



Review

Hyaluronan-CD44 axis orchestrates cancer stem cell functions

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ABSTRACT

The prominent role of CD44 in tumor cell signaling together with its establishment as a cancer stem cell (CSC) marker for various tumor entities imply a key role for CD44 in CSC functional properties. Hyaluronan, the main ligand of CD44, is a major constituent of CSC niche and, therefore, the hyaluronan-CD44 signaling axis is of functional importance in this special microenvironment. This review aims to provide recent advances in the importance of hyaluronan-CD44 interactions in the acquisition and maintenance of a CSC phenotype. Hyaluronan-CD44 axis has a substantial impact on stemness properties of CSCs and drug resistance through induction of EMT program, oxidative stress resistance, secretion of extracellular vesicles/exosomes and epigenetic control. Potential therapeutic approaches targeting CSCs based on the hyaluronan-CD44 axis are also presented.

1. Introduction

Stem cells are embedded within dynamic specialized environments, the niches, that are composed of specific cellular and molecular constituents. The composition of the niches is tissue-dependent giving rise to defined signals that regulate stem cell fate [1]. The specific cellular composition of the niches orchestrates the secretion of factors (such as growth factors, chemokines, cytokines, Wnts, Hedgehog, hormones and exosomes) that are important for the maintenance of the balance between quiescence, self-renewal and differentiation of stem cells. These factors collectively regulate stemness through modulation of self-renewal pathways, including Notch, Hedgehog and Wnt/ β -catenin pathways or by interference with key transcriptional regulators, such as Oct4, Sox2 and NANOG, which are essential for the reprogramming of differentiated cells into an embryonic stem cell phenotype [2–4].

The maintenance of stem cell properties is critically regulated by the tight interaction of stem cells with their surrounding extracellular matrices (ECMs) through a number of cell surface receptors, including integrins and membrane-bound glycoproteins and proteoglycans [5]. The relevance of ECMs in regulating stem cell properties is evidenced by studies where decellularized tissues that maintained their ECM were able to drive stem cell differentiation into cell types that originally resided in these tissues [6–8].

Integrins, which are heterodimeric transmembrane receptors, connect cellular cytoskeleton to the extracellular milieu thus regulating stem cell proliferation, migration, differentiation, adhesion, anchorage

and homing. Although integrins do not possess any endogenous enzymatic activity, they regulate multiple downstream signaling pathways in numerous stem cell types – depending on the specific composition of their niches – thus modulating their stemness properties [5,9]. Several studies have shown that specific integrins are essential in regulating the properties of certain stem cell types. For example, β 1 integrins regulate the proliferation, self-renewal and differentiation of intestinal, mammary and neural stem cells via modulation of the activity of Notch, EGFR and Hedgehog signaling [10–12]. Moreover, α 6 β 1 integrin is required for the adhesion and homing of spermatogonial and neural stem cells in their niches [13,14], while the interaction of α 8 β 1 integrin with nephronectin maintains the proper position and function of hair follicle stem cells [15]. Recent findings support a model in which stem cells remodel their microenvironment to maintain integrin α 6 expression, inhibit β 1/FAK activation and preserve the expression of Oct4 and Sox2 transcription factors which are both associated with self-renewal and pluripotency [16]. Notably, integrin α 6 links stem cells to laminin which is a common ECM protein in multiple niches produced by both non-stem and stem cells promoting their self-renewal. Integrins may also direct stem cell fate via mechanical stimuli, termed mechanotransduction. Recent mechanistic studies demonstrated that integrin α 5 controls the fate of pancreatic progenitors via F-actin-YAP1-Notch mechanosignaling axis [17].

Apart from integrins, stem cell surface glycoproteins and proteoglycans are able to avidly bind extracellular messenger molecules, such as growth factors, cytokines/chemokines and other ligands,

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regulating their local concentration, distribution and activation in a cell context-dependent manner. Transmembrane syndecans and the GPI-anchored glypicans capture basic growth factors and morphogens from the extracellular milieu through their negatively charged heteropolysaccharide side chains and present them to their cognate cellular receptors, generating long range maintenance of gradients during embryogenesis and regeneration processes like tissue remodeling in inflammation and cancer [18]. Their involvement in the maintenance of stem cells has been shown in several stem cell types. For example, the expression of specific members of the syndecan and glypican families as well as their biosynthetic enzymes (exostoses, *N*-deacetylase, *N*-2-*O*/6-*O*-sulfotransferases, C5-epimerase) mediate human neural stem cells maintenance and lineage potential [19].

Among stem cell surface receptors, CD44 proteins comprise a major class of single-pass transmembrane glycoproteins that, similarly to integrins, do not possess endogenous enzymatic activity while they connect the extracellular matrix to the cellular cytoskeleton and organize receptor platforms on cell surfaces coordinating multiple downstream signaling pathways [20–23]. CD44 proteins arise from a single gene which in humans contains 19 exons. The alternative splicing of exons 6 to 15 (designated as variant exons v1-v10) produce multiple CD44 isoforms (CD44v), while their exclusion results in the formation of the standard form of CD44 (CD44s). The heterogeneity of CD44 proteins is further promoted by multiple post-translational modifications, such as glycosylation (N- and O-linked oligosaccharides) and, under particular developmental or pathological conditions, the covalent attachment of a glycosaminoglycan (chondroitin or heparan sulfate) side chain that classifies CD44 as a part-time proteoglycan [21]. Importantly, CD44 is a prominent marker for both normal and cancer stem cells (CSCs) in combination with other cell surface molecules, such as the detoxifying aldehyde dehydrogenase 1 (ALDH1) enzyme, CD133 and others [24]. The prominent role of CD44 in cell signaling together with its utilization as a stem cell marker imply also a functional role for CD44 in stem cells [21,25].

As mentioned above, stem cells fate is critically determined by their cross-talk with the cellular and non-cellular constituents of their niches. Hyaluronan, a non-sulfated glycosaminoglycan, is a major component of stem cell niches and the main ligand for the stem cell receptor/marker CD44. Despite its simple structure composed of a repeating disaccharide unit ($\rightarrow 4$ -D-glucuronic acid- $\beta 1 \rightarrow 3$ -N-acetyl-D-glucosamine- $\beta 1 \rightarrow$)_n, hyaluronan is synthesized by three synthase isoforms (HAS1-3) and degraded by five hyaluronidases (HYAL1-4, PH-20) suggesting complex metabolic regulation and biological functions. The relative expression levels of hyaluronan metabolic enzymes and, thus, hyaluronan concentration and size are tissue-dependent and highly related to specific physiological and pathological conditions [21,26]. In cancer, most studies suggest a strong correlation between high hyaluronan concentration, especially in the tumor stroma, and pro-tumorigenic and pro-metastatic mechanisms and tumor progression [27]. Notably, in addition to extracellular hyaluronan-rich matrices, hyaluronan can be also present in intracellular compartments in normal and pathological processes with potentially important regulatory roles in cellular functions [28]. Moreover, different hyaluronan polymers have different roles in controlling the hallmarks of cancer cells. Small fragments show pro-inflammatory, pro-angiogenic and tumorigenic functions by altering selective signaling pathways, while high molecular weight hyaluronan possess anti-inflammatory and anti-angiogenic properties [27,29].

Since hyaluronan is the major ligand of stem cell marker CD44, their specific interactions are important for the maintenance of the proper stem cell niche. Hyaluronan matrices produced by HAS2 and HAS3 in *Xenopus laevis* are essential for the development of intestinal stem cells through a positive feedback loop and for the formation of the niche during metamorphosis [30]. In humans, bone marrow-derived mesenchymal stem cells (hMSCs) have the potential for autocrine maintenance of the hyaluronan niche which is important for their stemness

properties. It has been shown that hMSCs form extensive pericellular CD44-bound hyaluronan coats produced by all three HASs which are highly expressed in these cells [31]. Another study demonstrated that hyaluronan and CD44 cooperate with stromal cell-derived factor-1 (SDF-1) for the transendothelial migration of human CD34+ stem/progenitor cells and their final anchorage within bone marrow niches [32]. Furthermore, hyaluronan-CD44 interactions have been shown to modulate neural stem cell differentiation and quiescence [33]. Notably, hyaluronan has been successfully used as 3D scaffolds for the chondrogenic and osteogenic differentiation of stem cells [34]. Therefore, hyaluronan and CD44 are primary functional ECM components in normal stem cell niche.

2. Hyaluronan-CD44 interactions promote tumor malignancy by regulating CSC functions

Cancer stem cells (CSCs) differ from the normal stem cells in their ability to alter their pluripotency and lineage-dependent differentiation, while they have been shown to be resistant to radiation and chemotherapy making them capable of repopulating a tumor after treatment resulting in cancer relapse [35,36]. Similarly to normal stem cell niches, hyaluronan matrices create the ideal microenvironment for self-renewal and maintenance of CSCs. Hyaluronan interactions with its main receptor CD44 have been shown to regulate various aspects of tumorigenesis and cancer progression. Specifically, hyaluronan binding to CD44 modulates oncogenic signaling pathways through activation of multiple cell surface receptors, such as c-MET, EGFR, ErbB2 and TGF- β RI by direct or indirect interactions. Hyaluronan through CD44 activates ERM, Vav2, Grb2, Gab-1, Src and Rac GTPase families to regulate cell migration. Moreover, hyaluronan-CD44 interactions drive drug resistance through activation and stabilization of multidrug and metabolic transporters [20,22]. Given the multitude of cellular functions in which CD44 and its ligand hyaluronan participate, it is not surprising that they have been linked with regulation of CSC properties such as self-renewal, survival, tumor initiation, anchorage-independent growth, chemoresistance and anti-apoptotic signaling. CD44 serves as a widely accepted cancer stem cell marker for various cancer types such as breast, pancreatic, colorectal, hepatocellular, lung, and head and neck cancer. Therefore, CD44 is frequently used for isolation of CSCs from the respective tumors [37]. Albeit hyaluronan and CD44 have been identified as main components of CSC niche, their functional importance for the pathobiology of CSCs remains largely obscure, owing to the multifaceted involvement of CD44 in the regulation of a large variety of signaling pathways.

In breast cancer, hyaluronan production induces aggressive properties of breast cancer cells in vitro [38] and negatively correlates with patient outcome [39]. Moreover, the presence of CD44 correlates with decreased overall survival of breast cancer patients. Importantly, CD44^{High}CD24^{Low} populations have been isolated from breast tumors and exert stem-like properties, such as self-renewal, tumor-initiating and metastatic capacities [40–42]. Mechanistically, Δ Np63 transcriptional program induces the expression of HAS3, HYAL-1 and CD44 to drive the formation of a hyaluronan-rich microenvironment, which in turn promotes proliferation and stemness of breast cancer cells [43]. Hyaluronan promotes acquisition of stemness as shown by experiments with niche mimetic hyaluronan-based multilayer nanofilms. In particular, when pancreatic carcinoma cells were placed in hyaluronan-containing niche mimetics, the expressions of Oct4, CXCR4 and CD44v6 were upregulated in the formed colonies. Moreover, E-cadherin, HIF1 α and vimentin levels were also increased suggesting the existence of a hybrid and metastable epithelial/mesenchymal phenotype [44]. In pancreatic cancer, CD44 promotes stem-like properties and metastatic behavior. KLF4 - a tumor suppressor gene with low expression in pancreatic cancer - binds to CD44 promoter inhibiting its expression and subsequently inhibiting the acquisition of pancreatic cancer stemness and metastasis [45]. In hepatocellular carcinoma, hyaluronan

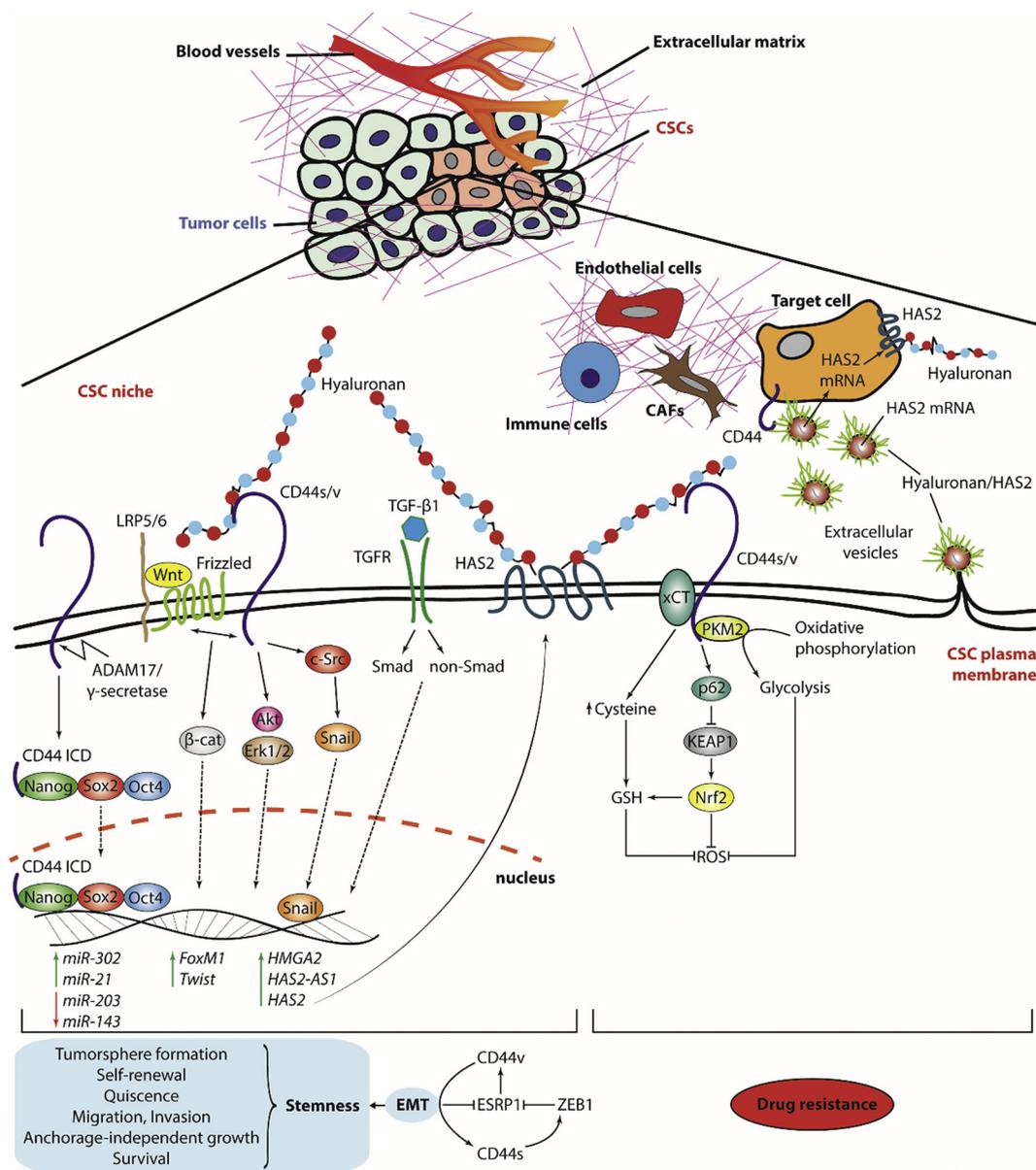


Fig. 1. Hyaluronan-CD44 axis regulates CSC stemness properties through induction of EMT program, oxidative stress resistance, epigenetic control and secretion of extracellular vesicles. For details, refer to text.

synthesis inhibition by 4-MU in CD133⁺ CD90⁺ EpCAM⁺ CD44⁺ CD13⁺ CSCs, reduced CD47 expression to promote phagocytosis by antigen presenting cells [46]. In the clinic, CD44 overexpression in lung cancer patients correlates with higher metastatic probability. CD44 has been shown to activate Wnt/ β -catenin signaling to upregulate FoxM1 and its transcriptional target Twist, promoting acquisition of stemness and metastatic activity of CD133⁺CD44⁺ lung adenocarcinoma cells (Fig. 1) [47]. β -catenin can be also activated by seryglycin-induced MAPK signaling to drive the expression of CD44 to promote stemness in nasopharyngeal carcinoma [48]. In malignant mesothelioma (MM) stem cells, inhibition of hyaluronan synthesis or CD44 with gene knockdown or specific antibodies abolished sphere-forming capacity and maintenance of CSCs highlighting the central role of hyaluronan-CD44 axis in MM onset and progression [49]. In oral squamous cell carcinoma, CD44⁺ circulating tumor cells have the ability to form spheres and display intrinsic chemoresistance and self-renewal properties [50]. These tumors exhibited increased expression of CD44v3, CD44v6, Nanog and Bmi1 and significant downregulation of PTEN and ATM. Pathway analysis of these markers demonstrated a CSC regulatory

mechanism by miRNA and cytokine network. The authors showed that the CD44v6-Nanog-PTEN axis is targeted by miR542-3p and miR34a, while cytokine profiling revealed increased levels of IL-6 and IL-8, which were strongly associated with CD44v6 [51]. Adding to the complexity of the biological role of CD44, it has been observed to compensate for ICAM1, which regulates PTTG1IP-p53-DNMT1 pathway to maintain stemness of esophageal squamous cell carcinoma [52]. Collectively, hyaluronan/CD44 play critical roles in the maintenance of CSC biology and the promotion of the metastatic potential of multiple carcinoma types.

2.1. Acquisition of CSC signatures by hyaluronan/CD44-driven EMT

Epithelial-to-mesenchymal transition (EMT) has been shown to coincide with acquisition of CSC properties [53] and increased metastatic incidents in breast cancer [54]. Hyaluronan molecular system exerts an important role in the regulation of the EMT process. Specifically, HAS2 protein is required for proper TGF- β -induced EMT in mammary epithelial cells [55]. Recently, it was discovered that TGF- β induces HAS2-

AS1 to drive the expression of *HAS2*, *HMGA2* and *FN1* [56]. *HAS2*-AS1 is a natural antisense transcript that is a long non coding RNA synthesized by the opposite genomic DNA strand at the *HAS2* locus. Nuclear *HAS2*-AS1 regulates *HAS2* transcription by altering chromatin organization around *HAS2* promoter, while cytoplasmic *HAS2*-AS1 may influence several processes such as *HAS2* mRNA stabilization, RNA editing and interactions with microRNAs suggesting important roles in tumor biology [27,57]. The subsequent production of hyaluronan by *HAS2* leads to the activation of Akt and Erk1/2 and ultimately EMT and acquisition of stem cell characteristics. Of note, CD44 was found to be important for EMT induction by TGF- β (Fig. 1) [56]. In another study, hyaluronan overproduction by *HAS2* accelerated breast cancer progression by promoting the enrichment of a CD44^{high}/CD24^{low} CSC-like subpopulation through the induction of EMT and the EMT-related factors Snail and Twist, and the activation of TGF β -Snail signaling axis [58]. Okuda and colleagues showed that upregulation of *HAS2* occurred in highly metastatic CD44+/CD24-/ESA+ breast CSCs and massive hyaluronan accumulation was critical for the interaction of CSCs with their niche leading to enhanced secretion of PDGF-BB from tumor associated macrophages which subsequently activated stromal cells to promote CSC self-renewal [59]. The critical involvement of hyaluronan in this process was demonstrated by the finding that hyaluronan synthesis inhibition by 4-MU resulted in drastic reduction of metastatic lesions in vivo [59]. Recently, Nguyen and colleagues showed that intracellular hyaluronan produced by *HAS1* induced EMT, increased centrosomal abnormalities and micronucleation allowing for the creation of a suitable niche of CSCs [60]. During EMT process, CD44 expression switches from CD44v to CD44s and this shift is important for the regulation of stemness during cancer progression. ESRP1 -which controls CD44 alternative splicing- levels are reduced during EMT, leading to the expression of the standard isoform (Fig. 1). CD44s is essential for cells to undergo EMT probably through activation of Akt signaling. When transplanted in mice, cells expressing CD44s lead to the formation of tumors with mesenchymal characteristics [61]. In CSCs, *ESRP1* expression is inhibited by *ZEB1* -a crucial transcriptional factor required for the EMT process- to control the switch from CD44v to CD44s. CD44s in turn induces *ZEB1* expression to provide a self-sustaining loop that further promotes EMT, allowing cancer cells to acquire stemness independently of external stimuli (Fig. 1) [62]. The relevance of CD44 in the maintenance of stemness and mammosphere formation as a readout of CSC activity was evident in head and neck squamous cell carcinoma cells in vivo where CD44 ectodomain cleavage by ADAM17 protease resulted in enhanced sphere-forming capacity and tumorigenesis of these cells [63]. Furthermore, cleavage of CD44 intracellular domain (ICD) by γ -secretase leads to its nuclear translocation together with Nanog/Sox2/Oct4 transcription factors to induce breast cancer tumorigenesis and sphere-forming capacity (Fig. 1) [64]. In glioma, CD44 ICD binds to HIF2 α (but not HIF1 α) to modulate pseudo-hypoxic phenotype of stem-like glioma cells independently of oxygen. Moreover, CD44 ICD enhances HIF1 α -target gene expression irrespective of HIF1 α and is required for hypoxia-induced stemness [65,66].

2.2. Hyaluronan/CD44 confers CSCs with resistance to oxidative stress

Reactive oxygen species (ROS) accumulation induces apoptosis in both normal and cancer cells [67,68]. CSCs are able to limit ROS production at low level, which is critical in preserving stem cell properties, quiescence induction and enhanced drug resistance [69,70]. A major transcription factor that regulates ROS levels is Nrf2. High Nrf2 expression was observed in CD44^{High}CD24^{Low} cell populations isolated from a drug-resistant breast cancer cell line. Mechanistically, hyaluronan through CD44, upregulates p62, which deactivates KEAP1 to promote Nrf2 activation and subsequent transcription of antioxidant response genes to inhibit ROS accumulation and induce drug resistance in breast cancer cells (Fig. 1) [71]. The synthesis of reduced glutathione

(GSH), a major intracellular antioxidant, is enhanced by several downstream target genes of Nrf2 (Fig. 1) [72]. GSH levels are also modulated by CD44 since the expression of CD44v8-10 regulates the glutamate-cystine xCT transporter, which promotes cystine uptake, to modulate glutathione synthesis and inhibit ROS accumulation, leading to drug resistance in urothelial and triple-negative breast cancer (Fig. 1) [73,74]. CD44v9 has been also correlated with Nrf2 activation and poor overall survival of patients with hepatocellular carcinoma [75].

A protective role of CD44 against the ROS damaging actions in CSCs is also probably mediated by the modulation of glucose metabolism by CD44. A proteomic screen of CD44 intracellular binding proteins revealed that pyruvate kinase M2 binds to the C-terminal tail of CD44 [76]. This interaction promoted the metabolic flux from mitochondrial respiration to glycolysis in cancer cells that were either deficient in p53 or hypoxic resulting in reduced ROS production (Fig. 1) [77]. Notably, elevated hyaluronan production enhanced HIF1 α signaling via hexosamine biosynthetic pathway, which could constitute a feed-forward signaling mechanism driving metabolic reprogramming towards glycolysis and the expression of CSC-like properties [78]. Overall, these data suggest that CSCs might develop multiple hyaluronan/CD44-mediated defensive mechanisms in order to gain a survival and oxidative stress-resistant advantage.

ROS regulation is important for the maintenance of drug resistance of CSCs by enhancing DNA repair. Importantly, hyaluronan was shown to exert important anti-oxidant roles to protect cells from DNA damage [79]. The mechanism of this protective effect may either involve entrapment of iron ions to inhibit the Fenton's reaction that produces secondary oxidative species and/or directly scavenging of primary and secondary ROS, as an antioxidant, resulting in hyaluronan degradation [80]. Activation of CD44 by hyaluronan facilitates DNA repair in T-cell lymphoma after treatment with chemotherapeutic agents and ionizing radiation [81]. CD44 is also implicated in modulation of the DNA repair process through activation of Chk1/2 and ATM pathways in prostate [82] and breast [83] cancer, respectively. Taken together, these data support a role of hyaluronan/CD44 in DNA repair after treatment with DNA-damaging anti-cancer drugs to maintain CSCs and drive chemoresistance.

2.3. Hyaluronan-CD44 interactions regulate epigenetic alterations in CSCs

HAS2-synthesized hyaluronan through CD44 contributes to silencing of ER α -driven genes by modulating the activity of nuclear histone deacetylases (HDACs) and histone acetyl-transferase EP300 [84]. Experiments performed in CD44⁺CD24⁻ breast CSCs showed that promoter CpG island methylation was different in relation to breast cancer subtype and stem cell phenotype of tumor, suggesting an underlying role of CD44 in DNA methylation to drive tumor stemness [85]. Interestingly, CD44 has been shown to modulate cytokine gene methylation in encephalitogenic T cells [86] and a similar model could be envisioned in maintenance of CD44⁺ CSCs through autocrine cytokine signaling. Importantly, CD44 itself as well as several other hyaluronan-related genes (*HYAL-1*, *HAS2*) expressions are controlled by epigenetic modifications such as histone deacetylation and CpG island methylation [87–92]. Hyaluronan through CD44 regulates histone modification (H3K79 methylation) through DOT1L to upregulate miR-10b expression, which activates downstream Rho GTPases to promote head and neck squamous cancer cell survival and resistance.

A wide panel of other miRNAs (miR-27b, miR-373, miR-181, miR-34a, miR145) have been identified to be induced by treatment with 200 kDa hyaluronan [93]. Hyaluronan/CD44 interactions also modulate c-Jun signaling to induce miR-21 expression leading to Bcl-2 expression and chemoresistance in breast cancer cells [94]. In colon cancer, hyaluronan binds to CD44 and activates c-Src to induce Snail nuclear translocation. Subsequently, nuclear Snail binds to E-box miR-203 (a stemness inhibitor) promoter to inhibit its expression and promote stemness marker expression, self-renewal, anchorage-independent

growth and tumor growth in animal xenografts (Fig. 1) [95]. The proposed mechanism of miR-203 is further supported by the findings of Chamnee and colleagues, which showed that high hyaluronan synthesis and accumulation promotes breast CSCs stemness by regulating the EMT-related transcriptional factors Snail and Twist [58]. Another study that shows the regulation of CSCs functions by hyaluronan-CD44 interactions in a miRNA-dependent manner was performed by Bourguignon and colleagues in a human oral squamous carcinoma cell line with high metastatic potential [96]. The authors demonstrated the presence of a CSC subpopulation expressing high levels of CD44v3 and, importantly, the interaction of hyaluronan with CD44v3 induced the formation and nuclear translocation of the Oct4-Sox2-Nanog tripartite complex, which is essential for CSC properties and controls the expression of miR-302 (Fig. 1). In contrast, the expression of miR-143 (a tumor suppressor) is down-regulated in CD44⁺ breast cancer cells and breast tumors compared to CD44⁻ cells and normal tissues, respectively (Fig. 1). The tumor-suppressive function of miR-143 depends on its ability to bind the 3'-untranslated region of CD44 inhibiting its expression and subsequently the stem cell properties of breast cancer cells [97]. For a comprehensive review of hyaluronan/CD44 role on epigenetic modifications and CSC biology, the reader is referred to an excellent review by Bourguignon and colleagues [93]. Therefore, the existence of an autocrine mechanism of hyaluronan to induce hyaluronan-related genes to promote a positive feedback loop for stemness acquisition through regulation of the epigenome is possible and remains to be experimentally proven.

2.4. Hyaluronan/CD44 drive CSC-derived extracellular vesicles/exosomes

CSC-derived extracellular vesicles (i.e. exosomes, microvesicles and apoptotic bodies) are important regulators of the communication between CSCs and their niche. They contain membrane trafficking regulators, cytoskeletal proteins, functional mRNAs and miRNAs, various angiogenic and immunosuppressive factors, as well as ECM effectors such as proteinases, glycosidases and hyaluronan/HAS/CD44 [98–100]. Hyaluronan is an important component of these entities as evidenced by its increased production and secretion by human mesenchymal stem cells, which are characterized by the massive shedding of hyaluronan-coated extracellular vesicles [31,101]. Although extracellular vesicles contain HAS proteins on their surface as well as functional HAS mRNAs, it is not known if they can actually produce hyaluronan [102,103]. As part of the communication of CSCs with their niche, it is likely that CSCs exchange HAS proteins with target cells that reside in the niche thereby promoting their hyaluronan-synthesizing capacity, while it is also possible that target cells receive the functional HAS mRNAs to translate them into newly synthesized HAS protein (Fig. 1).

Extracellular vesicles contain also CD44, which may either help to retain hyaluronan on the vesicles synergistically to HASs or transferred to the target cells. The latter was demonstrated by a recent study of Nakamura and colleagues, which indicated that ovarian cancer-derived exosomes transfer CD44 to human peritoneal mesothelial cells (HPMC) facilitating cancer invasion [104]. Mechanistically, CD44 was found to be enriched in the cancer cell-derived exosomes, transferred, and internalized to HPMCs, leading to high levels of CD44 in HPMCs. This increased CD44 expression in HPMCs promoted cancer invasion by inducing the secretion of MMP9 and by cleaving the mesothelial barrier for improved cancer cell invasion [104]. Moreover, the interaction of hyaluronan-coated CSC-derived extracellular vesicles with the cellular constituents of the niche could be facilitated and promoted by their specific binding to CD44 on the membranes of target cells resulting in the fusion and release of their cargo within the recipient cells of the niche thus contributing to niche remodeling and stemness promotion (Fig. 1). In pancreatic and colorectal cancers, cancer-initiating cells (CICs)-derived exosomes bearing CD44v6 were shown to transfer the migratory and invasive capacity to non-CICs and to regulate the

expression of integrins and proteases to promote cancer cell migration and invasiveness. Notably, CD44v6 knockdown altered the composition of the secreted tumor exosomes, which lost their ability to promote the malignant phenotype [105]. The finding that the induction of the pre-metastatic niche by exosomes is dependent on CD44v6 further supports the importance of hyaluronan-CD44 interactions on the role of extracellular vesicles in the promotion of tumor malignancy [106].

In various types of cancer, hyaluronan is also produced by stromal cells to support tumor growth [107–109]. Importantly, CD44⁺ cancer-associated fibroblasts (CAFs) are present in hypoxic and avascular areas of tumors. Kinugasa and colleagues were able to identify a positive role of CD44-expressing CAFs in the maintenance of drug resistance and stemness characteristics of cancer stem/initiating cells by utilizing co-culture systems and sphere formation assays [110]. Although further experiments are needed to identify the underlying molecular mechanisms responsible for this phenomenon, which could possibly involve extracellular vesicles, this study highlights the important role of niches' specific constituents in the acquisition of CSC stemness.

3. Relevance of CD44 isoform expression for the malignant CSC phenotype

CD44 has been identified as a potent CSC marker as discussed above, albeit important information is lacking regarding the specific isoforms expressed in each type of CSCs. Several CD44v have been recently identified as significant markers for the isolation of CSCs from various types of cancer. Importantly, specific CD44 isoforms appear to have significant roles in CSC functional properties, regulating several CSC features, such as self-renewal, high proliferative potential, apoptosis-resistance and chemoresistance [111]. During the last years, significant progress has been made for the identification of CD44v as CIC/CSC markers and the elucidation of their biological significance in CSCs.

In gastric cancer, CD44v8-10 was identified as the most frequent CD44v [112]. Subsequently, CD44v8-10 expression was correlated with advanced tumor initiation incidents of gastric cancer. Importantly, the expression of this CD44 isoform, but not CD44s, increased the frequency of tumor initiation in immunocompromised mice possibly through enhancing oxidative stress defense. These data demonstrated that CD44v8-10 could serve as a potent specific marker for gastric CSCs [112]. Furthermore, it was shown that IL-6/STAT3 can also induce CD44v6 expression to regulate tumor differentiation and lymph node metastasis in gastric cancer. Importantly, CD44v6 expression correlates with poor prognosis of gastric carcinoma patients [113]. Collectively, CD44v6 and CD44v8-10 could serve as potent CSCs markers in gastric carcinoma to predict metastasis and tumor recurrence.

CD44v3 and CD44v6 have been identified as CSC markers in oral squamous cell carcinomas and their functional importance in the regulation of IL-6 and IL-8 cytokine network through Nanog/PTEN axis has been recently proposed [51]. Moreover, in CD44v3⁺ALDH1⁺ CSCs, CD44v3 activates Oct4/Sox2/Nanog signaling to drive self-renewal/clonal formation and generate heterogenous cancer cell populations in head and neck squamous cell carcinoma [96]. CD44v6 expression has been also identified in CICs/CSCs from pancreatic and colorectal cancer and loading of CD44v6 on CIC-derived exosomes could transfer the migratory and invasive capacity to non-CIC cells residing within the niche thus regulating angiogenesis, invasion, migration and metastasis [105]. CD44v6 was found to be expressed by clonogenic colorectal CSCs and was required for their effective migration and formation of metastasis [114]. This study showed that secretion of HGF, osteopontin and SDF-1 from tumor associated cells induced the expression of CD44v6, which was low in primary tumors, by activating Wnt/ β -catenin pathway in CSCs promoting their metastatic potential. Therefore, the authors suggested that CD44v6 could serve as a marker of constitutive and reprogrammed CSCs driving colon cancer metastasis. CD44v6 has been also identified together with integrin β 4 and CD47 as

potent CSC markers to correlate with poor relapse-free survival in patients with posterior wall bladder cancer [115]. In urothelial cancer, CD44v9 upregulation was identified in tumor specimens and CD44v9 positivity was an independent risk factor of cancer-specific survival in patients that had received cisplatin and displayed recurrence and/or metastasis. It was proposed that CD44v9 inhibits intracellular ROS accumulation probably through the xCT transporter stabilization, which adjusts glutathione levels, thereby regulating chemoresistance to cisplatin [73]. A similar functional role was attributed to CD44v9-xCT system in hepatocellular carcinoma where its expression was found to be upregulated in patients treated with hepatic arterial infusion chemoembolization with cisplatin and could serve as an indicator of resistance to applied therapeutics [116]. Moreover, in hepatitis C virus-positive patients with resected hepatocellular carcinoma, CD44v9 and ALDH1 correlate with poor overall survival and recurrence-free survival as well as poor histological differentiation of the tumors [75].

Specific CD44 isoform expression can be considered a specific CSC signature in several tumor tissues and can be correlated with advanced recurrence and metastasis. Although further studies need to be conducted, the obtained data indicate a role of CD44v in the antioxidant responses of CSCs. Alternatively, CD44v, i.e. CD44v3/v6, could regulate CSCs functions through presentation of growth factors to their cognate receptors via their glycosaminoglycan side chains, thereby modulating various downstream signaling pathways.

Collectively, differential CD44v expression can be utilized as a strategy to detect CSC signatures in tumors that would enable the development of early detection tools and, therefore, effective early intervention and therapy.

4. Onco-suppressive approaches targeting hyaluronan-CD44 axis in CSCs

Hyaluronan, a major “poly-messenger” macromolecule of CSC niche, interacts with CD44 receptor on CSCs and the generated signals credit CSCs with unique functional properties that individuate them from the bulk of cancer cells within the tumor (Fig. 1). These specialized signals result in the development of drug resistance programs in CSCs that are responsible for their reconstitution after therapy and disease relapse. As described above, hyaluronan-CD44 axis affects major mechanisms of drug resistance in CSCs [117], including induction of EMT, overexpression of cell membrane transporters, hypoxia, regulation of ROS, shedding of extracellular vesicles/exosomes loaded with tumor-promoting effectors, and regulation of epigenetic factors. Hence, targeting hyaluronan-CD44 axis could constitute a valid and efficient approach to decline stemness properties of CSCs and important aspects of resistance to cancer therapies.

Resistance to HER2-targeted therapies - a major obstacle in the clinical management of HER2-positive breast cancer - is mediated mainly by CD44, independently of its role as a breast CSC marker, which is partly regulated by microRNAs. Knockdown of CD44 led to an increased response to trastuzumab while it blocked the invasiveness and anchorage-independent growth of trastuzumab-resistant cells [118]. Nrf2, a key regulator of antioxidant genes, has been linked to drug resistance in CD44^{high} breast CSCs while hyaluronan/CD44-mediated Nrf2 activation resulted in aggressive phenotype and tumor growth. Therefore, it was suggested that CD44-Nrf2 axis could be a candidate therapeutic target for the control of stress resistance and stemness properties of CD44^{high} CSC subpopulations within the bulk of breast tumor cells [71]. Moreover, since nuclear localization of cleaved CD44ICD is important for transcriptional activation of stemness factors (i.e. Nanog, Sox2, Oct4) and treatment with γ -secretase inhibitor blocked sphere formation, the utilization of this specific enzyme could effectively target CSC phenotype in breast cancer [64]. In patients with advanced HNSCC, concurrent chemoradiotherapy induced CD44v9 expression [119]. Therefore, targeting CD44v9 might enhance chemoradiation efficacy of this carcinoma type as well as organ

preservation and survival. Bourguignon and colleagues were able to demonstrate that hyaluronan-CD44v3 promoted miR-302 expression leading to cisplatin resistance in HNSCC. Therefore, anti-miR-302 inhibition could abrogate hyaluronan-CD44v3-mediated CSC functions making it a promising therapeutic approach against HNSCC CSC phenotype [96]. In another study, inhibition of PI3K selectively eliminated colorectal CSCs expressing CD44v6 that confers colorectal cancer cells with their high metastatic potential [114]. Interestingly, CD44 expressed on CAFs in CSC niche of melanoma is also of functional importance supporting stemness and drug resistance [110]. The authors of this study highlighted the possibility that drugs delivered specifically to CD44-positive CAFs could be a new promising candidate therapeutic approach for melanoma.

Rodriguez and colleagues suggested that a combined strategy involving hyaluronan biosynthesis inhibition by 4-MU with an adenovirus encoding IL-12 could represent a potent anti-tumor modality since it efficiently reduced hepatocellular carcinoma aggressiveness through induction of anticancer immunity and CSC targeting [46]. In particular, 4-MU when combined with adenovirus-encoding IL-12 gene induced potent cytotoxic-specific T cell responses. Moreover, hepatocellular carcinoma cells pretreated with 4-MU, when injected to animals displayed increased overall survival compared to non-treated. Targeting CSCs through specific receptor (CD44) – ligand (hyaluronan) interactions might also be an effective CSC-targeted therapeutic approach. Marengo and colleagues found that the use of hyaluronan decorated liposomal formulations containing diethyldithiocarbamate copper against pancreatic CSCs strongly reduced their proliferation and sphere formation through a ROS-mediated mechanism [120]. Since hyaluronan-modified nanoparticles exhibit good biocompatibility and aqueous solubility, their conjugation with chemotherapeutics constitute promising and effective drug delivery systems. In gastric cancer, hyaluronan-modified nanoparticles delivering rMETase suppressed tumor growth and tumorsphere formation damaging the mitochondrial function of CD44+ gastric CSCs [121]. In another study, nanovesicle-mediated miR-34a delivery attenuated proliferation and migration of gastric cells by repressing CD44 expression as well as Bcl-2, Oct4 and Nanog genes [122].

In malignant mesothelioma (MM), a novel therapeutic option was suggested involving targeting of CD44 and ALK4 signaling pathways that down-regulated sphere formation and maintenance of CIC compartment within MM cells [49]. In another line of research, as CD44v9-xCT system is involved in the resistance of hepatocellular carcinoma to hepatic arterial infusion chemoembolization with cisplatin, a combination treatment with sulfasalazine (SASP), a xCT inhibitor, has been suggested to overcome such drug resistance in this type of cancer [116]. In a similar fashion, knockdown of CD44v8-10 could offer an efficient therapeutic approach in urothelial cancer where this CD44 isoform contributes to ROS modulation through xCT [73]. This could be also potentiated by the combined application of SASP. In another line of research, the findings by Chu and colleagues that extracellular serglycin up-regulated CD44 to maintain nasopharyngeal carcinoma stemness and to promote chemoresistance suggest that manipulation of serglycin-CD44 axis could represent a promising therapeutic approach to target CSCs in nasopharyngeal carcinoma [48].

Hyaluronan, HAS and CD44 are major constituents of CSC-derived extracellular microvesicles and, thus, they may represent potential biomarkers of the pre-metastatic niche for the prediction of early metastasis or recurrence. The microvesicles have the potential to deliver the HAS machinery to target cells influencing niche remodeling and malignant properties of CSCs [102]. Since hyaluronan synthesis enhances shedding of plasma membrane-derived microvesicles [102], a promising therapeutic approach could be the blockade of their secretion through hyaluronan suppression using specific hyaluronan biosynthesis inhibitors (i.e. 4-MU [123–126]), HAS gene knockdown or hyaluronidases (i.e. PEGPH20 [127–129]). Similar approaches, which involved drugs that blocked H⁺/Na⁺ and Na⁺/Ca²⁺ channels (e.g.

dimethyl amiloride) or prevented biogenesis of exosomes (e.g. sphingomyelinase inhibitors), showed beneficial effects in metastatic tumors [130,131].

5. Conclusions

The use of CD44 as a main CSC marker for a large number of tumors combined with its key role in tumor cell signaling clearly suggests a functional link between them. Many studies, although initially solely focused on CD44 as a CSC marker, have now demonstrated the relevance of specific CD44v and hyaluronan-CD44 interactions in the maintenance of CSC properties. The observations of a switch from CD44v to CD44s during EMT, which is important for the acquisition of a CSC phenotype, possibly characterize CD44⁺ CSC subpopulations with different features within a tumor. Importantly, recent studies propose that CSCs constitute the main source of drug resistance, which is a leading cause of tumor recurrence and metastasis and, eventually, treatment failure. Hyaluronan-CD44 axis has nodal roles in key cell signaling pathways that underlie important aspects of resistance to tumor therapy in various cancers. Overall, the current literature suggests that hyaluronan-CD44 standard/variant axis is a very attractive target for the design and development of novel therapeutic strategies against metastatic tumors being in nascent stage.

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References

- [1] A. Rezza, R. Sennett, M. Rendl, Adult stem cell niches: cellular and molecular components, *Curr. Top. Dev. Biol.* 107 (2014) 333–372.
- [2] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, *Cell*. 126 (2006) 663–676.
- [3] A. Borah, S. Raveendran, A. Rochani, T. Maekawa, D.S. Kumar, Targeting self-renewal pathways in cancer stem cells: clinical implications for cancer therapy, *Oncogenesis*. 4 (2015) e177.
- [4] J. Kim, S.H. Orkin, Embryonic stem cell-specific signatures in cancer: insights into genomic regulatory networks and implications for medicine, *Genome Med.* 3 (2011) 75.
- [5] F. Gattazzo, A. Urciuolo, P. Bonaldo, Extracellular matrix: a dynamic micro-environment for stem cell niche, *Biochim. Biophys. Acta* 1840 (2014) 2506–2519.
- [6] K.H. Nakayama, C.A. Batchelder, C.I. Lee, A.F. Tarantal, Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering, *Tissue Eng. Part A* 16 (2010) 2207–2216.
- [7] A. Kurtz, S.J. Oh, Age related changes of the extracellular matrix and stem cell maintenance, *Prev. Med.* 54 (Suppl. S50-6) (2012).
- [8] G.C. Reilly, A.J. Engler, Intrinsic extracellular matrix properties regulate stem cell differentiation, *J. Biomech.* 43 (2010) 55–62.
- [9] P. Lu, V.M. Weaver, Z. Werb, The extracellular matrix: a dynamic niche in cancer progression, *J. Cell Biol.* 196 (2012) 395–406.
- [10] L.S. Campos, L. Decker, V. Taylor, W. Skarnes, Notch, epidermal growth factor receptor, and beta1-integrin pathways are coordinated in neural stem cells, *J. Biol. Chem.* 281 (2006) 5300–5309.
- [11] R.G. Jones, X. Li, P.D. Gray, J. Kuang, F. Clayton, W.S. Samowitz, B.B. Madison, D.L. Gumucio, S.K. Kuwada, Conditional deletion of beta1 integrins in the intestinal epithelium causes a loss of Hedgehog expression, intestinal hyperplasia, and early postnatal lethality, *J. Cell Biol.* 175 (2006) 505–514.
- [12] C. Briskin, S. Duss, Stem cells and the stem cell niche in the breast: an integrated hormonal and developmental perspective, *Stem Cell Rev.* 3 (2007) 147–156.
- [13] M. Kanatsu-Shinohara, M. Takehashi, S. Takashima, J. Lee, H. Morimoto, S. Chuma, A. Raducanu, N. Nakatsuji, R. Fassler, T. Shinohara, Homing of mouse spermatogenic stem cells to germline niche depends on beta1-integrin, *Cell Stem Cell* 3 (2008) 533–542.
- [14] Q. Shen, Y. Wang, E. Kokovay, G. Lin, S.M. Chuang, S.K. Goderie, B. Roysam, S. Temple, Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions, *Cell Stem Cell* 3 (2008) 289–300.
- [15] H. Fujiwara, M. Ferreira, G. Donati, D.K. Marciano, J.M. Linton, Y. Sato, A. Hartner, K. Sekiguchi, L.F. Reichardt, F.M. Watt, The basement membrane of hair follicle stem cells is a muscle cell niche, *Cell*. 144 (2011) 577–589.
- [16] L.G. Villa-Diaz, J.K. Kim, A. Laperle, S.P. Palecek, P.H. Krebsbach, Inhibition of focal adhesion kinase Signaling by integrin alpha6beta1 supports human pluripotent stem cell self-renewal, *Stem Cells* 34 (2016) 1753–1764.
- [17] A. Mamidi, C. Prawiro, P.A. Seymour, K.H. de Lichtenberg, A. Jackson, P. Serup, H. Semb, Mechanosignalling via integrins directs fate decisions of pancreatic progenitors, *Nature*. 564 (2018) 114–118.
- [18] N.K. Karamanos, Z. Piperigkou, A.D. Theocharis, H. Watanabe, M. Franchi, S. Baud, S. Brezillon, M. Gotte, A. Passi, D. Vigetti, S. Ricard-Blum, R.D. Sanderson, T. Neill, R.V. Iozzo, Proteoglycan chemical diversity drives multifunctional cell regulation and therapeutics, *Chem. Rev.* 118 (2018) 9152–9232.
- [19] L.E. Oikari, R.K. Okolicsanyi, A. Qin, C. Yu, L.R. Griffiths, L.M. Haupt, Cell surface heparan sulfate proteoglycans as novel markers of human neural stem cell fate determination, *Stem Cell Res.* 16 (2016) 92–104.
- [20] B.P. Toole, Hyaluronan-CD44 interactions in Cancer: paradoxes and possibilities, *Clin. Cancer Res.* 15 (2009) 7462–7468.
- [21] E. Karousou, S. Misra, S. Ghatak, K. Dobra, M. Gotte, D. Vigetti, A. Passi, N.K. Karamanos, S.S. Skandalis, Roles and targeting of the HAS/hyaluronan/CD44 molecular system in cancer, *Matrix Biol.* 59 (2017) 3–22.
- [22] S. Misra, P. Heldin, V.C. Hascall, N.K. Karamanos, S.S. Skandalis, R.R. Markwald, S. Ghatak, Hyaluronan-CD44 interactions as potential targets for cancer therapy, *FEBS J.* 278 (2011) 1429–1443.
- [23] V. Orian-Rousseau, CD44 acts as a Signaling platform controlling tumor progression and metastasis, *Front. Immunol.* 6 (2015) 154.
- [24] H. Clevers, The cancer stem cell: premises, promises and challenges, *Nat. Med.* 17 (2011) 313–319.
- [25] I. Morath, T.N. Hartmann, V. Orian-Rousseau, CD44: more than a mere stem cell marker, *Int. J. Biochem. Cell Biol.* 81 (Pt A) (2016) 166–173.
- [26] P. Heldin, C.Y. Lin, C. Kolliopoulos, Y.H. Chen, S.S. Skandalis, Regulation of hyaluronan biosynthesis and clinical impact of excessive hyaluronan production, *Matrix Biol.* 78–79 (2018) 100–117.
- [27] A. Passi, D. Vigetti, S. Buraschi, R.V. Iozzo, Dissecting the role of hyaluronan synthases in the tumor microenvironment, *FEBS J.* (2019), <https://doi.org/10.1111/febs.14847>.
- [28] S.S. Skandalis, T. Karalis, P. Heldin, Intracellular hyaluronan: Importance for cellular functions, *Semin. Cancer Biol.* (2019), <https://doi.org/10.1016/j.semcancer.2019.07.002> pii: S1044-579X(19)30130-0.
- [29] I. Caon, B. Bartolini, A. Parnigoni, E. Carava, P. Moretto, M. Viola, E. Karousou, D. Vigetti, A. Passi, Revisiting the hallmarks of cancer: the role of hyaluronan, *Semin. Cancer Biol.* (2019), <https://doi.org/10.1016/j.semcancer.2019.07.007> pii: S1044-579X(19)30042-2.
- [30] K. Fujimoto, T. Hasebe, M. Kajita, A. Ishizuya-Oka, Expression of hyaluronan synthases upregulated by thyroid hormone is involved in intestinal stem cell development during *Xenopus laevis* metamorphosis, *Dev. Genes Evol.* 228 (2018) 267–273.
- [31] C. Qu, K. Rilla, R. Tammi, M. Tammi, H. Kroger, M.J. Lammi, Extensive CD44-dependent hyaluronan coats on human bone marrow-derived mesenchymal stem cells produced by hyaluronan synthases HAS1, HAS2 and HAS3, *Int. J. Biochem. Cell Biol.* 48 (2014) 45–54.
- [32] A. Avigdor, P. Goichberg, S. Shvitiel, A. Dar, A. Peled, S. Samira, O. Kollet, R. Hershkovitz, R. Alon, I. Hardan, H. Ben-Hur, D. Naor, A. Nagler, T. Lapidot, CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34⁺ stem/progenitor cells to bone marrow, *Blood*. 103 (2004) 2981–2989.
- [33] W. Su, S.C. Foster, R. Xing, K. Feistel, R.H. Olsen, S.F. Acevedo, J. Raber, L.S. Sherman, CD44 transmembrane receptor and Hyaluronan regulate adult hippocampal neural stem cell quiescence and differentiation, *J. Biol. Chem.* 292 (2017) 4434–4445.
- [34] L. Zou, Y. Luo, M. Chen, G. Wang, M. Ding, C.C. Petersen, R. Kang, F. Dagnaes-Hansen, Y. Zeng, N. Lv, Q. Ma, D.Q. Le, F. Besenbacher, L. Bolund, T.G. Jensen, J. Kjems, W.T. Pu, C. Bunker, A simple method for deriving functional MSCs and applied for osteogenesis in 3D scaffolds, *Sci. Rep.* 3 (2013) 2243.
- [35] P. Dalerba, M.F. Clarke, Cancer stem cells and tumor metastasis: first steps into uncharted territory, *Cell Stem Cell* 1 (2007) 241–242.
- [36] A. Schulenburg, H. Ulrich-Pur, D. Thurnher, B. Erovic, S. Florian, W.R. Sperr, P. Kalhs, B. Marian, F. Wrba, C.C. Zielinski, P. Valent, Neoplastic stem cells: a novel therapeutic target in clinical oncology, *Cancer*. 107 (2006) 2512–2520.
- [37] B. Greve, R. Kelsch, K. Spaniol, H.T. Eich, M. Gotte, Flow cytometry in cancer stem cell analysis and separation, *Cytometry Part A* 81 (2012) 284–293.
- [38] A. Herrera-Gayol, S. Jothy, Effects of hyaluronan on the invasive properties of human breast cancer cells in vitro, *Int. J. Exp. Pathol.* 82 (2001) 193–200.
- [39] K.L. Schwertfeger, M.K. Cowman, P.G. Telmer, E.A. Turley, J.B. McCarthy, Hyaluronan, inflammation, and breast Cancer progression, *Front. Immunol.* 6 (2015) 236.
- [40] J. Shao, W. Fan, B. Ma, Y. Wu, Breast cancer stem cells expressing different stem cell markers exhibit distinct biological characteristics, *Mol. Med. Rep.* 14 (2016) 4991–4998.
- [41] W. Wei, H. Hu, H. Tan, L.W. Chow, A.Y. Yip, W.T. Loo, Relationship of CD44+CD24⁻/low breast cancer stem cells and axillary lymph node metastasis, *J. Transl. Med.* 10 (Suppl. 1) (2012) S6.
- [42] W. Li, H. Ma, J. Zhang, L. Zhu, C. Wang, Y. Yang, Unraveling the roles of CD44/CD24 and ALDH1 as cancer stem cell markers in tumorigenesis and metastasis, *Sci. Rep.* 7 (2017) 13856.
- [43] V. Gatti, C. Fierro, M. Compagnone, F. Giangrazi, E.K. Markert, L. Bongiorno-Borbone, G. Melino, A. Peschiaroli, DeltaNp63 regulates the expression of hyaluronic acid-related genes in breast cancer cells, *Oncogenesis*. 7 (2018) 65.
- [44] I.C. Lee, Y.C. Wu, W.S. Hung, Hyaluronic acid-based multilayer films regulate hypoxic multicellular aggregation of pancreatic Cancer cells with distinct Cancer stem-cell-like properties, *ACS Appl. Mater. Interfaces* 10 (2018) 38769–38779.
- [45] Y. Yan, Z. Li, X. Kong, Z. Jia, X. Zuo, M. Gagea, S. Huang, D. Wei, K. Xie, KLF4-mediated suppression of CD44 Signaling negatively impacts pancreatic Cancer Stemness and metastasis, *Cancer Res.* 76 (2016) 2419–2431.

- [46] M.M. Rodriguez, E. Fiore, J. Bayo, C. Atorrasagasti, M. Garcia, A. Onorato, L. Dominguez, M. Malvicini, G. Mazzolini, 4Mu decreases CD47 expression on hepatic Cancer stem cells and primes a potent antitumor T cell response induced by Interleukin-12, *Mol. Ther.* 26 (12) (2018) 2738–2750.
- [47] J. Su, S. Wu, H. Wu, L. Li, T. Guo, CD44 is functionally crucial for driving lung cancer stem cells metastasis through Wnt/beta-catenin-FoxM1-twist signaling, *Mol. Carcinog.* 55 (2016) 1962–1973.
- [48] Q. Chu, H. Huang, T. Huang, L. Cao, L. Peng, S. Shi, L. Zheng, L. Xu, S. Zhang, J. Huang, X. Li, C. Qian, B. Huang, Extracellular serglycin upregulates the CD44 receptor in an autocrine manner to maintain self-renewal in nasopharyngeal carcinoma cells by reciprocally activating the MAPK/beta-catenin axis, *Cell Death Dis.* 7 (2016) e2456.
- [49] Y. Ohno, S. Shingyoku, S. Miyake, A. Tanaka, S. Fudesaka, Y. Shimizu, A. Yoshifuji, Y. Yamawaki, S. Yoshida, S. Tanaka, K. Sakura, T. Tanaka, Differential regulation of the sphere formation and maintenance of cancer-initiating cells of malignant mesothelioma via CD44 and ALK4 signaling pathways, *Oncogene*. 37 (49) (2018) 6357–6367.
- [50] S. Patel, K. Shah, S. Mirza, K. Shah, R. Rawal, Circulating tumor stem like cells in oral squamous cell carcinoma: an unresolved paradox, *Oral Oncol.* 62 (2016) 139–146.
- [51] S. Patel, R. Rawal, Role of miRNA dynamics and cytokine profile in governing CD44v6/Nanog/PTEN axis in oral cancer: modulating the master regulators, *Tumour Biol.* 37 (2016) 14565–14575.
- [52] S.T. Tsai, P.J. Wang, N.J. Liou, P.S. Lin, C.H. Chen, W.C. Chang, ICAM1 is a potential Cancer stem cell marker of Esophageal squamous cell carcinoma, *PLoS One* 10 (2015) e0142834.
- [53] I. Fabregat, A. Malfettone, J. Soukupova, New insights into the crossroads between EMT and Stemness in the context of Cancer, *J. Clin. Med.* 5 (2016).
- [54] R. Lakhtakia, A. Aljarrah, M. Furrukh, S.S. Ganguly, Epithelial mesenchymal transition (EMT) in metastatic breast Cancer in Omani women, *Cancer Microenviron.* 10 (2017) 25–37.
- [55] H. Porsch, B. Bernert, M. Mehic, A.D. Theocharis, C.H. Heldin, P. Heldin, Efficient TGFbeta-induced epithelial-mesenchymal transition depends on hyaluronan synthase HAS2, *Oncogene*. 32 (2013) 4355–4365.
- [56] C. Koliopoulos, C.Y. Lin, C.H. Heldin, A. Moustakas, P. Heldin, Has2 natural antisense RNA and Hmga2 promote Has2 expression during TGFbeta-induced EMT in breast cancer, *Matrix Biol.* 80 (2018) 29–45.
- [57] D. Vignetti, M. Viola, E. Karousou, S. Deleonibus, K. Karamanou, G. De Luca, A. Passi, Epigenetics in extracellular matrix remodeling and hyaluronan metabolism, *FEBS J.* 281 (2014) 4980–4992.
- [58] T. Chanmee, P. Ontong, N. Mochizuki, P. Kongtawelert, K. Konno, N. Itano, Excessive hyaluronan production promotes acquisition of cancer stem cell signatures through the coordinated regulation of twist and the transforming growth factor beta (TGF-beta)-snail signaling axis, *J. Biol. Chem.* 289 (2014) 26038–26056.
- [59] H. Okuda, A. Kobayashi, B. Xia, M. Watabe, S.K. Pai, S. Hirota, F. Xing, W. Liu, P.R. Pandey, K. Fukuda, V. Mudur, A. Ghosh, A. Wilber, K. Watabe, Hyaluronan synthase HAS2 promotes tumor progression in bone by stimulating the interaction of breast cancer stem-like cells with macrophages and stromal cells, *Cancer Res.* 72 (2012) 537–547.
- [60] N. Nguyen, A. Kumar, S. Chacko, R.J. Ouellette, A. Ghosh, Human hyaluronan synthase-1 promotes malignant transformation via epithelial-to-mesenchymal transition, micronucleation and centrosome abnormalities, *Cell Commun. Signal.* 15 (2017) 48.
- [61] R.L. Brown, L.M. Reinke, M.S. Damerow, D. Perez, L.A. Chodosh, J. Yang, C. Cheng, CD44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression, *J. Clin. Invest.* 121 (2011) 1064–1074.
- [62] B.T. Preca, K. Bajdak, K. Mock, V. Sundararajan, J. Pfannstiel, J. Maurer, U. Wellner, U.T. Hopt, T. Brummer, S. Brabletz, T. Brabletz, M.P. Stemmler, A self-enforcing CD44s/ZEB1 feedback loop maintains EMT and stemness properties in cancer cells, *Int. J. Cancer* 137 (2015) 2566–2577.
- [63] P. Kamarajan, J.M. Shin, X. Qian, B. Matte, J.Y. Zhu, Y.L. Kapila, ADAM17-mediated CD44 cleavage promotes orasphere formation or stemness and tumorigenesis in HNSCC, *Cancer Med.* 2 (2013) 793–802.
- [64] Y. Cho, H.W. Lee, H.G. Kang, H.Y. Kim, S.J. Kim, K.H. Chun, Cleaved CD44 intracellular domain supports activation of stemness factors and promotes tumorigenesis of breast cancer, *Oncotarget.* 6 (2015) 8709–8721.
- [65] E. Johansson, E.S. Grassi, V. Pantazopoulou, B. Tong, D. Lindgren, T.J. Berg, E.J. Pietras, H. Axelson, A. Pietras, CD44 interacts with HIF-2alpha to modulate the hypoxic phenotype of Perinecrotic and perivascular glioma cells, *Cell Rep.* 20 (2017) 1641–1653.
- [66] K.E. Miletti-Gonzalez, K. Murphy, M.N. Kumaran, A.K. Ravindranath, R.P. Wernyj, S. Kaur, G.D. Miles, E. Lim, R. Chan, M. Chekmareva, D.S. Heller, D. Foran, W. Chen, M. Reiss, E.V. Bandera, K. Scotto, L. Rodriguez-Rodriguez, Identification of function for CD44 intracytoplasmic domain (CD44-ICD): modulation of matrix metalloproteinase 9 (MMP-9) transcription via novel promoter response element, *J. Biol. Chem.* 287 (2012) 18995–19007.
- [67] H. Wiseman, B. Halliwell, Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer, *Biochem. J.* 313 (Pt 1) (1996) 17–29.
- [68] T. Yamamori, H. Yasui, M. Yamazumi, Y. Wada, Y. Nakamura, H. Nakamura, O. Inanami, Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint, *Free Radic. Biol. Med.* 53 (2012) 260–270.
- [69] A. Carnero, Y. Garcia-Mayea, C. Mir, J. Lorente, I.T. Rubio, M.E. L. L., The cancer stem-cell signaling network and resistance to therapy, *Cancer Treat. Rev.* 49 (2016) 25–36.
- [70] D.W. Clark, K. Palle, Aldehyde dehydrogenases in cancer stem cells: potential as therapeutic targets, *Ann. Transl. Med.* 4 (2016) 518.
- [71] I.G. Ryoo, B.H. Choi, S.K. Ku, M.K. Kwak, High CD44 expression mediates p62-associated NFE2L2/NRF2 activation in breast cancer stem cell-like cells: implications for cancer stem cell resistance, *Redox Biol.* 17 (2018) 246–258.
- [72] C. Tonelli, I.L.C. Chio, D.A. Tuveson, Transcriptional regulation by Nrf2, *Antioxid. Redox Signal.* 29 (2018) 1727–1745.
- [73] M. Hagiwara, E. Kikuchi, N. Tanaka, T. Kosaka, S. Mikami, H. Saya, M. Oya, Variant isoforms of CD44 involves acquisition of chemoresistance to cisplatin and has potential as a novel indicator for identifying a cisplatin-resistant population in urothelial cancer, *BMC Cancer* 18 (2018) 113.
- [74] M. Hasegawa, H. Takahashi, H. Rajabi, M. Alam, Y. Suzuki, L. Yin, A. Tagde, T. Maeda, M. Hiraki, V.P. Sukhatme, D. Kufe, Functional interactions of the cysteine/glutamate antiporter, CD44v and MUC1-C oncoprotein in triple-negative breast cancer cells, *Oncotarget.* 7 (2016) 11756–11769.
- [75] A. Kakehashi, N. Ishii, E. Sugihara, M. Gi, H. Saya, H. Wanibuchi, CD44 variant 9 is a potential biomarker of tumor initiating cells predicting survival outcome in hepatitis C virus-positive patients with resected hepatocellular carcinoma, *Cancer Sci.* 107 (2016) 609–618.
- [76] S.S. Skandalis, I. Kozlova, U. Engstrom, U. Hellman, P. Heldin, Proteomic identification of CD44 interacting proteins, *IUBMB Life* 62 (2010) 833–840.
- [77] M. Tamada, O. Nagano, S. Tateyama, M. Ohmura, T. Yae, T. Ishimoto, E. Sugihara, N. Onishi, T. Yamamoto, H. Yanagawa, M. Suematsu, H. Saya, Modulation of glucose metabolism by CD44 contributes to antioxidant status and drug resistance in cancer cells, *Cancer Res.* 72 (2012) 1438–1448.
- [78] T. Chanmee, P. Ontong, T. Izumikawa, M. Higashide, N. Mochizuki, C. Chokchaitweesuk, M. Khansai, K. Nakajima, I. Kakizaki, P. Kongtawelert, N. Taniguchi, N. Itano, Hyaluronan production regulates metabolic and Cancer stem-like properties of breast Cancer cells via Hexosamine biosynthetic pathway-coupled HIF-1 Signaling, *J. Biol. Chem.* 291 (2016) 24105–24120.
- [79] G.M. Campo, A. Avenoso, S. Campo, A. D'Ascola, A.M. Ferlazzo, A. Calatroni, Reduction of DNA fragmentation and hydroxyl radical production by hyaluronic acid and chondroitin-4-sulphate in iron plus ascorbate-induced oxidative stress in fibroblast cultures, *Free Radic. Res.* 38 (2004) 601–611.
- [80] H. Zhao, T. Tanaka, V. Mitlitski, J. Heeter, E.A. Balazs, Z. Darzynkiewicz, Protective effect of hyaluronate on oxidative DNA damage in WI-38 and A549 cells, *Int. J. Oncol.* 32 (2008) 1159–1167.
- [81] C. Chen, M.C. Chang, R.K. Hsieh, Y.F. Chang, J. Lin, K.W. Tsan, Activation of CD44 facilitates DNA repair in T-cell lymphoma but has differential effects on apoptosis induced by chemotherapeutic agents and ionizing radiation, *Leuk. Lymphoma* 46 (2005) 1785–1795.
- [82] W. Xiao, P.H. Graham, C.A. Power, J. Hao, J.H. Kearsley, Y. Li, CD44 is a biomarker associated with human prostate cancer radiation sensitivity, *Clin. Exp. Metastasis* 29 (2012) 1–9.
- [83] H. Yin, J. Glass, The phenotypic radiation resistance of CD44 + /CD24 (– or low) breast cancer cells is mediated through the enhanced activation of ATM signaling, *PLoS One* 6 (2011) e24080.
- [84] M. Vanneste, V. Hanoux, M. Bouakka, P.J. Bonnamy, Hyaluronate synthase-2 overexpression alters estrogen dependence and induces histone deacetylase inhibitor-like effects on ER-driven genes in MCF7 breast tumor cells, *Mol. Cell. Endocrinol.* 444 (2017) 48–58.
- [85] S.Y. Park, H.J. Kwon, Y. Choi, H.E. Lee, S.W. Kim, J.H. Kim, I.A. Kim, N. Jung, N.Y. Cho, G.H. Kang, Distinct patterns of promoter CpG island methylation of breast cancer subtypes are associated with stem cell phenotypes, *Mod. Pathol.* 25 (2012) 185–196.
- [86] H. Guan, P.S. Nagarkatti, M. Nagarkatti, CD44 reciprocally regulates the differentiation of encephalitogenic Th1/Th17 and Th2/regulatory T cells through epigenetic modulation involving DNA methylation of cytokine gene promoters, thereby controlling the development of experimental autoimmune encephalomyelitis, *J. Immunol.* 186 (2011) 6955–6964.
- [87] N.S. Verkaik, J. Trapman, J.C. Romijn, T.H. Van der Kwast, G.J. Van Steenbrugge, Down-regulation of CD44 expression in human prostatic carcinoma cell lines is correlated with DNA hypermethylation, *Int. J. Cancer* 80 (1999) 439–443.
- [88] A. Canella, H. Cordero Nieves, D.W. Sborov, L. Cascione, H.S. Radomska, E. Smith, A. Stiff, J. Consiglio, E. Caserta, L. Rizzotto, N. Zanasi, V. Stefano, B. Kaur, X. Mo, J.C. Byrd, Y.A. Efebera, C.C. Hofmeister, F. Pichiorri, HDAC inhibitor AR-42 decreases CD44 expression and sensitizes myeloma cells to lenalidomide, *Oncotarget.* 6 (2015) 31134–31150.
- [89] I. Muller, F. Wischniewski, K. Pantel, H. Schwarzenbach, Promoter- and cell-specific epigenetic regulation of CD44, cyclin D2, GLP1R1 and PTEN by methyl-CpG binding proteins and histone modifications, *BMC Cancer* 10 (2010) 297.
- [90] S. Eberth, B. Schneider, A. Rosenwald, E.M. Hartmann, J. Romani, M. Zaborski, R. Siebert, H.G. Drexler, H. Quentmeier, Epigenetic regulation of CD44 in Hodgkin and non-Hodgkin lymphoma, *BMC Cancer* 10 (2010) 517.
- [91] V.B. Lokeshwar, P. Gomez, M. Kramer, J. Knapp, M.A. McCornack, L.E. Lopez, N. Fregien, N. Dhir, S. Scherer, D.J. Klumpp, M. Manoharan, M.S. Soloway, B.L. Lokeshwar, Epigenetic regulation of HYAL-1 hyaluronidase expression. identification of HYAL-1 promoter, *J. Biol. Chem.* 283 (2008) 29215–29227.
- [92] D. Vignetti, S. Deleonibus, P. Moretto, T. Bowen, J.W. Fischer, M. Grandoch, A. Oberhuber, D.C. Love, J.A. Hanover, R. Cinquetti, E. Karousou, M. Viola, M.L. D'Angelo, V.C. Hascall, G. De Luca, A. Passi, Natural antisense transcript for hyaluronan synthase 2 (HAS2-AS1) induces transcription of HAS2 via protein O-GlcNAcylation, *J. Biol. Chem.* 289 (2014) 28816–28826.

- [93] L.Y.W. Bourguignon, C. Earle, M. Shiina, Activation of matrix Hyaluronan-mediated CD44 Signaling, epigenetic regulation and Chemoresistance in head and neck Cancer stem cells, *Int. J. Mol. Sci.* 18 (2017).
- [94] L. Chen, L.Y. Bourguignon, Hyaluronan-CD44 interaction promotes c-Jun signaling and miRNA21 expression leading to Bel-2 expression and chemoresistance in breast cancer cells, *Mol. Cancer* 13 (2014) 52.
- [95] S.Y. Ju, S.H. Chiou, Y. Su, Maintenance of the stemness in CD44(+) HCT-15 and HCT-116 human colon cancer cells requires miR-203 suppression, *Stem Cell Res.* 12 (2014) 86–100.
- [96] L.Y. Bourguignon, G. Wong, C. Earle, L. Chen, Hyaluronan-CD44v3 interaction with Oct4-Sox2-Nanog promotes miR-302 expression leading to self-renewal, clonal formation, and cisplatin resistance in cancer stem cells from head and neck squamous cell carcinoma, *J. Biol. Chem.* 287 (2012) 32800–32824.
- [97] Z. Yang, D. Chen, J. Nie, S. Zhou, J. Wang, Q. Tang, X. Yang, MicroRNA143 targets CD44 to inhibit breast cancer progression and stem cell-like properties, *Mol. Med. Rep.* 13 (2016) 5193–5199.
- [98] K. Rilla, H. Siiskonen, M. Tammi, R. Tammi, Hyaluronan-coated extracellular vesicles—a novel link between hyaluronan and cancer, *Adv. Cancer Res.* 123 (2014) 121–148.
- [99] Y. Zhao, Q. Dong, J. Li, K. Zhang, J. Qin, J. Zhao, Q. Sun, Z. Wang, T. Wartmann, K.W. Jauch, P.J. Nelson, L. Qin, C. Bruns, Targeting cancer stem cells and their niche: perspectives for future therapeutic targets and strategies, *Semin. Cancer Biol.* 53 (2018) 139–155.
- [100] R.D. Sanderson, S.K. Bandari, I. Vlodavsky, Proteases and glycosidases on the surface of exosomes: newly discovered mechanisms for extracellular remodeling, *Matrix Biol.* 75–76 (2019) 160–169.
- [101] U.T. Arasu, R. Karna, K. Harkonen, S. Oikari, A. Koistinen, H. Kroger, C. Qu, M.J. Lammi, K. Rilla, Human mesenchymal stem cells secrete hyaluronan-coated extracellular vesicles, *Matrix Biol.* 64 (2017) 54–68.
- [102] K. Rilla, S. Pasonen-Seppanen, A.J. Deen, V.V. Koistinen, S. Wojciechowski, S. Oikari, R. Karna, G. Bart, K. Torronen, R.H. Tammi, M.I. Tammi, Hyaluronan production enhances shedding of plasma membrane-derived microvesicles, *Exp. Cell Res.* 319 (2013) 2006–2018.
- [103] B.S. Hong, J.H. Cho, H. Kim, E.J. Choi, S. Rho, J. Kim, J.H. Kim, D.S. Choi, Y.K. Kim, D. Hwang, Y.S. Gho, Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells, *BMC Genomics* 10 (2009) 556.
- [104] K. Nakamura, K. Sawada, Y. Kinose, A. Yoshimura, A. Toda, E. Nakatsuka, K. Hashimoto, S. Mabuchi, K.I. Morishige, H. Kurachi, E. Lengyel, T. Kimura, Exosomes promote ovarian Cancer cell invasion through transfer of CD44 to peritoneal mesothelial cells, *Mol. Cancer Res.: MCR* 15 (2017) 78–92.
- [105] Z. Wang, A. von Au, M. Schnolzer, T. Hackert, M. Zoller, CD44v6-competent tumor exosomes promote motility, invasion and cancer-initiating cell marker expression in pancreatic and colorectal cancer cells, *Oncotarget* 7 (2016) 55409–55436.
- [106] T. Jung, D. Castellana, P. Klingbeil, I. Cuesta Hernandez, M. Vitacolonna, D.J. Orlicky, S.R. Roffler, P. Brodt, M. Zoller, CD44v6 dependence of premetastatic niche preparation by exosomes, *Neoplasia* 11 (2009) 1093–1105.
- [107] S. Ghatak, V.C. Hascall, R.R. Markwald, S. Misra, Stromal hyaluronan interaction with epithelial CD44 variants promotes prostate cancer invasiveness by augmenting expression and function of hepatocyte growth factor and androgen receptor, *J. Biol. Chem.* 285 (2010) 19821–19832.
- [108] A. Josefsson, H. Adamo, P. Hammarsten, T. Granfors, P. Stattin, L. Egevad, A.E. Laurent, P. Wikstrom, A. Bergh, Prostate cancer increases hyaluronan in surrounding nonmalignant stroma, and this response is associated with tumor growth and an unfavorable outcome, *Am. J. Pathol.* 179 (2011) 1961–1968.
- [109] A. Kultti, X. Li, P. Jiang, C.B. Thompson, G.I. Frost, H.M. Shepard, Therapeutic targeting of hyaluronan in the tumor stroma, *Cancers (Basel)* 4 (2012) 873–903.
- [110] Y. Kinugasa, T. Matsui, N. Takakura, CD44 expressed on cancer-associated fibroblasts is a functional molecule supporting the stemness and drug resistance of malignant cancer cells in the tumor microenvironment, *Stem Cells* 32 (2014) 145–156.
- [111] L. Wang, X. Zuo, K. Xie, D. Wei, The role of CD44 and Cancer stem cells, *Methods Mol. Biol.* 1692 (2018) 31–42.
- [112] W.M. Lau, E. Teng, H.S. Chong, K.A. Lopez, A.Y. Tay, M. Salto-Tellez, A. Shabbir, J.B. So, S.L. Chan, CD44v8-10 is a cancer-specific marker for gastric cancer stem cells, *Cancer Res.* 74 (2014) 2630–2641.
- [113] Y.Y. Xu, M. Guo, L.Q. Yang, F. Zhou, C. Yu, A. Wang, T.H. Pang, H.Y. Wu, X.P. Zou, W.J. Zhang, L. Wang, G.F. Xu, Q. Huang, Regulation of CD44v6 expression in gastric carcinoma by the IL-6/STAT3 signaling pathway and its clinical significance, *Oncotarget* 8 (2017) 45848–45861.
- [114] M. Todaro, M. Gaggianesi, V. Catalano, A. Benfante, F. Iovino, M. Biffoni, T. Apuzzo, I. Sperduti, S. Volpe, G. Cocorullo, G. Gulotta, F. Dieli, R. De Maria, G. Stassi, CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis, *Cell Stem Cell* 14 (2014) 342–356.
- [115] L. Wang, J. Zhao, C. Yang, R. Kuang, G. Kazobinka, Z. Pang, T. Hou, Prognostic implication of urothelial stem cell markers differs according to primary tumour location in non-muscle-invasive bladder Cancer, *Cell. Physiol. Biochem.* 48 (2018) 2364–2373.
- [116] F. Wada, H. Koga, J. Akiba, T. Niizeki, H. Iwamoto, Y. Ikezono, T. Nakamura, M. Abe, A. Masuda, T. Sakaue, T. Tanaka, T. Kakuma, H. Yano, T. Torimura, High expression of CD44v9 and xCT in chemoresistant hepatocellular carcinoma: potential targets by sulfasalazine, *Cancer Sci.* 109 (2018) 2801–2810.
- [117] M. Prieto-Vila, R.U. Takahashi, W. Usuba, I. Kohama, T. Ochiya, Drug resistance driven by Cancer stem cells and their niche, *Int. J. Mol. Sci.* 18 (2017).
- [118] D.R. Boulbes, G.B. Chauhan, Q. Jin, C. Bartholomeusz, F.J. Esteve, CD44 expression contributes to trastuzumab resistance in HER2-positive breast cancer cells, *Breast Cancer Res. Treat.* 151 (2015) 501–513.
- [119] T. Aso, M. Matsuo, H. Kiyohara, K. Taguchi, F. Rikimaru, M. Shimokawa, Y. Segawa, Y. Higaki, H. Umeno, T. Nakashima, M. Masuda, Induction of CD44 variant 9-expressing cancer stem cells might attenuate the efficacy of chemoradioselection and Worsens the prognosis of patients with advanced head and neck cancer, *PLoS One* 10 (2015) e0116596.
- [120] A. Marengo, S. Forciniti, I. Dando, E. Dalla Pozza, B. Stella, N. Tsapis, N. Yagoubi, G. Fanelli, E. Fattal, C. Heeschen, M. Palmieri, S. Arpicco, Pancreatic cancer stem cell proliferation is strongly inhibited by diethylthiocarbamate-copper complex loaded into hyaluronic acid decorated liposomes, *Biochim. Biophys. Acta Gen. Subj.* 1863 (2019) 61–72.
- [121] Y.F. Li, H.T. Zhang, L. Xin, Hyaluronic acid-modified polyamidoamine dendrimer G5-entrapped gold nanoparticles delivering METase gene inhibits gastric tumor growth via targeting CD44+ gastric cancer cells, *J. Cancer Res. Clin. Oncol.* 144 (2018) 1463–1473.
- [122] E. Jang, E. Kim, H.Y. Son, E.K. Lim, H. Lee, Y. Choi, K. Park, S. Han, J.S. Suh, Y.M. Huh, S. Haam, Nanovesicle-mediated systemic delivery of microRNA-34a for CD44 overexpressing gastric cancer stem cell therapy, *Biomaterials* 105 (2016) 12–24.
- [123] H. Urakawa, Y. Nishida, J. Wasa, E. Arai, L. Zhuo, K. Kimata, E. Kozawa, N. Futamura, N. Ishiguro, 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* 130 (2012) 454–466.
- [124] R. Tamura, Y. Yokoyama, H. Yoshida, T. Imaizumi, H. Mizunuma, 4-Methylumbelliferone inhibits ovarian cancer growth by suppressing thymidine phosphorylase expression, *J. Ovarian Res.* 7 (94) (2014).
- [125] T.J. Yates, L.E. Lopez, S.D. Lokeshwar, N. Ortiz, G. Kallifatidis, A. Jordan, K. Hoye, N. Altman, V.B. Lokeshwar, Dietary supplement 4-methylumbelliferone: an effective chemopreventive and therapeutic agent for prostate cancer, *J. Natl. Cancer Inst.* 107 (2015).
- [126] T.T. Karalis, P. Heldin, D.H. Vynios, T. Neill, S. Buraschi, R.V. Iozzo, N.K. Karamanos, S.S. Skandalis, Tumor-suppressive functions of 4-MU on breast cancer cells of different ER status: regulation of hyaluronan/HAS2/CD44 and specific matrix effectors, *Matrix Biol.* 78–79 (2018) 118–138.
- [127] Z.K. Price, N.A. Lokman, C. Ricciardelli, Differing roles of Hyaluronan molecular weight on Cancer cell behavior and chemotherapy resistance, *Cancers* 10 (2018).
- [128] C.B. Thompson, H.M. Shepard, P.M. O'Connor, S. Kadhim, P. Jiang, R.J. Osgood, L.H. Bookbinder, X. Li, B.J. Sugarman, R.J. Connor, S. Nadsjombati, G.I. Frost, Enzymatic depletion of tumor hyaluronan induces antitumor responses in pre-clinical animal models, *Mol. Cancer Ther.* 9 (2010) 3052–3064.
- [129] S.R. Hingorani, L. Zheng, A.J. Bullock, T.E. Seery, W.P. Harris, D.S. Sigal, F. Braiteh, P.S. Ritch, M.M. Zalupski, N. Bahary, P.E. Oberstein, A. Wang-Gillam, W. Wu, D. Chondros, P. Jiang, S. Khelifa, J. Pu, C. Aldrich, A.E. Hendifar, HALO 202: randomized phase II study of PEGPH20 plus nab-paclitaxel/gemcitabine versus nab-paclitaxel/gemcitabine in patients with untreated, Metastatic Pancreatic Ductal Adenocarcinoma, *J. Clin. Oncol.* 36 (2018) 359–366.
- [130] M. Fabbri, A. Paone, F. Calore, R. Galli, E. Gaudio, R. Santhanam, F. Lovat, P. Fadda, C. Mao, G.J. Nuovo, N. Zanesi, M. Crawford, G.H. Ozer, D. Wernicke, H. Alder, M.A. Caligiuri, P. Nana-Sinkam, D. Perrotti, C.M. Croce, MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response, *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012) E2110–E2116.
- [131] F. Chalmin, S. Ladoire, G. Mignot, J. Vincent, M. Bruchard, J.P. Remy-Martin, W. Boireau, A. Rouleau, B. Simon, D. Lanneau, A. De Thonel, G. Multhoff, A. Hamman, F. Martin, B. Chaffert, E. Solary, L. Zitvogel, C. Garrido, B. Ryffel, C. Borg, L. Apetoh, C. Rebe, F. Ghiringhelli, Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells, *J. Clin. Invest.* 120 (2010) 457–471.