitsch G, Reinthaller A, et al. Prognostic value of CD44 splice variants in human stage III cervical cancer. *Eur J Cancer* 1995; 31A(10):1706–1709. doi:10.1016/0959- 8049(95)00353- K.

176.   Hsieh HF, Yu JC, Ho LI, Chiu SC, Harn HJ. Molecular studies into the role of CD44 variants in metastasis in gastric cancer. *Mol Pathol* 1999; 52(1): 25–28. doi:10.1136/mp.52.1.25.

177.   Shtivelman E, Bishop JM. Expression of CD44 is repressed in neuroblastoma cells. *Mol Cell Biol* 1991; 11(11): 5446–5453. doi:10.1128/mcb.11.11.5446

178.   Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F. et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell Stem Cell* 2007; 1(4): 389–402. doi:10.1016/j. stem.2007.08.001.

179.   Guo W, Frenette PS. Alternative CD44 splicing in intestinal stem cells and tumorigenesis. Oncogene 2014; 33(5): 537–538. doi:10.1038/onc.2013.260.

180.   Kainz C, Kohlberger P, Sliutz G, Tempfer C, Heinzl H, Reinthaller A, et al. Splice variants of CD44 in human cervical cancer stage IB to IIB. *Gynecol Oncol* 1995; 57(3): 383–387. doi:10.1006/gyno.1995.1159.

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

181.   Lesley J, Hyman R, English N, Catterall JB, Turner GA. CD44 in inflammation and metastasis. *Glycoconj J* 1997; 14(5): 611–622. doi:10.1023/A:1018540610858.

182.   De Marzo AM, Bradshaw C, Sauvageot J, Epstein JI, Miller GJ. CD44 and CD44v6 downregulation in clinical prostatic carcinoma: relation to gleason grade and cytoarchitecture. *Prostate* 1998;34(3): 162–8. doi:10.1002/(SICI) 1097- 0045(19980215)34:3< 162: AID- PROS2> 3.0.CO;2- K.

183.   Liao HX, Lee DM, Levesque MC, Haynes BF. N-terminal and central regions of the human CD44 extracellular domain participate in cell surface hyaluronan binding. *J Immunol* 1995;155(8): 3938–3945.

184.   Hsieh HF, Yu JC, Ho LI, Chiu SC, Harn HJ. Molecular studies into the role of CD44 variants in metastasis in gastric cancer. *Mol Pathol* 1999;52(1): 25–8. doi:10.1136/mp.52.1.25.

185.   Misra S, Hascall VC, Markwald RR, Ghatak S. Interactions between Hyaluronan and Its Receptors (CD44, RHAMM) Regulate the Activities of Inflammation and Cancer. *Front Immunol*. 2015; 6:201 doi:10.3389/fimmu.2015.00201

186.   Gul-e-Saba, Abdullah MA. Polymeric nanoparticle mediated targeted drug delivery to cancer cells. In: Thangadurai D, Sangeetha J (eds) Biotechnology and bioinformatics: advances and applications for bioenergy, bioremediation, and biopharmaceutical research. Apple Academic, NJ, 2015 pp 1–34

187.   Gul-e-Saba, Murni Ismail, Noraznawati Ismail, Habsah, TST Muhammad Induction of Apoptosis by Aaptos sp., fractions in human breast cancer. *Int J Res Pharm Sci* 2018; 9(2): 328-237.

188.   Chaudhry GE, Jan R, Habsah M, Mohammad TST. Vitex rotundifolia fractions induce apoptosis in the human breast cancer cell line, MCF-7, via extrinsic and intrinsic pathways. *Res Pharma Sci* 2019; 14(3): 273-285. doi: 10.4103/1735-5362.258496

189.   Chaudhry GE, Jan R, Naveed Zafar M, Mohammad H, Muhammad TST. Vitex rotundifolia fractions induced apoptosis in human breast cancer T-47D cell line via activation of extrinsic and intrinsic pathway. *Asian Pac J Cancer Prev* 2019; 20(12): 3555-3562. doi:10.4103/1735-5362.258496

190.   Hudaya T, Gul-e-Saba, Taib, M Ismail N, Mohammad TST. Methanol extract of four selected marine sponges induces apoptosis in human breast cancer cell line, MCF-7. *Int J Res Pharm Sci* 2017; 8(3): 667-675.

191.   Chaudhry GS, Murni NIK, Zafar MN, Habsah M, Andriani Y, Ismail N, TST Muhammad and Sung YY. Induction of apoptosis by Stichopus chloronotus and Holothuria nobilis fractions in the human cervical cancer cell line, HeLa. *Int J Res Pharm Sci* 2020; 11(1): 1238-1247. doi: 10.26452/ijrps. v11i1.1964.

192.   Chaudhry GS, Abdah A, Zafar MN, Abdullah MA, Sung YY, Muhammad TST. Induction of Apoptosis and role of PTX loaded HA-crosslinked nanoparticle in the regulation of AKT and RhoA. *J Adv Pharm Technol Res* In press

193.   Chaudhry GS, Sohimi NKA, Habsah M, Zafar MN, Ahmed A, Sung YY, et al. Xylocarpus moluccensis induces cytotoxicity in human hepatocellular carcinoma HepG2 cell line via activation of the extrinsic pathway. *Asia Pac J Cancer P*. In press

194.   Chaudhry GS, Zafar MN, Yeong Yik Sung, Muhammad TST Phytochemistry and Biological activity of Vitex rotundifolia L., *Res J Pharmacy Technol* In press

195.   Yunus U, Zulfiqar MA, Ajmal M, Bhatti MH, Chaudhry GS, Muhammad TST, et al. Targeted drug delivery systems: synthesis and in-vitro bioactivity and apoptosis studies of gemcitabine-carbon dots conjugates *Biomed Mater* 2020;10.1088/1748-605X/ab95e1. doi:10.1088/1748-605X/ab95e1

List of figures

Figure 1: ECM, potential site for HA-CD44 communication

Figure 2: Schematic presentation of HA binding to cytoplasmic domain of CD44

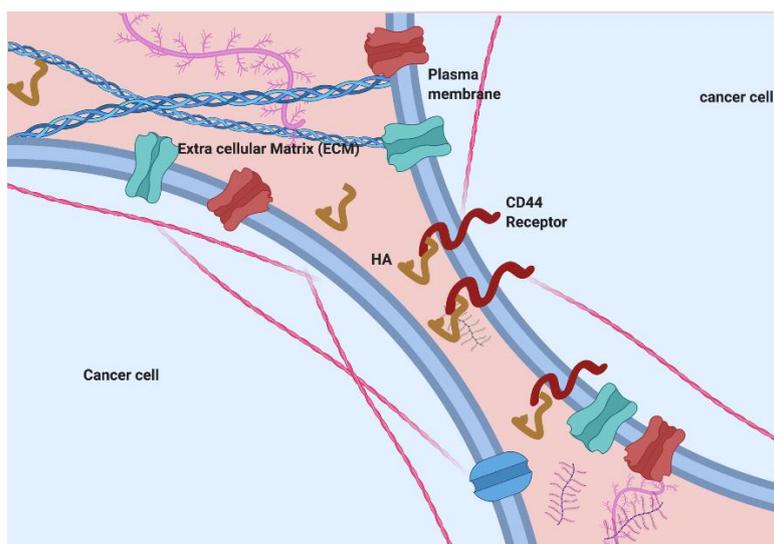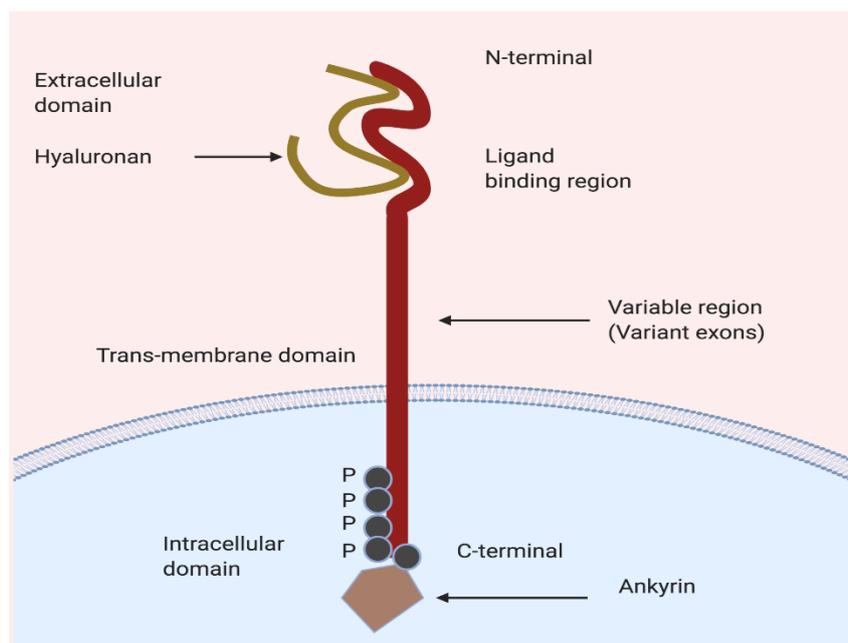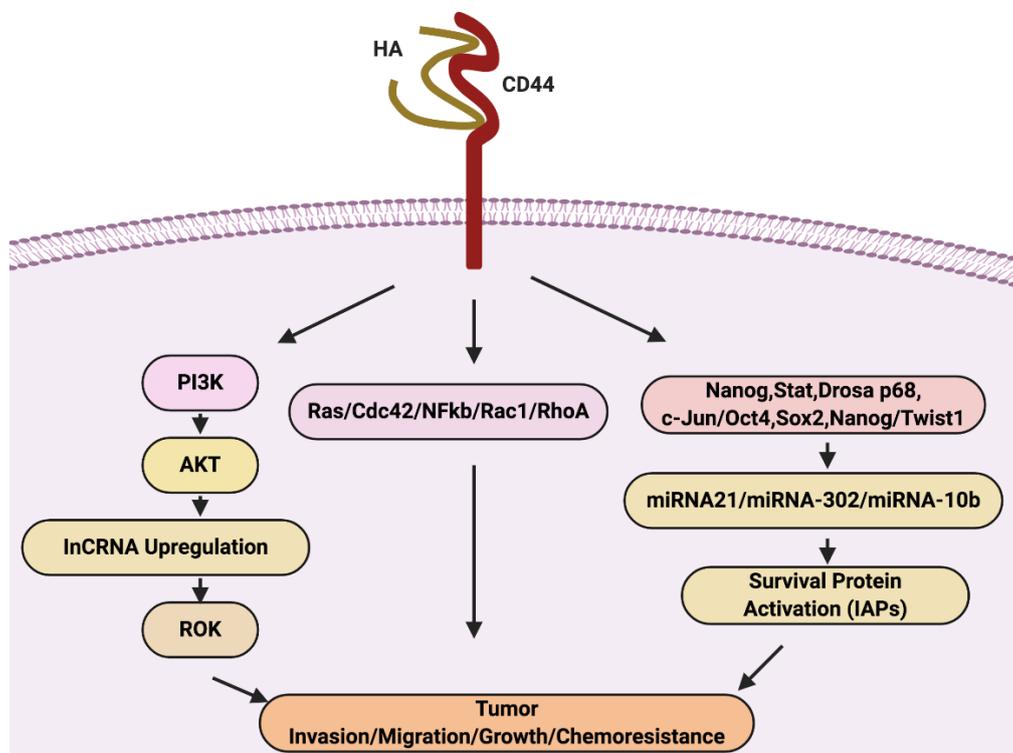**Figure 3: Therapeutic targets of tumor growth, invasion, metastasis and chemo resistance**