CD44, hyaluronan, the hematopoietic stem cell, and leukemia-initiating cells

Margot Zöller*

Department of Tumor Cell Biology, University Hospital of Surgery, Heidelberg, Germany

CD44 is an adhesion molecule that varies in size due to glycosylation and insertion of so-called variant exon products. The CD44 standard isoform (CD44s) is highly expressed in many cells and most abundantly in cells of the hematopoietic system, whereas expression of CD44 variant isoforms (CD44v) is more restricted. CD44s and CD44v are known as stem cell markers, first described for hematopoietic stem cells and later on confirmed for cancer- and leukemia-initiating cells. Importantly, both abundantly expressed CD44s as well as CD44v actively contribute to the maintenance of stem cell features, like generating and embedding in a niche, homing into the niche, maintenance of quiescence, and relative apoptosis resistance. This is surprising, as CD44 is not a master stem cell gene. I here will discuss that the functional contribution of CD44 relies on its particular communication skills with neighboring molecules, adjacent cells and, last not least, the surrounding matrix. In fact, it is the interaction of the hyaluronan receptor CD44 with its prime ligand, which strongly assists stem cells to fulfill their special and demanding tasks. Recent fundamental progress in support of this “old” hypothesis, which may soon pave the way for most promising new therapeutics, is presented for both hematopoietic stem cell and leukemia-initiating cell. The contribution of CD44 to the generation of a stem cell niche, to homing of stem cells in their niche, to stem cell quiescence and apoptosis resistance will be in focus.

Keywords: CD44, hematopoietic stem cells, leukemia-initiating cells, bone marrow niche, homing, adhesion, dormancy, apoptosis resistance

Introduction

CD44, first described as a lymphocyte homing receptor (1), is expressed by a wide range of hematopoietic and non-hematopoietic cells (2). Interest in CD44 increased considerably, when it was noted that the insertion of alternatively spliced exon products in the CD44 standard or CD44 hematopoietic isoform (CD44s) strikingly affects the molecules function, such that expression of CD44 variant isoforms (CD44v) induces a metastatic phenotype in locally growing tumor cells (2, 3).

Abbreviations: ASC, adult stem cells; bFGF, basic fibroblast growth factor; BM, bone marrow; BMP, bone morphogenetic protein; BM-Str, BM stroma cells; C, complement; CD44s, CD44 standard isoform; CD44v, CD44 variant isoforms; CIC, cancer initiating cells; ECM, extracellular matrix; ERM, ezrin, radixin, moesin; ESC, embryonic SC; FN, fibronectin; GAG, glucosaminoglycan; GEM, glycolipid enriched membrane microdomains; HA, hyaluronic acid; HAS, hyaluronan synthase; HGF, hepatocyte growth factor; HSC, hematopoietic SC; ICD, intracellular domain; kd, knockdown; ko, knockout; LIC, leukemia initiating cells; MSC, mesenchymal SC; Mϕ, macrophages; OPN, osteopontin; PCCD4, programmed cell death 4; PDGFR, platelet-derived growth factor receptor; RTK, receptor tyrosine kinase; TGF, transforming growth factor; TPO, thrombopoietin; VEGF, vascular endothelial growth factor; wt, wild type.
At the time, it was surprising that a leukocyte marker is engaged in solid tumor metastasis formation. As the hematopoietic system is the only organ that components repeatedly shift between sessile and mobile states, we argued that metastasizing tumor cells may transiently take over part of the program of hematopoietic cells and that this programmatic shift, which is independent of oncogene transformation, depends to a considerable degree on CD44 and its activities (4). This hypothesis received strong support by the recovery of cancer- and leukemia-initiating cells (CIC/LIC), which are defined by their capacity to take over part of the program of stem cells (SC). In fact, CD44s/CD44v are CIC/LIC markers (5, 6) and, most importantly, CD44 was the first marker defined as a CIC/LIC biomarker. This implies that CD44 is engaged in fulfilling special SC-related tasks in CIC/LIC (7). These particular tasks include, besides growth upon serial transplantation in xenogeneic models, self-renewal and recapitulation of the heterogeneous phenotype of the parental tumor, reflecting the differentiation capacity of CIC/LIC. It also includes, at least for a subset of CIC/LIC, the capacity of SC to transiently shift from a sessile toward a mobile state, which is required for metastasis formation (7–9). Furthermore, like SC, CIC/LIC are highly apoptosis resistant (6, 7) and may profit from the crosstalk with the surrounding (5, 6). Notably, too, CIC/LIC are heterogeneous (10) and genetically instable (11). This is in line with their disputed, not mutually exclusive origin from adult stem cells (ASC), from oncogene-transformed committed progenitors or from cell fusion particularly with macrophages (Mφ) (12). Despite their heterogeneous origin, CIC/LIC share many features with hematopoietic SC (HSC), like relative quiescence, longevity, drug resistance, and support by the surrounding that for SC is called the niche. Finally, there is strong evidence that CD44/CD44v is engaged in many of the activities, which CIC/LIC share with ASC (13).

CD44 is a quite abundant expressed molecule. Thus, the question arises, what qualifies CD44 for this multitude of very special tasks. This review outlines that two features of CD44 mostly account for the molecule’s contribution to SC maintenance: first and most important, CD44 crosstalks with the surrounding/the niche. Second, CD44 is located in membrane subdomains, which are particularly prone for collecting signal transduction molecules, proteases, and cytoskeletal components, and foster concerted activities. HSC and LIC were chosen as prominent examples. Based on the largely overlapping activities of CD44 in CIC and LIC, some references to CIC are included, as far as deeper insight was gained with the latter.

**CD44 Structure and Ligands**

CD44 is a member of the family of cartilage link proteins (15, 16). The N-terminal region forms a globular structure. Conserved cysteins are important for the stability of the extracellular domain, and two cysteins in the flanking region account for correct link domain folding (19). This globular structure contains binding sites (AA 32–132) for collagen, laminin, fibronectin (FN), and cell surface receptors like E-selectin and L-selectin (20–22). Importantly, CD44 also is the major receptor for hyaluronan (HA) (23). HA binds to a basic motif (AA 150–158) within the globular structure, but outside of the link domain (23, 24). Though the HA binding motif is present in all CD44 isoforms, not all CD44+ cells bind HA. However, HA-binding can be induced by CD44 cross-linking, which indicates that HA-binding depends on conformational changes or a redistribution of CD44 in the cell membrane (25). CD44 also has two binding sites for other glycosaminoglycans (GAG) (26). The N-terminal globular domain is followed by a stretch of 46 AA, which comprises exon products 5–7. This stretch of 46 AA forms a stalk like structure (27). It is heavily glycosylated and contains putative proteolytic cleavage sites (28). Variable exon products are inserted in the stalk like region (29). The transmembrane region supports CD44 oligomerisation and contributes to incorporation in glycolipid-enriched membrane microdomains (GEM) (30). The cytoplasmic tail of CD44 contains binding sites for the cytoskeletal proteins ankyrin and ezrin, radixin, moesin (ERM). Ankyrin mediates contact with spectrin and is involved in HA-dependent adhesion and motility (31). ERM proteins are engaged in regulating migration, cell shape, and protein resorting in the plasma membrane (32). The N-terminus of activated ERM proteins binds to a motif between the transmembrane region and the ankyrin binding site, and their C-terminus binds to F-actin. Thereby, ERM proteins link CD44 to the actin cytoskeleton (33).

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Finally, CD44 O-glycosylation, the transmembrane region, and the cytoplasmic tail affect the membrane subdomain localization. Depending on the activation state, CD44 is recruited into GEM (35), which has great bearing on the interaction of CD44 with extracellular ligands and the association with other transmembrane and cytoplasmic molecules (36, 37). These associations are most crucial for the accessory functions of CD44 in migration and signal transduction. This is a sequel of the inner membrane side organization of GEM, which favors harboring adapter and signal transducing molecules like src family members (38). Some of these cytoplasmic adapter and signaling molecules are constitutively associated with GEM-located CD44 (39). GEM are also prone for internalization (40).

Unlike CD44s, CD44v is only expressed on subpopulations of epithelial and hematopoietic cells, particularly during embryonic development, hematopoietic cell maturation and activation, and in some carcinoma and leukemia with a tendency toward overexpression in CIC/LIC (41, 42). Several of the CD44v exon products can contain specific post-translational modifications. These include a heparan-sulfate site in exon v3, which serves for the
binding of heparin-binding proteins like basic fibroblast growth factor (bFGF)\(^{43}\); CD44v6 contains a binding site for hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and osteopontin (OPN)\(^{44-46}\). OPN also binds to CD44v10\(^{47}\). Via these cytokine/chemokine binding sites, CD44v takes over a central and coordinating role in receptor tyrosine kinase (RTK) activation\(^{48}\).

Briefly, CD44 is the major HA receptor. It has a multitude of additional ligands and associates with transmembrane and cytoplasmic molecules. This is due to several glycosylation sites, variant exon product sequences, the insertion into GEM, and the cytoplasmic tail structure. Noteworthy, HA binding contributes to the GEM recruitment of CD44. Beyond forcing CD44 associations, this has bearing on CD44 internalization.

### HSC/LIC CD44 and Stem Cell Genes

CD44 is a marker of ASC, including HSC and of a large range of CIC and LIC\(^{7,49}\). In addition, there is some evidence for pronounced CD44v expression in SC\(^{50}\). Thus, the question arose, whether CD44 is engaged in stem cell gene expression and/or whether CD44/CD44v expression is regulated by stem cell genes.

Embryonic SC (ESC) are characterized by expression of a set of master SC transcription factors, Oct4, Sox2, and Nanog\(^{51}\), as well as distinct chromatin organization and epigenetic signatures, which govern the intrinsic ability to self renew and to differentiate into multiple lineages\(^{52}\). Polycomb genes, which have a role in transcriptional repression through histone modification, associate with the promoter and regulatory regions of target genes in ESC. Expression of master gene transcription factors and epigenetic regulation are maintained in HSC and LIC\(^{53,54}\), which share with ESC Oct4, Nanog, and Myc overexpression\(^{55,56}\), and Notch, Wnt, and Hedgehog signaling pathways, important in shaping tissue structure, cell fate, and identity\(^{57}\). In fact, leukemia recurrence was prevented by deletion of the polycomb gene Bmi1, important for HSC self-renewal\(^{58}\).

As discussed below in concern of HSC quiescence, there are links between CD44, Nanog, and Myc expression, and CD44 is a target of the Wnt and Notch pathways in HSC and LIC\(^{59-61}\). However, there is no evidence that CD44 plays a central role in regulating master gene transcription factors in HSC/LIC\(^{62-64}\).

Besides master SC transcription factors, miRNA were recognized as key regulators of self-renewal and SC fate\(^{65}\). This includes hematopoiesis, HSC being lost upon abrogation of Dicer, which was ascribed to miR-125a\(^{66}\). Additional miRNA overexpressed in HSC either promote HSC engraftment (miR-125b-5p, miR-126-3p, miR-155) or are disadvantageous (miR-196b, miR-181c, miR-542-5p, let7e)\(^{67}\). Notably, miRNA profiles differ significantly between HSC and lineage committed progenitors, and miRNA profiles in hematological malignancies differ from those of HSC and progenitor cells. In addition, miRNA profiles are selective for distinct leukemia [review in Ref. \((68-72)\)]. Nonetheless, there are some common trends: miR-15a, miR-29b, miR-34a, miR-151, and miR-204 frequently act as tumor suppressors, and miR-155, miR-96, miR-24, miR-21, miR-32, miR-106-25, and let-7 as oncomir\(^{73}\). However, the engagement of HA/CD44 on miRNA regulation in HSC and LIC remains to be elaborated in detail. So far, there are only sporadic hints toward a mutual impact.

HA-crosslinked CD44v3 binds Nanog, Oct4, and Sox2, which promotes miR-302 expression\(^{74}\) a key player in controlling SC self-renewal and pluripotency\(^{75}\). Also, binding of HER2 to CD44 leads to upregulation of MTAS-1 (metastasis-associated-1), which induces silencing of the miR-139 promoter, accompanied by increased CXCR4 expression\(^{76}\). HA-CD44v6 binding promotes PKCe activation, and this increases Nanog phosphorylation and nuclear translocation, where Nanog associates with Drosha and an RNA helicase p68, which leads to oncogenic miRNA-21 transcription and a reduction in the expression of the tumor suppressor programmed cell death 4 (PCD4)\(^{77}\). CD44v6-associated overexpression of miR-21\(^{78}\) induces pre-B-cell lymphoma\(^{79}\), and is frequently observed in CML\(^{80}\). Analyzing the impact of CD44v6 on the miRNA profile in metastasizing CIC\(^{81}\) revealed CD44v6-dependent downregulation of the tumor suppressors let-7b, let-7d, let-7e, miR-101, and miR-34a. The latter, which suppresses tumor growth by CD44 downregulation\(^{82}\), is abundantly expressed in CD44v6 knockdown (CD44v6\(^{4d}\)) cells, which argues for CD44v6 to be engaged in miR-34a silencing. On the other hand, metastasis-promoting miR-494 and miR-21 and apoptosis-regulating miR-24-1\(^{83-85}\) are abundant only in CD44v6-competent cells. miRNA transcription and/or posttranscriptional regulation also were affected by CD44v6-associated MET\(^{86}\), which supports miR-103 transcription\(^{87}\). MiR-103 expression was only high in CD44v6-competent cells.

We are also far away from a comprehensive view on the regulation of CD44 via miRNA. MiR-199a binding to the CD44 3′-UTR suppresses tumorigenicity, multidrug resistance, and migration\(^{88,89}\). The CD44 3′-UTR binds additional miRNA that target extracellular matrix (ECM) miRNA, like miR-328, miR-491, miR-671, and miR-512-3p. In fact, transfection-induced CD44 3′-UTR overexpression is accompanied by collagen I and FN upregulation\(^{90}\). However, stressing the need for further studies, opposing findings have also been reported, such as downregulation of CD44 by pro-metastatic miR-373/520c\(^{91}\).

Finally, aberrant and alternative splicing is frequently observed in CIC/LIC, and CD44 ranks first in the affected genes\(^{92}\). However, no mutations were found in cis acting CD44 splice elements\(^{93}\). Thus, a genetic basis for CD44 alternative splicing in malignancies remains questionable.

Taken together, though links between CD44 and master SC genes, dominating SC signaling pathways, and epigenetic regulation of SC genes were described, HSC do not essentially depend on CD44. This could have been expected, as HSC are not or not seriously affected in panCD44\(^{94}\), CD44v10\(^{95}\), CD44v7\(^{96}\), or CD44v6/v7\(^{96-98}\) mice.

On the other hand, it is already known since 1990 that CD44 is required for the development and maintenance of early hematopoietic progenitors. In long-term bone marrow (BM) cultures, tightly packed clusters of small cells, so called cobble stone areas, develop below a stroma layer. These cobble stones contain cells with the capacity for long-term reconstitution. When cultures contain anti-CD44, HSC clusters do not develop\(^{99}\). Furthermore, CD44 is a reliable LIC marker in many malignancies\(^{100}\).
and the first LIC biomarker that blockade severely affected LIC maintenance, e.g., anti-CD44 drives LIC into apoptosis (101, 102). Thus, the essential contribution of CD44 relies on the communication of SC/HSC and LIC with the surrounding. In the following sections, those features of HSC are discussed that depend on or are modulated by the surrounding. This includes the requirement for a niche to maintain quiescence and to receive signals that drive out of quiescence toward differentiation. The latter frequently is associated with changes in motility. Finally, HSC are relatively apoptosis resistant. It also will be discussed, where LIC, which resemble HSC in many respects, become less dependent on the surrounding or respond differently due to the oncogenic transformation.

The Endosteal Niche

The fate of a cell in the developing organism is determined by its position (103, 104). SC reside in specialized locations, the niches, which minutely regulate their activity (105). Niches are composed of epithelial and mesenchymal cells and extracellular substrates. They govern location, adhesiveness, retention, homing, mobilization, quiescence and activation, symmetric and asymmetric division, and differentiation (106). Accordingly, a niche might prevent tumorigenesis, which would argue against CIC/LIC profiling from a niche. However, there is ample evidence that a preformed niche supports CIC/LIC survival and homing (105) and regulates the balance between quiescence and growth (107). Beyond this, a niche can support reprogramming of non-CIC toward CIC by exposing them to an embryonic microenvironment (108). CD44 plays a central role in the crosstalk between SC/malignant SC and the niche, which includes an active contribution of CD44 in niche assembly.

The Composition of HSC and LIC Niches

A niche for HSC, where they receive instructions particularly in respect to their lifelong capacity for self-renewal, was first proposed by Schofield in 1978 (109). Only 25 years later, it was uncovered that osteoblasts lining the surface of the bone play a major role (110). Additional cellular components of the endosteal niche are mesenchymal stem cells (MSC), osteoclasts, Mϕs, fibroblasts, and adipocytes (111, 112). Interestingly, MSC, too, are influenced by their surrounding. Thus, it was expected that MSC from different tissue fulfill equivalent biological activities. On the contrary, when implanting MSC from BM, white adipose tissue, umbilical cord or skin, only BM-derived MSC spontaneously formed a BM cavity, which was progressively replaced by hematopoietic tissue and bone and permitted homing and maintenance of long-term murine and human HSC (113). Matrix components of the endosteal niche are HA, FN, laminin, and collagen that are secreted by endosteal niche cells and support HSC adhesion, quiescence, and self-renewal. Prominent cytokines and chemokines secreted by BM stroma cells (BM-Str) and/or captured by the BM stroma are thrombopoietin (TPO), SDF1, OPN, and parathyroid hormone. TPO promotes HSC quiescence (114). SDF1 supports quiescence and affects apoptosis resistance (115). OPN is engaged in lodging to the endostium (116), and parathyroid hormone supports trabecular network formation of osteoblasts and HSC expansion (117).

Hematopoietic SC avail on a second niche, the vascular niche, which is located in proximity to endothelial cells (118). Though components and activities of the endosteal and the vascular niche are partly overlapping (119), distinct to the endosteal niche, the vascular niche plays a major role in HSC homing and hematopoietic progenitor egress. The vascular niche also supports hematopoietic progenitor expansion and maturation. In line with these special duties, reticular cells in the vascular niche express IL6, HGF, OPN, and SDF1 at high or higher levels than cells in the osteogenic niche (120).

Thus, possibly distinct to ASC in solid organs, HSC dispose of two niches. The requirement for two niches might be linked to the general feature of cells of the hematopoietic system that are not sessile and circulate through the body to fulfill upon request their tasks in loco, and thereafter patrol again through the organism. Noteworthy, HSC/LIC CD44 contributes to the establishment of both BM niches.

CD44 Contributes to the Generation of the BM Niches

The Contribution of CD44 to Matrix Assembly

Stem cells niches, including the osteogenic niche in the BM, are particularly rich in HA (121). HSC synthesize and express HA, and HA expression correlates with HSC adhesion to the endosteal niche (122). Similarly, CD44 contributes to building an HA coat on endothelial cells, which facilitates binding of mobilized HSC to endothelial cells as well as HSC homing (123, 124). Furthermore, perturbation in matrix components alters cell shape and intracellular tension, which results in shifts in signaling events that affect gene expression (125). This could be particularly important in the BM niche, where HA delivery by HSC/LIC can induce expression of HA in niche cells (126). Furthermore, CD44 is involved in matrix assembly (127) such that the HA−CD44 association modifies the matrix to support colonization (128).

In concern about CD44v, there is evidence for an engagement of CD44v6 in matrix assembly. A CD44v6 knockdown (kd) in a highly metastatic tumor line revealed a striking reduction in metastatic capacity, which was, at least, partly due to an altered tumor matrix (81). CD44v6kd cells secrete a matrix not supporting adhesion of CD44vwt or CD44v6kd cells, whereas both cells readily adhere to the CD44vwt-matrix. In fact, HA synthesize 3 (HAS3) expression is strongly reduced in CD44v6kd cells (86), where high HAS3 expression frequently correlates with aggressiveness of carcinoma and leukemia (129). On the opposite, the CD44v6kd cells abundantly secrete hyaluronidase such that the matrix contains a lower amount of HA and exclusively low molecular weight HA (130), which significantly affects adhesion and the catcher activity of the matrix (Figure 1A).

Briefly, HSC CD44 contributes to the generation of HSC niches mostly via HA provision, where the composition of HA varies depending on the expression of CD44v isoforms. CD44/CD44v strengthens HAS3 expression and, by not yet defined mechanisms, prohibits hyaluronidase activity.

CD44 Contributes to the Catcher Activity of the Niche Matrix

CD44 is a transmembrane proteoglycan, which allows for the local concentration of glycosaminoglycan-associating proteins (131).
FIGURE 1 | The contribution of CD44 in HSC/LIC for assembly and modulation of the osteogenic niche. (A) HSC secrete large amounts of HA. Upon HA crosslinking, CD44 becomes activated, which supports transcription of HAS3, which strengthens the process of high MW HA deposition and incorporation of cytokines, chemokines, and proteases in the abundant HA coat. (B) HSC quiescence and LIC proliferation are supported by the catcher activity of CD44/CD44v6 and most pronounced CD44v3 associated GAG, which bind a large range of cytokines and chemokines including HGF, bFGF, OPN, and VEGF. (C) Particularly, LIC contribute to modulation of the matrix in the osteogenic niche. This is due to CD44-HA initiated transcription of MMP2, MMP9, uPAR, and cathepsinG. MMP2 and MMP9 bind to CD44; pro MMP2 is cleaved by CD44v6-associated MT1MMP, which concomitantly allows for focal matrix degradation. CathepsinG and MMP9-activated TGF\(\beta\) contributes to bone resorption and niche preparation for LIC.

Of special interest for HSC and LIC is the binding of OPN to CD44v3, CD44v6, and CD44v10 (47, 132, 133), where OPN secretion is further stimulated by HA (134). OPN is chemotactic and haptotactic, and as such important for the recruitment of HSC into the niche (135). On the other hand, the OPN–CD44 interaction exerts a feedback on the donor cell, which supports migration. Thus, p53\(^{ko}\)CD44\(^{ko}\) mice have the same rate of primary tumor development as p53\(^{ko}\) mice, but tumors do not metastasize (136). Similarly, a blockade of CD44v10 strongly reduced OPN delivery by leukemic cells, which was accompanied by pronounced retention of HSC in the niche (137). CD44v6 also binds VEGF and HGF (44, 45, 138, 139). In the hypoxic environment of the osteogenic niche, HIF1\(\alpha\) acts as a regulator to prevent HSC proliferation and exhaustion, where it is supported by VEGF, a target of HIF1\(\alpha\) (140). Instead, leukemic cells, which were supported by VEGF-activated endothelial cells in the vascular niche, gain in cytotoxic drug resistance (141). In concern about HGF, it is worthwhile noting that a subpopulation of HSC responds to HGF by migrating toward skeletal muscles (142). CD44v3 binds bFGF that stimulates proliferation of underlying mesenchymal cells in the developing limb and affects BM MSC (143, 144). This might be due to bFGF inducing changes in HAS and hyaluronidase isoform expression (145). A direct contribution of CD44v3-bound bFGF to the activity of bone MSC remains to be explored (Figure 1B).

Finally, CD44v6 can directly contribute to the composition of the niche matrix (130). CD44v6 supports transcription of hepatoma-derived growth factor, which stimulates the growth of fibroblasts, endothelial cells, and vascular smooth muscle cells, and recruits MSC (146). CD44v6 also promotes clusterin secretion that influences chemokine secretion and initiates stromal changes affecting intercellular communications (147). In addition, the complement (C) components 3a and 3b are absent in a CD44v6\(^{kd}\) matrix, but are abundantly delivered by CD44v6\(^{wt}\) cells (86). These findings are well in line with the innate immune system, particularly C3, cooperating with CD44 in HSC to strengthen the HSC CD44 – niche interaction [review in Ref. (148)] (Figure 1A).

CD44 Modulates HSC Niches
CD44 concentrates MMPs at the cell surface, where the production of uPAR, MMP2, and MMP9 is concomitantly stimulated by...
the interaction between HA and CD44 (86, 149). MMP9 transcription is actively supported by the CD44 intracellular domain (ICD), which binds to a MMP9 promoter response element (150). By a not yet defined mechanism, CD44v6 also is involved in uPAR transcription (86). CD44 aggregation via HA binding facilitates MMP binding (150). Furthermore, proMMP2 becomes activated through CD44v-associated MMP14, which is located in the leading lamella. As cell-bound MMPs are protected from their inhibitors, this allows for focal degradation of the ECM to form space for invading LIC (151). LIC also stimulate osteoclasts to secrete cathepsinG and MMP9 to resorb bone to create a niche. CathepsinG, primarily secreted by osteoclasts (152), is another transcriptional target of HA–CD44 signaling (153). Transforming growth factor β (TGFβ) activation through CD44-associated MMP9 promotes angiogenesis, invasion (154), and enhances osteoclast activity and bone resorption (155) (Figure 1C).

Taken together, HSC require a niche and CD44 contributes to niche assembly. The most prominent CD44 contribution relies on the stimulation of HA provision via pronounced HAS activation. CD44 also supports retaining growth factors and chemokines that are supplied by the different niche elements. This facilitates message delivery from the niche toward the HSC/LIC. There is evidence for a contribution of CD44v particularly in cytokine/chemokine retention. It remains to be explored whether this provides a pronounced profit from the niche for CD44v expressing LIC. CD44 also contributes to modulating the niche by hyaluronidases and proteases that transcription is promoted by CD44 or that become activated via direct or indirect associations with CD44. There is no evidence that LIC contribute to establishing a niche. Rather, LIC are suggested to make use of the HSC niche. It is still disputed whether LIC displace HSC from their niche or actively remodel/destroy the niche (156), such that HSC die by neglect. High hyaluronidase secretion by CD44v6+ LIC could favor the latter.

**CD44 Supports Adhesion, Homing, and Migration of HSC and LIC**

**CD44, HSC, and LIC Adhesion to the Bone Marrow Stroma**

One of the prime functions of the osteogenic niche is the retention of HSC to instruct for longevity and quiescence, which requires firm HSC adhesion. This task is mainly taken over by HA (157). HA binding initiates or, at least, influences most activities of CD44 (158). The importance of CD44 as an adhesion molecule for HSC and LIC has been amply demonstrated (157, 159). The particular engagement of the CD44–HA interaction was confirmed by the finding that HSC adhesion can be blocked by anti-CD44, soluble HA, or hyaluronidase (160).

CD44 adhesion to its ligand(s) induces up-regulation of additional adhesion molecules, mostly integrins, which strengthen HSC adhesion (161). This was intensely explored for the association of CD44 with α4β1 (162, 163). Upon activation by HA adhesion, the two molecules directly associate (164), which is accompanied by α4β1-promoted stronger adhesion to FN and laminin (165). HSC α4β1 additionally supports the direct contact with stroma cells via ICAM1 binding (166). In line with the contribution of CD44 and α4β1 to the adhesion of HSC to the osteogenic niche, anti-CD44 and anti-α4β1 dislodge HSC from the BM niche (167, 168) (Figure 2A).

CD44 also participates in LIC embedding into the endosteal niche, such that targeting CD44 is considered a new strategy to eliminate persistent and drug-resistant LIC. In AML, CD44 is required for the transport of LIC to the HSC niche, and anti-CD44 antibodies alter the fate of the LIC by inducing differentiation (101). In a mouse model of CML, BCR-ABL1-transduced progenitors from CD44-mutant donors were defective in BM homing, which resulted in decreased engraftment and impaired CML-like disease induction (100). These studies provided additional evidence that LIC may be more dependent on CD44 for settlement in the osteogenic niche than HSC (101). Studies in a murine model confirmed leukemia cell homing and growth retardation by a CD44-specific antibody (169). However, during reconstitution, a panCD44-specific antibody more efficiently interfered with HSC than leukemia cell settlement in the BM niche (82).

The latter topic, how to avoid replacement of HSC when attacking LIC, was recently approached in an elegant study aiming to find selective ligands for LIC in CML and AML. Alteration of the niche by osteoblastic cell-specific activation of the parathyroid hormone receptor attenuates BCR-ABL1 oncogene-induced CML-like myeloproliferative neoplasia, but enhances MLL-AF9 oncogene-induced AML, possibly through opposing effects of increased TGF-β1 on the respective LIC. These results, though providing a first and very important hint toward sparing niche embedded HSC, also demonstrate that niches differ for distinct LIC (170). The use of CD44v specific antibodies could be an alternative for blocking LIC, but sparing HSC embedding in the niche. Unfortunately, our data so far point toward a dominating role of CD44v in niche embedding. Thus, adhesion of CD44v6/v7ko and of CD44v7ko HSC to BM-Str is unimpaired (97, 171).

Facing competition for the niche, it should be remembered that LIC might remodel the niche such that it no longer serves the requirements of HSC (172). This possibility did not yet receive appropriate attention. Nonetheless, growing awareness and elaboration of the differentiation potential of distinct SC populations, particularly of BM derived MSC, might finally allow reconstituting/replacing a niche, which was distorted by LIC.

Distinct to the contribution of HSC/LIC CD44 on adhesion, little is known on the engagement of stroma cell provided CD44. Anti-CD44v6 strikingly hampered stroma formation in rat long-term BM cultures, but had no impact on HSC embedding in a preformed stroma (173). In addition, recovery of HSC from wt mice in the BM of CD44v7ko mice is severely impaired (97). In vitro studies confirmed that HSC poorly adhere to long term CD44v6/v7ko and CD44v7ko BM-Str (171, 174). Thus, BM-Str CD44 should not be neglected as it contributes to matrix assembly. A comprehensive evaluation is missing. However, the availability of conditional ko mice will facilitate answering the question.

There is overwhelming evidence confirming the important finding of Miyake that HSC require CD44 for embedding in the BM niche, and that dislodgement by anti-CD44 severely affects hematopoiesis. In view of the essential role of CD44 to anchor HSC in the osteogenic niche, care should be taken on the therapeutic use of anti-CD44 to drive LIC out of the niche. Despite promising results, further refinement is required to guarantee
unimpaired hematopoiesis. First trials to replace anti-CD44 have been successful, but point toward no single strategy being effective in distinct LIC. Additional studies are also needed to elaborate options for correcting niches, which were distorted by LIC.

**CD44 and HSC Mobilization**

Transplantation of hematopoietic progenitor cells provides in many instances an ultimate chance of curative leukemia therapy. It has become obvious that the transfer of peripheral blood HSC appears advantageous, yet it requires HSC mobilization. In 1976, Richman et al. described an increase of HSC in the blood of patients, who had undergone chemotherapy (175). Later, a similar increase was observed after the administration of recombinant growth factors (176). In fact, both G-CSF and chemotherapy mobilize HSC through the same mechanisms, with chemotherapy increasing the level of endogenous G-CSF (177). Though the precise mechanism remains to be elucidated, HSC mobilization obviously does not proceed directly, as HSC do not express the G-CSF receptor, which is expressed by BM macrophages (Mφ) (178). Activation of Mφ could result in a reduction of Nes+ MSC and SDF1-abundant reticular cells (CAR), and their provision of SDF1 such that the SDF1-CXCR4 bond becomes loosened (179, 180). SDF1 is secreted by several BM-Str and its interaction with CXCR4 on HSC plays a key role in retention and trafficking. CXCR4 expression is enhanced through signaling cascades involving cAMP, PI3K, several GTPases, and PKCζ (181). PKCζ induces...
motility, adhesion and survival, and MMP2 and MMP9 secretion (182). Disruption of the SDF1-CXCR4 axis is the major mechanism leading to HSC release from their niche (182, 183). Alternatively, not mutually exclusive, upregulated expression of proteases may be involved, which can affect SDF1 (184) via MMP9 (185) or CD26 (186), cathepsin G and K, and neutrophil elastase (183). The same proteases also may account for VCAM1, FN, and OPN degradation (187). In addition, CD44 cleavage via MMP14 can contribute to HSC mobilization, where G-CSF leads to increased MMP14 expression in HSC (188, 189). Activation of the C cascade and plasminogen also contributes to HSC mobilization (190, 191) (Figure 2B).

Although G-CSF efficiently mobilizes HSC in most instances, some patients do not or insufficiently respond. In addition, G-CSF treatment may be accompanied by maturation of the most primitive progenitors, and this impairs HSC homing and recovery of hematopoiesis. Therefore, additional approaches for HSC mobilization have been searched for, in particular, mobilization via a blockade of adhesion molecules expressed by CD34+ cells. As described above (167, 168), concomitant application of anti-CD44 and anti-α4β1 most efficiently mobilizes HSC. Notably, most of the mechanisms suggested accounting for G-CSF-induced HSC mobilization might be initiated directly via the CD44 blockade. First, as CD44 is associated with CXCR4, SDF1-CXCR4 binding becomes loosened by a CD44 blockade (192). Second, antibody crosslinking of CD44 contributes to the activation of MMP9 and MT1MMP, which both are associated with CD44 (193).

In brief, mobilization, though mostly approached via G-CSF, can be achieved by directly loosening adhesion of HSC to the niche via anti-CD44 and/or anti-α4β1.

CD44 and HSC Homing

With the therapeutic transfer of mobilized HSC, the question arose on their homing. Transferred HSC preferentially home into the BM, where they search for the osteogenic niche (194). Homing is facilitated by the unique feature of BM endothelium that constitutively expresses the endothelial P- and E-selectins and VCAM1 (195). Proinflammatory cytokines stimulate CD44 expression in endothelial cells and strengthen their binding to HA. This promotes the arrest of HSC, which bind via CD44 to HA captured by endothelial cells (196). HSC express P-selectin ligands and CD44 as well as VCAM1 receptors such as α4β1 (197–199). Function blocking antibodies and targeted deletions confirmed the contribution of these adhesion molecules to slow-down HSC, which allows for firm adhesion and extravasation (200, 201) (Figure 2C).

CD44 also is involved in the extravasation of the endothelial cell-attached HSC (202). CD44–HA binding initiates the interaction of the CD44 cytoplasmic tail with the actin cytoskeleton through ankyrin and ERM proteins (196, 203), guiding CD44 to the leading edge of migrating cells (204). Thus, cells expressing CD44 with a truncated cytoplasmic tail retain HA-binding capacity, but loose the capacity to migrate on HA (204). One of the central events in CD44-mediated cytoskeletal reorganization appears to be Rac1 activation. Lamellipodia formation on HA-coated plates can be inhibited by a CD44 blocking antibody, but also by transfection of a dominant-negative mutant form of Rac1 (205). Upstream regulators of Rac1 are Vav1 and Vav2, phosphotyrosine-dependent guanine exchange factors of Rho GTPases. Vav phosphorylation is mediated by src kinases (206). As GEM-located CD44 associates with src (207), cytoskeleton reorganization most likely is initiated via src activation. Another mediator of CD44 signaling is RhoA. The RhoA-specific p115RhoGEF interacts with CD44 and regulates HA-mediated CD44 signaling via the serine-threonine Rho-Kinase (ROK), a downstream target of RhoA. ROK phosphorylates CD44, which promotes enhanced ankyrin binding (208). Another pathway of CD44 promoted motility proceeds via the association with α4β1. By associating with α4β1, CD44 gains access to FAK (focal adhesion kinase) and α4β1 gains access to src kinases and ERM proteins, such that the integrin–paxillin association becomes weakened and the GEM-integrated CD44-ezrin-integrin-FAK complex moves toward the leading edge (164, 209).

Cell motility is additionally supported by CD44 cleavage via a disintegrin and metalloproteinase domain (ADAM) protein and MMP-14 (210). CD44 cleavage is stimulated by Ca2+ influx, which triggers ADAM10 activation after proADAM10 dissociation from calmodulin. ADAM17, which colocalizes with CD44 at Rac-regulated membrane ruffling areas, becomes activated by PKC and Rac and contributes to CD44v cleavage (211, 212). Thus, the rapid activation of membrane-integrated proteases by CD44–HA binding contributes to a shift toward motility by CD44 cleavage. CD44 cleavage is tightly regulated, in part, by the missing activation of CD44-associated proteases after CD44 cleavage, and in part by cleavage-promoted CD44 transcription. After ectodomain cleavage, CD44 becomes accessible to the presenilin/γ-secretase complex, which triggers intramembrane CD44 cleavage, setting free the CD44 ICD (CD44-ICD). CD44-ICD acts as a cotranscription factor that potentiates CD44 (28), MMP9, and MMP3 transcription (150) (Figure 2D).

As leukemia therapy frequently relies on autologous HSC transplantation, it became important to know whether LIC compete with HSC not only for the niche but also for homing. The described signaling pathways promoting HSC extravasation do not fundamentally differ for LIC. However, there are subtle differences. This was explored in an elegant study for BCR-ABL1-induced CML-like myeloproliferative neoplasia. Expression of α4β1, α5β1, LFA1, and CXCR4 did not differ between BCR-ABL1(+) progenitors and HSC, but expression of P-selectin glycoprotein ligand-1 and of 1-selectin was lower than in HSC. Deficiency of E-selectin in the recipient BM endothelium significantly reduced engraftment by BCR-ABL1-expressing SC. Destruction of selectin ligands on leukemic progenitors by neuraminidase reduced engraftment. BCR-ABL1-expressing 1-selectin-deficient progenitors were also defective in homing and engraftment, and an 1-selectin-specific antibody decreased the engraftment of BCR-ABL1-transduced SC. These results establish that BCR-ABL1(+) LIC rely to a greater extent on selectins and their ligands for homing and engraftment than HSC. Thus, a selectin blockade may be beneficial in autologous HSC transplantation for CML and perhaps other leukemia (213).
After extravasation, transplanted HSC should reach the osteogenic niche. As mentioned, HSC synthesize HA (214), and HA expression supports HSC migration toward the endosteal niche (189). In the endosteal niche, SDF1 promotes adhesion through CD44-associated CXCR4 accompanied by rac1 and cdc42 activation (182, 192, 215). These findings confirm the key role of HA and CD44 in SDF1-dependent HSC anchorage within specific niches (189). Finally, space is created by activated ROK, which phosphorylates the Na-H-exchanger1. Hyaluronidase-2 and cathepsinB become activated in the acidic milieu and support ECM degradation (206, 207).

Unfortunately, AML share the homing mechanisms with HSC (216, 217). However, the problem can possibly be circumvented in leukemia highly expressing CD44v6, as CD44v6 expression is low in HSC and HSC homing is dominated by CD44s. Analyzing migration of HSC from CD44v6/v7ko and CD44v7ko mice toward HA and BM-Str revealed impaired migration toward FN, possibly due to CD44v6 directly binding to FN (218) or being promoted by the CD44v6-αβ1 association (174). Furthermore, HSC migration toward IL6 is strikingly impaired by anti-CD44v6 (219). Migration of CD44v7ko and CD44v6/v7ko HSC toward SDF1 was reduced to background levels, which indicates major importance of these two splice variants in migration along a SDF1 gradient (220). Binding and migration toward OPN is also impaired in CD44v6/v7ko HSC. The finding fits the selective CD44v6 binding of OPN, which triggers migration and invasion (221, 222). Finally, BM-Str CD44v7 supports HSC migration (97). Thus, CD44v6/v7 are engaged in HSC migration toward chemokines/cytokines and BM-Str. Another protein selectively trapped by CD44v6 is C3 (81, 130). As elegantly elaborated by the group of Ratajczak [review in Ref. (223)], C3 can drive CXCR4 into lipid rafts, where it associates with CD44v6. Thereby, the CXCR4–SDF1 axis becomes strengthened, which helps retaining HSC in the niche. In a similar attempt elaborating homing of multiple myeloma to the BM, the authors explored differences in a stroma-dependent and a stroma-independent line. Only the stroma-dependent line expressed IGF-1R and CD44v6, where IGF-1 promoted chemotaxis toward BM-Str and CD44v6 supported adhesion. By modulating the culture conditions, the authors demonstrated that BM-Str promotes up-regulation of IGF-1R and CD44v6 in multiple myeloma, which facilitate homing and support adhesion to BM-Str (224).

Thus, the particular BM endothelium supports the egress of transplanted HSC into the BM. The engagement of CD44 relies on the provision of HA and the binding of CD44 to s-selectin, binding being supported by the association with integrins. Once attached to the endothelium, CD44 promotes activation of rac and rho, which initiate the shift toward a migratory phenotype. Migration is strengthened by activation of CD44-associated proteases. The proteases create space and cleave adhesion molecules including CD44, which fosters migration toward the endosteal niche. Settlement in the niche follows the path that underlies the preferential retention of HSC in the osteogenic niche. In most instances, LIC and HSC use the same adhesion molecules and signaling pathways for migration. Nevertheless and notably, there are some discrete differences between HSC and LIC, which may help elaborating protocols for preferential homing of transplanted HSC.

**CD44 and the Crosstalk Between HSC and the Niche**

CD44 does not only contribute to niche assembly but, importantly, there is a feedback from the niche toward HSC and LIC, which also involves CD44. Two aspects of this crosstalk will be in focus, the engagement of CD44 in (i) HSC quiescence and (ii) stress resistance, which both are linked to the osteogenic niche.

Different to the engagement of CD44 in HSC and LIC homing and migration, mostly HSC rely on CD44-promoted quiescence. Instead, both HSC and LIC profit from CD44 in apoptosis resistance. Though the CD44-mediated crosstalk with the niche and the GEM location of CD44 are important for HSC and LIC apoptosis resistance (225, 226), the dominating mechanisms differ.

In advance of discussing the impact of the niche on CD44-promoted quiescence and apoptosis resistance, exosomes need to be mentioned. Unfortunately, their impact on HSC and LIC has not yet been explored in detail. Many cells including HSC and LIC secrete small vesicles, called exosomes, which are supposed to be most efficient intercellular communicators (227–229), where miRNA transfer via exosomes can lead to target cell reprogramming (230). This was demonstrated for ESC exosomes reprogramming hematopoietic progenitors through miRNA delivery (231), and for the transfer of miRNA between different cells of the hematopoietic system as well as from CIC into BM-Str (232). Although the impact of CD44 on the exchange of exosomal miRNA between HSC/LIC and niche cells has not been elaborated, the impact of exosomal CD44v6 on miRNA transfer points toward the engagement of CD44 (78) and unquestionable demonstrated the strong impact of exosomal miRNA. Thus, a more comprehensive knowledge on the transfer of HSC exosomal miRNA should be approached, and can be expected to open new therapeutic options.

**CD44 and HSC Quiescence**

The quiescent state is critical for preserving self-renewal capacity and stress resistance of HSC. Besides intrinsic regulatory mechanisms, where p53 plays a dominant role, there are extrinsic microenvironmental regulatory mechanisms, which include angiopoetin-1, TGFβ, bone morphogenetic proteins (BMP), TPO, N-cadherin, integrins, Wnt/β-catenin, and OPN (233).

Angiopoietin is secreted by osteoblasts and binds to Tie2 on HSC, which supports maintenance of quiescence and prevents cell division. Furthermore, the Tie2-angiopoietin interaction promotes cobblestone formation in long-term BM cultures (234). Besides strengthening adhesion of HSC to BM-Str (234), possibly via CD44, I am not aware of a particular linkage between the angiopoietin-Tie2 axis and CD44 signaling.

TGFβ are potent inhibitors of HSC proliferation. TGFβ disruption increases circulating progenitor cells, and a bolus injection of TGFβ1 inhibited early progenitor proliferation. TGFβ-mediated quiescence of HSC may be due to alteration in cytokine receptor expression and upregulation of cyclin-dependent kinase inhibitors (235). The engagement of CD44 relies on its interaction with the TGFβR1 (236). TGFβ cooperates with HA-activated CD44 to induce expression of the NADPH oxidase (237), which could help regulating redox signals in HSC.
Bone morphogenetic proteins, secreted by osteoblasts (238), potently inhibit HSC proliferation. BMP bind to their serine threonine kinase receptors on HSC, which leads to transphosphorylation and kinase domain activation, initiating phosphorylation of Smad 1, 5, and 8 that concomitantly associate with Smad4 and translocate to the nucleus. In the nucleus, they act as cotranscription factor regulating expression of target genes such as Runx1 and GATA2, which operate during specification of hematopoiesis (239) and regulate HSC quiescence (110, 240, 241). The linkage to CD44 is based on the association of CD44 with Smad1 (242). Alternatively, and BMP-independent, Smad1 can become phosphorylated via galectin-9, where galectin-9 binding to CD44 promotes formation of a CD44/BMP receptor complex with concomitant BMP receptor activation (243).

Binding of TPO to its ligand (MPL) is critically involved in HSC steady-state maintenance with an over 150-fold reduction of HSC in TPOko mice. Posttransplantation HSC expansion was highly MPL-and TPO-dependent. Accelerated HSC cell-cycle kinetic in TPOko mice is accompanied by reduced cyclin-dependent kinase inhibitor p57kip2 and p19Ink4d expression (244). The activity of TPO becomes strengthened by glucosaminoglycans in the matrix. Though this was demonstrated for megakaryocytopoiesis (245), it may have bearing on TPO affecting HSC embedded in the osteogenic niche.

Wnt signaling has emerged as an important factor in HSC quiescence, self-renewal, and differentiation (246). Wnt, secreted glycoproteins, binds to their sevenpass transmembrane receptors (Frizzled) (247) and low-density lipoprotein receptors LRPS5and LPR6 (248), which become phosphorylated and form a activated Frizzled/LPR receptor complex. The Frizzled/LPR receptor complex promotes dephosphorylation of β-catenin, which in the absence of Wnt signaling is phosphorylated and associated with a so-called destruction complex (249). Dephosphorylated β-catenin translocates to the nucleus, and together with LEF/TCF initiates transcription of Wnt target genes. In the non-canonical pathway, mostly Wnt5a signals via Frizzled using as coreceptor ROR, which leads to Rhôa/Rac and JNK activation. In the Wnt–Ca2++ pathway, G-protein signaling is activated with upregulation of IP3-mediated release of intracellular Ca2++, and activation of PKC, which triggers nuclear translocation of NFAT and NFkB (246). Though Wnt is a potent morphogen (250), Wnt effects are highly context and dose-dependent (251), which makes it difficult to define precisely its role in HSC maintenance. To circumvent these difficulties, the group of Scadden used an osteoblast-specific promoter for expression of the Wnt paninhibitor Dickkopf1 (Dkk1). Binding to the coreceptor LRPS5/6 leads to internalization of the complex (252). Inhibition of Wnt signaling in HSC resulted in reduced p21Cip1 expression, increased cell cycling, and a continuing decline in the reconstitution capacity of HCS. Notably, though the effect on HSC was microenvironment-dependent, HSC did not recover, when transferred in a normal host (253). Furthermore, Wnt-inhibition affected activation of the Notch target, Hes-1. This finding suggests that Notch and Wnt coordinate regulation HSC quiescence. Indeed, elevated Hes-1 and p21 expression correlate with the maintenance of HSC quiescence (254). The importance of Notch signaling was confirmed by inhibition of Notch signaling diminishing the capacity of HSC to maintain an undifferentiated state. As proliferation and survival were not affected, the authors suggest that Notch “may act as a “gatekeeper” between self-renewal and commitment” (255).

Wnt signaling, in fact, is not only important for HSC [review in Ref. (62)], but the association of CD44 with Wnt signaling is also amply demonstrated for LIC [review in Ref. (61)]. However, as mentioned, Wnt effects are context dependent. Highlighting the differences to HSC, one example will be given. The cytoplasmic domain of GEM-located CD44 associates with the Wnt receptor LRPS6, whereby LRPS6 becomes recruited into the plasma membrane, which strengthens Wnt signaling and the accumulation of β-catenin in the nucleus, where down- and upregulation of CD44 directly affected Wnt signaling. Importantly, this activity of CD44 does not require CD44–HA crosslinking (256).

Osteopontin also negatively regulates the number of HSC in the BM niche. OPNko mice display a significantly increased number of HSC, but not of committed progenitors (257). OPN also can modify primitive hematopoietic cell number and function in a stem cell–non-autonomous manner. This conclusion derived from the observation that the BM microenvironment of OPNko mice was sufficient to increase the number of HSC, which was accompanied by an increase in stromal lagged1 and angioipoietin-1 expression and a reduction of primitive hematopoietic cell apoptosis (116). The authors discuss that the ECM plays a dynamic role in governing HSC responsiveness to expansion signals. Whether the signals are transferred via CD44-associated integrins (258) or directly via OPN binding to CD44 (133) remain to be explored. Instead, OPN binding to CD44v6 in CIC promotes activation of the PI3K/Akt pathway and promotes tumor growth (259). This is mentioned to remember that in concern of signal transduction, the peculiarities of HSC frequently do not allow a direct comparison to oncogene transformed CIC or LIC.

Finally, the interaction of SDF1, expressed by developing stroma in fetal bones, with HSC CXCR4 is critically for retaining HSC in the quiescence-protecting niche (260). Originally, it was noted that SDF1ko and CXCR4ko embryos have greater impairment of myelopoiesis in the BM than in the fetal liver, which suggested that SDF1 and CXCR4 are primarily involved in colonization of the BM by HSC during embryogenesis (261). Later on, elegant work with conditional CXCR4ko mice implicate stromal SDF1 and its receptor in maintaining the pool of quiescent HSC. Conditional CXCR4ko mice have a significantly increased pool of HSC in G1 compared to wt mice. This may be due to an altered environment with upregulation of cytokines, which promote HSC cycling and differentiation (115). Interestingly, actively signaling CXCR4 is associated with GEM localization (262). This implies that CXCR4 signaling sensitivity can be modulated by colocalization with other signaling molecules, including Rac1 (263). Notably, HA-crosslinked, GEM-located CD44 directly interacts with CXCR4, such that SDF1–CXCR4 signaling is abrogated in CD44-ko cells (264). Less is known about the impact of the niche on the resting versus cycling state of leukemia. However, it can be expected that due to oncogene transformation LIC are less susceptible to quiescence promoting signals from the niche or may even distinctly respond.

Figure 3 summarizes those signaling pathways in HSC, where CD44 is actively involved in maintaining HSC quiescence and...
HSC stress protection. Thus, CD44 becomes stimulated by several key molecules engaged in HSC quiescence. Alternatively, CD44, particularly when crosslinked by HA, associates with quiescence regulating molecules.

Last, not least, the importance of the CD44-HA interaction was also demonstrated with HAS3ko mice. HSC homing into the osteogenic niche depends on the HA coat of endothelial cells, and is significantly retarded in HAS3ko mice (265). Furthermore, HA is required for the generation of HSC during differentiation of ESC (266). Finally, HSC seeded on HA rarely proliferate and retain multipotency (267, 268). All these findings strengthen the upmost importance of the CD44-HA crosstalk in HSC maintenance.

**CD44 and HSC Stress Resistance**

The distinction between HSC and LIC also accounts for a second phenomenon, the response to low oxygen pressure according to the location of HSC in niches (110), characterized by low oxygen concentration (269). Though LIC compete with HSC for the niche, they are not dependent on low oxygen and instead remodel the niche toward accumulation of inflammatory myeloid-birotic cells, which drive LIC expansion, but compromise HSC maintenance (270). Notably, too, the metabolic status of HSC residing in a hypoxic BM environment also differs from that of their differentiated progeny (271).

Hematopoietic SC maintains redox homeostasis by low oxygen production due to the minimal metabolic rate (271, 272). Low metabolic rate maintenance further relies on asymmetric cell division, where the daughter cell, which remains in the SC state, inherits a very low level of energized mitochondria (273, 274). Furthermore, HSC generate energy mainly via anaerobic metabolism maintaining a high rate of glycolysis, which limits the production of reactive oxygen species. The hypoxia responsive regulatory pathways in HSC resembles that in other cells, HIF1α being the master regulator driving the metabolic machinery toward anaerobic glycolysis (275). HIF1α, stabilized under hypoxic conditions (276), reprograms glucose metabolism via transcriptional activation of genes encoding glucose transporters, glycolytic enzymes, and metabolic regulatory enzymes, thereby switching from oxidative to glycolytic metabolism (277). One
of the mechanisms proceeds via the HSP GRP78 and its ligand Cripto, HIF1α binding to the Cripto promoter (278). The importance of HIF1α orchestrating molecular responses, which maintain redox homeostasis in the face of changing O2 levels, was demonstrated by the loss of reconstitution capacity of HSC in HIF1αko mice (279). HSC dispose on additional regulatory molecules, including polycomb, DNA damage-related, and antioxidant proteins that participate in ROS regulation (280). Notably, maintenance of the hypoxic state of HSC is not restricted to the location in a poorly vascularized niche, but is dictated by cell-specific mechanisms derived from their glycolytic metabolic profile (281). Finally, the cotranscription factor CD44–ICD promotes expression of HIF2α (282), as well as of additional hypoxia-related genes, like aldolase c, 6-phosphofructose-2-kinase, pyruvate dehydrogenase kinase-1, and pyruvate dehydrogenase, which are directly associated with aerobic glycolysis (283–285). The authors point out that the repeatedly observed impact of the CD44-ICD on HIF expression in SC suggests a more active role of CD44-ICD in SC maintenance and protection as previously anticipated (282).

In concern about the engagement of CD44 and its ligands in protection from oxidative stress, it also was described that neural SC reside undifferentiated in a HA rich matrix, but proliferate and differentiate upon hyaluronidase upregulation (286). Additional contributions of CD44 and HA on stress protection may be shared by HSC and LIC and are described below.

Taken together, HSC circumvent stress, which would drive them into proliferation and exhaustion, mostly by a minimal metabolic rate and the generation of energy via anaerobic metabolism. The main contribution of CD44 relies on the cotranscription factor activity of CD44-ICD.

**CD44, LIC, and Apoptosis Resistance**

Besides contributing to circumvent stress, CD44 also actively promotes apoptosis resistance. From this activity of CD44, which does not appear to be of major importance for HSC stress resistance, LIC make profit. As to my knowledge, CD44-mediated apoptosis resistance proceeds in LIC and CIC via overlapping pathways, some examples of CIC will be included, where corresponding experiments have not yet been performed with LIC. There are two major mechanisms of CD44-mediated apoptosis protection: (i) initiation of signal transduction by CD44 croslinking via HA, which frequently involves CD44v and associated RTK (287), and (ii) the crosstalk of CD44 with multidrug resistance genes that also is HA-dependent (288).

Apoptosis resistance initiated by the cooperation of CD44- or CD44v with RTK mostly proceeds via activation of anti-apoptotic proteins. CD44 coimmunoprecipitates with all ERBB family members. The association of CD44 with ERBB2 and ERBB3 mediates heterodimerization and activation of the receptor in response to neuregulin, which strongly promotes CIC apoptosis resistance (289, 290). The impact of CD44 on ERBB2 activation is strikingly HA-dependent. CD44 croslinking via HA initiates association of CD44 with ERBB2, which becomes phosphorylated. The complex, located in lipid rafts, includes ezrin, the chaperones HSP90 and CDC37, and PI3K, which accounts for drug resistance via activation of anti-apoptotic proteins. Apoptosis resistance is not seen when the HA–CD44 interaction is blocked, which causes complex disassembly and inactivation of ERBB2 (291). The authors also unraveled activation of an ERBB2–PI3K/Akt–β-catenin axis, which contributes to COX2 expression and COX2-promoted suppression of caspase3 activation. The data argue for a feedback loop, whereby COX2 strengthens HA production and promotes prostaglandin E2 expression (292). An additional feedback loop proceeds via formation of the ERBB2/ERBB4–CD44 complex, which via ERK activation promotes HA production by HAS1, -2, and -3 phosphorylation/activation (293) (Figure 4A).

Another, well-known pathway of apoptosis resistance involves the CD44v association with MET, which is initiated by HGF binding to CD44v3 or CD44v6 (138, 294), and promotes MET phosphorylation. MET phosphorylation requires the cytoplasmic tail of CD44 and the interaction with ERM proteins for activation of the Ras-MAPK pathway (294). CD44v6 binding to the ECM also activates the PI3K–Akt pathway and Wnt/β-catenin signaling (130, 295) and regulates MET transcription (86, 296). Similar observations account for insulin-like growth factor-1 receptor and PDGFR activation through HA-stimulated CD44 in transformed cells (133, 297, 298) (Figure 4A).

Importantly, CD44v6 promoted apoptosis resistance can also rely on the association of GEM-located CD44v6 with FAS. This association prevents trimerization of FAS upon ligand binding. Notably, apoptosis susceptibility strongly increases by a blockade of CD44v6 (299). Promoting FAS trimerization by a CD44v6 antibody blockade presents a new and interesting option in the therapy of CD44v6 expressing leukemia.

The interaction between CD44 and RTK can also proceed via proteases. CD44v3 binds the proform of the heparin binding epidermal growth factor (HB-EGF), which is cleaved by CD44-recruited MMP7. Cleaved HB-EGF binds and activates ERBB4, which signals for cell survival (300, 301). The interaction of CD44 with MMP9 leads to apoptosis protection independent of RTK. CD44 and MMP9 expression are interdependent (149), and in CLL patients with poor prognosis CD44, CD49, and MMP9 are physically associated (302, 303). CD44-associated MMP14 accounts for proMMP9 cleavage (191). Activated MMP9 can interfere with TGFβ activation, whereby several mechanisms of TGFβ-promoted apoptosis become silenced (304, 305).

An additional pathway of CD44-promoted resistance to reactive oxygen- and cytotoxic drug-induced stress under physiological and pathological conditions proceeds via the mammalian Hippo signaling pathway. In the resting state, CD44-associated merlin accounts for JNK, p53, and p21 upregulation, and YAP as well as cIAP1/2 downregulation, which jointly promote caspase3 activation and apoptosis. When CD44 becomes activated by HA binding, merlin is phosphorylated and dissociates from CD44. In the absence of merlin, CD44 directly regulates YAP expression via active RhoA. Thereby, the HIPPO pathway becomes blocked, which results in increased apoptosis resistance (306, 307). In a feedback loop, activated YAP binds to the promoter of RHAMM, thereby inducing RHAMM transcription (308, 309) (Figure 4B).

Besides via CD44-associating molecules, the direct interaction between CD44 and HA strongly affects apoptosis resistance. Exploring the effect of HA on the extent of DNA damage induced
by exogenous and endogenous oxidants revealed that CD44 in SC internalizes HA by endocytosis. One of the functions of the internalized HA is the protection of DNA from oxidants. The authors propose entrapment of iron ions. Thereby the Fenton’s reaction, which produces secondary oxidative species becomes inhibited. Particularly in HSC residing in the osteogenic niche, it has been observed that CD44-attached highMW HA can become internalized together with CD44, where HA acts as a catcher of ROI, which protects DNA from damage. (D) MDR proteins are associated with activated CD44 (highMW HA-igated and associated with pERM). Instead, lowMW HA promotes CD44 and concomitantly MDR internalization, which is accompanied by a significant increase in cytotoxic drug sensitivity. Maintenance of the complex between CD44, pERM, actin, and MDR-1, as well as the recovery of the S100A/AnnexinII complex indicates internalization within caveolae and/or GEM. As far as LIC compete with HSC for the osteogenic niche, they will profit from the highMW HA matrix that via CD44 stabilizes MDR proteins within the cell membrane contributing to drug resistance.

Drug resistance can also be promoted through CD44v3–HA binding, which via the Oct4-Sox2-Nanog complex induces miR-302 transcription (74). The CD44–HA induced nuclear translocation of Nanog also leads to miR-21 production and upregulation of apoptosis inhibitors and MDR1 (77, 313).

Last, the interplay between CD44 and HA accounts for rapid drug elimination via drug transporters (288), which creates a major obstacle in leukemia and cancer therapy (314). Both CD44 and HA contribute to drug resistance. MDR genes are associated with CD44 and CD44 regulates expression of drug transporters. This likely is due to HA-activated CD44 binding to Gab1, which...
promotes PI3K activation. Activated PI3K stimulates HA production as well as MDR transporter expression (315, 316). Alternatively, though not mutually exclusive, HA binding to CD44 up-regulates p300 expression and its acetyltransferase activity. This, in turn, promotes acetylating β-catenin and NFκB-p65. Activated β-catenin and NFAT act as cotranscription factors with NFκB in MDR1 transcription (317). The direct involvement of HA was demonstrated by replacing high MW by low MW HA. In the presence of high MW, HA activated CD44 is predominantly recovered in GEM and is associated with ERM and actin. MDR1 is associated with CD44 and the association stabilizes MDR1 expression. Instead, low MW HA does not stabilize the complex, but rather supports internalization such that all components of the complex including S100A and Annexin II are recovered in the cytoplasm (318). Whether the internalized complex becomes degraded or released as exosomes has not been explored. Independent of the answer to this question, the reduction of MDR1 in the cell membrane is accompanied by increased drug susceptibility (319). Notably, the three hyaluronan synthases are supposed to produce HA of different size. However, this as well as the major transcription factors engaged in HAS1, -2, and -3 transcription remain to be defined. Transcriptional regulation of hyaluronidases also awaits unraveling (320, 321). Taking into account that hyaluronidase as well as small HA oligosaccharides can improve drug efficacy (318), and that HA-CD44 cross-linking regulates expression of drug transporters (315, 316), filling this gap becomes demanding to improve therapeutic targeting of HA [review in Ref. (322, 323)] (Figure 4D).

The major importance of CD44 in apoptosis resistance relies (i) on the association of activated (HA-crosslinked) CD44/CD44v with RTK, which promote activation of anti-apoptotic signaling cascades, (ii) on interferences of CD44v6 with FAS trimerization, and (iii) the engagement of CD44 in regulating drug-resistance gene expression. The impact of CD44 is efficiently reinforced by high MW, but not low MW HA, where the latter may open a therapeutic window.

Open Questions

This review highlights the importance of CD44 in the crosstalk between HSC/LIC and the surrounding matrix. However, due to space constraints, this review does not cover the role of CD44 in all aspects of hematopoiesis and leukemia induction; this can be found within an excellent review which also focuses on signal transduction and transcription (324). Furthermore, while CD44-based therapeutic concepts have been highlighted in individual sections within this review, there is some excellent literature which describes CD44 antibody and vaccination-based therapeutic concepts that may be of further interest to the reader (325–332). However, there are a number of key issues/questions that need to be addressed before a major therapeutic breakthrough can be achieved:

1. More information on the active contribution of the niche is required as well as the contribution of the individual components. This includes the possible transfer of information, comprising miRNA, via exosomes and accounts for HSC and LIC.
2. There is insufficient information on the modulation of the osteogenic niche by LIC, to safely protect HSC, if LIC was to be targeted, and to reconstruct a destroyed niche for unimpaired hematopoiesis.
3. The generation of LIC is not well understood. Again, the possibility has to be taken into account that LIC are instructed by surrounding cells, preferably HSC or MSC, via exosomes. Furthermore, there is a paucity of knowledge on the decision for self-renewal versus differentiation, which to some degree also accounts for HSC.
4. Much progress has been made in homing, migration, and signal transduction, which could well become the first question to be comprehensively answered.

In summary, the specific therapeutic targeting of LSC is still very much a field in its infancy (323). However, there is justified hope that this may change in the near future.

Conclusion

Stem cells require a niche, which has been particularly well explored for HSC, where the central importance of the CD44–HA interaction, including more recently the cellular stroma elements, is amply demonstrated. The CD44–HA crosstalk promotes adhesion and via cytokines/chemokines harbored in the BM-Str, homing, and migration of HSC as well as HSC quiescence and resistance to low oxygen pressure. LIC share with HSC the requirement for the crosstalk with the stroma to promote adhesion, homing, and migration. Apoptosis resistance of LIC, though strikingly dependent on the CD44–HA crosstalk, proceeds differently to that of HSC predominantly via CD44v and the cooperation with RTK, proteases, and drug transporters.

The abundant array of HA-bound CD44-initiated activities relies on the cooperation of CD44 with multiple membrane molecules including integrins, chemokine receptors, RTK, and proteases as well as its transient association with cytoskeletal linker molecules and cytoplasmic signal transducers, which includes central stem cell fate regulators. The multitude of interactions is fostered by the GEM location of CD44, which also promotes the proximity to proteases. Proteases facilitate CD44 cleavage, where the CD44-ICD acts as a cotranscription factor. It has been suggested, but needs further approval, that CD44-ICD also regulates miR transcription/repression of CD44 cooperation partners, thereby creating an additional feedback loop.

In view of the most promising results in leukemia therapy by blocking CD44, awareness increased on possible selective differences between HSC and LIC in the crosstalk with the osteogenic niche. Several elegant and sophisticated studies clearly demonstrated the existence of differences not only between HSC and LIC but also between distinct leukemia. Progress in this field will greatly facilitate selective therapeutic interference with LIC homing and may allow for corrections of the LIC-distorted osteogenic niche. The latter as well as the interaction with the vascular niche and homing into the osteogenic niche is of particular interest in view of the HSC transfer being frequently a last chance for curative therapy. Further clarifying HA production and degradation may also open new avenues for a therapeutic dissection between the HSC- and the LIC–HA crosstalk. Last, not least, uncovering the
importance of miRNA and the role of exosomes in miRNA transfer will add optimizing the HSC–HA crossstalk in the osteogenic niche and will allow interfering with niche destructing activities of LIC.

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References

1. Gallatin WM, Weissman IL, Butcher EC. A cell-surface molecule involved in organ-specific homing of lymphocytes. Nature (1983) 304:30–4. doi:10.1038/30430a0
2. Günther U, Hofmann M, Rudy W, Reber S, Zöller M, Hausmann I, et al. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell (1991) 65:13–24. doi:10.1016/0092-8674(91)90043-I
3. Naor D, Wallach-Dayan SB, Zalaka MA, Sionov RV. Involvement of CD44, a molecule with a thousand faces, in cancer dissemination. Semin Cancer Biol (2008) 18:260–7. doi:10.1016/j.semcancer.2008.03.015
4. Herrlich P, Zöller M, Pals ST, Ponta H. CD44 splice variants: metastases meet lymphocytes. Immunol Today (1993) 14:395–9. doi:10.1016/0167-6999(93)90141-7
5. Ratajczak MZ. Cancer stem cells – normal stem cells “Jedi” that went over to the “dark side”. Folia Historia Cytoptob (2005) 43:175–81.
6. Zöller M. CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? Nat Rev Cancer (2011) 11:254–67. doi:10.1038/nrc3203
7. Sales KM, Winslet MC, Seifalian AM. Stem cells and cancer: an overview. Stem Cell Rev (2007) 3:249–55. doi:10.1007/s12015-007-9002-0
8. Pei D. Regulation of pluripotency and reprogramming by transcription factors. J Biol Chem (2009) 284:3365–9. doi:10.1074/jbc.R800063200
9. Făbăin A, Barok M, Verbe G, Szollősi J. Die hard: are cancer stem cells the Bruce Willies of tumor biology? Cytometry A (2009) 75:67–74. doi:10.1002/cyt.a.20690
10. Adams JM, Strasser A. Is tumor growth sustained by rare cancer stem cells or dominant clones? Cancer Res (2008) 68:4018–21. doi:10.1158/0008-5472. CAN-07-6334
11. Conway AE, Lindgren A, Galic Z, Pyle AD, Wu H, Zack JA, et al. A pluripotency and self-renewal program controls the expansion of genetically unstable cancer stem cells in pluripotent stem cell-derived tumors. Stem Cells (2009) 27:18–28. doi:10.1634/stemcells.2008-0529
12. Wang JC. Good cells gone bad: the cellular origins of cancer. Trends Mol Med (2010) 16:45–51. doi:10.1016/j.molmed.2009.11.001
13. Alison MR, Guppy NJ, Lim SM, Nicholson LJ. Finding cancer stem cells: the “dark side”. J Pathol (2010) 222:335–44. doi:10.1002/path.2772
14. Sceatton GR, Bell MV, Bell JI, Jackson DG. Identification of mRNA that encodes an alternative proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. J Biol Chem (2001) 276:755–62. doi:10.1038/jbc.200108159
15. Calina Z, Zayakin P, Silina K, Line A. Alterations of pre-mRNA splicing in cancer. Genes Chromosomes Cancer (2005) 42:342–57. doi:10.1002/gcc.20156
16. Liu D, Sy MS. Phorbol myristate acetate stimulates the dimerization of CD44 involving a cysteine in the transmembrane domain. J Immunol (1997) 159:2702–11.
17. Lokeshwar VB, Fregien N, Bourquinon LY. Akinyrin-binding domain of CD44(Gp85) is required for the expression of hyaluronic acid-mediated adhesion function. J Biol Chem (1994) 269:1099–109. doi:10.1038/jcb.126.4.1099
18. Felton RG, McClatchey AI, Bretscher A. Organizing the cell cortex: the role of ERM proteins. Cell (2004) 117:263–7. doi:10.1016/j.cell.2004.05.019
19. Morri T, Kitao K, Terawaki S, Maesaki R, Fukami Y, Hakoshima T. Structural basis for CD44 recognition by ERM proteins. J Biol Chem (2008) 283:29602–12. doi:10.1038/jbc.M803660200
20. Stanenkov I, Yu Q, Merlin, a “magic” linker between extracellular cues and intracellular signaling pathways that regulate cell motility, proliferation, and survival. Curr Protein Pept Sci (2010) 11:471–84. doi:10.2174/138920310791824011
21. Oliferenko S, Paiha K, Harder T, Gerke V, Schwärlch C, Schwarz H, et al. Analysis of CD44-containing lipid rafts: recruitment of annexin II and stabilization by the actin cytoskeleton. J Cell Biol (1999) 146:843–54. doi:10.1083/jcb.146.4.843
22. Neame SJ, Isacce CM. The cytoplasmic tail of CD44 is required for basolateral localization in epithelial MDCK cells but does not mediate association with the detergent-insoluble cytoskeleton of fibroblasts. J Cell Biol (1993) 121:1299–309. doi:10.1083/jcb.121.6.1299
23. Zabock A, Ley K, McEver RP, Hidalgo A. Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow. Blood (2011) 118:6743–51. doi:10.1182/blood-2011-07-343566
24. Röy T, Vattulaínen I. Cholesterol, sphingolipids, and glycolipids: what do we know about their role in raft-like membranes? Chem Phys Lipids (2014) 184:82–104. doi:10.1016/j.chemphysil.2014.10.004
25. Föger N, Marhaba R, Zöller M. Involvement of CD44 in cytoskeleton rearrangement and raft reorganization in T cells. J Cell Sci (2001) 114:1169–78.
26. Slote JP. Biological functions of sphingomyelins. Prog Lipid Res (2013) 52:424–37. doi:10.1016/j.plipres.2013.05.001
27. Rutz P, Schwärlch C, Günther U. CD44 isoforms during differentiation and development. Bioessays (1995) 17:17–24. doi:10.1002/bies.950170106
42. Zöller M. CD44: physiological expression of distinct isoforms as evidence for organ-specific metastasis formation. J Mol Med (1995) 73:425–38.
43. Bennett KL, Jackson DG, Simon JC, Tanczos E, Peach R, Modrell B, et al. CD44 isoforms containing exon v3 are responsible for the presentation of heparin-binding growth factor. J Cell Biol (1995) 128:687–98. doi:10.1083/jcb.128.4.687
44. Orian-Rousseau V, Ponta H. Adhesion proteins meet receptors: a common theme? Adv Cancer Res (2008) 101:65–92. doi:10.1016/S0065-230X(08)00431-1
45. Tremmel M, Matzke A, Albrecht I, Laib AM, Olaku V, Ballmer-Hofer K, et al. A CD44v6 peptide reveals a role of CD44 in VEGF-R2 signaling and angiogenesis. Blood (2009) 114:5236–44. doi:10.1182/blood-2009-04-219204
46. Todaro M, Gaggianesi M, Catalanvo V, Benfante A, Iovino F, Biffoni M, et al. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. Cell Stem Cell (2014) 14:342–56. doi:10.1016/j.stem.2014.01.009
47. Megapathè AP, Erb U, Büchler MW, Zöller M. CD44v10, osteospin in lymphoma growth retardation by a CD44v10 specific antibody. Immunol Cell Biol (2014) 92:709–20. doi:10.1038/icb.2014.47
48. Williams K, Motiani K, Girdhar PV, Kasper S. CD44 integrates signaling in normal stem cell, cancer stem cell and (pre)metastatic niches. Exp Biol Med (2013) 238:324–38. doi:10.1177/1535370213480714
49. Zou GM. Cancer stem cells in leukemia, recent advances. J Cell Physiol (2007) 213:440–4. doi:10.1002/jcp.21140
50. Nagano O, Okazaki S, Saya H. Redox regulation in stem-like cancer cells by CD44 variant isoforms. Oncogene (2013) 32:5191–8. doi:10.1038/onc.2012.658
51. Tarnok A, Ulrich H, Bocci J. Phenotypes of stem cells from diverse origin. Cytotherapy (2010) 12:76–10. doi:10.1016/j.cytot.200844
52. Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S, Miyazono K. Modulation of microRNA processing by p53. Nature (2009) 460:529–33. doi:10.1038/nature08119
53. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature (2010) 464:1071–6. doi:10.1038/nature08975
54. Messaoudi-Aubert SE, Nicholls J, Maertens GN, Brookes S, Bernstein E, Peters K, et al. A CD44v6 peptide reveals a role of CD44 in VEGF-R2 signaling and angiogenesis. Blood (2009) 114:5236–44. doi:10.1182/blood-2009-04-219204
55. Courrier P, Fatemi B, Kazi Y, Zöller M. CD44 in cancer stem cells. Curr Stem Cell Res Ther (2009) 4:203–16. doi:10.2174/157488908784911750
56. Ali S, Almhanna K, Chen W, Philip PA, Sarkar FH. Differentially expressed miRNAs in the plasma may provide a molecular signature for aggressive pancreatic cancer. Am J Transl Res (2010) 2:8–27.
57. Liu L, Jiang Y, Zhang H, Greenlee AR, Han Z. Overexpressed miR-194 down-regulates PTEG1 gene expression in cells transformed by
Monoclonal antibodies to Pgp-1/CD44 block lympho-hemopoiesis in long-term human acute myeloid leukemic stem cells.

Experimental colitis correlates with increased apoptosis in mice deficient for CD44 variant isoforms control experimental autoimmune encephalomyelitis - initiating cells.

Targeting the open reading frame (ORF) region of FAF1 in cancer cells.

Maintenance of the hematopoietic stem cell pool by affecting the lifespan of the pathogenic T cells.

Hyaluronan anchored to activated CD44 on central nervous system surface of tissue-specific endothelial cell lines.

Poncin is a hematopoietic stem cell niche component that negatively regulates hematopoietic stem cell pool size.

Hematopoietic stem cell niche component that negatively regulates hematopoietic stem cell pool size.

The vascular niche: home for normal and malignant hematopoietic stem cells.

The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood (1978) 47: 4–7.

Identification of the haemopoietic stem cell niche and control of the niche size. Nature (2003) 425:836–41. doi:10.1038/nature02041.

 Stromal stem cells: marrow-derived osteogenic precursors. Ciba Found Symp (1988) 136:12–60.

Thrombopoietin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. J Exp Med (2005) 201:1781–91. doi:10.1084/jem.20041992.

What is the true nature of the osteoblastic hematopoietic stem cell niche? Trends Endocrinol Metab (2009) 20:303–9. doi:10.1016/j.ten.2009.03.004.

Steroid receptor association with cancer and “acid mucopolysaccharidases”: an old concept comes of age, finally. Semin Cancer Biol (2008) 18:238–43. doi:10.1016/j.semcancer.2008.03.014.

Replicating metastatic tumour cells with embryonic microenvironment.

Regulating hematopoietic progenitor distribution, granuloma formation, and osteoblastic hematopoietic stem cell niche.

Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. Blood (2014) 125(2):249–60. doi:10.1182/blood-2014-04-752255.

Hematopoiesis, leukemia and CD44.
127. Girish KS, Kemaparaju K. The magic glue hyaluronan and its erase hyaluronidase: a biological overview. Life Sci (2007) 80:1921–43. doi:10.1016/j.lfs.2007.02.037

128. Kuhn NZ, Tuan RS. Regulation of stemness and stem cell niche of mesenchymal stem cells: implications in tumorigenesis and metastasis. J Cell Physiol (2010) 222:268–77. doi:10.1002/jcp.21940

129. Adami S, Maxwell CA, Pilarski LM. Hyaluronan and hyaluronan synthase: potential therapeutic targets in cancer. Curr Drug Targets Cardiovasc Haematol Disord (2005) 5:3–14. doi:10.2174/15680060535005563

130. Jung T, Gross W, Zoller M. CD44v6 coordinates tumor matrix-triggered motility and apoptosis resistance. J Biol Chem (2011) 286:15862–74. doi:10.1074/jbc.M110.208421

131. Couchman JR. Transmembrane signaling proteoglycans. Annu Rev Cell Dev Biol (2010) 26:89–114. doi:10.1146/annurev-cellbio-100109-104126

132. Weber GF. Molecular mechanisms of metastasis. Cancer Lett (2008) 270:181–90. doi:10.1016/j.canlet.2008.04.030

133. Katagiri YU, Sleeman J, Fuji H, Herrlich P, Hotta H, Tanaka K, et al. CD44 variants but not CD44s cooperate with beta1-containing integrins to permit cells to bind to osteopontin independently of arginine-glycine-aspartic acid, thereby stimulating cell matrix motility and chemotaxis. Cancer Res (1999) 59:219–26.

134. Kim MS, Park MJ, Moon EI, Kim SJ, Lee CH, Yoo H, et al. Hyaluronic acid induces osteopontin via the phosphatidylinositol 3-kinase/Akt pathway to initiate osteoclastogenesis. Blood Cells Mol Dis (2009) 43:256–63. doi:10.1016/j.bcmd.2009.08.005

135. Weber GF, Bronson RT, Iagdan J, Cantor H, Schmitt R, Mak TW. Absence of the CD44 gene prevents sarcoma metastasis. Cancer Res (2002) 62:2281–6.

136. Erb U, Megapcthe AP, Gu X, Bächler MW, Zöller M. CD44 standard and CD44V10 isoform expression on leukemia cells distinctly influences niche embedding of hematopoietic stem cells. J Hematol Oncol (2014) 7:29. doi:10.1186/1756-8722-7-29

137. van der Voort R, Taheer TE, Wielenga VJ, Spaargaren M, Prevo R, Smit L, et al. 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 (2012) 287:18995–9007. doi:10.1074/jbc.M111.318774

138. Yu Q, Stamenkovic I. Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. Dev Dyn (1999) 113:35–48. doi:10.1002/gado.1131.35

139. Wilson TJ, Nunnuru KC, Futakuchi M, Sadanandam A, Singh RK. Cathepsin G enhances mammary tumor-induced osteolysis by generating soluble receptor activator of nuclear factor-kappaB ligand. Cancer Res (2008) 68:5803–11. doi:10.1158/0008-5472.CAN-07-5889

140. Hill A, McFarlane S, Johnston PW, Waugh DJ. The emerging role of CD44 in regulating skeletal micrometastasis. Cancer Lett (2006) 237:1–9. doi:10.1016/j.canlet.2005.05.006

141. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Dev Dyn (2000) 14:163–76.

142. Wilson TJ, Nunnuru KC, Singh RK. Cathepsin G-mediated activation of pro-matrix metalloproteinase 9 at the tumor-bone interface promotes transforming growth factor-beta signaling and bone destruction. Mol Cancer Res (2009) 7:1224–33. doi:10.1158/1541-7786.MCR-09-0028

143. Chen Q, Yuan Y, Chen T. Morphology, differentiation and adhesion molecule expression changes of bone marrow mesenchymal stem cells from acute myeloid leukemia patients. Mol Med Rep (2014) 9:293–8. doi:10.3892/mmr.2013.1789

144. Almond A. Hyaluronan. Cell Mol Life Sci (2007) 64:1591–6. doi:10.1007/s00018-007-0702-z

145. Toole BP. Hyaluronan: from extracellular glue to pericellular cue. Nat Rev Cancer (2004) 4:528–39. doi:10.1038/nrc1391

146. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? Blood (2005) 106:1901–10. doi:10.1182/blood-2005-04-1417

147. Liu J, Jiang G. CD44 and hematologic malignancies. Cell Mol Immunol (2006) 3:359–65.

148. Lundell BI, Mccarthy JB, Kovach NL, Verfaillie CM. Activation of beta1 integrins on CML progenitors reveals cooperation between beta1 integrins and CD44 in the regulation of adhesion and proliferation. Leukemia (1997) 11:822–9. doi:10.1038/sj.leu.240053

149. Carstanjen D, Gross A, Kosova N, Fichtner I, Salama A. The alpha5beta1 and alpha6beta1 integrins mediate engraftment of granulocyte-colony-stimulating factormobilized human hematopoietic progenitor cells. Transfusion (2005) 45:1192–200. doi:10.1111/j.1537-2995.2005.00172.x

150. Wiernega PK, Weersing E, Don'tje B, de Haan G, van Os R. Differential role for CD44 in activating cell surface protein tyrosine phosphorylation in human hematopoietic progenitor cells. Bone Marrow Transplant (2006) 38:789–97. doi:10.1038/sj.bmt.1705534

151. Marhaba R, Frenschmidt-Paul P, Zöller M. In vivo CD44v5 beta1 complex formation in autoimmune disease has consequences on T cell activation and apoptosis resistance. Eur J Immunol (2006) 36:3017–32. doi:10.1002/eji.200631585

152. Prosper F, Verfaillie CM. Regulation of hematopoiesis through adhesion receptors. J Leukoc Biol (2001) 69:307–16.

153. Ford CD, Greenwood J, Anderson J, Handrahan D, Petersen FB. Good and poor mobilizing patients differ in mobilized CD34+ cell adhesion molecule profiles. Transfusion (2004) 44:1769–73. doi:10.1111/j.1537-2995.2004.04035.x

154. Christ O, Kronenwett R, Haas R, Zöller M. Combining G-CSF with a blockade of adhesion strongly improves the reconstitutive capacity of mobilized hematopoietic progenitor cells. Exp Hematol (2001) 29:380–90. doi:10.1016/S0301-472X(00)00674-3
granulocyte colony-stimulating factor. Blood (2001) 98:1289–97. doi:10.1182/blood.V98.5.1289
118. Vagima Y, Avigdor A, Goichberg P, Shavit S, Tesio M, Kalinkovich A, et al. MT1-MMP and RECK are involved in human CD34+ progenitor cell retention, egress, and mobilization. J Clin Invest (2009) 119:492–503. doi:10.1172/JCI36541
119. Avigdor A, Goichberg P, Shavit S, Dar A, Peled A, Samira S, et al. CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34+ stem/progenitor cells to bone marrow. Blood (2004) 103:2981–9. doi:10.1182/blood-2003-10–3611
120. Ratajczak MZ, Reca R, Wysoczynski M, Kucia M, Baran JT, Allenford DJ, et al. Transplantation studies in C3-deficient animals reveal a novel role of the third complement component (C3) in engraftment of bone marrow cells. Leukemia (2004) 18:1482–90. doi:10.1038/sj.lup.4903446
121. Tjwa M, Janssens S, Carmeliet P. Plasmipin therapy enhances mobilization of HPCs after G-CSF. Blood (2008) 112:4048–50. doi:10.1182/blood-2008-07-166587
122. Basak P, Chatterjee S, Das M, Das P, Pereira JA, Dutta RK, et al. Phenotypic alteration of bone marrow HSC and microenvironmental association in experimentally induced leukemia. Curr Stem Cell Res Ther (2010) 5:379–86. doi:10.2174/157488810793351677
123. Chellaiah MA, Ta M. Membrane localization of membrane type 1 matrix metalloproteinase by CD44 regulates the activation of pro-matrix metalloproteinase 9 in osteoclasts. Biomed Res Int (2013) 2013:302392. doi:10.1155/2013/302392
124. Raaijmakers MH. Regulating traffic in the hematopoietic stem cell niche. Haematologica (2010) 95:1439–41. doi:10.3324/haematol.2010.027342
125. Sipkins DA, Wei X, Wu JW, Rummels JM, Côté D, Means TK, et al. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. Nature (2005) 435:969–73. doi:10.1038/nature03703
126. Lesley J, English NM, Gál I, Mikecz K, Day AJ, Hyman R. Hyaluronic binding properties of a CD44 chimera containing the link module of TSG-6. J Biol Chem (2002) 277:26600–8. doi:10.1074/jbc.M201068200
127. Katayama Y, Hidalgo A, Chang I, Peired A, Frenette PS. CD44 is a physiological E-selectin ligand on neutrophils. J Exp Med (2005) 201:1183–9. doi:10.1084/jem.20042014
128. Papayannopoulou T, Craddock C, Nakamoto B, Priestley GV, Wolf NS. The VLA/A5CAM-1 adhesion pathway defines contrasting mechanisms of lodging of transplanted murine hematopoietic progenitors between bone marrow and spleen. Proc Natl Acad Sci U S A (1995) 92:9647–51. doi:10.1073/pnas.92.21.9647
129. Lévesque JP, Levesley DL, Niutta S, Vadas M, Simmons PJ. Cytokines increase human hematopoietic cell adhesiveness by activation of very late antigen (VLA)-4 and VLA-5 integrins. J Exp Med (1995) 181:1805–15. doi:10.1083/jem.181.5.1805
130. Mazo IB, Gutierrez-Ramos JC, Frenette PS. Hynes RO, Wagner DD, von Andrian UH. Hematopoietic progenitor cell rolling in bone marrow microvessels: parallel contributions by endothelial selectins and vascular cell adhesion molecule 1. J Exp Med (1998) 188:465–74. doi:10.1084/jem.188.3.465
131. Katayama Y, Hidalgo A, Furie BC, Vestweber D, Furie B, Frenette PS. PSGL-1 participates in E-selectin-mediated progenitor homing to bone marrow: evidence for cooperation between E-selectin ligands and alpha4 integrin. Blood (2003) 102:2060–7. doi:10.1182/blood-2003-04–1212
132. Siegelman MH, Stanescu D, Estess P. The CD44-initiated pathway of T-cell extravasation uses VLA-4 but not LFA-1 for firm adhesion. J Clin Invest (2000) 105:683–91. doi:10.1172/JCI8692
133. Lamontagne CA, Grandbois M. PKC-induced stiffening of hyaluronan/CD44 linkage: local force measurements on gloma cells. Exp Cell Res (2008) 314:227–36. doi:10.1016/j.yexcr.2007.07.013
134. Thomas L, Byers HR, Vink J, Stamenkovic I. CD44H regulates tumor cell migration on hyaluronate-coated substrate. J Cell Biol (1992) 118:971–7. doi:10.1083/jcb.118.4.971
135. Ofierenko S, Kaverina I, Small JV, Huber LA. Hyaluronic acid (HA) binding to CD44 activates Rac1 and induces lamellipodia outgrowth. J Cell Biol (2000) 148:1159–64. doi:10.1083/jcb.118.4.971
136. Bustelo XR. Regulatory and signaling properties of the Vav family. Mol Cell Biol (2000) 20:1461–77. doi:10.1128/MB.20.5.1461–1477.2000
137. Bourguignon LY, Singleton FA, Zhu H, Diedrich E. Hyaluronic-mediated CD44 interaction with RhöGf2 and Rho kinase promotes Grb2-associated activity.
hinder-1 phosphorylation and phosphatidylinositol 3-kinase signaling leading to cytokine (macrophage-colony stimulating factor) production and breast tumor progression. *J Biol Chem* (2003) **278**:29420–34. doi:10.1074/jbc.M301885200

208. Bourguignon LY. Hyaluronan-mediated CD44 activation of RhodGTPase signaling and cytoskeletal function promotes tumor progression. *Semin Cancer Biol* (2008) **18**:251–9. doi:10.1016/j.semcancer.2008.03.007

209. Singh V, Erb U, Zöller M. Cooperativity of CD44 and α4β1 in leukemia cell homing, migration, and survival offers a means for therapeutic attack. *Immunol* (2013) **191**:3304–16. doi:10.4049/immunol.1301543

210. Nagano O, Saya H. Mechanism and biological significance of CD44 cleavage. *Cancer Sci* (2004) **95**:930–5. doi:10.1111/j.1349-7006.2004.tb03179.x

211. Nakamura H, Suenaga N, Tanikawa K, Matsuki H, Yonezawa K, Fuji M, et al. Constitutive and induced CD44 shedding by ADAM-like proteases and membrane-type 1 matrix metalloproteinase. *Cancer Res* (2004) **64**:876–82. doi:10.1158/0008-5472.CAN-03–3502

212. Sugahara KN, Hiranra T, Tanaka T, Ogino S, Takeda M, Terawasa H, et al. Chondroitin sulfate E fragments enhance CD44 cleavage and CD44-dependent motility in tumor cells. *Cancer Res* (2008) **68**:7191–9. doi:10.1158/0008-5472.CAN-07–6198

213. Krause DS, Lazarides K, Lewis JR, von Andrian UH, van Etten RA. Selectins and their ligands are required for homing and engraftment of BCR-ABL1+ leukemic stem cells. *Blood* (2014) **123**:1361–71. doi:10.1182/blood-2013-11-538694

214. Ratajczak MZ, Kucia M, Majka M, Reca R, Ratajczak J. Heterogeneous populations of bone marrow stem cells – are we spotting on the same cells from the different angles? *Folia Histochem Cytobiol* (2004) **42**:139–46.

215. Lang L, Wang L, Geiger H, Canela JA, Mo J, Zheng Y. Rho GTPase Cdc42 coordinates hematopoietic stem cell quiescence and niche interaction in the bone marrow. *Proc Natl Acad Sci U S A* (2007) **104**:5091–6. doi:10.1073/pnas.0610819104

216. Vaiselbuh SR, Edelman M, Lipton JM, Liu JM. Ectopic human mesenchymal stem cell-coated scaffolds in NOD/SCID mice: an in vivo model of the leukemia niche. *Tissue Eng Part C Methods* (2010) **16**:1523–31. doi:10.1089/ten.tec.2010.0179

217. Tavor S, Petit I. Can inhibition of the SDF-1/CXCR4 axis eradicate acute leukemia? *Semin Cancer Biol* (2010) **20**:178–85. doi:10.1016/j.semcancer.2010.07.001

218. Naor D, Sionov RV, Ish-Shalom D. CD44: structure, function, and association with the malignant process. *Adv Cancer Res* (1997) **71**:241–319.

219. Yamamara O, Okishi H, Kasaya M, Nito K, Shibasaki T, Sugimoto Y. Evidence for horizontal transfer of mRNA and protein delivery. *Non-coding RNAs and extracellular vesicles – diagnostic and therapeutic implications (review).* Int J Oncol (2015) **46**:27–32. doi:10.3892/ijon.2014.2712

220. Ratajczak J, Mietkus K, Kucia M, Zhang J, Reca R, Dvorak P, et al. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* (2006) **20**:847–56. doi:10.1038/leu.2004.131

221. Okuyama K, Ogata J, Yamakawa N, Chanda B, Kotani A. Small RNA as a regulator of hematopoietic development, immune response in infection and tumorigenesis. *Int J Hematol* (2014) **99**:553–60. doi:10.1007/s12188-014-1564-4

222. Li L, Bhatia R. Stem cell quiescence. *Clin Cancer Res* (2011) **17**:4936–41. doi:10.1158/1078-0432.CCR-10-1499

223. Arai F, Hiroa A, Ohmura M, Sato H, Matsuoka S, Takubo K, et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* (2004) **118**:149–61. doi:10.1016/j.cell.2004.07.004

224. Blank U, Karlsson G, Karlsson S. Signaling pathways governing stem-cell fate. *Blood* (2008) **111**:492–503. doi:10.1182/blood-2007-07-075168

225. Bourguignon LIV, Singleton PA, Zou H, Zhou B. Hyaluronan promotes signaling interaction between CD44 and the transforming growth factor beta receptor I in metastatic breast tumor cells. *J Biol Chem* (2002) **277**:39703–12. doi:10.1074/jbc.M204320200

226. Basoni C, Reuzeau É, Croft D, Génost E, Kramer IM. CD44 and TGFbeta1 synergize to induce expression of a functional NADPH oxidase in promyelocytic cells. *Biochem Biophys Res Commun* (2006) **343**:609–16. doi:10.1016/j.bbrc.2006.03.003

227. Robin C, Durand C. The roles of BMP and IL-3 signaling pathways in the control of hematopoietic stem cells in the mouse embryo. *Int J Dev Biol* (2010) **54**:1189–200. doi:10.1387/ijdb:090340cr

228. Pinmanda JE, Oettersbach K, Knezovic K, Kinston S, Chan WY, Wilson NK, et al. Gata2, Fli1, and Scl form a recursively wired gene-regulatory circuit during early hematopoietic development. *Proc Natl Acad Sci U S A* (2007) **104**:10736–41. doi:10.1073/pnas.0610819104

229. Calvi LM, Adams GB, Weibricht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* (2003) **425**:841–6. doi:10.1038/nature02400

230. Marshall CJ, Sinclair JC, Thrasher AJ, Kinnon C. Bone morphogenetic protein 4 modulates c-Kit expression and differentiation potential in murine embryonic aorta-gonad-mesonephros haematopoiesis in vitro. *Br J Haematol* (2007) **139**:321–30. doi:10.1111/j.1365-2457.2007.06795.x

231. Peterson RS, Andhare RA, Rousche KT, Knudson W, Wang W, Grossfeld JB, et al. CD44 modules Smad1 activation in the BMP-7 signaling pathway. *J Cell Biol* (2004) **166**:1081–91. doi:10.1083/jcb.200402138

232. Tanikawa R, Tanikawa T, Hirashima M, Yamauchi A, Tanaka Y. Galexin-9 induces osteoblast differentiation through the CD44/Smad signaling path. *Biochem Biophys Res Commun* (2010) **394**:317–22. doi:10.1016/j.bbrc.2010.02.175

233. Qian H, Buza-Vidas N, Hyland CD, Jensen CT, Antonchuk J, Månsson R, et al. Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. *Cell Stem Cell* (2007) **1**:671–84. doi:10.1016/j.stem.2007.10.008

234. Kashiwakura I, Teramachi T, Kakizaki I, Takagi Y, Takahashi TA, Takagaki K. The effects of glycaminoglycans on thrombopoietin-induced megakaryocytopenia. *Haematologica* (2006) **91**:445–51.

235. Cain CJ, Manilay JO. Hematopoietic stem cell fate decisions are regulated by Wnt antagonists: comparisons and current controversies. *Exp Hematol* (2013) **41**:3–16. doi:10.1016/j.exphem.2012.09.006

236. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Dev* (1997) **11**:3286–305. doi:10.1101/gad.11.24.3286
embryonic stem cells. Required for generation of hematopoietic cells during differentiation of human hematopoietic stem cells from the bone marrow and its level in peripheral blood increases in inhibits SDF-1 signaling of lipid raft-associated Rac1/PI3K/Akt signaling complexes by curcumin chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. The pathway regulates growth of brain tumor stem cells partially through the AKT-mediated quiescence through interactions with Hsc70. Cell Stem Cell (2011) 2:5–7. 10.1016/j.stem.2011.07.003

Suda T, Nakao K, Senmen GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell (2011) 2:298–310. 10.1016/j.stem.2011.09.010

Zou P, Yoshihara H, Hosokawa K, Tai I, Shimmyouzi K, Tsukahara F, et al. p57(Kip2) and p27(Kip1) cooperate to maintain hematopoietic stem cell quiescence through interactions with Hsc70. Cell Stem Cell (2011) 2:247–61. 10.1016/j.stem.2011.07.003

Mantel C, Messina-Graham S, Broxmeyer HE. Upregulation of nascent mitochondrial biogenesis in mouse hematopoietic stem cells parallels upregulation of CD34 and loss of pluripotency: a potential strategy for reducing oxidative risk in stem cells. Cell Cycle (2010) 9:2088–17. 10.4161/cc.9.10.11733

Darzykiewicz Z, Balaza EA. Genomic integrity, stem cells and hyalurhan. Aging (2012) 4:78–88.

Simsek T, Kocabas E, Zheng J, Deberardinis RJ, Mahmoud AI, Olson EN, et al. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. Cell Stem Cell (2010) 7:380–90. 10.1016/j.stem.2010.07.011

Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell (2008) 30:393–402. 10.1016/j.molcel.2008.04.009

Wheaton WW, Chandel NS. Hypoxia. 2. Hypoxia regulates mitochondrial metabolism. Am J Physiol Cell Physiol (2011) 300:C385–93. 10.1152/ajpcell.00485.2010

Miharada K, Karlsson G, Rehn M, Rörby E, Siva K, Cammenga J, et al. Cripto related protein mediates Wnt signalling in mice. Nat Immunol (2004) 5:73–80. 10.1038/ni0404-73

Wang A, de la Mote C, Lauer M, Hascall V. Hyaluronan matrices in pathobiological processes. FERS J (2011) 278:1412–8. 10.1111/j.1744-4658.2011.08069.x

Mohyelidin A, Garzón-Muñiz T, Quiñones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. Cell Stem Cell (2010) 7:150–61. 10.1016/j.stem.2010.07.007

Schepers K, Pieters EM, Reynaud D, Flach J, Binnewies M, Garg T, et al. Osmoprotective neoplasia remodels the endothelial bone marrow niche into a self-reinforcing leukemic niche. Cell Stem Cell (2013) 13:285–99. 10.1016/j.stem.2013.06.009

Toole BP, Slomiany MG. Hyaluronan: a constitutive regulator of chemoresistance and malignancy in cancer cells. Semin Cancer Biol (2008) 18:24–50. 10.1016/j.semcancer.2008.03.009

Wang SJ, Bouguignoux L. Hyaluronan and the interaction between CD44 and epidermal growth factor receptor in oncogenic signaling and chemotherapy
resistance in head and neck cancer. *Arch Otolaryngol Head Neck Surg* (2006) 132:771–8. doi:10.1001/archotol.132.7.771

291. Sherman LS, Rizvi TA, Karyala S, Ratner N. CD44 enhances neuregulin signaling by Schwann cells. *J Cell Biol* (2000) 150:1071–84. doi:10.1083/jcb.150.5.1071

292. Ghatak S, Misra S, Toole BP. Hyaluronan constitutively regulates ErbB2 phosphorylation and signaling complex formation in carcinoma cells. *J Biol Chem* (2005) 280:8875–83. doi:10.1074/jbc.M410882200

293. Misra S, Haskell VC, De Giovani C, Markwald RR, Ghatak S. Delivery of CD44 shRNA/nanoparticles within cancer cells: perturbation of hyaluronan/CD44V6 interactions and reduction in adenoma growth in Apc Min/+ Mice. *J Biol Chem* (2009) 284:12432–46. doi:10.1074/jbc.M806772200

294. Bourguignon LY, Gilad E, Peyrollier K. Heregulin-mediated ErbB2-ERK signaling activates hyaluronan syntheses leading to CD44-dependent ovarian tumor cell growth and migration. *J Biol Chem* (2007) 282:19424–46. doi:10.1074/jbc.M610054200

295. Orian-Rousseau V, Morrison H, Matzke A, Kastilan T, Pace G, Herrlich P, et al. Hepatocyte growth factor-like growth factor in cutaneous squamous cell carcinoma. *J Biol Chem* (2004) 279:22775–82. doi:10.1074/jbc.M400923200

296. Zhao H, Tanaka T, Mitilinski V, Heeter J, Balazs EA, Darzynkiewicz Z. Protective effect of hyaluronate on oxidative DNA damage in WI-38 and A549 cells. *Arch Biochem Biophys* (2008) 473:1159–67.

297. Thankamony SP, Knudson W. Acylation of CD44 and its association with lipid rafts are required for receptor and hyaluronan endocytosis. *J Biol Chem* (2006) 281:34601–9. doi:10.1074/jbc.M601530200

298. Campo GM, Avenoso A, Campo S, D’Ascola A, Ferlazzo AM, Calatroni A. Reduction of DNA fragmentation and hydroxyl radical production by hyaluronic acid and chondroitin-4-sulphate in iron plus ascorbate-induced oxidative stress in fibroblasts. *Free Radic Res* (2004) 38:601–11. doi:10.1080/10715760410001694017

299. Bourguignon LY, Earle C, Wong G, Spevak CC, Krueger K. Stem cell marker (Nanog) and Stat3 signaling promote microRNA-21 expression and chemoresistance in hyaluronan/CD44-activated head and neck squamous cell carcinoma cells. *Oncogene* (2012) 31:149–60. doi:10.1038/onc.2011.222

300. Kathawala RJ, Gupta P, Ashby CR Jr, Chen Z. The modulation of ABC transporter-mediated multidrug resistance in cancer: a review of the past decade. *Drug Resist Updat* (2014) 17:78–87. doi:10.1016/j.drup.2014.11.002

301. Misra S, Ghatak S, Toole BP. Regulation of MDR1 expression and drug resistance by a positive feedback loop involving hyaluronan, phosphoinositide 3-kinase, and ErbB2. *J Biol Chem* (2005) 280:20316–5. doi:10.1074/jbc.M405073200

302. Liu CM, Chang CH, Yu CH, Hsu CC, Huang LL. Hyaluronan substratum induces multidrug resistance in human mesenchymal stem cells via CD44 signaling. *Cell Tissue Res* (2009) 336:465–75. doi:10.1007/s00441-009-0780-3

303. Bourguignon LY, Xia W, Goyen N. Hyaluronan-mediated CD44 interaction with p300 and SIRT1 regulates beta-catenin signaling and NF-kappaB-specific transcription activity leading to MDR1 and Bcl-xL gene expression and chemoresistance in breast tumor cells. *J Biol Chem* (2009) 284:2657–71. doi:10.1074/jbc.M806708200

304. Slomiany MG, Dai L, Bomar PA, Knackstedt TJ, Kranc DA, Tolliver L, et al. Abrogating drug resistance in malignant peripheral nerve sheath tumors by disrupting hyaluronan-CD44 interactions with small hyaluronan oligosaccharides. *Cancer Res* (2009) 69:1992–8. doi:10.1158/0008-5472.CAN-09-0143

305. Negi LM, Talegaonkar S, Jaggi M, Ahmad FJ, Iqbal Z, Khar RK. Role of CD44 in tumour progression and strategies for targeting. *J Drug Target* (2012) 20:561–73. doi:10.1080/1061186X.2012.702767

306. Midgley AC, Bowen T. Analysis of human hyaluronan synthase gene transcriptional regulation and downstream hyaluronan cell surface receptor mobility in myofibroblast differentiation. *Methods Mol Biol* (2015) 1229:605–18. doi:10.1007/978-1-4939-1714-3_47

307. Kobayashi K, Hara R, Takenoshita T, Goto K, Yasuda K, et al. Hyaluronan activates the Akt/c-Akt survival signaling pathway in glioblastoma cells. *Cancer Biol Ther* (2015) 16:861–6. doi:10.4161/cbj.69820

308. Majeti R. Monoclonal antibody therapy directed against human acute myeloid leukemia stem cells. *Oncogene* (2011) 30:1009–19. doi:10.1038/onc.2010.511
329. Konopleva MY, Jordan CT. Leukemia stem cells and microenvironment: biology and therapeutic targeting. *J Clin Oncol* (2011) 29:591–9. doi:10.1200/JCO.2010.31.0904

330. Brayer JB, Pinilla-Ibarz J. Developing strategies in the immunotherapy of leukemias. *Cancer Control* (2013) 20:49–59.

331. Bourguignon LY, Gunja-Smith Z, Iida N, Zhu HB, Young LJ, Muller WJ, et al. CD44v(3,8-10) is involved in cytoskeleton-mediated tumor cell migration and matrix metalloproteinase (MMP-9) association in metastatic breast cancer cells. *J Cell Physiol* (1998) 176:206–15. doi:10.1002/(SICI)1097-4652(199807) 176:1<206::AID-JCP22>3.0.CO;2-3

332. D’Arena G, Calapai G, Deaglio S. Anti-CD44 mAb for the treatment of B-cell chronic lymphocytic leukemia and other hematological malignancies: evaluation of WO2013063498. *Expert Opin Ther Pat* (2014) 24:821–8. doi:10.1517/13543776.2014.915942

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