Review

Understanding the Dual Nature of CD44 in Breast Cancer Progression

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Abstract

CD44 has been the subject of extensive research for more than 3 decades because of its role in breast cancer, in addition to many physiological processes, but interestingly, conflicting data implicate CD44 in both tumor suppression and tumor promotion. CD44 has been shown to promote protumorigenic signaling and advance the metastatic cascade. On the other hand, CD44 has been shown to suppress growth and metastasis. Histopathological studies of human breast cancer have correlated CD44 expression with both favorable and unfavorable clinical outcomes. In recent years, CD44 has garnered significant attention because of its utility as a stem cell marker and has surfaced as a potential therapeutic target, necessitating a greater understanding of CD44 in breast cancer. In this review, we attempt to unify the literature implicating CD44 in both tumor promotion and suppression, and explain its dualistic nature. *Mol Cancer Res; 9(12); 1573–86. ©2011 AACR.*

Introduction

CD44 is a member of a large family of cell adhesion molecules that is responsible for mediating communication and adhesion between adjacent cells and between cells and the extracellular matrix (ECM). Cell adhesion molecule–mediated organization is a basic feature of normal breast histology and is essential for maintaining tissue integrity. Disruption or misregulation of these adhesive relationships causes a loss of tissue architecture and is a feature of neoplastic transformation. In addition to its role in cellular adhesion, CD44 can direct intracellular signaling for growth and motility, and thus it is involved in many types of cancers, including breast, lung, prostate, ovarian, cervical, and colorectal cancers and neuroblastoma (1). In prostate cancer and neuroblastoma, *CD44* has been dubbed a metastasis suppressor gene (2, 3), although it was recently shown to promote prostate cancer growth and metastasis in a xenograft model (4). Its role in breast cancer, however, is unclear and controversial. CD44 expression in breast cancer has been correlated with both poor and favorable outcomes. It mediates both pro- and antitumoral signaling *in vitro*, and it can inhibit and promote metastatic progression *in vivo*. Although researchers often focus on one or another aspect of

CD44-mediated biology, it is important to understand its dualistic nature if it is to be used as a diagnostic and therapeutic tool. Here we review the pro- and antitumoral signaling events that are mediated by CD44, and we discuss its expression in human breast cancer and its use as a therapeutic target. CD44 has been examined in many cancer types; however, we will focus primarily on evidence derived from breast cancer. Furthermore, although CD44 is used as a stem cell marker in breast cancer (5), its role in that context is beyond the scope of this review, and the reader is directed to previous excellent reviews for an evaluation of this topic (6, 7).
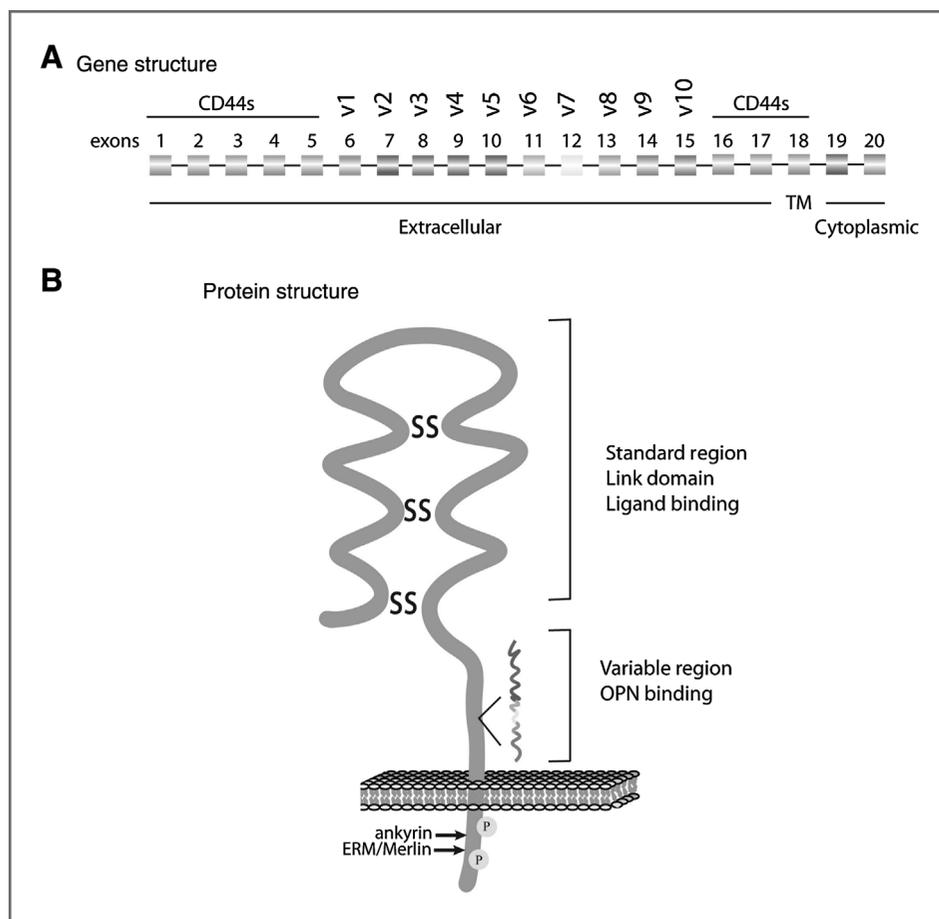
CD44 Structure

CD44 is encoded by a single, highly conserved gene, spanning ~50 kilobases. It is located on chromosome 11 in humans and chromosome 2 in mice, and it encodes a group of proteins ranging from 80 to 200 kDa in size. The heterogeneity of this group is due to posttranscriptional regulation, including alternative splicing and protein modification (8). The *CD44* gene contains 20 exons, which encode ~20 *CD44* isoforms (9). Exons 1–5 and 16–18 are constant, whereas exons 6–15 and 19–20 are variants and inserted by alternative splicing (ref. 10; Fig. 1A). The nonvariant standard isoform, denoted *CD44s*, is encoded by the constant exons, is the smallest and most widely expressed isoform, and is present on the surface of most vertebrate cells (8). Inclusion of the variant exons lengthens the extracellular membrane-proximal stem structure of CD44 (11), creating larger isoforms and exposing binding sites for additional posttranslational modifications and ligand-binding sites. Variant expression is regulated by tissue and environment-specific factors, and oncogenic pathways such as the Ras-MAPK cascade

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Figure 1. *CD44* gene and protein structure. A, the *CD44* gene is encoded by 20 exons, the first 5 of which are constant for all *CD44* isoforms. Exons 6–15 are encoded by alternative splicing, 16–18 are constant, and 19–20 are inserted by alternative splicing. The first 17 exons comprise the extracellular region, exon 18 encodes the transmembrane domain, and exons 19 and 20 encode the cytoplasmic tail. B, the CD44 protein consists of a globular extracellular domain that is stabilized by disulphide bonding of 6 cysteine residues and contains binding sites for hyaluronan (a region known as the link domain) and other CD44 ligands. Insertion of variant exons lengthens the stalk structure and exposes binding sites for additional glycosaminoglycans.

can regulate alternative splicing during cancer progression (12, 13).

CD44 Protein Family

As depicted in Fig. 1B, the first 5 N-terminal exons of *CD44* encode the extracellular region, which contains 6 cysteine amino acids that stabilize a globular domain and form a structure that includes a conserved link-module for hyaluronan binding (14–16), binding sites for other CD44 ligands (discussed below), and sites for *O-* and *N*-linked glycosylation and chondroitin sulfate binding. A span of 46 amino acids in the membrane-proximal region contains several putative proteolytic cleavage sites (17, 18) and can be lengthened by the insertion of variant exons that form a heavily glycosylated stalk-like structure. This then exposes binding domains for additional glycosaminoglycans and heparan sulfate binding (11, 19). The transmembrane region contains 23 hydrophobic amino acids and 1 cysteine residue, and is thought to be involved in CD44 oligomerization and association with lipid rafts (20, 21).

The cytoplasmic tail of CD44 spans 72 amino acids and contains motifs that direct CD44 basolateral localization or subdomain localization during cell migration, and it mediates CD44 interactions with intracellular binding partners.

Although CD44 has no intrinsic kinase activity, the cytoplasmic tail interacts with a variety of signaling mediators and contains binding sites for the actin-cytoskeleton adaptor proteins ankyrin and members of the band 4.1 family ERM (ezrin/radixin/moesin), which direct reorganization of the actin cytoskeleton and mediate cell adhesion and motility (22–25). Alternatively, CD44 can interact with Merlin, which does not link to actin but mediates contact inhibition and growth arrest (26). The cytoplasmic tail contains 6 potential serine phosphorylation sites that are phosphorylated by protein kinase C and Rho kinase (8). Ser325 is phosphorylated in the resting state and is dephosphorylated upon PKC activation, which then phosphorylates Ser291 (27). The phosphate switch enhances intracellular association with ERM proteins. Activation by Rho kinase is thought to promote ankyrin binding and cell motility (28).

CD44 Proteolytic Cleavage

CD44 is subject to proteolytic cleavage in the extracellular membrane-proximal region and in the intracellular cytoplasmic domain. Extracellular cleavage is accomplished by proteases, including members of the ADAM (a disintegrin and metalloprotease) family, and by membrane type I MMP (18). Extracellular CD44 cleavage triggers presenilin-

dependent γ-secretase cleavage of the cytoplasmic tail, which can then be translocated to the nuclease where it can mediate gene transcription of genes containing a TPA-response element or act as a coactivator of CBP/P300 target genes, and even promote its own transcription (29–31). Of interest, CD44 cleavage increases in cancer in response to production of hyaluronan oligosaccharides, fluctuation of extracellular Ca^{++}, and activation of PKC and Ras, resulting in enhanced cellular migration (17, 32, 33).

CD44 Ligands

CD44 mediates epithelial stromal interactions with the extracellular microenvironment to direct intracellular signaling as well as organization and modification of the ECM. The CD44 extracellular domain can bind to numerous ECM components, including collagen, laminin, fibronectin, and hyaluronan (34–36). In addition, CD44 contains binding sites for a number of glycosaminoglycans, including osteopontin (37). Osteopontin selectively binds to CD44 variants v6 and v7, triggering signaling that promotes cell survival, migration, and invasion, and angiogenesis (38).

Hyaluronan is the best-characterized CD44 ligand and has an immense repertoire of biological functions (39, 40). Hyaluronan is a cell-surface–associated glycosaminoglycan that is ubiquitous in extracellular and pericellular matrices. It is synthesized and simultaneously secreted by transmembrane hyaluronan synthases as an extremely high molecular mass polymer of $\sim10^6$ to 10^7 MDa (41). However, it is subject to cleavage by hyaluronidases, which results in hyaluronan species of varying sizes, sometimes as small as a few disaccharides (42). Hyaluronan influences intracellular signaling by binding to cell-surface receptors, namely CD44 and RHAMM, but it also has context- and size-specific biological activities (39). For example, high molecular weight (HMW) hyaluronan has been shown to inhibit tumorigenesis by promoting cell-cycle arrest under conditions of high cell density, to inhibit CD44-mediated cell invasion in breast cancer cell lines, and to be antiangiogenic and antiinflammatory (26, 43–47). In contrast, low molecular weight (LMW) hyaluronan oligomers can promote cell motility, CD44 cleavage, and angiogenesis (33, 48, 49). Thus, hyaluronan-mediated biological functions are strongly size dependent. Although previous research has not defined the molecular weight that differentiates LMW from HMW hyaluronan, for the purpose of this review, LMW refers to hyaluronan species that are $<10^6$ Da, and HMW refers to species that are $>10^6$ Da. It should be noted, however, that in some studies the size of the hyaluronan was not closely monitored or defined.

CD44-Mediated Cell Signaling

Uncontrolled growth, evasion of apoptosis, angiogenesis, and cell motility and invasion are hallmarks of cancer progression (50). CD44 can promote these functions, either independently or in collaboration with other cell-surface receptors, and it can also inhibit these functions. CD44 has

been shown to activate a number of central signaling highways, including Rho GTPases and the Ras-MAPK and the PI3K/AKT pathways, but it has also been shown to act as a growth/arrest sensor that, in response to cues from the microenvironment, can arrest growth, promote apoptosis, and inhibit angiogenesis and invasion (1, 51, 52). While CD44 signaling initiates upon binding to various ligands the ECM, signaling induced by hyaluronan is the most extensively characterized.

CD44 has no intrinsic kinase activity; thus, it induces signaling by recruiting intracellular kinases and adaptor proteins that link the CD44 cytoplasmic tail to the actin cytoskeleton and induce signaling cascades. Alternatively, CD44 can act as coreceptor through interactions with other cell-surface receptors. As mentioned above, CD44 is subject to biological cleavage in both its extracellular domain and cytoplasmic tail, through which CD44 can influence paracrine signaling events and transcription (31). Additionally, CD44 can influence signaling by harboring cell-surface–associated growth factors, enzymes, and cytokines (51).

It should be noted that much of our knowledge about CD44's role in cell signaling comes from *in vitro* studies of human cancer cell lines. Although they can provide valuable insights, cell culture studies of CD44 are particularly difficult to perform, because cultivation conditions have been shown to upregulate cell-surface CD44 and splice variants, resulting in the expression of new isoforms in noncancerous cell lines similar to their cancerous counterparts (8). Additionally, CD44-mediated signaling is heavily dependent on extracellular conditions and can vary significantly among various cell types or even in the same cell (1). In addition, the CD44 ligand hyaluronan can exist in species ranging in size from megadaltons to small fragments of only a few disaccharides. It can also be present in a matrix-embedded state or presented to cells in soluble form, the variability of which affects intracellular signaling (41, 53). Such factors should be taken into consideration, but despite this variability, researchers have amassed a considerable body of knowledge about CD44-mediated cell signaling.

Direct Signal Transduction

CD44 modulates many signaling activities through interactions in its cytoplasmic tail (Fig. 2). Treatment with soluble LMW or HMW hyaluronan has been shown to induce cell invasion and migration through CD44-mediated activation of the Rho family of GTPases. Various studies have shown that Hyaluronan-CD44 interactions initiate recruitment of signaling molecules including Tiam1, p115, Rac1, Rho Gefs, Rho-associated protein kinase, and cSrc. Interactions with signaling molecules leads to activation of the PI3K pathway and a number of cellular outputs, namely survival and cell invasion (54–56). CD44 was also shown to interact with and activate RhoA independently of hyaluronan binding, which enhances CD44 association with ankyrin, leads to the formation of membrane projections, and induces migration (28). Conversely, hyaluronan oligomers were shown to inhibit PI3K activation and AKT
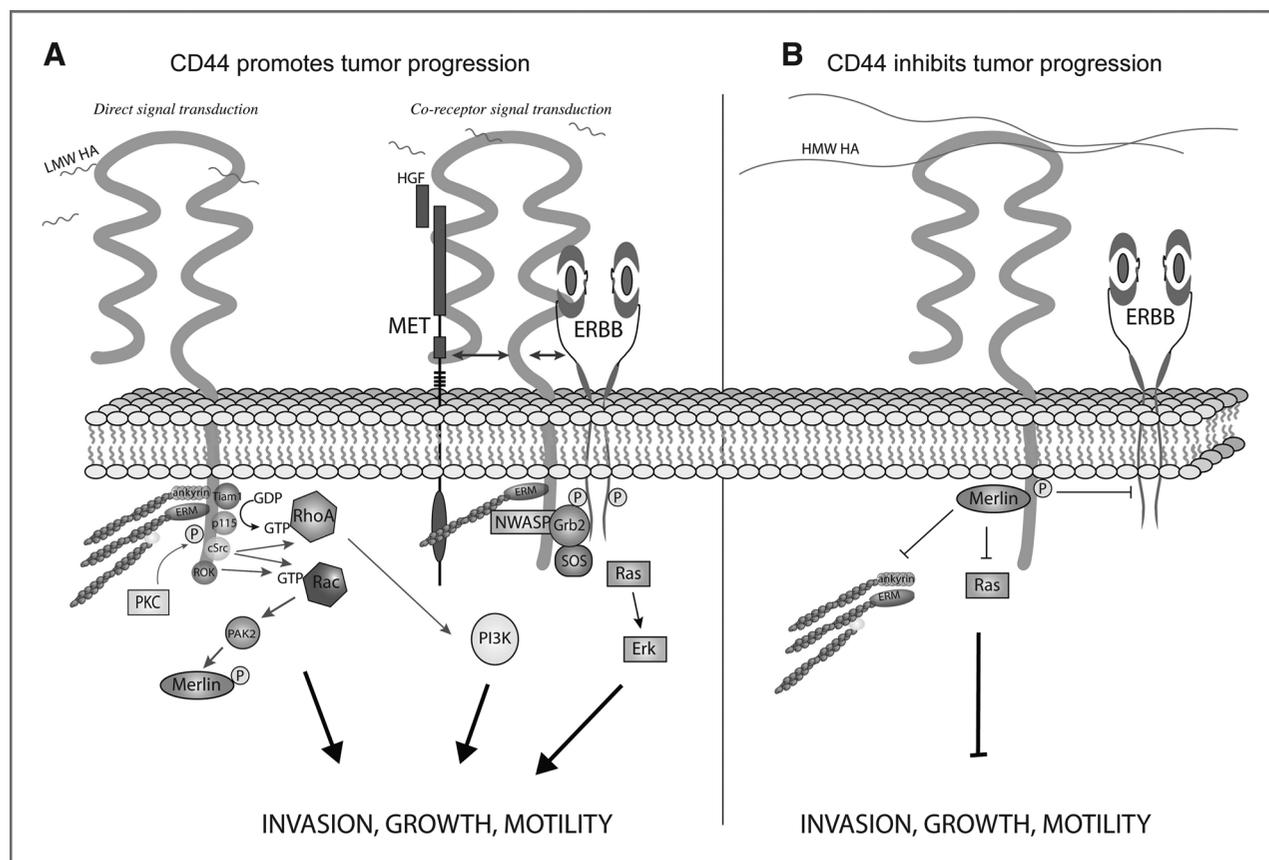
Figure 2. CD44 activates and inhibits oncogenic signaling. A, CD44 promotes tumor progression. CD44 directly mediates signal transduction through activation of LMW hyaluronan, which upon binding recruits signaling mediators to the CD44 cytoplasmic tail. This then activates signaling pathways that promote cell migration and invasion. Alternatively, CD44 can promote signaling by acting as a coreceptor to oncogenes such as c-Met and ErbB receptors. These interactions promote activation of signaling pathways that promote growth and cellular invasion. B, CD44 inhibits tumor progression in response to extracellular cues, primarily binding to HMW hyaluronan. CD44's interaction with HMW hyaluronan promotes its interaction with hypophosphorylated Merlin, inhibits Ras activation, and inhibits CD44–ERM interactions. Additionally, the interaction between CD44 and HMW hyaluronan suppresses EGFR activation.

phosphorylation while stimulating apoptosis and upregulating expression of the tumor suppressor phosphatase PTEN in TA3/St murine mammary carcinoma cells (57). Thus, CD44 activation of Rho GTPases and the PI3K pathway is highly dependent on microenvironmental cues. More recently, CD44 was shown to contribute to chemoresistance and to upregulate expression of the multidrug resistance receptor by activating the stem cell marker Nanog. This in turn activates expression of miR-21, which has been shown to increase expression of the multidrug resistance receptor (58). Drug resistance can additionally be activated downstream of CD44 through the Stat3 pathway (59).

CD44 mediates actin cytoskeleton remodeling and invasion through interaction with ERM proteins, which link CD44 indirectly to the actin cytoskeleton and promote cytoskeletal remodeling and invasion. ERM proteins, however, compete for binding sites on the cytoplasmic tail with Merlin, an ERM-related protein that functions as a tumor suppressor. ERM and Merlin may compete for CD44 binding to either accomplish growth and migration or inhibit growth and migration. In response to high cell density and HMW hyaluronan, Merlin binds to CD44,

displacing ERM and thereby inhibiting Ras-activated cell growth (26). Conversely, activation of PI3K leads to the phosphorylation and deactivation of Merlin by p21-associated kinase (Pak2), which inhibits its binding to CD44. This leaves ankyrin and ERM proteins free to link CD44 cytoplasmic to the actin cytoskeleton, which in turn promotes cytoskeletal reorganization and increases cellular invasion (26, 60, 61).

CD44 as a Coreceptor

The role of CD44 in the metastatic cascade is tightly coupled to its interaction and collaboration with other cell-surface receptors (Fig. 2A). The extracellular domain of CD44 can bind coreceptors, initiating recruitment and activation of signaling cascades. Significant evidence supports its interaction with and influence on the ErbB family of receptor tyrosine kinases. Epidermal growth factor receptor (EGFR)/ErbB1 and ErbB2/Her2 are key regulators of metastatic disease, and their expression is associated with the most aggressive forms of breast cancer (62, 63). CD44 colocalizes and coimmunoprecipitates with EGFR and

ErbB2 in numerous breast cancer cell lines and in cytology samples from patients with metastatic breast cancer; however, research varies on this point because no correlation between CD44 and EGFR or ErbB2 expression has been reported (64, 65). In addition, LMW hyaluronan binding induces interactions with Grb2 and p185Her2, and it promotes CD44 binding to N-Wasp, leading to activation of Ras- and SOS-mediated growth and invasion (66, 67).

In addition to the ErbB receptors, CD44 mediates signaling through the oncogene c-Met. Met, the receptor for the hepatocyte growth factor (HGF), is overexpressed in 20% to 30% of breast cancers, is associated with poor clinical outcome (68, 69), and of importance, requires CD44v6 to become fully activated. CD44v6-specific antibodies have been shown to block Met activation in many different cancer cell lines and primary cells, and loss of CD44 in mice correlates with c-Met haploinsufficiency (70, 71). Furthermore, CD44v6–ERM interaction is required for activation of c-Met and subsequent downstream activation of the Ras-SOS signaling cascade (72). CD44v6 is thought to cooperate with Met both by binding to extracellular HGF and by recruiting ERM proteins to the CD44 cytoplasmic tail, which in turn catalyzes the activation of Ras (73).

In addition to Met and ErbB receptors, CD44 has been shown to interact with TGFβ receptors 1 and 2, which promotes ankyrin–CD44 interaction and leads to Smad-dependent invasion (74). CD44v6 has been shown to activate endothelial cell migration, sprouting, and tubule formation through activation of c-Met and VEGFR-2 in response to HGF or VEGF-A (75). This activation is thought to require CD44-intracellular interactions with ERM proteins (76). Additionally, a recent study identified FKBPL, a member of the FK506 binding proteins, as an endogenously secreted antiangiogenic protein that inhibits angiogenesis by suppressing CD44 activation of Rac1 in prostate cell human tumor xenografts and in human breast cancer cell lines (77).

In addition, CD44 can alter angiogenesis differentially when coexpression of the hyaluronidase hyal2 occurs. CD44 forms a complex with the transmembrane sodium-hydrogen exchanger, NHE1, and hyal2 (47). NHE1 acidifies the microenvironment, activates Cathepsin B, and promotes invasion. Hyal2 promotes cleavage and catabolism of HMW hyaluronan to small oligomerized hyaluronan disaccharides, which are thought to promote invasion and have been shown to preferentially stimulate angiogenesis (41).

CD44 Promotes Cancer Progression

CD44 is capable of promoting tumorigenic signals through a variety of major signaling networks, including activation of Rho GTPases, which promote cytoskeletal remodeling and invasion, and the PI3K/AKT and MAPK-Ras pathways, which promote growth, survival, and invasion. CD44 complexes with key oncogenes to augment their activity and promote tumorigenesis and angiogenesis, and it can even modify the tumor microenvironment by promoting cleavage of hyaluronan to support tumor pro-

gression. In addition, CD44 serves as a docking site for matrix metalloproteases (MMP), matrix-modifying enzymes that degrade basement membrane and promote cell migration. Specifically, CD44 promotes docking of the collagen-specific MMP9, whose localization to the leading edge of migrating cells promotes collagen degradation and invasion and is also capable of TGFβ cleavage, which promotes angiogenesis and invasion (78, 79).

Recent evidence showing that CD44 is transcriptionally repressed by the tumor suppressor p53 suggests that it promotes survival. p53 binding to the CD44 promoter enables cells to respond to stress-induced p53-dependent apoptotic signals that, in the absence of p53, enhance CD44 expression and evade apoptosis (80).

In addition to the extensive *in vitro* research on CD44 in prometastatic signaling, several groups have assessed the role of CD44 in breast cancer progression *in vivo* using xenograft or transgenic mouse models. One of the earliest indications of CD44's role in metastasis came not from breast cancer but from pancreatic cancer. Transfection of CD44 variants into a nonmetastatic rat pancreatic carcinoma cell line conferred metastatic potential in these cells when injected into syngeneic rats (81), which could be blocked by treatment with anti-CD44v6 monoclonal antibody (82). Studies in breast cancer have produced similar, albeit conflicting, results. Researchers developed a tetracycline-inducible *CD44s* in the weakly metastatic MCF7 breast cancer cell line and found that induction of *CD44s*, in addition to promoting aggressive characteristics *in vitro* (83), promoted metastasis to the liver when injected into immunodeficient mice, although it did not affect growth rate or local invasion (84). In another study using a xenograft tumor model in which aggressive primary tumors from human breast were transplanted into the mammary fat pad of mice, treatment with a CD44-blocking monoclonal antibody, P245, not only dramatically inhibited tumor growth but also prevented recurrence in a triple-negative xenograft after treatment with doxorubicin/cyclophosphamide (85).

CD44 Inhibits Cancer Progression *In Vitro*

Although the majority of *in vitro* research supports the role of CD44 in cancer progression, numerous reports have shown that CD44 can respond to cues from the microenvironment, often in response to HMW hyaluronan, to inhibit growth and invasion in cancer cells (Fig. 2B). Correspondingly, the loss of CD44 is associated with transformation, particularly in Burkitt's lymphomas, neuroblastomas, and prostate cancers (1); its associations in breast cancer are more varied (discussed below). CD44 binding to Merlin acts as a growth/arrest sensor in response to cues from the microenvironment and plays a role in contact inhibition, a capability that tumor cells have overridden or lost (61). In addition, we have found that type I collagen-embedded HMW hyaluronan can inhibit invasion of several metastatic breast cancer cell lines and that blocking the CD44–hyaluronan interaction with a functional blocking antibody (KM201) releases this inhibition (46). Similarly, our

laboratory also showed that collagen-embedded HMW hyaluronan can hamper the activation of EGFR and prevent filopodia formation on collagen in MDA-MB-231 cells (53).

Tumor inhibition by CD44 does not solely depend on HMW hyaluronan, as oligomerized hyaluronan (3–10 disaccharide units) can promote apoptosis through activation of caspase-3, and similarly, it can inhibit PI3K activation and AKT phosphorylation in murine mammary carcinoma cells and in HCT116 colon cancer cells (57). Similarly, treatment with hyaluronan oligos or CD44-blocking antibody stimulates production of the PTEN phosphatase (57).

Of interest, the SWI/SNF chromatin remodeling complex, the loss of which is associated with malignant transformation, has been shown to positively regulate *CD44* expression. The SWI/SNF subunits BRG-1 and BRM promote expression of *CD44* while inhibiting cyclin A expression. Concurrent with this, forced expression of cyclin E abrogates Brg-1 activity, a component of the SWI/SNF complex, and downregulates *CD44* expression (86–88).

CD44 has been implicated in the inhibition of angiogenesis, particularly by HMW hyaluronan engagement. HMW hyaluronan can inhibit induction of the immediate early genes c-fos and c-jun, and it can inhibit migration of cultured bovine aortic endothelial cells (43).

CD44 has also been shown to inhibit tumorigenesis during *in vivo* transformation. SV40-transformed *CD44*-null fibroblasts injected subcutaneously into nude BALB/C mice were highly tumorigenic, whereas the introduction of *CD44s* into these fibroblasts resulted in a dramatic inhibition of tumor growth (89). In our laboratory, we examined tumorigenesis and metastasis in *CD44*-null mice in the MMTV-PyV MT model, and we found that the loss of CD44, in contrast to the tumorigenicity of *CD44*-null fibroblasts, had no effect on tumor onset or growth but dramatically increased metastasis to the lung, suggesting that CD44 inhibits metastasis without regulating transformation (46). Of interest, we found that MMTV-PyV MT mice, which develop multifocal and highly metastatic mammary tumors, show strong expression of CD44 throughout the tumor epithelium of large tumors, a dichotomy that currently is not well understood.

CD44 Duality in Cancer Progression

The evidence reported here shows that CD44 supports signaling that both inhibits and promotes cancer progression. There are coherent themes, though, that suggest that pro- or antitumoral signaling is dictated by stromal cues. For example, HMW hyaluronan has been shown in several instances to enhance the metastasis-suppressing activity of CD44, whereas LMW hyaluronan does the opposite. Discrepancies stem in part from differences in cell line usage, antibody variability, culture conditions, and other experimental variability, but ultimately they reflect the inherent duality of this molecule and its function as a matrix-sensing molecule. Additional discrepancies among *in vivo* studies may stem from researchers examining CD44 at different stages of tumor progression. *In vivo* studies that showed a

protumorigenic role for CD44 focused on tumor progression in animals injected with cancer cells. In contrast, studies of CD44 in which tumorigenesis was driven in the background of a *CD44*-null mutation showed a protective role for CD44 in breast cancer, suggesting that CD44 may influence tumor growth or metastasis differently at different phases of tumor progression. CD44 may play cancer-type–specific roles in tumorigenesis and metastasis, however, because the loss of CD44 abrogated osteosarcoma metastasis in mice with the min mutation of the *APC* gene or the tm1 mutation of the *p53* gene (90).

Variability in CD44-mediated biology is also due to the expression of alternatively spliced isoforms. Some research suggests that variant expression is linked to increased metastatic behavior. For example, transfection of *CD44* variants into a nonmetastatic rat pancreatic carcinoma cell line rendered cells metastatic (81). In addition, CD44-mediated signaling has been linked to variant expression. CD44v3 was shown to interact with Rac and Rho Gef to promote cell migration and invasion (56). Conversely, another study showed that *CD44* variant expression is downregulated in human mammary epithelial cells induced to undergo an epithelial–mesenchymal transition, whereas the standard isoform is upregulated and required for epithelial–mesenchymal transitions in this system (91). The expression of *CD44* variants has also produced conflicting results with no definitive correlation between expression and clinical outcomes (discussed below). Although research shows that oncogenic signaling can promote alternative splicing of *CD44* (13), a full understanding of how variant expression is regulated under different conditions and how the *CD44* variants modulate cellular behavior has not yet emerged. The extensive splicing of this molecule makes CD44 difficult to study and undoubtedly contributes to some of the variability in research. For a more thorough discussion of this topic, the reader is directed to previous reviews (1, 52).

Histopathological CD44 in Human Cancer

Many histopathological studies have attempted to correlate CD44 expression patterns with breast cancer progression and metastasis, ultimately yielding contradictory results. This variability may be due to differences in histological technique and antibody usage, but more significantly, different groups have compared different types of mammary tumors. For example, some researchers graded invasive tumors, whereas others correlated CD44 expression with lymph node status. Furthermore, patients received different treatments, which were not always reported, and certain chemotherapeutics have been shown to alter the expression of CD44 (92). Given the high variability among studies thus far, CD44 expression may not be reliably used as a diagnostic tool; however, information garnered from these studies does provide valuable clues about the tumor-promoting and tumor-suppressing activities of CD44.

CD44 expression in tissues has primarily been detected by immunohistochemistry (IHC) and RT-PCR. IHC is less sensitive, but it allows the identification and enumeration of

cells. In neoplastic tissue, investigators have correlated CD44 isoform expression with overall survival (OS), disease-free survival (DFS), tumor grade (e.g., noninvasive, invasive, or invasive and node-negative or node-positive), and occasionally, histological grade. These studies are summarized in Table 1, which notes the tumor grade examined (e.g., benign or invasive) and the CD44 isoforms examined when that information was provided by the authors. Of interest, 8 out of 10 studies that focused on mammary tumors classified as either noninvasive or lymph node–negative showed that CD44s (and in some cases CD44v6) expression correlates with favorable prognosis or cellular differentiation, indicative of antitumoral activity (91, 93–99). The exception to this is a 1995 study by Kaufmann and colleagues (64), who examined tumors graded as node-negative and reported that CD44v6 expression correlates with poor OS. Six out of 11 studies that focused on mammary tumors classified as invasive, malignant, or with lymph node–positive status showed a correlation with unfavorable outcome, suggesting that CD44 promotes tumor progression (64, 91, 99–102). In 3 out of 11 studies, investigators found no correlation between CD44 expression and clinical outcome, but they did see increases in CD44 variant expression that correlated with increased malignancy (103–105). Of note, CD44 is often highly expressed in invasive cancer, but it does not correlate with clinical outcome, leaving open the question of the role of CD44 in metastatic progression.

CD44 as a Therapeutic Target

Research showing an association between CD44 and metastatic disease prompted several groups to target it therapeutically with monoclonal antibodies, mimetic peptides, or more recently, miRNA therapies that regulate *CD44* expression. The Met receptor, for example, is potent mediator of metastasis whose activation depends on CD44v6. The use of a CD44v6 exon–specific monoclonal antibody was shown to be extremely effective against metastasis in a rat model of pancreatic cancer (82). Based on these findings and the association between CD44v6 expression and tumor progression in squamous cell carcinoma (106), a humanized monoclonal antibody targeting CD44v6, bivatuzumab, coupled to a cytotoxic drug, mertansine, was used in phase I dose escalation studies in patients with head and neck squamous cell carcinomas. It was reported that 2 out of 20 of patients experienced stabilization and regression of tumors with low toxicity, and 1 patient died of toxic epidermal necrolysis, upon which the trial was terminated (73). A radiolabeled humanized CD44v6 antibody was also used in a pharmacodynamic study of patients with early-stage breast cancer, and it was well tolerated. Accumulation of the antibody was detected in nontumor areas, and as the antibody did not affect CD44v6 expression or tumor burden, it did not progress further (73, 107).

Additionally, the CD44v6 amino acid motif required for c-Met activation was identified (108), and a small peptide scanning this sequence completely abrogated c-Met activation and resulting cell migration. *CD44v6*-induced expression in nonmetastatic BSp73AS cells induced lung metastasis when injected into syngeneic rats, yet treatment with the CD44v6 peptides completely abolished metastatic dissemination (73). Similarly, the CD44v6 peptide was used to inhibit the cooperation of CD44v6 with Met and VEGFR2 in endothelial cells (75). The v6 blocking peptide effectively inhibited migration and tubular network formation in human umbilical vein endothelial cells (HUVEC), and it dramatically blocked vascularization of VEGF-stimulated HUVECs in matrigel plugs injected subcutaneously into nude mice. The peptide was also effective against angiogenesis and metastasis of pancreatic carcinoma cells in xenograft tumors, but it has not been used in clinical trials.

The miRNA miR34a was recently identified as a regulator of CD44. miR34a expression results in the degradation of CD44, resulting in decreased tumor growth and metastasis in mouse models of prostate treatment (4). Of interest, treatment with miR34a was found to increase survival in these mice, showing promise as a potential therapeutic target against CD44-driven tumors (4). This miRNA is thought to target the 3′UTR of CD44, which is a mechanism by which CD44 can increase its own translation while also binding to and inactivating multiple miRNAs. Conversely, the 3′-UTR of CD44 was recently found to inhibit tumorigenesis and angiogenesis and to increase cell sensitivity to docetaxel in MT-1 breast cancer cells (109).

Discussion of CD44's Role in Cancer

CD44 regulates critical aspects of metastatic disease, including transformation, growth, cell invasion and motility, and chemoresistance, and it is a marker of breast cancer stem cells (5). It is important to understand the complexities of this molecule given its ability to function at the center of multiple signaling highways and to act as a tumor microenvironment sensory tool. However, CD44-mediated biology goes beyond the complexity of a molecule that either promotes or inhibits cancer, because CD44 regulates cellular processes that can do both. Decades of research have shown that CD44 participates in major oncogenic signaling networks and complexes with oncogenes that promote every aspect of tumor progression. Conversely, CD44 signaling also mediates contact inhibition and inhibits cell invasion and angiogenesis. CD44 is extremely sensitive to changes in the microenvironment, and although a great deal is known about its biology, its reaction to changing extra- and intracellular conditions is still the subject of active research.

Although many of the contradictory findings published to date may be due to experimental and technical differences among studies, a picture has emerged suggesting that CD44 may function differently at different stages of cancer progression. For example, mice with germline disruptions of *CD44* display relatively mild phenotypes compared with mice in which tissue-specific CD44 function is disrupted at later phases of development or in adulthood, suggesting that the absence of CD44 in early development and a loss of CD44 function late in development are tolerated differently (52). In breast cancer, CD44 often correlates with a favorable

Table 1. The histopathology of CD44 in breast cancer

References	Benign or noninvasive	Invasive and node-negative	Invasive and node-positive	Isoforms examined	Correlations and conclusions	CD44 association
Joensuu et al. (101)	No noninvasive tumors were evaluated.	Evaluated 106 node-negative invasive carcinomas.	Evaluated 75 node-positive carcinomas	Examined CD44s expression.	Sixteen percent of the tumors examined had >90% positive expression for CD44s, and those tumors that were >90% CD44-positive were more often poorly differentiated, had higher mitotic counts, and were often ER-negative.	Unfavorable
Kaufmann et al. (64)	Three tumors with low histological grade.	Thirty-three lymph node–negative tumors; local invasion not reported.	Evaluated 100 primary invasive tumors, 12 local recurrences, and 18 lymph node metastases.	Examined CD44v3, v5, and v6.	CD44v6 was expressed in 84% of primary tumors and 100% of metastases and recurrences. CD44v6 expression correlated with poor OS. There was no correlation between other variants and OS.	Unfavorable
Friedrichs et al. (97)	Evaluated 108 node-negative samples by IHC.	Not reported.	119 node-positive patients by IHC and 43 high-risk cases by RT-PCR. Follow-up 7 years.	Examined CD44s, v4, v6, and v9.	No significant correlations between CD44s and CD44v9 with DFS or OS were observed, but they were more often expressed in lower pathological grade tumors. CD44v6 was associated with less aggressive tumors but did not correlate with OS or DFS.	Favorable
Diaz et al. (95)	NA	Evaluated 230 lymph node–negative invasive tumors.	NA	Examined CD44s and CD44v6.	High CD44s expression was correlated with increased DFS. CD44v6 expression	Favorable

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Table 1. The histopathology of CD44 in breast cancer (Cont'd)

References	Benign or noninvasive	Invasive and node-negative	Invasive and node-positive	Isoforms examined	Correlations and conclusions	CD44 association
		Mean follow-up of 15 years.			was not associated with clinical outcomes.	
Jansen et al. (98)	Evaluated 183 lymph node–negative samples.	Evaluated 136 node-positive samples. Mean follow-up of 128 months.		Examined CD44v6.	CD44v6 expression correlated with smaller tumor size and lymph node–negative status.	Favorable
Tokue et al. (99)	Examined breast tumors from 95 patients by RT-PCR and IHC. Did not mention tumor grade or invasive status.		Examined CD44v6 and CD44v2.		CD44v6 was expressed in 73% of tumors, and CD44v2 was expressed in 35% of tumors. CD44v6 expression was correlated with OS, whereas v2 expression was correlated with reduced OS.	Dependent on variant expression.
Bánkfalvi et al. (100)	Evaluated 152 breast carcinomas, including 20 DCIS and 19 LCIS.	Did not report lymph node status.	Evaluated 152 breast carcinomas, including 56 IDC and 17 ILC. Mean follow-up was 72 months.	Examined CD44v3, v4, v6, v7 and v9.	The loss of CD44v6 expression correlated with poorly differentiated tumors (grades 3 and 4) but was associated with favorable overall survival. Expression of CD44v4 and v7 correlated with lymph node–positive status, but it did not correlate with patient survival.	Dependent on variant expression.
Foekens et al. (96)	Evaluated 72 \noninvasive tumors.	Evaluated 165 node-negative invasive cases.	Evaluated 230 node-positive primary cases.	Examined CD44v6, v7/9, v9, and v10.	CD44v6 expression was associated with a favorable prognosis in node-negative patients. The other variants were not significantly associated with relapse-free survival.	Favorable

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Table 1. The histopathology of CD44 in breast cancer (Cont'd)

References	Benign or noninvasive	Invasive and node-negative	Invasive and node-positive	Isoforms examined	Correlations and conclusions	CD44 association
Bànkfalvi et al. (93)	Evaluated 142 breast carcinomas, including 19 DCIS and 9 LCIS.	Did not report lymph node status.	Evaluated 142 breast carcinomas, including 44 IDC and 17 ILC. Mean follow-up was 72 months.	Examined CD44v4, v6, and v7.	Lack of CD44v6 expression correlated with poor survival.	Favorable
Berner et al. (104)	Evaluated 59 pleural and peritoneal effusions, including benign effusions.	NA	Effusions including malignant or atypical cells.	Examined CD44s and CD44v3–10.	CD44s expression was positive in 94% of benign cells and 23% of malignant or atypical cells. CD44v3–10 was positive in 6% of benign cells and 55% of malignant or atypical cells. Expression of variants was higher in breast cancer than in corresponding normal cells.	Neutral
Morris et al. (105)	Evaluated 109 patients with stage 2 cancer, with a minimum 5-year follow-up, but did not differentiate between size and lymph node status.		Examined CD44s and CD44v6.		CD44s was detected in 26% of tumors and v6 was detected in 80% of tumors, independently of lymph node status. No association was observed between CD44s or v6 expression with DFS or OS.	Neutral
Berner et al. (94)	Evaluated 40 node-negative tumors, including histological grades 1–3.	N/A	Evaluated 68 node-positive tumors. Mean follow-up time was 67 months.	Examined CD44s, v5, v6, v7, and v3–10.	Increased CD44s mRNA correlated with lower pathological grade, DFS, and OS. CD44s and v6 mRNA correlated with lower pathological grade. The other variants did not correlate with histological subtype, OS, or DFS.	Favorable

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Table 1. The histopathology of CD44 in breast cancer (Cont'd)

References	Benign or noninvasive	Invasive and node-negative	Invasive and node-positive	Isoforms examined	Correlations and conclusions	CD44 association
Auvinen et al. (103)	Evaluated 15 benign and 6 premalignant breast tumors.	Evaluated 30 cases of IDC, 12 cases of LDC, and 12 other invasive breast tumors.		Examined CD44s, v3, and v6.	CD44s and v3 were lowly expressed in benign or premalignant tumors, and v6 was expressed in 20–30% of ductal epithelium. CD44s, v3, and v6 were upregulated in invasive carcinomas, but the authors reported no correlation with DFS or OS.	Not assessed for clinical outcome.
Yu et al. (102)	None evaluated.	Evaluated 60 invasive node-negative carcinomas.	Evaluated 38 node-positive invasive ductal carcinomas.	Examined CD44v6 and found 38.8% of samples positive for CD44v6 expression.	CD44v6-positive cells correlated with shorter DFS and OS, and they were an independent biological marker for prognosis.	Unfavorable
Brown et al. (91)	Evaluated 5 normal samples and breast tumors graded 1.	Evaluated 27 tumors, including grades 2 and 3.		Examined CD44s and CD44v5, v6.	CD44s did not differ between normal breast tissue and grade 1 tumors. CD44s was highly elevated in grades 2 and 3 tumors. CD44v5 and v6 expression did not differ between tumor grades.	Unfavorable

Abbreviations: DCIS, ductal carcinoma *in situ*; IDC, invasive ductal carcinoma; LCIS, lobular carcinoma *in situ*; LDC, lobular invasive carcinoma.

prognosis in early noninvasive cancer, and indeed, CD44 may not function as a marker of tumor-initiating cells at this phase in breast cancer progression (110). CD44 is not a consistent cancer stem cell marker in luminal breast cancer subtypes, but it was shown in several studies to be highly overexpressed and to serve as a cancer stem cell marker in basal (particularly triple-negative) subtypes (110, 111). Of interest, myoepithelial cells isolated from salivary myoepitheliomas shed extracellular CD44, which contributes to the anti-invasive and antiangiogenic properties of this cell type (112). Although its role is not fully understood, the myoepithelium may serve a protective function in early stages of transformation (113). Our current understanding of the hierarchy of cancer progression suggests that basal subtypes arise from luminal progenitors (114). Although the intermediate steps of this transition are not defined, the dualistic nature of CD44 suggests that the cell-type–specific expression of oncogenic mediators may regulate this transition, or that luminal and basal breast cancers represent distinct diseases with unrelated cellular origins.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Understanding the Dual Nature of CD44 in Breast Cancer Progression

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