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REVIEW ARTICLE

Proteoglycans and glycosaminoglycans as regulators of cancer stem cell function and therapeutic resistance

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In contrast to the bulk of the tumor, a subset of cancer cells called cancer stem cells (CSC; or tumor-initiating cells) is characterized by self-renewal, unlimited proliferative potential, expression of multidrug resistance proteins, active DNA repair capacity, apoptosis resistance, and a considerable developmental plasticity. Due to these properties, CSCs display increased resistance to chemo- and radiotherapy. Recent findings indicate that aberrant functions of proteoglycans (PGs) and glycosaminoglycans (GAGs) contribute substantially to the CSC phenotype and therapeutic resistance. In this review, we summarize how the diverse functions of the glycoproteins and carbohydrates facilitate acquisition and maintenance of the CSC phenotype, and how this knowledge can be exploited to develop novel anticancer therapies. For example, the large transmembrane chondroitin sulfate PG NG2/CSPG4 marks stem cell (SC) populations in brain tumors. Cell surface heparan sulfate PGs of the syndecan and glypican families modulate the stemness-associated Wnt, hedgehog, and notch signaling pathways, whereas the interplay of hyaluronan in the SC niche with CSC CD44 determines the maintenance of stemness and promotes therapeutic resistance. A better understanding of the molecular mechanisms by which PGs and GAGs regulate CSC function will aid the development of targeted therapeutic approaches which could avoid relapse after an otherwise successful conventional therapy. Chimeric antigen receptor T cells, PG-primed dendritic cells, PG-targeted antibody–drug conjugates, and inhibitory peptides and glycans have already shown highly promising results in preclinical models.

Introduction

Differentiated tissues self-renew and regenerate due to the activity of stem cells (SCs), a long-lived subpopulation of cells capable of asymmetric cell division. This process generates a daughter SC and a cell

Abbreviations
ABC, ATP-binding cassette; ALDH, aldehyde dehydrogenase; CAR, chimeric antigen receptor; CS, chondroitin sulfate; CSC, cancer stem cell; DS, dermatan sulfate; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; ESC, embryonic stem cell; FGF, fibroblast growth factor; GAG, glycosaminoglycan; HA, hyaluronan; HAS, hyaluronan synthase; HS, heparan sulfate; LOX, lysyl oxidase; MDR, multidrug resistance; MSC, mesenchymal stem cell; PG, proteoglycan; Pgp, P-glycoprotein; SDF-1, stromal cell-derived factor 1; SP, side population; TAM, tumor-associated macrophage; TGF, transforming growth factor.

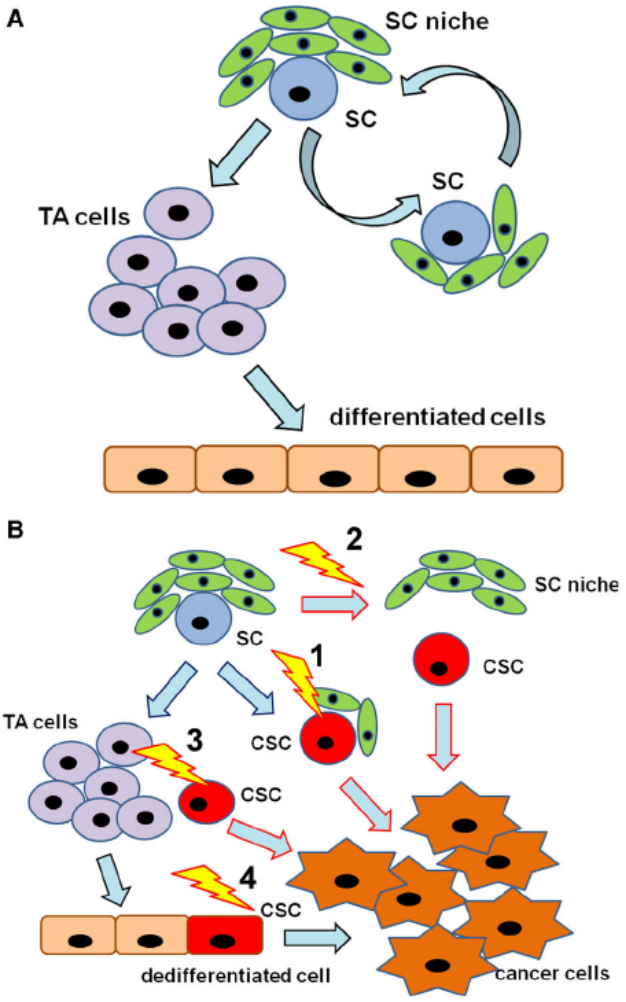

Fig. 1. The CSC concept. (A) Physiological stem cell function. SCs reside in a SC niche consisting of cells and an ECM environment that provides signals which keep the SC in an undifferentiated state. Asymmetric cell division of the SC results in a generation of a daughter cell with SC properties, and a second daughter cell which can progressively undergo differentiation via a transient amplifying (TA) state, ultimately generating a differentiated cell type which fulfils a defined function within the body (e.g., an epithelium, as depicted here). (B) Different mechanisms are thought to generate CSCs. CSCs may arise through (1) direct conversion of a SC into a CSC capable of generating bulk tumor cells, (2) mechanisms that confer niche independence to the CSC, (3) mutations in TA cells may lead to the acquisition of a CSC state, and (4) mutations in differentiated cells may lead to the acquisition of (cancer) SC-like properties.

which can undergo transient amplifications and ultimately differentiation into a given cell type or tissue [1] (Fig. 1A). Several independent cell tracing and lineage studies strongly support that malignancies originate from a small population of SC-like cells with increased tumor-seeding ability. Such cells are referred to as tumor-initiating cells or cancer SCs (CSCs) [1,2]. CSCs are the subpopulation of cells within a tumor that can self-renew, differentiate into multiple lineages, and drive tumor growth. As CSCs exhibit drug resistance due to a high DNA repair capacity, ATP-binding cassette (ABC) transporter expression, and apoptosis resistance, they may represent a subpopulation of tumor cells particularly therapeutic resistant to chemotherapy/radiotherapy [1,2]. Tumors are embedded in an extracellular matrix (ECM) consisting of a network of proteoglycans (PGs), large glycoproteins such as collagen, fibronectin, or laminin, they are normally excellently connected to the vascular system and additionally interact with different cells of their microenvironment like fibroblasts, macrophages, and lymphocytes [3,4]. This complex network causes and determines an organ-like structure with tumor cells at different stages of (de)differentiation. The main part of a tumor, the so-called tumor bulk refers to tumor growth whereas some rare tumor cells which reside in special niches often rarely or even do not divide and in so far do not contribute to long-term tumor growth. These cells possess SC characteristics combined with explicit strategies to survive therapeutic interventions [2]. Similar to normal SCs, the CSCs bear self-renewal potential, can differentiate, and are able to develop tumor tissue. The origin of CSCs is still under discussion. According to current hypotheses, they can arise by acquisition of cumulative mutations and epigenetic modifications of normal tissue SCs or differentiated somatic cells [1,2]. CSCs can also develop from non-stem cancer cells which dedifferentiate, this could happen spontaneously or induced by cytokines such as IL-6 [5,6]. Finally, a loss of dependency on the SC niche may be an important step in tumor progression [1] (Fig. 1B). In an experimental setting, a strong indication for the stemness of a cancer cell is its repeated adoptive transfer into immunocompromised mice (NOD/SCID mice). The inoculation of some few CSCs in this xenotransplantation assay is enough to generate a tumor with all its facets, whereas thousands of non-CSCs could not [1,2]. CSCs possess characteristic features which enable their identification and isolation out of tumor samples and cell lines. The plethora of markers defining CSCs is highly variable for different cancer types and comprise of cell surface markers as well as specific physiological features [e.g., expression of CD133, CD44, CD90, ESA, aldehyde dehydrogenase (ALDH), and multidrug resistance boxes, etc.]. Even in an entity such as breast cancer, the variability for CSC characteristics persists and both ESA+/CD44+/CD24−/low/lin- and CD90low/CD44+ and CD44+/CD24−/low/ALDHhigh define stemness of breast cancer cells [2]. CSCs reside in specific niches inside the tumor, composed of fibroblasts, tumor-

associated monocytes and macrophages (TAMs) and regulatory T cells as well as defined growth factors and cytokines. Together, these components form an optimal microenvironment responsible for intercellular communication, cell nutrition, signal transduction, and cell fate [1,3]. Especially TAMs were described to create a breast CSC niche through juxtacrine signaling with CSCs [3,7]. Also, cancer-associated fibroblasts, one of the most influential cell types in the tumor stroma, can promote a CSC-like phenotype [8]. Furthermore, the special configuration of the SC niche contributes to the resistance of CSCs to standard chemotherapy and radiotherapy and thus to relapse after treatment [1,2,8]. As will be demonstrated in the following sections, PGs and glycosaminoglycans (GAGs) have emerged as important modulators of the cancer SC phenotype, and as novel therapeutic targets in malignant disease.

Proteoglycans and glycosaminoglycans—versatile multifunctional integrators of signal transduction and extracellular matrix function

Proteoglycans are glycoproteins of cell surfaces and the ECM which are characterized by the covalent modification with a carbohydrate chain of the GAG type [4]. GAGs are non-branched linear chains of repetitive disaccharide units, including heparan sulfate (HS; *N*-acetylglucosamine-α-L-iduronic acid/β-D-glucuronic acid), chondroitin sulfate (CS), and dermatan sulfate (DS, *N*-acetyl-β-D-galactosamine-D-glucuronic acid; DS is derived from CS by C5-epimerization of the β-D-glucuronic acid residue), keratan sulfate (*N*-acetyl-β-D-glucosamine-β-D-galactose), and hyaluronic acid/hyaluronan (HA, *N*-acetyl-β-D-glucosamine-D-glucuronic acid), the only GAG that is synthesized at the plasma membrane. Through variable sulfation reactions occurring in a sequential manner in the Golgi apparatus, and/or due to the presence of carboxylate residues, GAGs have a highly negative charge that enables the binding of a plethora of positively charged ligands [4,9]. PGs can be grouped according to their GAG substitution, their core protein sequence, and their subcellular localization [4]. As reviewed in more detail elsewhere [3,4,9], PGs and GAGs have numerous cellular functions, many of which are linked to their capability to physically and functionally interact with signaling molecules and their receptors (ranging from receptor tyrosine kinases to G-protein–coupled receptors), and with a multitude of ECM proteins [4]. Notably, several PGs and GAGs are misexpressed in cancers, constituting prognostic markers, and contributing to tumor progression via the modulation of virtually all Hallmarks of Cancer [9]. Due to space limitations, we will focus on selected examples with relevance to SC function. Indeed, several reports showed a contribution of PGs to SC maintenance and differentiation: In embryonic SCs (ESCs), HSPG expression is involved in the self-renewal and also in the differentiation of committed cells to specific lineages through a promotion of signaling via the Wnt, fibroblast growth factor (FGF), and BMP pathways [10,11]. For example, HSPG expression levels and GAG composition were crucial for the maintenance of self-renewal of pluripotent cells and also for differentiation to a specific cell fate [12–15]. During the transition of ESCs from an undifferentiated to a committed cell type, the level of HS sulfation was increased, whereas inhibition of sulfation accelerated neural differentiation [10,16]. Moreover, murine ES cells carrying mutations in HS biosynthetic genes (Ext1) were not capable to differentiate upon withdrawal of leukemia inhibitory factor. Supporting this observation, deletion of Ndst1/2 in murine ES cells reduced FGF binding due to reduced sulfation, and cells were not able to differentiate into a specific lineage fate [10]. Overall, these data underscore the importance of PGs and GAGs in physiological SC function. In the following section, we will present selected examples of PGs and GAGs with a well-documented role in CSC function.

Proteoglycans as modulators of the cancer stem cell phenotype and therapeutic resistance

Syndecan-1—a multifunctional integrator of signaling at the cell surface

Via interactions with cytokines and morphogens, cell surface PGs integrate multiple signaling events relevant to SC function, including the Notch, Wnt, and hedgehog signaling pathways [3,4,9]. Several studies have revealed a functional role of the transmembrane HSPG syndecan-1 (Sdc-1, CD138) in CSCs. siRNA-mediated knockdown of Sdc-1 in the human breast cancer cell lines MCF-7 (an estrogen-receptor positive, low invasive, cell line which retains epithelial characteristics) and MDA-MB-231 (an aggressive, highly dedifferentiated triple-negative cell line) resulted in a significant reduction of several stemness-related phenotypic characteristics, including the side population (SP) phenotype, ALDH-1 activity, the CD44(+)CD24(−/low) phenotype (in MDA-MB-231 cells), and the capacity to form 3-dimensional spheres under nonadherent cell culture conditions [17]. At the molecular

level, the reduction of syndecan-1 expression was associated with reduced expression of a coreceptor for Wnt signaling, LRP-6, and reduced expression and activation of components of the IL-6/LIF-signaling pathway, in accordance with the coreceptor role of syndecan-1 for cytokine-mediated signaling. While Sdc-1-depleted MDA-MB-231 cells show increased resistance to radiotherapy *in vitro* [6], this effect may be due to an increased activation of focal adhesion kinase rather than an altered CSC phenotype. In contrast, high expression of Sdc-1 is associated with a poor response to chemotherapy in breast cancer [18], which would be consistent with the *in vitro* results regarding a reduction of SP phenotype of Sdc-1-depleted cells [17]. In an extension of this work, the authors studied the role of syndecan-1 in triple-negative inflammatory breast cancer, a particularly aggressive form of breast cancer [19]. Comparing the expression of Sdc-1 and the CSC markers CD44, Notch-1 and -3 by immunohistochemistry and qPCR in tumor specimens of inflammatory breast cancer and noninflammatory breast cancer patients, the authors found an upregulation and significant positive correlation of Sdc-1 expression with CD44, Notch-1, and Notch-3 in inflammatory compared to non-inflammatory breast cancer. At the functional level, Ibrahim *et al.* [19] demonstrated that siRNA knockdown of Sdc-1 in the inflammatory breast cancer cell line SUM-149 and the HER2-positive cell line SK-BR3 resulted in reduced 3D spheroid and colony formation, a reduction of ALDH1 activity, and a reduction of the CD44(+)CD24(−/low) subset as CSC markers. Furthermore, expression of Notch-1, -2, -3, -4 and of their downstream target, the transcription factor Hey-1, was reduced in SUM-149 cells following syndecan-1 knockdown. Pharmacological inhibitor assays demonstrated that syndecan-1 knockdown resulted in a downregulation of components of the IL-6-/STAT3-/NF-KB/ and Akt-signaling pathways in a Notch activation-dependent manner, providing a functional link between syndecan-1-modulated signaling pathways and the stemness-associated notch pathway.

Apart from a molecular role between Sdc-1 and the notch signaling pathway, an important function in Wnt-signaling has been documented. Caroline Alexander and coworkers crossed Sdc-1-deficient mice to a mouse model breast carcinogenesis, which transgenically overexpressed the Wnt1 oncogene in a tissue-specific manner in the mammary glands [20,21]. Extending their initial observation that the absence of Sdc-1 reduced Wnt-induced hyperplasia by 70%, and reduced tumor formation to an even larger extent, they could show that the number of cells responsive to beta-catenin/TCF transactivation was reduced by 50% in Sdc-1 knockout mice, and that the number of SP cells was significantly reduced in these mice, suggesting that Sdc-1 is required to create or stabilize the beta-catenin/TCF-responsive CSC precursor cells in the murine mammary gland [20]. While specific sulfation patters of HS (in particular increased 3-*O*-sulfation) have been shown to increase expression of beta-catenin and the Wnt-dependent transcription factor TCF4 in human breast cancer cells [22], Sdc-1 knockout mouse cells showed compensatory effects with respect to the substitution of other PGs with GAG chains [20]. Finally, the authors showed that Sdc-1 is heterogeneously expressed in the hematopoietic SC fraction, and that juvenile Sdc-1-deficient cells are resistant to carcinogen-induced tumor formation not only in the mammary gland but also to skin, lung, and liver cancer and lymphoma, confirming its role as an important modulator of CSC function [21]. Indeed, a role for syndecan-1 in CSC function has not only been demonstrated for breast cancer: In prostate cancer, Sdc-1 is required for maintaining the stability of tumor-initiating clonogenic cells *in vitro*, and for the CD133(+)/CD44(+) cell population *in vivo* [23]. Notably, the authors found that Sdc-1 gene knockdown significantly enhanced chemotherapeutic efficacy by inhibiting the docetaxel-induced increase in the CSC population *in vivo*. Sdc-1 has been suggested as a colon CSC marker due to its expression in a colon cancer-initiating cell line, and in liposarcoma, where it promoted undifferentiated adipocyte progenitor cell proliferation and inhibited their differentiation [2,24]. In multiple myeloma, one of the most frequent hematological malignancies, Sdc-1/CD138 serves as a prognostic marker; however, its role regarding a CSC phenotype is controversial [25–27]. Different studies suggested that a clonogenic Sdc-1-low/- cell type serves as a precursor for more differentiated Sdc-1+ cells. These cells are more resistant to chemotherapy due to high drug efflux and intracellular drug detoxification activity as determined by SP and ALDH assay, and are more capable of forming tumors *in vivo* [25,26]. Hypoxia and bidirectional myeloma–stroma cell interactions have been identified as factors responsible for Sdc-1 downregulation [25,27]. In contrast, other studies could not find a difference in the stemness-associated properties of Sdc-1 positive and negative multiple myeloma cell populations, showed the presence of Sdc-1 positive rather than negative cells in the multiple myeloma cell (line) SP and in clonogenic cells, enrichment for pathogenetic properties in the Sdc-1 positive cell population, and responsiveness of the Sdc-1-positive cell population to the stemness-associated hedgehog and IL-6 signaling pathways, which were associated

with chemotherapeutic resistance [25,28,29]. The kinetics of conversion of a Sdc-1-low into a Sdc-1-positive population and the modulation of Sdc-1 expression and shedding status by the HS-degrading enzyme heparanase may partially account for these seemingly contradictory data [30].

The functional involvement of Sdc-1 as a modulator of CSC functions marks this HSPG as a potential therapeutic target. Indeed, a therapeutic targeting of Sdc-1 by antibody–drug conjugates has been proposed as a therapy for myeloma already 20 years ago: Post *et al*. [31] demonstrated that a combination of the chemotherapeutic doxorubicin with an anti-Sdc-1 monoclonal antibody–immunotoxin conjugate showed additive therapeutic effects with the chemotherapy toward myeloma cell lines and patient-derived myeloma cells, and beneficial effects particularly on drug-resistance tumor cells. Later studies demonstrated a substantial increase in survival of multiple myeloma xenografted mice subjected to anti-Sdc-1-radioimmunotherapy [32]. Notably, an independent pilot study demonstrated the feasibility of anti-Sdc-1 radioimmunotherapy in multiple myeloma. However, besides the promising observation of a remission of one of four patients, the need to eliminate side effects of the treatment became apparent [33]. Finally, inhibition of Sdc-1 in a melanoma xenograft model resulted in a substantial reduction of vascular mimicry, and in a highly synergistic therapeutic effect with the L19-IL2 immunocytokine in this model [34].

Glypicans—membrane-anchored modulators of morphogen signaling

Apart from the transmembrane-anchored syndecans, the glycosylphosphatidylinositol-anchored glypicans constitute a major family of six cell surface PGs with relevance to CSC function [3,4]. During development, both GAG chains and core proteins of glypicans support the generation of morphogen gradients and modulate signaling through the stemness-associated Wnt, hedgehog, and FGF pathways [4,9]. For example, the two *Drosophila* glypicans differentially regulate follicle SC maintenance and competitiveness for niche occupancy via the Jak/Stat, Wingless, and Hedgehog pathways [35], whereas 2-*O*-sulfated HS chains attached to glypican 5 promote the proliferation of neural progenitor cells by acting as coreceptors for the sonic hedgehog pathway [36]. These functions are not only relevant for physiological SC function but also in the context of CSCs: Based on studies in glypican-4 mutant murine ESCs, it has been proposed that this HSPG plays a role in fine-tuning and preserving self-renewal and pluripotency through the modulation of Wnt/beta-catenin signaling [37]. Notably, the authors could demonstrate that while impairment of glypican-4 function in ES cells disrupts teratoma formation, it does not compromise pluripotency *in vivo*, suggesting that this PG is capable of uncoupling pluripotent SC differentiation from tumorigenic potential in this experimental model. While a recent study in pancreatic cancer cells confirmed a role for glypican-4 in Wnt/beta catenin signaling, the authors could demonstrate that a targeting of the HSPG resulted in an attenuation of SC properties, which was associated with an increased susceptibility to 5-fluorouracil chemotherapy [38]. It has, however, to be considered that in the first case, ES cells were studied, whereas the latter study was investigating cells that had undergone malignant transformation (and were thus harboring major genetic alterations). Another member of the glypican family, glypican-3 is not only coexpressed with SC markers in ES cells, hepatic progenitor cells, and rhabdoid and germ cell tumors [4,9,39], but it has also been proposed to be the target for malignant transformation in an animal model of chemically induced hepatic carcinogenesis, following the classical concept of conversion of a SC into a CSC through mutagenesis [39]. However, as a caveat, it has to be noted that there is no clear evidence so far that therapies targeting glypican-3 in hepatocellular carcinoma are acting on the CSC population, as this PG is widely expressed in the bulk of liver tumor cells. As dysregulation of glypican expression is mechanistically related to tumor progression in a variety of tumor entities [3,4,9], their targeting has emerged as an approach to overcome therapeutic resistance. In this context, several studies have explored the effect of an anti-glypican-3 primed immunotherapy. A therapeutic effect was demonstrated in the case of glypican-3-expressing ES-cell-derived dendritic cells in a model of murine melanoma [40], of primed dendritic cells in a xenograft model of hepatocellular carcinoma [41], and in a patient-derived xenograft model of hepatocellular carcinoma subjected to anti-Glypican-3 chimeric antigen receptor (CAR) T-cell treatment [42].

NG2/CSPG4—a giant proteoglycan marks cancer stem cells in the brain

Apart from HSPGs, selected CSPGs play a role in CSCs. The 2322 amino acid large type I transmembrane CSPG NG2/CSPG4 serves as a marker for tumor-initiating cells in glioblastoma [43]. Apart from its CS GAG substitution, the large ectodomain is characterized by laminin-like, cadherin-like, and

carbohydrate-substituted domains, allowing for a multitude of ligand interactions. These include numerous ECM molecules, integrins, and growth factors such as FGF and PDGF [4]. Indeed, a coreceptor function of NG2 for PDGFR appears to be important for the responsiveness of progenitor cells to PDGF [44]. In an experimental model of *N*-ethyl-*N*-nitrosourea-induced gliomagenesis, NG2-positive glial precursor cells were identified as a key target cell population [4]. Interestingly, aberrant regulation of NG2 endocytosis by knockout of the tumor suppressor Lgl1 in murine oligodendrocyte precursor cells *in vivo* induces gliomagenesis, thus offering an additional mechanism by which benign precursor cells can be converted into a CSC [45]. NG2-expressing precursor cells show a considerable developmental plasticity: Activation of notch signaling was shown to induce pericyte-like differentiation in NG2-positive glioblastoma CSCs, thus contributing to vessel stabilization during tumor angiogenesis [46]. While these data provide strong evidence for a role of NG2 in CSCs, it is not fully clear if the GAG moieties, or one or more of the other functional domains of this giant PG are responsible for the stemness-related functions. Regarding a possible link of the stemness-associated functions of NG2 to therapeutic resistance, it could be shown that a high expression of NG2 is associated with a poor prognosis and resistance to radiotherapy in glioblastoma, characterized by induction of reactive oxygen species scavenging enzymes and enhanced DNA damage signaling [43]. Moreover, NG2 promoted chemoresistance via activation of integrin-mediated PI3K/Akt signaling [47]. It remains to be shown which proportion of the observed therapeutic resistance is due to an association of NG2 with the CSC phenotype.

Several preclinical studies have successfully demonstrated antitumoral effects of a targeting of NG2. These include the successful combination of anti-NG2 antibodies with induction chemotherapy in B-cell acute lymphoblastic leukemia [48] and NG2-directed CAR-T cells which proved to efficiently target glioblastoma CSCs [49].

Hyaluronic acid–CD44 interactions in cancer stem cell function

The stemness properties and subsequent growth of CSC are highly influenced by the tumor microenvironment [1]. Among the different components of tumor microenvironment, HA constitutes a major GAG. In contrast to other GAGs, HA is not covalently bound onto a core protein to form a PG. HA can interact with numerous cell membrane receptors such as receptor for HA-mediated motility, CD44, LYVE-1, among others; CD44 being the main HA receptor and the furthest studied in cancer progression [50]. Moreover, some PGs can bind to HA by link modules, forming supramolecular complexes [51]. Notably, elevated levels of HA and its receptors correlate with poor prognosis and survival in cancer patients [50]. Moreover, it has been amply demonstrated that HA promotes tumor development and angiogenesis by enhancing the recruitment of stromal cells, such as fibroblasts, macrophages, endothelial, and mesenchymal SCs (MSCs) [52].

CD44 is a type I transmembrane protein that is expressed by numerous healthy and malignant cell types [50]. The single gene encoding CD44 is comprised of 20 exons. Structural diversity is generated by alternative splicing of 10 of these exons, thus generating CD44 variants (CD44v). CD44 protein is composed of an N-terminal extracellular domain, which is followed by a membrane-proximal stalk-like region encoded by variant exons, a single transmembrane region and a cytoplasmic tail. [50]. The extracellular domain contains multiple *N*- and *O*-glycosylation sites and consensus sequences for documented GAG-attachment. The non-variable first five CD44 exons binding sites for several ECM constituents, thus enabling interactions with HA and other GAGs [50,52]. A single point mutation in the most N-terminal HA binding motif abolishes both HA and CS binding, suggesting that the HA binding motifs are responsible for the majority of CD44-GAG interactions at the cell surface [53]. Usage of alternatively spliced exons alters the size of CD44 and modifies signaling events downstream of CD44. Of note, some CD44v isoforms contain specific post-translational modifications. For example, CD44v3 can be substituted with HS, allowing for interactions with heparin-binding proteins such as FGF-2 [54], whereas CD44v6 harbors a binding site for the proangiogenic growth factors, HGF and VEGF [50,52]. While there is ample evidence for the association of defined CD44 variants with specific tumor entities and their initiation and progression, CD44v isoform expression in CSCs has not yet been fully defined. However, CD44 has been identified as a typical stem and CSC surface marker in several types of cancer, individually or in combination with other markers [50]. Importantly, an increase in CD44 expression correlates with tumor recurrence after antitumoral treatment in breast cancer xenografts [53,55].

Interactions between HA and CD44 have been shown to play essential roles in tumor cell survival, angiogenesis, metastasis, and drug resistance. Even more, a HA-rich tumor microenvironment affects

several processes that favor the self-renewal, migration, and establishment of CSC in different niches. Figure 2 summarizes key functions of HA–CD44 interactions in CSCs, as discussed in the following sections of the review.

HA–CD44 interaction in EMT and maintenance of stemness

Epithelial-to-mesenchymal transition (EMT) disrupts epithelial polarity and cell–cell adhesion that lead to a SC-like phenotype, providing tumor cells with migratory and invasive properties. The EMT program requires extensively regulated changes in gene expression that are directly influenced by signaling pathways that respond to extracellular cues [1,6,9,56]. EMT and CSCs generation have been related to transforming growth factor-beta (TGF-β) signaling. Notably, Bourguignon *et al.* have demonstrated that TGF-βRI contains a CD44-binding site, and the HA-CD44 interaction induces the formation of a complex between CD44 and TGF-βRI that activates the serine/threonine kinase activity of TGF-βRI in metastatic breast cancer cells. Most importantly, TGF-βRI kinase activated by HA phosphorylates CD44, which enhances its binding interaction with the cytoskeletal protein ankyrin, leading to HA-mediated cell migration, and favoring EMT in breast tumor cells [56]. Functionally, treatment with TGF-β1 induced the mesenchymal phenotype in CD44+ hepatocellular carcinoma cells, and loss of CD44 inhibited TGF-β-mediated vimentin expression, mesenchymal morphology, and tumor invasiveness [57].

The expression of EMT-associated transcription factors dynamically changes during HA-induced EMT. El-Haibi *et al.* have demonstrated that the interaction

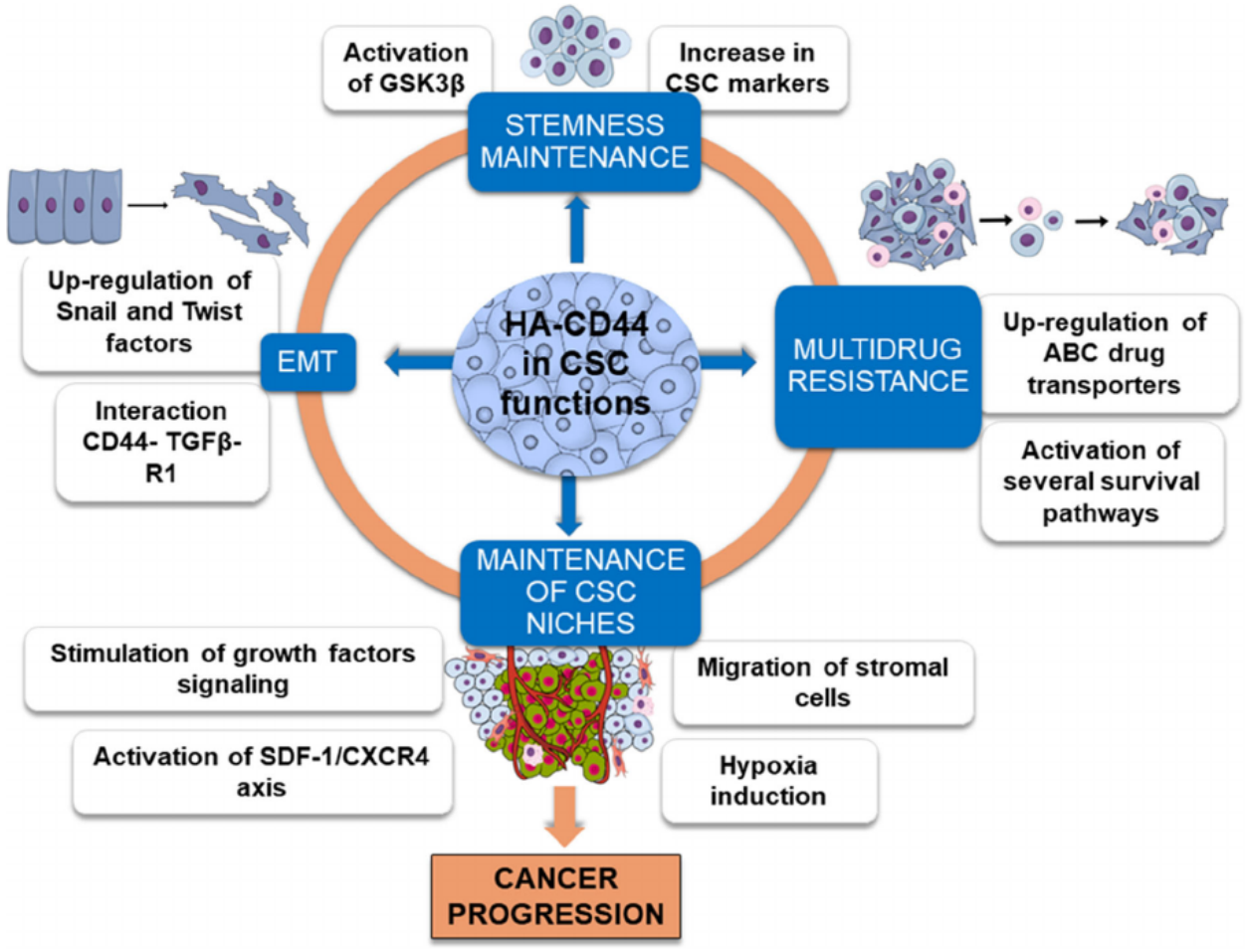

Fig. 2. Role of the HA–CD44 axis in CSC functions. The interaction between HA and CD44 plays essential roles in tumor cell survival, drug resistance, and metastasis. Even more, a HA-rich tumor microenvironment affects several processes that favor the self-renewal, migration, and establishment of CSCs in different niches.

between bone marrow-derived human MSC and breast cancer cells promotes *de novo* production of lysyl oxidase (LOX) by carcinoma cells. They also observed that extracellular HA from MSCs induces Twist expression, the nuclear translocation of CD44, and the activation of the LOX promoter in cancer cells. In turn, processed and enzymatically active LOX stimulates Twist transcription, which mediates the MSC-triggered EMT of breast carcinoma cells [58]. In agreement, HA-overproducing breast tumor cells acquired stemness through the upregulation of TGF-β and induction of Snail and Twist, while a loss of EMT by inhibition of TGF-β-Snail signaling or Twist knockdown markedly reduced CSC subpopulations [59].

Transforming growth factor-beta favors EMT by increasing the expression of HA synthase 2 (HAS-2). Porsch *et al*. demonstrated that the regulatory mechanisms of TGF-β-induced EMT required HAS2 expression in mammary epithelial cells. This stimulatory effect requires the kinase active TβRI and the activation of Smad and p38 MAPK. Furthermore, the silencing of HAS-2 inhibited the TGF-β-induced EMT diminishing the expression of EMT markers, such as fibronectin, Snail, and Zeb1 [60]. In addition, HA overproduction in transgenic mice overexpressing *Has2* resulted in a loss of the epithelial phenotype in spontaneous mammary tumors by reducing E-cadherin expression and by inducing nuclear translocation of β-catenin [61].

Stem-cell-specific transcription factors (as Sox2, Oct4, and Nanog), and their transcriptional networks are crucial for the development and maintenance of CSCs [1,2]. Several studies have recently emphasized the key role of CD44 in self-renewal and maintenance of CSCs [50]. For example, Shigeishi *et al*. [62] have demonstrated that CD44-mediated GSK3β activation is required for the maintenance of CSC properties in head and neck cancers: Inhibitory phosphorylation of GSK3B reduced the formation of tumor spheres and the expression of Oct4, Sox2, and Nanog.

HA–CD44 interaction in multidrug resistance

Multidrug resistance (MDR) is a major obstacle for an effective cancer therapy. The drug resistance in CSCs can be attributed to several mechanisms: the activation of survival/anti-apoptotic signals; autophagy induction; active DNA repair capacity; higher expression of ABC transporters; among others [1,2]. Overexpression of different ABC efflux transporters has been reported in several cancers and more predominantly in CSCs, since the ability of these transporters to expulse antitumoral drugs from tumor cells has been demonstrated [2].

In the last years, the role of HA and CD44 in drug resistance has been widely studied. It has been determined that HA–CD44 interaction promote drug resistance by activating survival pathways in several cancer types, including breast, lung, head, and neck carcinomas, as well as in a lymphoma T model [50]. HA–CD44 interactions also regulate the expression of different multidrug transporters in several types of cancer cells. Bourguignon *et al*. [63] demonstrated that HA binding to CD44 induced the association of Nanog and Stat-3 to stimulate Stat-3-dependent MDR1 gene expression in breast and ovarian cancer cells. In agreement with these findings, it has been shown that in response to carboplatin chemotherapy, ovarian cancer cells induced HA production favoring chemoresistance by regulating ABC transporter expression [64]. Even more, the systemic administration of CD44 targeted HA-MDR1 siRNA nanoparticles shown to enhance the sensitivity to paclitaxel and to decrease drug resistance in ovarian cancer [65]. In agreement with the data previously discussed, results from our laboratory demonstrate that co-treatment with low-molecular-weight HA and doxorubicin favored drug resistance in lymphoma T, osteosarcoma, and breast cancer cells by activating survival pathways and promoting angiogenesis *in vitro* and *in vivo* [66].

Mechanistically, the stabilization of ABC transporters by CD44 at the membrane of different tumor cells may be responsible for this important observation. For example, CD44 shows co-localization with BCRP and P-glycoprotein (Pgp) and in the plasma membrane of malignant peripheral nerve sheath tumor cells. Importantly, HA antagonist treatment induced internalization of the ABC transporters and CD44 in these cells [67]. Moreover, Pgp and CD44 could be coimmunoprecipitated in carcinoma and melanoma cells, suggesting a direct or indirect physical interaction [50,68]. Overall, these data document an important role for CD44–HA binding interactions in the development of multidrug resistance of cancer cells and CSCs.

As many tumors overexpress HA receptors, the coupling of cytotoxic drugs to HA emerges as a promising strategy for a modified chemotherapy, where a more efficient targeting of the drug to tumor cells allows to use lower treatment doses. Indeed, several studies have shown that the conjugation of antitumor drugs with HA reduced side effects of the therapy and increased cancer cell specificity [50,69]. For instance, doxorubicin-HA liposomes showed higher cytotoxicity compared with the drug alone and more than 100-fold

increased activity compared to liposomes devoid of HA [70].

Role of HA in CSC niches

Stem cells reside in a special microenvironmental niche that provides the major cues for their survival and maintenance, and similar conditions apply to CSCs [1–3]. HA is a primary ECM component of these stem-cell niches. For example, in hematopoietic malignances, ECM remodeling is important for continued recruitment of hematopoietic progenitor cells to the premetastatic niche [50].

CD44 acts also as co-receptor for several ECM components (FGF, VEGF, TGF-β, and MMPs among others), playing pivotal roles in intercellular communication within CSC niches [50]. While CD44v3 serves as a co-receptor for FGF and VEGF, CD44v6 functions as an EGF and HGF co-receptor to potentiate receptor tyrosine kinase-mediated or CD44-mediated signaling critical for maintenance of a CSC phenotype [71]. Furthermore, it has been demonstrated that CD44 functions as receptor for osteopontin, enhancing the aggressiveness of glioma SCs in the perivascular niche [72]. Hypoxia is one of the fundamental processes implicated in tumor growth, and in recent years has also been proposed as a new mechanism to develop drug resistance: The hypoxic microenvironment promotes glioblastoma SC maintenance, and aids reprogramming to a CSC phenotype [73,74]. Indeed, multiple HIF-regulated genes are preferentially expressed in CSCs compared with differentiated tumor cells [73]. Specifically, HIF-1a was found to upregulate CD44s, CD44v6, and CD44v7/8 expression in triple negative breast cancer, suggesting that CD44 plays a central role in signaling regulation circuits for the maintenance of cancer stemness under hypoxic conditions [74].

Signaling mediated by stromal cell-derived factor-1 (SDF-1/CXCL12) and its heptahelical chemokine receptor CXCR4 has a crucial role in mediating the interaction between CSCs and supporting cells within the CSC niche. The interaction of SDF-1 and CXCR4 during tracking and homing of CSCs of several tumors in different niches, as well as the relation with other CSC markers such as CD44, has been widely studied. Avigdor *et al*. have shown that HA is highly enriched in human bone marrow endosteal regions, particularly in sinusoidal endothelium and endosteum, regions where SDF-1 is abundant. The proximity of HA and SDF-1 facilitated the trans-endothelial migration and the anchorage of hematopoietic SCs to their microenvironmental niches, inducing the adhesion of hematopoietic SCs to HA, and CD44 distribution to the leading edge of the migrating cells [75]. Consistently, it has been shown that CD44 is an essential regulator of cell homing in microenvironmental niches and the maintenance of SC properties in acute myeloid leukemia (AML) progression. Jin *et al*. demonstrated that the alteration of CD44 function with the mAb H90 resulted in marked reduction of the leukemic burden NOD-SCID mice transplanted with primary AML cells. Specifically, it has been proposed that treatment with the H90 mAb altered the destination and homing of AML SCs, leading to their eradication [76].

In agreement, CD44 and CXCR4 were found to physically interact in glioblastoma cells in the presence of SDF-1α ligand, and that interaction could be inhibited by small HA oligosaccharides [77]. Even more, it was demonstrated that invasion ability of glioblastoma SCs was reduced by blockade of CXCR4, SDF-1, and/or CD44, suggesting that glioblastoma SC invasion may be mediated simultaneously by both mechanisms [78]. In concordance, Faber *et al*. have demonstrated that CD44 and CXCR4 are located mainly at the borderline of head and neck squamous tumor nests on particular niches, but not in the tumor stroma. They also proposed that the SDF-1-CXCR4 axis is a critical in cell trafficking in the CSC niche, while SDF-1 was not detected in the peripheral blood of patients [79].

Conclusions and perspective

Proteoglycans and GAGs are not only important regulators of physiological SC function during embryonic development and tissue regeneration, their aberrant expression in cancer cells and within cells and the ECM of the tumor microenvironment (and CSC niches) makes an important contribution to tumor progression and recurrence by modulating CSC properties. In this context, selected PGs and GAGs emerge as therapeutic targets to induce differentiation of CSCs, and to guide therapeutics to the CSC population in order to prevent recurrence after a conventional radio or chemotherapy. While data generated in preclinical models show highly promising results in this respect, potential side effects of such therapies need to be carefully evaluated, specifically for PGs/GAGs with a widespread tissue distribution and for PGs/GAGs with highly context-dependent functions.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

DV, LA, and MG wrote major parts of the manuscript and designed figures; SKK and BG wrote additional parts of the manuscript; BJ and E-SO performed literature searches and helped to conceptualize the manuscript; LA, E-SO, and MG supervised DV, BJ, and SKK, respectively.

References

1 Batlle E & Clevers H (2017) Cancer stem cells revisited. *Nat Med* **23**, 1124–1134.

2 Greve B, Kelsch R, Spaniol K, Eich HAT & Götte M (2012) Flow cytometry in cancer stem cell analysis and separation. *Cytometry A* **81**, 284–293.

3 Götte M & Kovalszky I (2018) Extracellular matrix functions in lung cancer. *Matrix Biol* **73**, 105–121.

4 Karamanos NK, Piperigkou Z, Theocharis AD, Watanabe H, Franchi M, Baud S, Brézillon S, Götte M, Passi A, Vigetti D *et al.* (2018) Proteoglycan chemical diversity drives multifunctional cell regulation and therapeutics. *Chem Rev* **118**, 9152–9232.

5 Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, Brooks M, Reinhardt F, Su Y, Polyak K *et al.* (2011) Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci USA* **108**, 7950–7955.

6 Hassan H, Greve B, Pavao MS, Kiesel L, Ibrahim SA & Götte M (2013) Syndecan-1 modulates β-integrin-dependent and interleukin-6-dependent functions in breast cancer cell adhesion, migration, and resistance to irradiation. *FEBS J* **280**, 2216–2227.

7 Lu H, Clauser KR, Tam WL, Fröse J, Ye X, Eaton EN, Reinhardt F, Donnenberg VS, Bhargava R, Carr SA *et al.* (2014) A breast cancer stem cell niche supported by juxtacrine signalling from monocytes and macrophages. *Nat Cell Biol* **16**, 1105–1117.

8 Najafi M, Farhood B & Mortezaee K (2019) Cancer stem cells (CSCs) in cancer progression and therapy. *J Cell Physiol* **234**, 8381–8395.

9 Ibrahim SA, Hassan H & Götte M (2014) MicroRNA regulation of proteoglycan function in cancer. *FEBS J* **281**, 5009–5022.

10 Holley RJ, Meade KA & Merry CL (2014) Using embryonic stem cells to understand how glycosaminoglycans regulate differentiation. *Biochem Soc Trans* **42**, 689–695.

11 Kraushaar DC, Rai S, Condac E, Nairn A, Zhang S, Yamaguchi Y, Moremen K, Dalton S & Wang L (2012) Heparan sulfate facilitates FGF and BMP signaling to drive mesoderm differentiation of mouse embryonic stem cells. *J Biol Chem* **287**, 22691–22700.

12 Johnson CE, Crawford BE, Stavridis M, Ten Dam G, Wat AL, Rushton G, Ward CM, Wilson V, van Kuppevelt TH, Esko JD *et al.* (2007) Essential alterations of heparan sulfate during the differentiation of embryonic stem cells to Sox1-enhanced green fluorescent protein-expressing neural progenitor cells. *Stem Cells* **25**, 1913–1923.

13 Kraushaar DC, Dalton S & Wang L (2013) Heparan sulfate: a key regulator of embryonic stem cell fate. *Biol Chem* **394**, 741–751.

14 Kraushaar DC, Yamaguchi Y & Wang L (2010) Heparan sulfate is required for embryonic stem cells to exit from self-renewal. *J Biol Chem* **285**, 5907–5916.

15 Sasaki N, Okishio K, Ui-Tei K, Saigo K, Kinoshita-Toyoda A, Toyoda H, Nishimura T, Suda Y, Hayasaka M, Hanaoka K *et al.* (2008) Heparan sulfate regulates self-renewal and pluripotency of embryonic stem cells. *J Biol Chem* **283**, 3594–3606.

16 Sasaki N, Hirano T, Kobayashi K, Toyoda M, Miyakawa Y, Okita H, Kiyokawa N, Akutsu H, Umezawa A & Nishihara S (2010) Chemical inhibition of sulfation accelerates neural differentiation of mouse embryonic stem cells and human induced pluripotent stem cells. *Biochem Biophys Res Commun.* **401**, 480–486.

17 Ibrahim SA, Hassan H, Vilardo L, Kumar SK, Kumar AV, Kelsch R, Schneider C, Kiesel L, Eich HT, Zucchi I *et al.* (2013) Syndecan-1 (CD138) modulates triple-negative breast cancer stem cell properties via regulation of LRP-6 and IL-6-mediated STAT3 signaling. *PLoS ONE* **8**, e85737.

18 Götte M, Kersting C, Ruggiero M, Tio J, Tulusan AH, Kiesel L & Wülfing P (2006) Predictive value of syndecan-1 expression for the response to neoadjuvant chemotherapy of primary breast cancer. *Anticancer Res* **26**, 621–627.

19 Ibrahim SA, Gadalla R, El-Ghonaimy EA, Samir O, Mohamed HT, Hassan H, Greve B, El-Shinawi M, Mohamed MM & Götte M (2017) Syndecan-1 is a novel molecular marker for triple negative inflammatory breast cancer and modulates the cancer stem cell phenotype via the IL-6/STAT3, Notch and EGFR signaling pathways. *Mol Cancer* **16**, 57.

20 Liu BY, Kim YC, Leatherberry V, Cowin P & Alexander CM (2003) Mammary gland development requires syndecan-1 to create a beta-catenin/TCF-responsive mammary epithelial subpopulation. *Oncogene* **22**, 9243–9253.

21 McDermott SP, Ranheim EA, Leatherberry VS, Khwaja SS, Klos KS & Alexander CM (2007) Juvenile syndecan-1 null mice are protected from carcinogen-induced tumor development. *Oncogene* **26**, 1407–1416.
22 Vijaya Kumar A, Salem Gassar E, Spillmann D, Stock C, Sen YP, Zhang T, Van Kuppevelt TH, Hülsewig C, Koszlowski EO, Pavao MS *et al.* (2014) HS3ST2 modulates breast cancer cell invasiveness via MAP kinase- and Tcf4 (Tcf7l2)-dependent regulation of protease and cadherin expression. *Int J Cancer* **135**, 2579–2592.
23 Shimada K, Anai S, Fujii T, Tanaka N, Fujimoto K & Konishi N (2013) Syndecan-1 (CD138) contributes to prostate cancer progression by stabilizing tumour-initiating cells. *J Pathol* **231**, 495–504.
24 Zaragosi LE, Dadone B, Michiels JF, Marty M, Pedeutour F, Dani C & Bianchini L (2015) Syndecan-1 regulates adipogenesis: new insights in dedifferentiated liposarcoma tumorigenesis. *Carcinogenesis* **36**, 32–40.
25 Reijmers RM, Spaargaren M & Pals ST (2013) Heparan sulfate proteoglycans in the control of B cell development and the pathogenesis of multiple myeloma. *FEBS J* **280**, 2180–2193.
26 Kawano Y, Fujiwara S, Wada N, Izaki M, Yuki H, Okuno Y, Iyama K, Yamasaki H, Sakai A, Mitsuya H *et al.* (2012) Multiple myeloma cells expressing low levels of CD138 have an immature phenotype and reduced sensitivity to lenalidomide. *Int J Oncol* **41**, 876–884.
27 Fuhler GM, Baanstra M, Chesik D, Somasundaram R, Seckinger A, Hose D, Peppelenbosch MP & Bos NA (2010) Bone marrow stromal cell interaction reduces syndecan-1 expression and induces kinomic changes in myeloma cells. *Exp Cell Res* **316**, 1816–1828.
28 Paíno T, Sarasquete ME, Paiva B, Krzeminski P, San-Segundo L, Corchete LA, Redondo A, Garayoa M, García-Sanz R, Gutiérrez NC *et al.* (2014) Phenotypic, genomic and functional characterization reveals no differences between CD138++ and CD138low subpopulations in multiple myeloma cell lines. *PLoS ONE* **9**, e92378.
29 Wu W, Ma D, Wang P, Cao L, Lu T, Fang Q, Zhao J & Wang J (2016) Potential crosstalk of the interleukin-6-heme oxygenase-1-dependent mechanism involved in resistance to lenalidomide in multiple myeloma cells. *FEBS J* **283**, 834–849.
30 Mahtouk K, Hose D, Raynaud P, Hundemer M, Jourdan M, Jourdan E, Pantesco V, Baudard M, De Vos J, Larroque M *et al.* (2007) Heparanase influences expression and shedding of syndecan-1, and its expression by the bone marrow environment is a bad prognostic factor in multiple myeloma. *Blood* **109**, 4914–4923.
31 Post J, Vooijs WC, Bast BJ & De Gast GC (1999) Efficacy of an anti-CD138 immunotoxin and doxorubicin on drug-resistant and drug-sensitive myeloma cells. *Int J Cancer* **83**, 571–576.
32 Chérel M, Gouard S, Gaschet J, Saï-Maurel C, Bruchertseifer F, Morgenstern A, Bourgeois M, Gestin JF, Bodéré FK, Barbet J *et al.* (2013) Bi radioimmunotherapy with an anti-mCD138 monoclonal antibody in a murine model of multiple myeloma. *J Nucl Med* **54**, 1597–1604.
33 Rousseau C, Ferrer L, Supiot S, Bardiès M, Davodeau F, Faivre-Chauvet A, Baumgartner P, Wijdenes J, Lacombe M, Barbet J *et al.* (2012) Dosimetry results suggest feasibility of radioimmunotherapy using anti-CD138 (B-B4) antibody in multiple myeloma patients. *Tumor Biol* **33**, 679–688.
34 Orecchia P, Conte R, Balza E, Pietra G, Mingari MC & Carnemolla B (2015) Targeting Syndecan-1, a molecule implicated in the process of vasculogenic mimicry, enhances the therapeutic efficacy of the L19-IL2 immunocytokine in human melanoma xenografts. *Oncotarget* **6**, 37426–37742.
35 Su TY, Nakato E, Choi PY & Nakato H (2018) Drosophila glypicans regulate follicle stem cell maintenance and niche competition. *Genetics* **209**, 537–549.
36 Witt RM, Hecht ML, Pazyra-Murphy MF, Cohen SM, Noti C, van Kuppevelt TH, Fuller M, Chan JA, Hopwood JJ, Seeberger PH *et al.* (2013) Heparan sulfate proteoglycans containing a glypican 5 core and 2-O-sulfo-iduronic acid function as Sonic Hedgehog co-receptors to promote proliferation. *J Biol Chem* **288**, 26275–2688.
37 Fico A, De Chevigny A, Egea J, Bösl MR, Cremer H, Maina F & Dono R (2012) Modulating Glypican4 suppresses tumorigenicity of embryonic stem cells while preserving self-renewal and pluripotency. *Stem Cells* **30**, 1863–1874.
38 Cao J, Ma J, Sun L, Li J, Qin T, Zhou C, Cheng L, Chen K, Qian W, Duan W *et al.* (2018) Targeting glypican-4 overcomes 5-FU resistance and attenuates stem cell-like properties via suppression of Wnt/β-catenin pathway in pancreatic cancer cells. *J Cell Biochem* **119**, 9498–9512.
39 Grozdanov PN, Yovchev MI & Dabeva MD (2006) The oncofetal protein glypican-3 is a novel marker of hepatic progenitor/oval cells. *Lab Invest* **86**, 1272–1284.
40 Motomura Y, Senju S, Nakatsura T, Matsuyoshi H, Hirata S, Monji M, Komori H, Fukuma D, Baba H & Nishimura Y (2006) Embryonic stem cell-derived dendritic cells expressing glypican-3, a recently identified oncofetal antigen, induce protective immunity against highly metastatic mouse melanoma, B16–F10. *Cancer Res* **66**, 2414–2422.
41 Wang Y, Wang Y, Mu H, Liu T, Chen X & Shen Z (2015) Enhanced specific antitumor immunity of dendritic cells transduced with the glypican 3 gene and

co-cultured with cytokine-induced killer cells against hepatocellular carcinoma cells. *Mol Med Rep* **11**, 3361–3367.

42 Jiang Z, Jiang X, Chen S, Lai Y, Wei X, Li B, Lin S, Wang S, Wu Q, Liang Q *et al.* (2017) Anti-GPC3-CAR T cells suppress the growth of tumor cells in patient-derived xenografts of hepatocellular carcinoma. *Front Immunol* **7**, 690.

43 Svendsen A, Verhoeff JJ, Immervoll H, Brøgger JC, Kmiecik J, Poli A, Netland IA, Prestegarden L, Planagumà J, Torsvik A *et al.* (2011) Expression of the progenitor marker NG2/CSPG4 predicts poor survival and resistance to ionising radiation in glioblastoma. *Acta Neuropathol* **122**, 495–510.

44 Nishiyama A, Lin XH, Giese N, Heldin CH & Stallcup WB (1996) Interaction between NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells is required for optimal response to PDGF. *J Neurosci Res* **43**, 315–330.

45 Daynac M, Chouchane M, Collins HY, Murphy NE, Andor N, Niu J, Fancy SPJ, Stallcup WB & Petritsch CK (2018) Lgl1 controls NG2 endocytic pathway to regulate oligodendrocyte differentiation and asymmetric cell division and gliomagenesis. *Nat Commun* **9**, 2862.

46 Guichet PO, Guelfi S, Teigell M, Hoppe L, Bakalara N, Bauchet L, Duffau H, Lamszus K, Rothhut B & Hugnot JP (2015) Notch1 stimulation induces a vascularization switch with pericyte-like cell differentiation of glioblastoma stem cells. *Stem Cells* **33**, 21–34.

47 Chekenya M, Krakstad C, Svendsen A, Netland IA, Staalesen V, Tysnes BB, Selheim F, Wang J, Sakariassen PØ, Sandal T *et al.* (2008) The progenitor cell marker NG2/MPG promotes chemoresistance by activation of integrin-dependent PI3K/Akt signaling. *Oncogene* **27**, 5182–5194.

48 Lopez-Millan B, Sanchez-Martinez D, Roca-Ho H, Gutierrez-Aguera F, Molina O, Diaz de la Guardia R, Torres-Ruiz R, Fuster JL, Ballerini P, Suessbier U *et al.* (2019) NG2 antigen is a therapeutic target for MLL-rearranged B-cell acute lymphoblastic leukemia. *Leukemia*. [Epub ahead of print]. doi: 10.1038/s41375-018-0353-0

49 Beard RE, Zheng Z, Lagisetty KH, Burns WR, Tran E, Hewitt SM, Abate-Daga D, Rosati SF, Fine HA, Ferrone S *et al.* (2014) Multiple chimeric antigen receptors successfully target chondroitin sulfate proteoglycan 4 in several different cancer histologies and cancer stem cells. *J Immunother Cancer* **2**, 25.

50 Karousou E, Misra S, Ghatak S, Dobra K, Götte M, Vigetti D, Passi A, Karamanos NK & Skandalis SS (2017) Roles and targeting of the HAS/hyaluronan/CD44 molecular system in cancer. *Matrix Biol* **59**, 3–22.

51 Iozzo RV & Schaefer L (2015) Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. *Matrix Biol* **42**, 11–55.

52 Spinelli FM, Vitale DL, Demarchi G, Cristina C & Alaniz L (2015) The immunological effect of hyaluronan in tumor angiogenesis. *Clin Transl Immunology* **4**, e52.

53 Zöller M (2011) CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? *Nat Rev Cancer* **11**, 254–267.

54 Bennett KL, Jackson DG, Simon JC, Tanczos E, Peach R, Modrell B, Stamenkovic I, Plowman G & Aruffo A (1995) CD44 isoforms containing exon V3 are responsible for the presentation of heparin-binding growth factor. *J Cell Biol* **128**, 687–698.

55 Marangoni EN, Lecomte N, Durand L, de Pinieux G, Decaudin D, Chomienne C, Smadja-Joffe F & Poupon MF (2009) CD44 targeting reduces tumour growth and prevents post-chemotherapy relapse of human breast cancers xenografts. *Br J Cancer* **100**, 918–922.

56 Bourguignon LY, Singleton PA, Zhu H & Zhou B (2002) Hyaluronan promotes signaling interaction between CD44 and the transforming growth factor beta receptor I in metastatic breast tumor cells. *J Biol Chem* **277**, 39703–39712.

57 Mima KH, Okabe H, Ishimoto T, Hayashi H, Nakagawa S, Kuroki H, Watanabe M, Beppu T, Tamada M, Nagano O *et al.* (2012) CD44s regulates the TGF-beta-mediated mesenchymal phenotype and is associated with poor prognosis in patients with hepatocellular carcinoma. *Cancer Res* **72**, 3414–3423.

58 El-Haibi CP, Bell GW, Zhang J, Collmann AY, Wood D, Scherber CM, Csizmadia E, Mariani O, Zhu C, Campagne A *et al.* (2012) Critical role for lysyl oxidase in mesenchymal stem cell-driven breast cancer malignancy. *Proc Natl Acad Sci USA* **109**, 17460–17465.

59 Chanmee T, Ontong P, Mochizuki N, Kongtawelert P, Konno K & Itano N (2014) 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**, 26038–26056.

60 Porsch H, Bernert B, Mehić M, Theocharis AD, Heldin CH & Heldin P (2013) Efficient TGFbeta-induced epithelial-mesenchymal transition depends on hyaluronan synthase HAS2. *Oncogene* **32**, 4355–4365.

61 Koyama H, Hibi T, Isogai Z, Yoneda M, Fujimori M, Amano J, Kawakubo M, Kannagi R, Kimata K, Taniguchi S *et al.* (2007) Hyperproduction of hyaluronan in neu-induced mammary tumor accelerates angiogenesis through stromal cell recruitment: possible involvement of versican/PG-M. *Am J Pathol* **170**, 1086–1099.

62 Shigeishi H, Biddle A, Gammon L, Emich H, Rodini CO, Gemenetzidis E, Fazil B, Sugiyama M, Kamata N & Mackenzie IC (2013) Maintenance of stem cell self-renewal in head and neck cancers requires actions of

GSK3beta influenced by CD44 and RHAMM. *Stem Cells* **31**, 2073–2083.
63 Bourguignon LY, Peyrollier K, Xia W & Gilad E (2008) Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. *J Biol Chem* **283**, 17635–17651.
64 Ricciardelli C, Ween MP, Lokman NA, Tan IA, Pyragius CE & Oehler MK (2013) Chemotherapy-induced hyaluronan production: a novel chemoresistance mechanism in ovarian cancer. *BMC Cancer* **13**, 476.
65 Yang X, Iyer AK, Singh A, Choy E, Hornicek FJ, Amiji MM & Duan Z (2015) MDR1 siRNA loaded hyaluronic acid-based CD44 targeted nanoparticle systems circumvent paclitaxel resistance in ovarian cancer. *Sci Rep* **5**, 8509.
66 Vitale DL, Spinelli FM, Del Dago D, Icardi A, Demarchi G, Caon I, García M, Bolontrade MF, Passi A, Cristina C *et al.* (2018) Co-treatment of tumor cells with hyaluronan plus doxorubicin affects endothelial cell behavior independently of VEGF expression. *Oncotarget* **9**, 36585–36602.
67 Slomiany MG, Dai L, Bomar PA, Knackstedt TJ, Kranc DA, Tolliver L, Maria BL & Toole BP (2009) Abrogating drug resistance in malignant peripheral nerve sheath tumors by disrupting hyaluronan-CD44 interactions with small hyaluronan oligosaccharides. *Cancer Res* **69**, 4992–4998.
68 Colone M, Calcabrini A, Toccacieli L, Bozzuto G, Stringaro A, Gentile M, Cianfriglia M, Ciervo A, Caraglia M, Budillon A *et al.* (2008) The multidrug transporter P-glycoprotein: a mediator of melanoma invasion? *J Invest Dermatol* **128**, 957–971.
69 Yip GW, Smollich M & Götte M (2006) Therapeutic value of glycosaminoglycans in cancer. *Mol Cancer Ther* **5**, 2139–2148.
70 Peer D & Margalit R (2004) Tumor-targeted hyaluronan nanoliposomes increase the antitumor activity of liposomal Doxorubicin in syngeneic and human xenograft mouse tumor models. *Neoplasia* **6**, 343–353.
71 Zhao P, Damerow MS, Stern P, Liu AH, Sweet-Cordero A, Siziopikou K, Neilson JR, Sharp PA & Cheng C (2013) CD44 promotes Kras-dependent lung adenocarcinoma. *Oncogene* **32**, 5186–5190.
72 Pietras A, Katz AM, Ekström EJ, Wee B, Halliday JJ, Pitter KL, Werbeck JL, Amankulor NM, Huse JT & Holland EC (2014) Osteopontin-CD44 signaling in the glioma perivascular niche enhances cancer stem cell phenotypes and promotes aggressive tumor growth. *Cell Stem Cell* **14**, 357–369.
73 Mohyeldin A, Garzon-Muvdi T & Quinones-Hinojosa A (2010) Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell* **7**, 150–161.
74 Krishnamachary B, Penet MF, Nimmagadda S, Mironchik Y, Raman V, Solaiyappan M, Semenza GL, Pomper MG & Bhujwalla ZM (2012) Hypoxia regulates CD44 and its variant isoforms through HIF-1alpha in triple negative breast cancer. *PLoS ONE* **7**, e44078.
75 Avigdor A, Goichberg P, Shivtiel S, Dar A, Peled A, Samira S, Kollet O, Hershkoviz R, Alon R, Hardan I *et al.* (2004) CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34+ stem/progenitor cells to bone marrow. *Blood* **103**, 2981–2989.
76 Jin L, Hope KJ, Zhai Q, Smadja-Joffe F & Dick JE (2006) Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med* **12**, 1167–1174.
77 Fuchs K, Hippe A, Schmaus A, Homey B, Sleeman JP & Orian-Rousseau V (2013) Opposing effects of high- and low-molecular weight hyaluronan on CXCL12-induced CXCR77 signaling depend on CD44. *Cell Death Dis* **4**, e819.
78 Kingsmore KM, Logsdon DK, Floyd DH, Peirce SM, Purow BW & Munson JM (2016) Interstitial flow differentially increases patient-derived glioblastoma stem cell invasion via CXCR78, CXCL12, and CD44-mediated mechanisms. *Integr Biol (Camb)* **8**, 1246–1260.
79 Faber A, Goessler UR, Hoermann K, Schultz JD, Umbreit C & Stern-Straeter J (2013) SDF-1-CXCR79 axis: cell trafficking in the cancer stem cell niche of head and neck squamous cell carcinoma. *Oncol Rep* **29**, 2325–2331.