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REVIEW

Hyaluronic acid metabolism and chemotherapy resistance: recent advances and therapeutic potential

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Hyaluronic acid (HA) is a major component of the extracellular matrix, providing essential mechanical scaffolding for cells and, at the same time, mediating essential biochemical signals required for tissue homeostasis. Many solid tumors are characterized by dysregulated HA metabolism, resulting in increased HA levels in cancer tissues. HA interacts with several cell surface receptors, such as cluster of differentiation 44 and receptor for hyaluronan-mediated motility, thus co-regulating important signaling pathways in cancer development and progression. In this review, we describe the enzymes controlling HA metabolism and its intracellular effectors emphasizing their impact on cancer chemotherapy resistance. We will also explore the current and future prospects of HA-based therapy, highlighting the opportunities and challenges in the field.

1. Introduction

While chemotherapy is a common and effective treatment option for cancer patients, contributing to kill cancer cells and preventing them from spreading, the response to chemotherapy is often transient. The development of chemoresistance is, indeed, a major challenge in cancer treatment and multiple molecular mechanisms underlie the tumor response to chemotherapy, including the circuits controlling the homeostasis of the tumor microenvironment (TME) [1–6]. In solid tumors, TME consists of an intricate network of tumor cells, stromal cells, immune cells, and endothelial cells embedded in the extracellular matrix (ECM). The interactions between these components contribute to the development of a complex network that supports tumor growth, invasion, and metastasis. Moreover, TME can also cause drug resistance through various

Abbreviations
4-MU, 4-methylumbelliferone; CD44, cluster of differentiation 44; CSCs, cancer stem cells; ECM, extracellular matrix; EGF-R, epidermal growth factor receptor; ERK1, extracellular response kinase 1; ERK2, extracellular response kinase 2; FOLFIRI, folinic acid, 5-FU and irinotecan; GlcNAc, *N*-acetyl-D-glucosamine; GlcUA, D-glucuronic acid; GSH, glutathione; HA, hyaluronic acid; HAases, hyaluronidases; HARE, HA receptor for endocytosis; HAS, hyaluronan synthases; HMW-HA, high-molecular weight HA; HNSCC, head and neck squamous cell carcinoma; IFP, interstitial fluid pressure; LMW-HA, low-molecular weight HA; LYVE-1, lymphatic vessel endothelial hyaluronan receptor 1; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; NFE2L2, nuclear factor erythroid 2-like 2; PAG, nab-paclitaxel/gemcitabine; PDA, pancreatic ductal adenocarcinoma; PEGPH20, PEGylated human recombinant PH20 hyaluronidase; PFS, progression-free survival; PKC, protein kinase C; PKM2, pyruvate kinase M2; RAR, retinoic acid receptor; RHAMM, receptor for hyaluronan-mediated motility; RNS, reactive nitrogen species; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; TGFβ, transforming growth factor-β; TLR-2, toll-like receptor 2; TLR-4, toll-like receptor 4; TME, tumor microenvironment; UGT, UDP-glucuronosyltransferase.

mechanisms, including altered drug metabolism, reduced drug uptake, and activation of signaling pathways that promote cell survival and proliferation [7–9].

Changes in the composition and structure of the ECM in the TME contribute to chemotherapy resistance in cancer cells. The main components of ECM include collagen, noncollagenous glycoproteins, glycosaminoglycans, and proteoglycans. Hyaluronic acid (HA) is one of the most abundant glycosaminoglycans present in the ECM, playing important physiological functions in many biological processes such as embryonic morphogenesis, cellular regeneration, and wound healing [10]. In addition to providing structural and mechanical support and protection to resident cells, HA impacts a variety of cellular processes, including cell growth, cell survival, migration, invasion, cell fate determination, and tissue morphogenesis.

Hyaluronic acid is typically produced by tumor and stromal cells, and its molecular weight determines whether it plays pro-tumorigenic or antitumorigenic roles. High-molecular weight HA (HMW-HA) is predominant in homeostatic tissues. It protects cells from mechanical damage and has anti-inflammatory and antiproliferative properties, thus hampering any pro-tumorigenic events [11]. During tumor initiation and progression, HA is rapidly degraded by hyaluronidases into small fragments of different lengths, which possess pro-inflammatory and pro-angiogenic functions promoting tumor growth, metastasis, and therapy resistance [12] (Fig. 1).

In this review, we will provide a comprehensive overview of the current knowledge on the role of HA in cancer chemotherapy and its underlying molecular mechanisms. We will also examine the status and

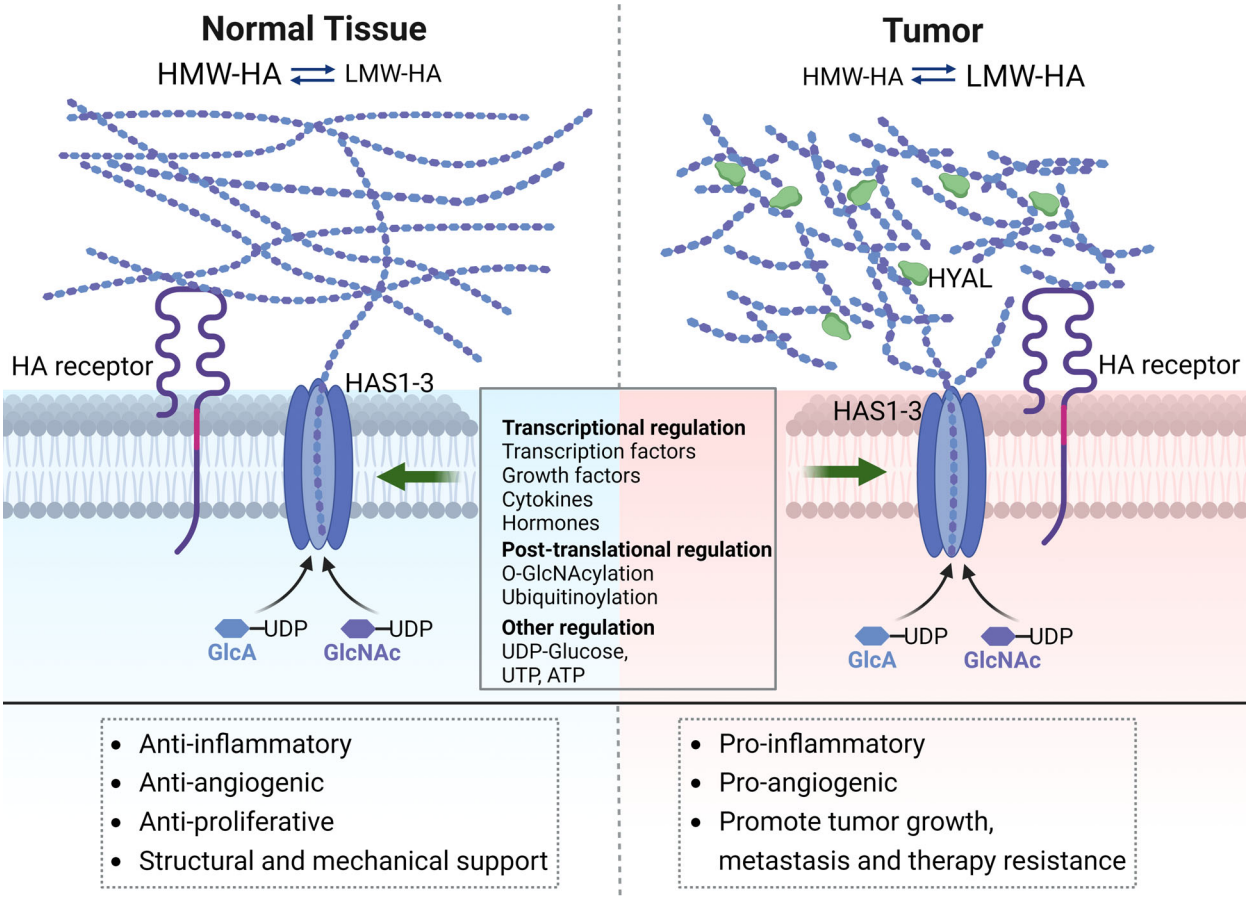

Fig. 1. Hyaluronic acid (HA) synthesis, catabolism, regulation, and functions. HA is synthesized at the plasma membrane by the transmembrane hyaluronan synthases (HAS): HAS1, HAS2, and HAS3, using the UDP-GlcNAc and UDP-GlcUA as precursors. Generally, high-molecular weight HA (HMW-HA) is predominant in normal tissues. During tumor initiation and progression, HMW-HA is rapidly degraded by hyaluronidases (HYAL) into low-molecular weight HA (LMW-HA) and small fragments of different lengths, which may possess pro-inflammatory and pro-angiogenic functions, promoting tumor growth, metastasis, and therapy resistance. This figure was created with BioRender.com.

future prospects of HA-based cancer therapy, highlighting the opportunities and challenges in this field.

2. Biology of HA

2.1. Basic information about HA

Hyaluronic acid is a glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid (GlcUA) and *N*-acetyl-D-glucosamine (GlcNAc) linked by glycosidic bonds. According to the length of disaccharide units, HA molecular weight ranges from 10^3 to 10^7 Da with a variable length of 2–25 μm. The purification of HA was first reported in 1934 when Karl Meyer and John Palmer isolated HA from the vitreous body of the bovine eye [13] while its structure was resolved later on in 1970. HA is present in many bacteria strains and is ubiquitous among all vertebrates, where it is particularly rich in embryonic tissue, for example, umbilical cord, and in adult soft connective tissues such as synovial fluid and vitreous body.

A prominent physico-chemical property of HA is its capacity to absorb water up to 1000 times its molecular weight. Therefore, HA acts as a biological lubricant of the joints, keeping moisture retention and reducing friction [14], and hydrates the ECM, reducing tissue compression. HA can be produced with a variable molecular weight and different tissues and fluids might contain HA with different size.

Generally, HMW-HA (1000–6000 kDa) is mainly synthesized under normal conditions and healthy tissues such as the epidermis contain high amount of HMW-HA [15]. Low-molecular weight HA (LMW-HA, $\leq$ 250 kDa) are physiologically present at low level in fluids such as milk, blood, saliva [16,17]. The ratio of these different HA species does not stay invariant and HA polymers with different lengths inside the HA mixture are dynamically regulated in response to physiological and pathological conditions. For instance, during cancer progression or during wound healing, HMW-HA is progressively degraded by hyaluronidase enzymes (HAases, see Section 2.3) into LMW-HA, in order to sustain the inflammatory phase [18,19]. The dynamic regulation of HA metabolism suggests that distinct species of HA could modulate distinct biological outcomes. Indeed, the cellular response activated by HA species largely depends on their specific size and the cell types the HA fragments interact with. Generally, HMW-HA is involved in maintaining tissue integrity and has antiproliferative, antiangiogenic, and immunosuppressive properties [12,20–25] (Fig. 1). However, HMW-HA can exert also a pro-proliferative function in vascular muscle cells and fibroblasts [26,27]. LMW-HA can also modulate proliferation and inflammation depending on cell type and the specific molecular size of the LMW-HA species. For instance, LMW-HA with a 35-kDa weight induces breast cancer cell migration and invasion while a 117-kDa HA fragment shows the opposite effects [28]. Regarding the inflammation response, LMW-HA is widely recognized to be an important mediator of the inflammatory response [24]. However, several studies also suggest that small HA molecules can attenuate the inflammatory process and induce pro-resolving responses [29]. Therefore, the precise biological outcome exerted by HA species is quite complex and can be cell-type dependent.

In the next paragraphs, we will describe how the synergistic activity of biosynthetic and degradation processes together with the activity of specific HA receptors modulate HA continent and its biological outcomes.

2.2. HA regulation

In mammals, HA is synthesized by one of three distinct transmembrane hyaluronan synthases (HAS): HAS1, HAS2, and HAS3. These enzymes utilize UDP-GlcNAc and UDP-GlcUA as precursors to catalyze the synthesis of HA polymer chain, which is then transferred into the extracellular space through the pores in HAS structures along with its elongation [30]. Some studies have also shown that HA might be also secreted by the multidrug transfer protein in vertebrate cells [31,32]. Although the three HAS enzymes have a similar structure and share 50–70% of similarity in their amino acid sequence, they possess specific enzymatic properties and are differently expressed during morphogenesis and pathological conditions.

HAS1 exhibits a slower rate for HA synthesis and produces the smallest polymer (0.12×10^6 Da). Conversely, HAS2 produces the largest HA polymers (over 3.9×10^6 Da) and accounts for the majority of cellular HA production. Accordingly, *Has2*$^{-/-}$ mice have severe cardiac and vascular abnormalities and die during midgestation. Conversely, *Has1*$^{-/-}$ and *Has3*$^{-/-}$ animals are viable and fertile, indicating that HAS2 is the only HAS enzymes required during embryogenesis [33]. HAS3 is the most active enzyme and produces intermediate-length HA ($0.12–1 \times 10^6$ Da) [23].

HAS enzymes are differentially expressed in human tissues. HAS1 and HAS2 are highly expressed in adipose tissue, whereas HAS3 expression level is relatively abundant in the esophagus [34]. In mouse embryo, HAS2 is most abundant in mesenchymal tissues and heart while HAS3 staining is relatively low in mouse embryonic

tissues [35]. However, it is worth noting that the lack of reliable antibodies against HAS proteins together with their low expression levels in many cell types may limit our knowledge on HAS's tissue distribution.

In several cancers, HAS expression correlates with cancer grade and can predict patient survival. In breast cancer cells, HAS1-3 levels in stromal and malignant cells are related to tumor aggressiveness and poor patient outcome [36]. Similarly, in colon and ovarian cancers, increased HAS1 mRNA expression and protein correlates with poor survival of patients [37].

HAS expression is regulated both at transcriptional and post-transcriptional levels and distinct growth factors and cytokines as well as glycolytic metabolites modulate HAS expression and activity in response to microenvironment changes (Fig. 1). At transcriptional level, various transcription factors interact with the promoter regions of HAS genes to influence their transcription and subsequent expression. HAS2 promoter region is characterized by binding sites for several transcription factors such as CREB1, retinoic acid receptor (RAR), SP1, STAT1, YY1, and FOXOs [38,39]. Similarly, HAS1 expression is regulated by the transcription factors Smad3 and SP3 [40], while HAS3 is influenced by factors such as CEBP, SP1, and NFκB [41]. In head and neck squamous cell carcinoma (HNSCC), the transcription factor ΔNp63, a member of the p53 family that is widely expressed and deregulated in cancer [42–49], binds to a specific p63-binding site (p63 BS) in the promoter region of the HAS3 gene. This binding event enhances the expression of HAS3, thereby modulating the amount of HA synthetized by HNSCC cells [50,51].

Post-translational modifications include O-GlcNAcylation of HAS2 and HAS3 and protein ubiquitylation of HAS2, which modulates enzymatic activity and protein stability, respectively [52–54]. The availability of HA precursors (UDP-GlcUA and UDP-GlcNAc) controls the levels of HA synthesis [55]. In addition, UTP, ATP, and their degradation products released from stressed cells can cause a transient upregulation of HAS expression [56–58]. Conversely, during energy restriction HAS2 is inactivated by a mechanism involving AMPK [59]. Different growth factors and cytokines, such as keratinocyte growth factor, epidermal growth factor, transforming growth factor-β, interleukin-1β or interferon-c, and hormones (e.g., prostaglandins, corticosteroids, and retinoids) present in TME influence HAS expression [38].

2.3. HA catabolism

Under normal conditions, the synthesis and degradation of HA are tightly regulated. Pathological conditions, such as cancer and inflammation, promote extensive HA remodeling, mainly by inducing endogenous HMW-HA degradation by hyaluronidases, resulting in increased levels of LMW-HA species.

There are seven different hyaluronidases (HAases) in humans: HYAL1, HYAL2, HYAL3, HYAL4, PH-20, Hyalp-1, and HYBID [60,61]. Among these HAases, the most studied are HYAL1, HYAL2, and PH-20. HYAL1 and HYAL2 are widely expressed in most tissues. HYAL2 is a glycosyl phosphatidylinositol (GPI)-anchored cell surface enzyme able to bind to and internalize HA into vesicles, where it catalyzes the digestion of HA into 20 kDa fragments. The 20 kDa HA fragments are subsequently internalized into the lysosome where HYAL1 further processes them into oligosaccharides, which eventually can be further degraded into monosaccharides by exoenzymes [62]. PH-20, the most active mammalian hyaluronidase, is necessary for fertilization of the oocyte by sperm and possesses signaling properties [63].

HA fragments of different sizes may also be generated by reactive oxygen species (ROS) or reactive nitrogen species (RNS), mainly during inflammatory processes and tissue repair [64]. ROS-generated HA species are molecularly distinct from those raising from the activity of HAases since they are generated randomly with a polydisperse size. ROS-mediated degradation of HA is mainly involved in the wound healing process as it enhances the inflammatory response which in turn stimulates HA synthesis and dermal fibroblasts proliferation with the subsequent formation of a new ECM [65,66].

HAase expression is generally elevated in a variety of carcinomas and is considered a tumor biomarker. However, in some carcinomas, HAase expression is inversely correlated with tumor grade [67]. For instance, higher HYAL2 expression is associated with poor prognosis of triple-negative breast cancer patients and silencing of HYAL2 expression reduces tumorigenicity in a tumor xenograft model [68]. In terms of tumor treatment strategies, since HA accumulation is often observed in the tumor stroma where it contributes to cancer progression, PEGylated human recombinant PH20 hyaluronidase (PEGPH20) has been developed as an anticancer therapy and is currently in clinical trials [69]. We will describe this HA-based approach in Section 4.

2.4. HA receptors

HA receptors are ubiquitously expressed in a variety of different cell types, such as endothelial cells, epithelial cells, and immune cells. The main HA receptors

include the cluster of differentiation 44 (CD44), the receptor for hyaluronan-mediated motility (RHAMM), the HA receptor for endocytosis (HARE), the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), toll-like receptor 2 and 4 (TLR-2 and TLR-4) and layilin. Here, we briefly describe CD44 and RHAMM since evidence have demonstrated their crucial involvement in the tumor pathogenesis (for an extensive review on HA receptors, please see [70]).

CD44, a nonkinase transmembrane glycoprotein, acts as an important signaling hub that initiates diverse intracellular signaling cascades affecting multiple processes (e.g., survival, inflammation, cytoskeleton remodeling and motility, and epithelial to mesenchymal transition). CD44 gene undergoes complex alternative splicing events which generate multiple variants such as CD44v3 and CD44v6 isoforms, which are critically involved in regulating cancer stem cell homeostasis contributing thus to tumor chemioresistance [71,72]. CD44 proteins have distinct structural domains that include an N-terminal HA-binding link-homology module, which is responsible for HA interaction, a stem region, a transmembrane domain, and a short C-terminal cytoplasmic domain, which is responsible for the activation of intracellular signaling pathways upon HA-CD44 interaction [73]. The downstream signaling routes activated by HA-CD44 association are mediated by the activation of diverse cytoskeletal proteins and intracellular signaling components, such as the Rho-family GTPases, Src family kinases, and associated molecules, which play a role in regulating cell adhesion and migration [74].

In addition to initiate intracellular signaling, CD44-HA interaction might also modulate the activity of growth factor receptors. At functional level, HA-CD44 interaction mediates cell proliferation and survival of different cancer cells, mainly through the activation of the EGFR/AKT/ERK signaling pathway [75]. In breast cancer [76] and HNSCC cells [77,78], HA-CD44 interaction promotes chemoresistance by enhancing c-Jun signaling pathways and microRNA-21 expression. Furthermore, HA-CD44 interaction has been implicated in mediating epithelial–mesenchymal transition in many cancer cells [79,80].

Similar to CD44, RHAMM, also termed CD168, encodes alternative splicing isoforms (e.g., $RHAMM^{A}$ and $RHAMM^{B}$). RHAMM is expressed at low levels in normal tissues and its expression is transiently induced upon specific signals or during several biological processes, such as wound repair [81]. Interestingly, recent evidence demonstrated that RHAMM is more sensitive to the density of HA than CD44 in breast cancer cell lines, and its surface expression can be enhanced to compensate the pro-tumorigenic signaling mediated by CD44 when CD44 is blocked [82]. In various carcinomas (e.g., colorectal cancer [83], pancreatic ductal adenocarcinoma (PDA) [84], breast cancer [85], and multiple myeloma [86]), RHAMM is overexpressed and commonly associated with poor prognosis [87,88]. At the functional level, in tumor cells RHAMM-HA interaction elicits multiple signaling cascades that regulate the following tumor-related features: (a) it enhances cell motility and invasion, mainly by activating protein kinase C (PKC) [89–91]; (b) it boosts cell proliferation via regulating the expression of mitogen-activated protein kinase (MAPK) [85], and (c) it induces epithelial-to-mesenchymal transition and multidrug resistance by increasing TGFβ/smad2 expression [92].

3. HA-mediated chemoresistance in tumors

HA is a significant component of TME and influences tumor development and prognosis [93]. The involvement of HA in tumor chemoresistance is mainly mediated by the CD44 receptor. The interaction between HA and CD44 promotes antiapoptotic pathways induced by receptor tyrosine kinase (RTK) activation, enhances transporter activities, and confers cancer stem cells (CSCs) with resistance to oxidative stress. Additionally, HA, as biophysical barrier, impairs vascular function and drug delivery, further contributing to chemotherapy resistance (Fig. 2). Understanding the molecular mechanisms by which HA mediates chemotherapy resistance can provide insights into novel therapeutic strategies to overcome resistance and improve drug sensitivity in chemotherapy-tolerant patients.

3.1. HA regulates receptor tyrosine kinases activation and antiapoptotic signaling pathways

Enhanced activation of cell survival/antiapoptotic pathways is a common phenomenon in tumors and significantly contributes to drug resistance [94]. By acting as plasma membrane receptors of growth factors, RTKs activate antiapoptotic signal transduction pathways [95]. Many studies have demonstrated that HA is able to modulate RTK activation, thus eliciting prosurvival and antiapoptotic signaling. In detail, the following intracellular cascades are influenced by HA-mediated signaling:

3.1.1. Phosphoinositide 3-Kinase (PI3K) pathway

In breast cancer cells, a positive feedback loop coupling CD44 splice isoform and HAS2 results in increased

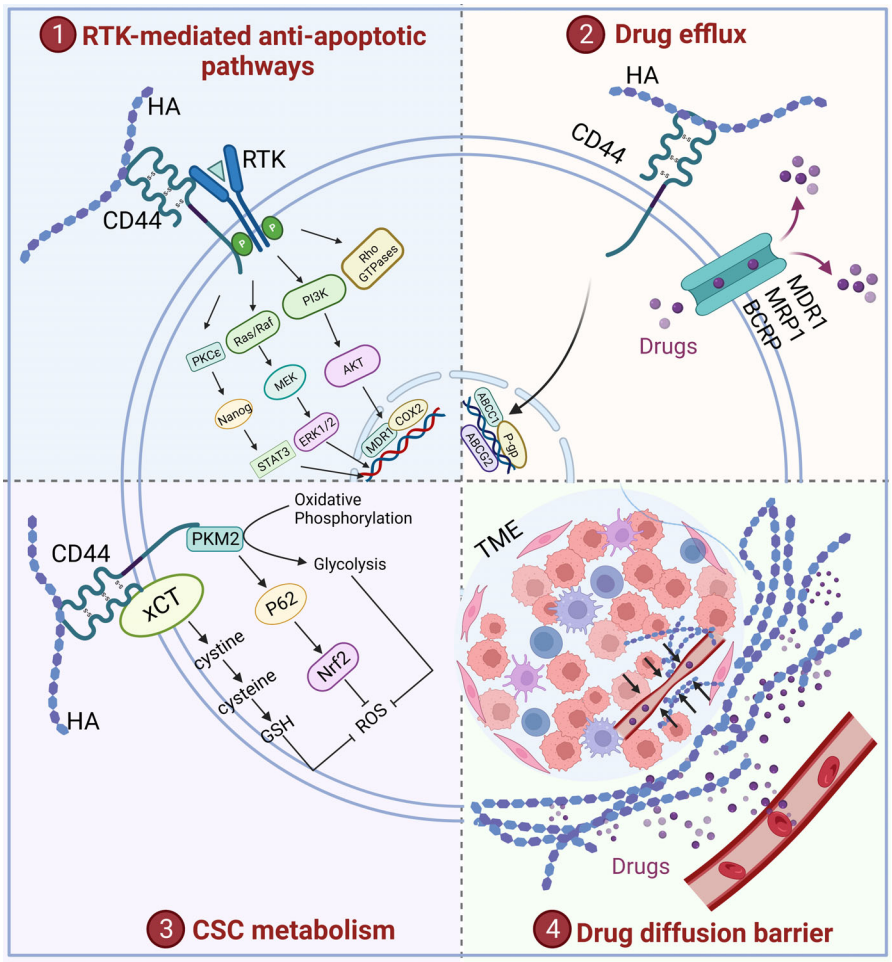

Fig. 2. Mechanisms of hyaluronic acid (HA)-mediated chemoresistance in tumors. (1) CD44 regulates receptor tyrosine kinase (RTK) activation and modulates different antiapoptotic signaling pathways, which promote chemoresistance. (2) HA-CD44 interaction regulates multidrug resistance in cancer cells. (3) HA-CD44 axis regulates cancer stem cell (CSC) properties through induction of oxidative stress resistance. (4) HA acts as a biophysical barrier impairing vascular function and drug delivery in tumor cells. This figure was created with BioRender.com.

PI3K/Akt activation which ultimately leads to cell death resistance [96]. In colon and breast carcinoma cells, elevated levels of HA result in the formation of a signaling complex involving CD44 and ErbB2, along with PI3K, ezrin, HSP90, and CDC37. This complex leads to the activation of the AKT pathway and stimulates the expression of the multidrug resistance gene MDR1 [97,98]. In HA overexpressing intestinal epithelial and colon carcinoma cells, HA-CD44 interaction activates ErbB2-PI3K/AKT-β-catenin signaling and enhances tumor cell growth and survival by inducing COX-2 expression [99]. In HNSCC, HA-CD44 promotes Rho kinase- and PI3K-mediated oncogenic signaling and cisplatin resistance [100].

3.1.2. MAPK pathway

The MAPK pathway is a crucial signal transduction pathway that involves the activation of RAS GTPase. This activation leads to the activation of downstream kinases, including RAF, MEK, and extracellular response kinase 1 and 2 (ERK1 and ERK2), which regulate cell survival, proliferation, differentiation, and apoptosis [101]. In HNSCC, HA-CD44 promotes EGF-R activation, which in turn activates ERK1 and 2 to promote tumor cell growth, migration, and chemoresistance [102]. Another study demonstrated that in breast cancer cell lines, HA/CD44/RHAMM forms a signaling complex with ERK-1,2 to sustain tumor invasiveness [103].

3.1.3. Rho GTPase signaling

Rho GTPases are a family of small GTP-binding proteins that act as molecular switches inside cells, regulating various cellular processes such as cytoskeletal organization, cell migration, proliferation, and gene expression. The Rho GTPases family includes several members, including Rho, Rac, and Cdc42, which are the most extensively studied [104]. In cancer cells, Rho GTPase signaling promotes cell motility via actin polymerization, which can affect cell stiffness and mediate chemoresistance [105]. Several lines of evidence suggest that HA-CD44 association might mediate the activation of Rho GTPase signaling and cytoskeleton rearrangement, which in turn can promote tumor progression and chemoresistance [106]. For instance, in HNSCC cells, HA-CD44 mediates Rho GTPase activation by a DOT1L/H3K79-dependent mechanism and confers cisplatin resistance [100,107].

3.1.4. Other HA-mediated antiapoptotic signaling pathways

In addition to regulating RTK signaling, HA activates other anti-apoptotic signaling pathways. In breast tumor cells, HA-CD44 promotes PKCepsilon activation, which induces Nanog nuclear translocation. Nuclear Nanog associates with RNase III DROSHA and the RNA helicase p68, leading to induction of microRNA-21 expression and repression of the pro-apoptotic protein PDCD4. These events ultimately promote tumor cell survival and chemotherapy resistance [108]. HA-CD44-mediated Nanog-Stat-3 signaling pathways have a similar effect in ovarian tumor and breast tumor cells [109]. The HA receptor- RHAMM confers resistance to 5-FU via TGFβ/Smad2-induced EMT in gastric cancer [92]. HA-CD44 also regulates the Hippo signaling pathway, which modulates tumor cell death and proliferation [68,110].

Therefore, targeting HA-CD44-mediated signaling by disruption of the HA-CD44 complex (see Section 4) might be an effective strategy to dampen tumor chemoresistance.

3.2. HA-mediated multidrug resistance

Tumor chemoresistance is the result of diverse molecular pathways. One of the most important mechanisms underlying tumor drug resistance is related to the increased efflux rate of antineoplastic drugs by members of the ATP-binding cassette (ABC) transporters family. There are 48 members of this protein family, of which the most extensively studied are MDR1 (also known as P-glycoprotein or ABCB1), MRP1 (also known as ABCC1), and BCRP (also known as ABCG2) [111].

MDR1 (P-gp, ABCB1) was the first ABC transporter to be identified. It is overexpressed in many solid tumors and its expression can be induced by chemotherapy, thus causing intrinsic and acquired chemoresistance [112]. In breast cancer cells, a positive feedback loop involving HA, PI3K, and ErbB2 strongly amplifies the expression of MDR1 and increases resistance to doxorubicin. This HA-mediated effect has also been reported in another study showing that HA-CD44 stimulates MDR1 expression and induces resistance to doxorubicin, paclitaxel, and etoposide [108,109,113].

Similar to MDR1, MRP1 (ABCC1), the second member of the ABC transporter family to be identified [114], has been correlated with chemoresistance in many types of solid cancers. As mentioned before, in HNSCC cells the oncogenic activity of transcription factor ΔNp63 sustains the HA levels and signaling by transcriptionally regulating the expression of HAS3, HYAL-1, HYAL-3, and CD44. This ΔNp63-driven pathway leads to the HA-dependent activation of EGF-R and the induction of ABCC1 expression. Notably, in HNSCC tumors p63 expression is positively correlated with the expression of CD44, HAS3, and ABCC1 while the p63-HA pathway acts as a negative prognostic factor for the survival of HNSCC patients [50]. Similar to ABCC1, ABCC2 expression is also modulated by HA. In ovarian cancer, carboplatin significantly increases the expression of HAS2, HAS3, and ABCC2. Along the same line, in nonsmall cell lung cancer cells HA promotes the expression of ABCC2 and induces resistance to cisplatin [115]. Furthermore, HA treatment markedly induces the expression of ABCB3, ABCC1, ABCC2, and ABCC3 [116].

BCRP (ABCG2) was the third MDR drug efflux pump to be identified. In malignant gliomas, HA oligomers inhibit activation of EGFR and AKT, decrease BCRP expression, and increase methotrexate cytotoxicity [117,118].

In addition to regulating the expression of ABC members at the transcriptional level, HA-mediated signaling might also impact ABC transporter expression by stabilizing their levels in the plasma membrane, thereby enhancing their activity [119]. In malignant peripheral nerve sheath tumor cells, CD44 interacts with BCRP and P-glycoprotein and HA oligomers treatment induces internalization of CD44, BCRP, and P-glycoprotein, leading to decreased plasma membrane localization of drug transporters and enhanced sensitivity to doxorubicin [118]. Another study also

confirmed the physical and genetic interaction between CD44s and P-glycoprotein, which results in invasion and multidrug resistance in cancer cells [119].

Another interesting point about HA and ABC transporters family is that HA might be secreted through multidrug transporters in vertebrate cells [31]. Thus, drugs that inhibit multidrug transporters may also inhibit HA secretion and could represent an alternative strategy to dampen HA-mediated signaling.

3.3. Regulation of cancer stem cell properties by HA-CD44 signaling

CSCs are a subpopulation of cells that possess stem cell-like characteristics, such as self-renewal and differentiation potential, and are thought to be responsible for tumor initiation, maintenance, progression, and therapeutic resistance [120,121]. In human cancers, CSCs have been identified through different biomarkers. CD44 is a marker of CSCs in almost all tumors and, as primary receptor of HA, has a significant impact on the characteristics of CSCs including resistance. For instance, in ovarian cancer and breast cancer cells, HA interacts with CD44 to promoting the formation of a complex between CD44, Nanog, and STAT-3, which in turn induces the expression of SOX2, REX1, and MDR1, enhancing thus resistance to doxorubicin and paclitaxel [109]. In HNSCC, HA significantly promotes the upregulation of a subset of antiapoptotic proteins and, as a consequence, CSCs resistance to cisplatin [122]. Here, we mainly describe how the HA-CD44 axis affects CSC therapeutic resistance through oxidative stress resistance.

The buildup of ROS triggers apoptosis in both normal and cancer cells. CSCs are characterized by upregulation of several antioxidant pathways able to maintain low ROS intracellular levels [123–125]. Therefore, CSCs are relatively more resistant to radiation- or chemotherapy-induced cell death compared with nontumor cells [126]. One of the main antioxidant pathways upregulated in CSCs is mediated by the nuclear factor erythroid 2-like 2 (NFE2L2; NRF2), a transcription factor able to induce the expression of key antioxidant genes [127–129]. NRF2 is highly expressed in $CD44^{high}CD24^{low}$ CSC subpopulation isolated from doxorubicin-resistance breast cancer cells. CD44 overexpression induces the deregulation of hypoxia, upregulation NRF2, and HA itself can enhance NRF2 activation. Functionally, the molecular mechanism of CD44-mediated NRF2 activation involves high expression of p62 [130]. Once activated, NRF2 induces the expression of many genes encoding multiple antioxidant proteins, for example, glutathione (GSH)-generating enzymes. In turn, CD44 interacts with xCT, a subunit of a glutamate-cystine transporter, thereby promoting the uptake of cystine for GSH synthesis. In human gastrointestinal cancer cells, high level of CD44 expression is associated with an enhanced capacity of GSH synthesis and defense against ROS. These findings suggest that CD44-targeted therapy may impair the ROS defense ability of CSCs thereby sensitizing them to cancer therapy [131]. Similar results were also demonstrated in urothelial [132] and triple-negative breast cancer cells [133]. Another mechanism whereby CD44 enhances cellular defense against ROS-mediated cellular damage in CSCs is the regulation of glucose metabolism. By performing a proteomic screen of the binding proteins of CD44, it was discovered that pyruvate kinase M2 (PKM2) binds to the C-terminal tail of CD44 [134]. The interaction between CD44 and PKM2 enhances the glycolytic phenotype of cancer cells. CD44 ablation shifts tumor cell metabolism toward mitochondrial oxidative phosphorylation, resulting in increased ROS production, decreased level of the intracellular GSH, and higher sensitivity to ROS-mediated antineoplastic drugs [135].

3.4. HA as biophysical barrier impairs vascular function and drug delivery

Pancreatic ductal adenocarcinoma is one of the human malignancies whose TME is characterized by vascular dysfunction and extensive ECM deposition. HA is highly abundant in the ECM of PDA, and mediates PDA chemoresistance mainly by physical mechanisms [136]. Provenzano et al. [136] showed that HA significantly increases the interstitial fluid pressure (IFP) in PDA and induces vascular collapse, creating substantial barriers to perfusion, diffusion, and convection of small molecule drugs. By using a clinically formulated PEGylated human recombinant PH20 hyaluronidase (PEGPH20) to deplete HA, the authors showed that HA degradation significantly impacts IFP leading to a dramatic increase in vessel diameter, which in turn allows high concentrations of chemotherapy to reach the tumor, thereby improving the cytotoxic effects of the antineoplastic drugs. Similar evidence has also been provided by Jacobetz et al. [137]. The authors found that depletion of HA by PEGPH20 induces the re-expansion of PDA blood vessels and the increase in the delivery of two chemotherapeutic agents, doxorubicin, and gemcitabine. In addition, PEGPH20 induces ultrastructural changes in tumor endothelial cells, leading to a significant increase in the fenestration of the endothelial barrier, which is a phenotype associated

with high permeability. Furthermore, the combination therapy of PEGPH20 and gemcitabine inhibits the growth of PDA tumors and prolongs survival compared with gemcitabine monotherapy. Similar results have also been obtained in prostate cancer. In high-HA prostate PC3 tumors, administration of PEGPH20 via intravenous injection results in depletion of tumor HA, reduction in tumor stromal pressure and water content, decrease in the tumor vascular pressure, and more than a threefold increase in tumor vascular area. Moreover, PEGPH20 boosts the efficacy of docetaxel and liposomal doxorubicin in PC3-derived tumors [138]. Based on these preclinical observations, PEGPH20 efficacy and toxicity have been evaluated in clinical trials. We will provide a detailed overview of these findings in the following sections.

In addition to affect drug delivery and chemotherapy efficacy, HA can directly bind to doxorubicin and form strong interactive forces, which hinders its cellular entry in the tumor tissue [139].

4. Potential applications of targeting HA in cancer therapy

The role of HA in promoting tumor chemoresistance suggests that HA is a valuable therapeutic target for treating cancer and enhancing the efficacy of chemotherapy. Several strategies have been employed to target HA in cancer, including targeting HA synthesis, degradation, and its interaction with the receptors (Fig. 3).

4.1. Targeting HA synthesis

Given the contributions of HA and its synthesis to tumor progression and chemotherapy tolerance, there is significant interest in finding pharmacological methods to impede HA synthesis. So far, the only available inhibitor of HA synthesis is 4-methylumbelliferone (4-MU). 4-MU, a coumarin derivative, is present in various Chinese herbal medicines. 4-MU is also called

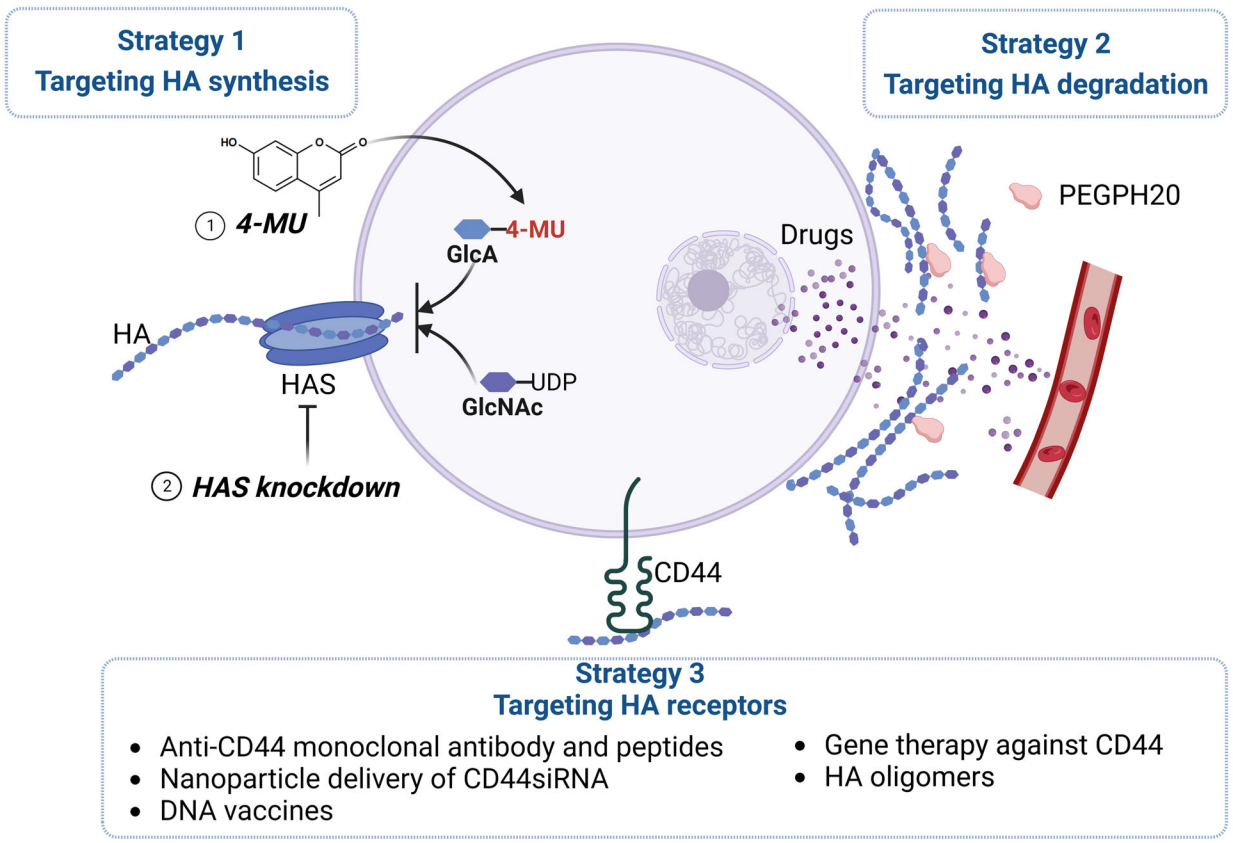

Fig. 3. Potential applications of targeting hyaluronic acid (HA) in cancer therapy. Three strategies have been employed to target HA in cancer: targeting HA synthesis (1), targeting HA degradation (2), and targeting HA receptors (3). This figure was created with BioRender.com.

'hymecromone', and it is clinically utilized to treat biliary spasm in Europe and Asia [140].

4-MU inhibits HA synthesis by two different mechanisms. Firstly, 4-MU is a competitive substrate for UDP-glucuronosyltransferase (UGT), an enzyme involved in HA synthesis [141]. Secondly, 4-MU reduces the expression of HAS mRNA expression [142]. In agreement with the critical role of HA metabolism in controlling tumor behavior, 4-MU treatment exerts antitumorigenic effects in multiple cancer cell types mainly by an HA-dependent mechanism [143–149]. For example, in glioblastoma cells, the ability of 4-MU to decrease HA synthesis, proliferation, cell migration, and induce apoptosis is dependent on HA [146].

Treatment with 4-MU can also enhance the efficacy of chemotherapy and immunotherapy. In ovarian cancer, 4-MU inhibits HA production and tumor cell survival in both chemo-resistant and chemo-sensitive cells. In chemotherapy-resistant tumor cells, the combination of 4-MU and carboplatin inhibits cell survival more effectively than carboplatin alone. This result has also been validated by using an *ex vitro* explant assay demonstrating that the combined treatment of 4-MU with carboplatin significantly increases apoptosis and reduces proliferation in tissues from chemo-resistant patients [150]. In malignant pleural mesothelioma, the combination of trametinib and 4-MU treatment has a greater inhibitory effect in comparison to trametinib monotherapy [151]. 4-MU also boosts the antitumor effect of immunotherapy. In murine colorectal carcinoma, 4-MU significantly reduces the level of tumoral HA, decreases the tumor interstitial pressure, and also increases the number of cytotoxic T lymphocytes reaching the tumor [152]. Furthermore, antitumoral efficacy of cyclophosphamide + interleukin-12 is improved by 4-MU [152]. Several clinical trials have also demonstrated the excellent safety profile of 4-MU during short-term administration [153,154]. Thus, 4-MU has the potential to be an effective agent for cancer inhibition by enhancing the efficacy of chemotherapy and immunotherapy.

It is worth noting that other evidence indicates that 4-MU might exert its antitumor effect independently of HA [155,156]. For instance, 4-MU inhibits the growth of ovarian cancer cells by suppressing thymidine phosphorylase expression [156]. Furthermore, 4-MU may impact the synthesis of additional ECM components, such as versican and fibronectin, and other glycosaminoglycans, such as chondroitin and heparin sulfates [157,158]. During the ECM remodeling induced by liver fibrosis, the synthesis of collagen 1A is also affected by 4-MU treatment [159]. In addition to ECM components, 4-MU can regulate the activity of the matrix metalloproteinases (MMPs), a family of enzymes capable to degrade many ECM components leading to tissue degradation and remodeling in an HA-independent manner [160,161]. Notably, the action of 4-MU on MMPs is different when comparing normal vs tumor tissue. More specifically, in human skin fibroblasts, 4-MU induces MMP2 activation [162], while in human carcinoma cells, 4-MU inhibits MMP9 [163]. Altogether, these studies suggest that although the main action exerted by 4-MU is HA synthesis inhibition, other HA-independent effects of 4-MU need to be considered.

In addition to dampen tumor progression, 4-MU has been recently utilized as a potential drug for COVID-19 therapy. Lungs of critically ill patients with COVID-19 infection are characterized by the presence of clear liquid jelly mainly consisting of HA [164]. By reducing the accumulation of HA and favoring the clearance of the jelly, 4-MU might restore proper alveoli function, reducing the need of ventilators for patient care [165]. This hypothesis has also been validated in clinical trials [166].

Another approach to dampen HA synthesis is by inhibiting HAS expression. There are many studies that have shown that HAS knockdown can induce apoptosis, inhibiting both tumor growth and angiogenesis in multiple cancer types [153].

4.2. Targeting HA degradation

Targeting the HA catabolism process has also been proposed to prevent HA accumulation in tumor cells and improve the efficacy of chemotherapeutic agents. HYAL (mainly PEGPH20) has been evaluated in several clinical trials in combination with other therapies (such as chemotherapy, immunotherapy, or radiotherapy) in different types of cancer [167–174] (Table 1). In an early study of 40 pediatric brain cancer patients, the combination of HYAL with standard chemotherapeutics (carboplatin and etoposide) significantly improved event-free survival and overall survival [172]. This reduced recurrence was also seen in a study of HNSCC [170]. In 2013, the safety, pharmacokinetic, and pharmacodynamic profile of PEGPH20 in patients with a variety of solid tumors was demonstrated in a phase I clinical study. The results demonstrate a reduction in HA levels within the tumor, improved tumor perfusion, and decreased tumor metabolic activity. These findings provide support for further evaluation of the combination of PEGPH20 with anticancer therapies in patients with advanced solid tumors [69]. In 2016, Hingorani et al. [168] reported the results of a phase 1b study of PEGPH20 in combination with gemcitabine to treat stage IV metastatic PDA.

Table 1. Clinical trials of hyaluronidase-targeted therapies.

Study	Trial type	Tumor type	Chemotherapy	No. of patients	Results
Smith KJ et al., 1997, (124)	Phase I	Kaposi's sarcoma	Vinblastine	6	Enhances drug efficacy and reduces recurrence
Pillwein K et al., 1998 (123)	Phase II	Malignant brain	Carboplatin/etoposide	40	Event-free survival and overall survival at 36 months were significantly improved
Klocker J et al., 1998, (125)	Phase II	Advanced squamous cell carcinoma of the head and neck	Irradiation + cisplatin/ vindesine	48	The disease-free survival rate at 5 years was 47%
Baumgartner G et al., 1998, (126)	Phase III	Bladder cancer	Mitomycin C	56	Reduced recurrence
Hingorani SR et al., 2016, (102)	Phase Ib	Pancreatic ductal adenocarcinoma	Gemcitabine	28	PEGPH20 in combination with Gem was well tolerated and has therapeutic benefit
Hingorani SR et al., 2018, (103)	Phase II	Pancreatic ductal adenocarcinoma	Nab-paclitaxel/ gemcitabine	279	Progression-free survival was significantly improved
Van Cutsem E et al., 2020, (127)	Phase III	Metastatic pancreatic adenocarcinoma	Nab-paclitaxel/ gemcitabine	494	Increased the ORR but did not improve OS or PFS
Ko AH et al., 2023, (128)	Phase Ib/II	Pancreatic ductal adenocarcinoma/ gastric cancer	Atezolizumab	108	Atezolizumab plus PEGPH20 showed limited clinical activity in patients with PDAC and none in patients with GC

According to clinical trial, the combination of PEGPH20 and gemcitabine is well tolerated and could be an effective treatment option for patients with advanced PDA, particularly those with high levels of HA in tumors. In 2018, they reported the results of a phase II study of PEGPH20 in combination with nab-paclitaxel/gemcitabine (PAG) in 279 patients with metastatic PDA. PEGPH20 combination therapy consistently improved the progression-free survival (PFS; overall) [169]. Although these phase I/II results were encouraging, the subsequent HALO-301 phase III trial did not meet its primary endpoint of OS [175,176]. In detail, this study, which enrolled patients with stage IV PDA expressing high amount of HA, compared PEGPH20 in combination with PAG to PAG alone. No improvement in duration of response, PFS, or OS was seen in PEGPH20-treated patients. The failure of this phase III study together with the high toxicity observed in the PEGPH20-FOLFIRINOX (folinic acid fluorouracil irinotecan oxaliplatin) combination [177] suggest that targeting HA might not be sufficient for eradicating tumor cells. Other intrinsic factors such the high complexity of TME and the low tumor mutational burden may considerably concur to the elevated chemoresistance of PDA. Furthermore, it is worth noting that disruption of the tumor stroma might also facilitate the metastatic capacity of tumor cells, as described for PDA deficient in Sonic Hedgehog signaling [178]. Therefore, further research and evaluation need to be directed to understand how the complex interplay between HA dysregulation, TME complexity, and metastatic potential in order to increase the efficacy and safety of HA-based drugs.

4.3. Targeting HA receptors

The interaction between HA and CD44, as we described above, modulates chemoresistance of tumors through multiple mechanisms. Therefore, CD44 is an attractive target to modulate HA-dependent signaling. The therapeutic approaches aimed to interfere with CD44 signaling are mainly based on targeting either CD44-HA interaction or CD44 itself through different strategies, such as antiCD44 monoclonal antibody [179], peptides [180], nanoparticle delivery of CD44 siRNA [181], DNA vaccines [182], or gene therapy against CD44 [183]. For example, monoclonal antibodies targeting CD44v6 have been demonstrated to be effective in hampering tumor growth in preclinical studies [180]. However, phase I clinical studies failed due to the severe skin reactions likely caused by the physiological expression of CD44v6 antigen in normal squamous cells [184]. Hence, the employment of the CD44 targeting approach in cancer therapy would require meticulous assessment before implementation [79,184].

Alternative approaches aimed at blocking CD44-mediated signaling include the usage of soluble CD44 or HA oligomers which can act as competitors of CD44 thus impeding HA-CD44 interaction. In melanoma cells, soluble CD44 acts as a competitor of CD44 and blocks HA-CD44 interaction, thereby inhibiting tumor growth and metastasis [185]. In the

plasma membrane of malignant peripheral nerve sheath tumor cells, HA oligosaccharides disrupt the complex between CD44, BCRP (ABCG2), and P-glycoprotein (ABCB1), thereby inhibiting drug transporter activity and increasing doxorubicin sensitivity [118]. HA oligomer treatment also significantly inhibits the growth of melanoma [186] and glioma tumor cells [117]. In HepG2iso cells and endothelial cells, HA oligomers inhibit cell motility and angiogenesis by interfering with HMW-HA-CD44 interaction [187]. It has also been reported that HA oligosaccharides impact on the anchorage-independent tumor cell proliferation by suppressing the PI-3K/Akt cell survival pathway [188]. Overall, these results demonstrate that HA oligomers might be potentially exploited as an approach to dampen HA-mediated pro-tumorigenic effects.

The interaction between HA and its receptor CD44, together with the excellent HA biocompatibility, biodegradability, and nonimmunogenicity, has also been exploited to increase the delivery of conventional antineoplastic drugs into tumor cells. HA can be either conjugated directly with antitumor drugs or used in several types of nanomaterials, such as micelles and hydrogels [189,190].

A promising approach for treating colon cancer patients was based on the noncovalent conjugation of Irinotecan with a domain of HA. Although this approach successfully passed phase I and phase II trials [191,192], multicenter phase III study failed to meet statistical significance between HA–Irinotecan-based FOLFIRI (folinic acid, 5-FU, and irinotecan) versus standard FOLFIRI in metastatic colon cancer patients [193]. The failure of this phase III study might be due to intrinsic factors such as the upregulation of drug efflux channels which might impact the intracellular concentration of irinotecan [194], or the decreases of CD44 expression which would render the tumor cells largely less responsive to CD44-targeted treatment [195]. Alternatively, a more rigorous enrollment criterion based for instance on the analysis of CD44 expression levels could facilitate a better stratification of the patients and decrease patient heterogeneity.

5. Conclusion

Chemotherapy resistance represents a major cause of therapeutic failure and mortality in cancer patient. HA accumulation in the TME is able to promote either tumor growth, or recurrence and therapy resistance, according to distinct molecular circumstances. Mechanistically, HA acts as a pro-chemo-resistant factor by regulating multiple pathways, such as activation of RTKs and antiapoptotic signaling, induction of drug transporter expression, regulation of cancer stem cell properties and by acting as a barrier impairing vascular function and drug delivery. Hence, HA-based strategies have been developed to enhance the effectiveness of chemotherapeutic agents and overcome chemoresistance in multiple types of cancers. Nevertheless, HA-based cancer therapy faces challenges. HA synthesis and degradation is a complex circuit and the proportion of HA polymers with different lengths inside the HA mixture is dynamically regulated. Understanding the detailed proportion of HA polymers under certain circumstances is pivotal to predict the cellular response of HA dysregulation on a specific biological process. HA abundance and chain lengths dynamically remodel the mechanical and biological properties of the TME and affect the intracellular signaling cascades during tumor progression; it is important to understand HA homeostasis in a dynamic manner in order to better interpret HA-mediated signaling. Furthermore, the detection of the serum concentration of specific HA fragments would provide a more meaningful prognosis predictor and diagnostic index in certain human diseases. In conclusion, future research effort should be focused on tackling these challenges to provide a better know-how of the clinical application of HA-based therapy.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

ZL and AP wrote the manuscript. ZL prepared the figures. PH, JF, CS, and YS provide comments. GM revised the manuscript. All authors have approved this submitted version.

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