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REVIEW

The role of CD44 in epithelial–mesenchymal transition and cancer development

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Abstract: CD44, a multi-structural and multifunctional transmembrane glycoprotein, was initially identified as a receptor for hyaluronan that participates in both physiological and pathological processes. CD44 is found to be closely linked to the development of various solid tumors. Molecular studies have revealed that high CD44 expression was correlated with the phenotypes of cancer stem cells and epithelial–mesenchymal transition, thereby contributing to tumor invasion, metastasis, recurrence, and chemoresistance. Correspondingly, blockade of CD44 has been demonstrated to be capable of attenuating the malignant phenotype, slowing cancer progression, and reversing therapy resistance. Clinical analyses showed that high CD44 expression is associated with poor survival of various cancer patients, indicating that CD44 can be a potential prognostic marker. In this review, we summarize recent research progress of CD44 on tumor biology and the clinical significance of CD44.

Keywords: CD44, epithelial–mesenchymal transition, tumor progression, prognosis

Introduction

CD44, a complex transmembrane glycoprotein, also called Hermes antigen, homing cell adhesion molecule, HUTCH-1, phagocytic glycoprotein-1, lymphocyte-homing receptor, and ECM-III, is encoded by the *CD44* gene on chromosome 11,[1] which consists of 20 exons.[2] Transcripts for the *CD44* gene undergo complex alternative splicing, which results in many functionally distinct isoforms, such as CD44 standard isoform (CD44s) and CD44 variant isoform (CD44v).[3] The smallest CD44s is encoded by constant exons 1–5 and 16–20 and translated into a polypeptide of a molecular mass of 80–85 kDa (Figure 1).[4] Exon 1 is an N-terminal signal sequence, exons 2 and 3 are a link module that binds to hyaluronic acid (HA), exons 4, 5, 16, and 17 compose a stem region, exon 18 makes up a single-pass transmembrane domain, and exon 20 forms a cytoplasmic domain.[4] Exon 19 is spliced out in all forms of *CD44* cDNAs.[4] Alternative splicing is the basis not only for the structural but also for functional diversity of this protein. Multiple CD44v is produced by insertion of variant exons (v1–v10) at the proximal plasma membrane external region (Figure 1).[4] CD44s is found in most cells,[5] while CD44v is expressed primarily on cells during inflammation and on tumor cells.[6–8] CD44 protein consists of a short C-terminal cytoplasmic domain, a transmembrane domain, and seven extracellular domains which contains an N-terminal HA-binding link-homology module and stem region (Figure 2).[9,10]

CD44 was initially identified as a receptor for HA and lymphocyte-homing receptor[11] that participates in both physiological and pathological processes, including cell adhesion, angiogenesis, inflammation, and tumor development.[12,13] Since CD44 expression in several tumor types was found to be different from that in normal counterparts, studies have been subsequently carried out to investigate the role of
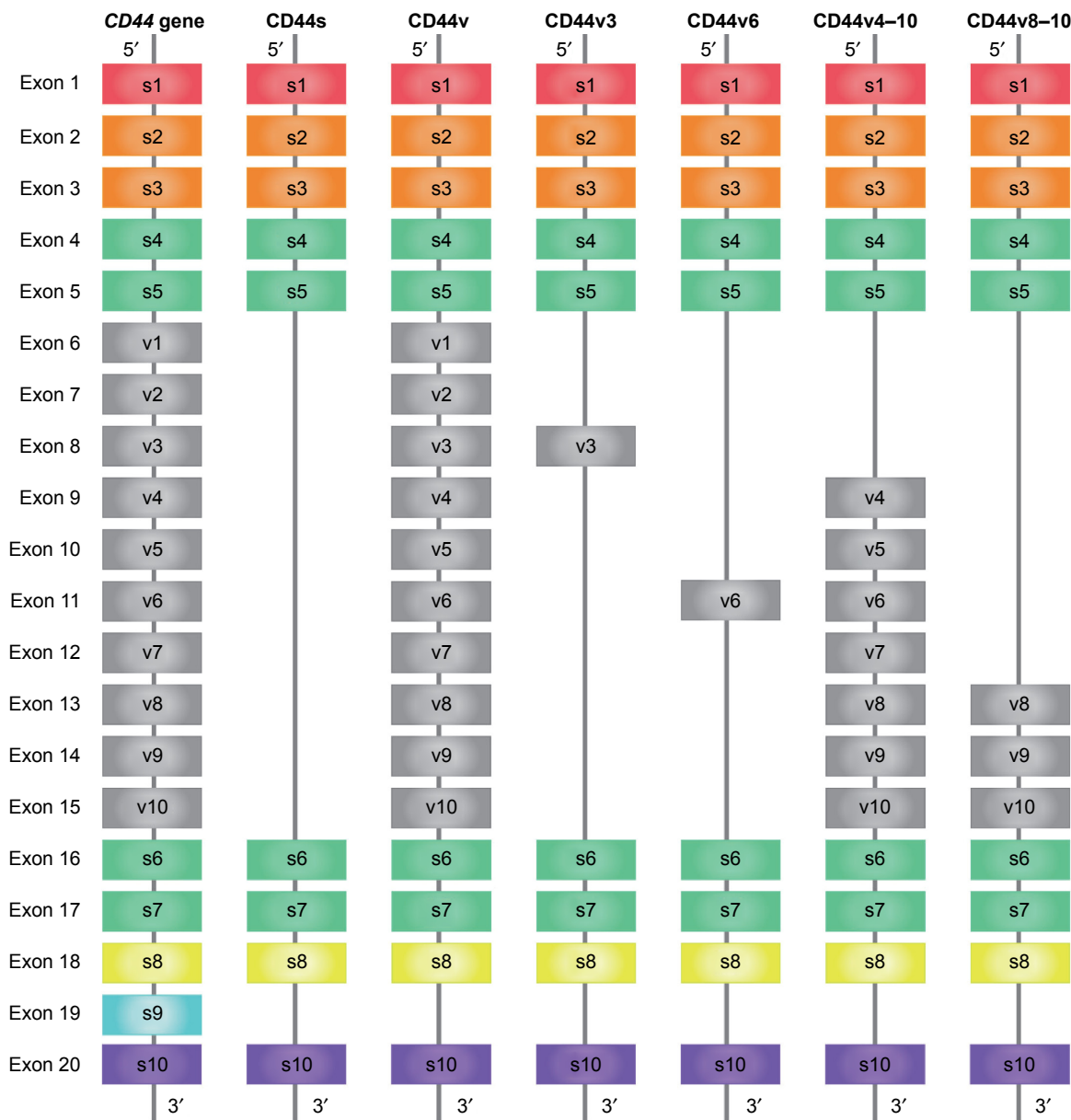
Figure 1 Schematic structures of alternative splicing in CD44.

Notes: *CD44* gene contains 20 exons.[10] Alternative splicing gives rise to CD44s and CD44v. Exon 1 is an N-terminal signal sequence, exons 2 and 3 are a link module that binds to HA, exons 4, 5, 16, and 17 compose a stem region, exon 18 makes up a single-pass transmembrane domain, and exon 20 forms a cytoplasmic domain.[10] Exon 19 is spliced out in all forms of *CD44* cDNAs.[4] The smallest CD44s is encoded by constant exons 1–5 and 16–20.[10] Multiple CD44v is produced by insertion of variant exons (exons 6–15), typically identified as v1–v10. CD44 variant can contain one or more variant regions, such as CD44v3, CD44v6, CD44v4–10, or CD44v8–10. Figure adapted from Chanmee T, Ontong P, Kimata K, Itano N. Key roles of hyaluronan and its CD44 receptor in the stemness and survival of cancer stem cells. *Front Oncol*. 2015;5:180. Available from: http://journal.frontiersin.org/article/10.3389/fonc.2015.00180/abstract. Copyright © 2015 Chanmee, Ontong, Kimata and Itano.[10]

Abbreviations: CD44s, CD44 standard; CD44v, CD44 variant; HA, hyaluronic acid; s, standard; v, variant.

CD44 in cancer development for decades. The expression of CD44 is regulated by many extracellular or intracellular factors. For example, CD44 is a target of the Wnt pathway.[14] Currently, extensive research reveals that CD44 is critical in epithelial–mesenchymal transition (EMT). CD44 has also been reported to be one of the key biomarkers for isolation and characterization of cancer stem cells (CSCs). Recent studies showed that specific targeted knockdown of CD44 attenuated cancer progression,[15,16] which suggests that CD44 may be a promising target of cancer treatment. Most studies indicate that high CD44 expression is closely linked to clinical parameters,[17–19] and a promising prognostic indicator in several solid tumors, including lung cancer,[20] breast cancer,[21] prostate cancer,[22] gastric cancer,[23] colon cancer,[24] malignant glioma,[25] and ovarian cancer.[19] In this review, we summarize new insights into the regulation of CD44, the involvement of CD44 in EMT and CSCs, as well as the relevance of CD44 for clinical outcome.
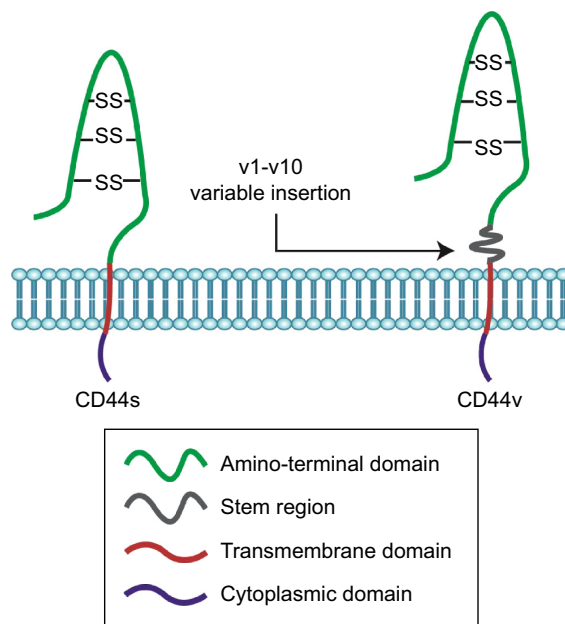
Figure 2 Key domains of CD44.
Notes: CD44 protein consists of a short C-terminal cytoplasmic domain, a transmembrane domain, and seven extracellular domains which contain an N-terminal HA-binding link-homology module and stem region. In CD44v, variant exons are inserted into the stem region at the proximal plasma membrane external region. Figure adapted from Misra S, Hascall VC, Markwald RR, Ghatak S. Interactions between hyaluronan and its receptors (CD44, RHAMM) regulate the activities of inflammation and cancer. *Front Immunol.* 2015;6:201. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2015.00201/abstract. Copyright: © 2015 Misra, Hascall, Markwald and Ghatak.[4]
Abbreviations: HA, hyaluronic acid; CD44s, CD44 standard; CD44v, CD44 variant; SS, disulfide bond; v, variant.

Regulation of CD44 expression

CD44 expression is regulated by many extracellular or intracellular factors during tumor development. Activation of STAT3 signaling promotes stem cell-like traits by upregulating CD44, and a feedback regulation between STAT3 and CD44 is observed. Interleukin-6 exposure activated interleukin-6/STAT3 signaling in CD44(−) T47D cells and induced upregulation of CD44 protein expression, resulting in the enrichment of CD44(+) cell population.[18] In breast cancer MDA-MB-231 cells, CD44 promoted the phosphorylation of STAT3 by interacting with STAT3, and then the pSTAT3 moved to nucleus and combined with NF-κB to activate hTERT, which in turn increased CD44 expression (Figure 3).[26] Shang et al revealed that transforming growth factor β1 (TGF-β1) upregulated CD44 expression in prostate cancer LNCaP and CWR22RV1 cells.[22] In agreement with these findings, blocking TGF-β1 signaling by using SB431542 in these cells decreased CD44 expression.[22] Similar phenomena were also reported in hepatocellular carcinoma (HCC) and oral cancer.[27,28]

MicroRNA (miRNA) profiling has revealed that several miRNAs are involved in the regulation of CD44 expression in a variety of cancer cells. Song et al have demonstrated that miR-9 promotes CD44 expression by inducing β-catenin nuclear translocation. Correspondingly, knocking down miR-9 in esophageal squamous cell carcinoma (SCC) KYSE30 and KYSE510 cells decreased the CD44 protein abundance.[29] However, some other members of the miRNA family, including miR-34a[17] and miR-203,[30] regulate CD44 in an opposite manner. For example, miR-34a negatively correlated with the expression of CD44.[17] Restoration of miR-34a was capable of decreasing CD44 protein in bladder cancer cells by directly and specifically interacting with the target site in the CD44′-UTR.[17] However, a specific miRNA-targeting CD44 expression has not been fully identified yet.

There are many other molecules regulating CD44 expression, such as VCAM-1 (CD106),[31] actin-binding Fascin,[32] and cyclin-dependent kinase-like 2.[33] Generally speaking, CD44 expression is regulated by a complicated network through interacting with other pathways to convey its function in many tumor types.

CD44 and EMT

EMT, a tightly regulated and highly conserved cellular process for a cell type changing from an epithelial phenotype to a mesenchymal phenotype, results in the cell acquiring fibroblast-like properties. It plays a crucial role not only in normal embryogenesis and tissue remodeling but also in the progression of various diseases including inflammation, fibrosis, and especially, in tumor proliferation, invasion, metastasis, recurrence, and drug resistance.[34–38] EMT is involved in the acquisition of stemness of epithelial tumor cells, which confers cells with aggressive traits and an invasive phenotype that may result in tumor recurrence and metastasis.[33,39] EMT promotes CD44 expression. Mesenchymal genes, such as *TWIST1*,[34,40] *SNAI1*,[41,42] *ZEB1*,[15,43] and *SLUG*,[44] are positively correlated with CD44 expression. Li and Zhou revealed that expression of Twist dramatically elevated the level of CD44 in cervical carcinoma HeLa cells and breast cancer MCF-7 cells through activation of β-catenin and the Akt pathway.[34] Twist1-induced CD44 expression is also found in head and neck SCC.[40] Knockdown of *ZEB1* by siRNA reduced CD44 expression in prostate cancer DU-145R and PC-3R cells and prostate tumor samples.[43] Furthermore, overexpression of Slug in CD24+/CD44− breast cancer MCF-10A and MCF-7 cells gave rise to a subpopulation of CD24−/CD44+ cells, and this phenotype conferred enhanced mammosphere forming ability.[44] In contrast, E-cadherin, an epithelial marker, participates in the negative regulation of CD44 expression. In prostate cancer PC3 cells, stable knockdown of E-cadherin increased the CD44 protein abundance.[42]
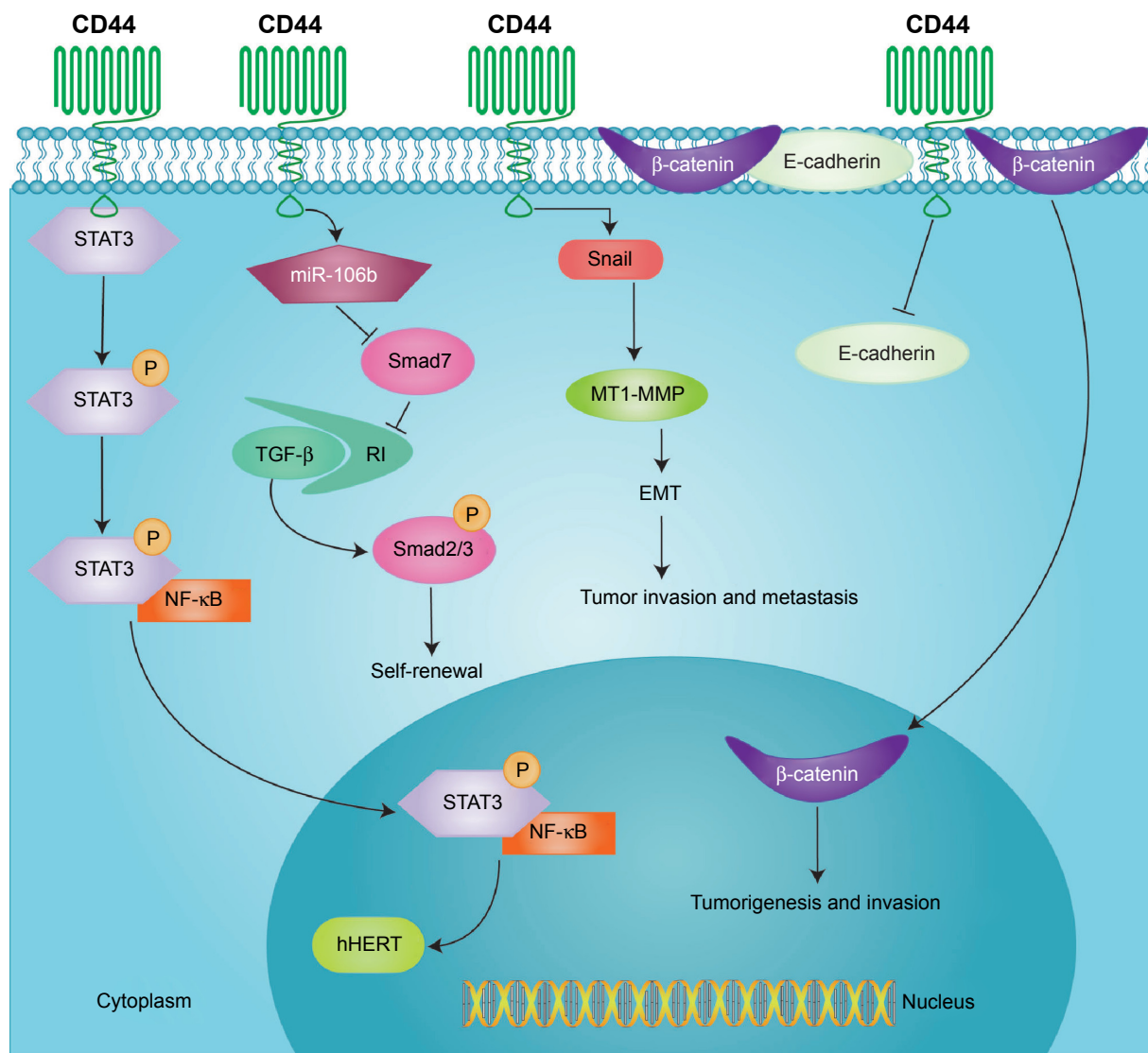
Figure 3 Representative signal pathways induced by CD44.

Notes: CD44 promotes phosphorylation of STAT3, leading to the nuclear translocation of pSTAT3 and activation of hHERT.[26] Upregulation of miR-106b family by CD44 represses inhibitory Smad7, which inhibits TGF-β/Smad2/3 signaling by suppressing TGF-β receptor I (RI) and then enhances self-renewal of cancer cells;[47] Snail1 upregulates the expression of membrane type 1-matrix metalloproteinase (MT1-MMP), which promotes tumor invasion.[50] CD44 disassociates the formation of the membrane-associated E-cadherin–β-catenin complex, releasing β-catenin translocates into nucleus and activating genes related to cell invasion and migration.[46]

Abbreviations: TGF-β, transforming growth factor β; EMT, epithelial–mesenchymal transition; miR, micro RNA; P, phosphorous status of moleculars; RI, receptor I; MT1-MMP, membrane type 1-matrix metalloproteinase.

On the other hand, CD44 also promotes EMT in many cancer types, such as colon cancer,[45,46] gastric cancer,[47] pancreatic cancer,[48–50] prostate cancer,[22] liver cancer,[28] and glicoma,[51] by upregulating mesenchymal markers and downregulating epithelial markers. For example, ectopic CD44 expression in SW480 cells induced the EMT phenotype, while knockdown of CD44 attenuated EMT. CD44 upregulated the expression of EGFR, leading to the activation of PI3K/Akt and expression of glycogen synthase kinase-3-beta. CD44 inhibited the formation of the membrane-associated E-cadherin–β-catenin complex, which resulted in the nuclear translocation of β-catenin and

transcriptional activation of genes related to cell invasion and migration (Figure 3).[46] However, a different mechanism was observed in gastric cancer. The miR-106b family, including miR-106b, miR-93, and miR-25, was increased in stem-like cells with CD44(+) phenotype compared with CD44(−) cells. The upregulation of the miR-106b family repressed inhibitory Smad7, resulted in the activation of TGF-β/Smad signaling, and enhanced EMT in CD44(+) cells (Figure 3).[47] In a subset of human PanIN cells which are capable of invading the surrounding stroma, oncogenic K-Ras upregulated ATDC, and then ATDC increased CD44 via activation of β-catenin signaling, leading to the induction

of an EMT phenotype characterized by expression of Zeb1 and Snail1.[48]

In addition, different isoforms, including CD44s[21,52,53] and CD44v6,[54] were reported to determine the regulation of EMT. For example, a switch in CD44 isoform expression from CD44v to CD44s was essential for EMT in breast cancer cells. When nontumorigenic epithelial cell lines were induced to go through EMT with different EMT triggers, isoform transition from CD44v to CD44s was found in all these cells by various approaches. Switch from CD44v to CD44s activated Akt signaling, which activated EMT.[21] Similarly, a high CD44s expression in HCC was significantly associated with the EMT expression profile.[52] In addition, CD44v6 expression inversely correlates with E-cadherin expression and positively correlates with Vimentin expression in colon cancer.[54]

CD44 displays a close association with EMT by combination with other molecules such as CD29[55] and CD24.[56–58] CD29^{high}/CD44^{high} cells display molecular traits of EMT in oral SCC.[55] CD24^{+}/CD44^{+} phenotype is also positively correlated with EMT in pancreatic cancer.[56] In breast cancer cell lines SUM149, HCC1954, and MCF-7, gene-expression profiling revealed that CD24^{–}/CD44^{+} cells were enriched for expression of EMT-associated genes, including Vimentin, Zeb1, Zeb2, β-catenin, and matrix metalloproteinase-1.[57]

CD44 in self-renewal and tumorigenesis

Strong evidence supports that CSC drives tumor initiation and metastasis.[59] CD44 is selected as a surrogate marker for CSC in many types of cancers. Previous studies have shown that as few as 100 CD44(+) colorectal cancer cells isolated from patients were able to develop into a heterogeneous tumor, and that spheroids derived from a single CD44(+) cancer cell could recapitulate the heterogeneous hierarchy of tumor cells.[60,61] CD44(+) colon cancer cell population displayed higher soft agar colony-forming ability and tumorigenicity in vivo, compared with CD44(–) cells.[45,62] When CD44(+) or CD44(–) breast cancer cells were injected into the mammary fat pads of nonobese diabetic/severe combined immune-deficient (NOD/SCID) mice, significantly enhanced tumorigenic and proliferation potential was observed in CD44^{+} cell, compared to CD44^{–} cells.[18] CD44 level was higher in the lower chamber cells which displayed high tumorigenic characteristics, compared with the upper chamber cells and the bulk pancreatic cancer Panc-1 cells. Molecular analysis showed that self-renewal pathway (Notch, hedgehog, and Wnt)-related proteins were upregulated in

the lower chamber cells.[49] High CD44 expression is closely correlated with enhanced spheroid colony formation in bladder cancer[15] and gastric cancer.[47]

CD44 isoforms were reported to be involved in tumorigenesis. Immunostaining for CD44v6 on formalin-fixed, paraffin-embedded sections of colorectal carcinomas showed that the upregulation of CD44v6 through nuclear β-catenin activation may contribute to the formation of tumorbudding.[63] Lau et al showed that ectopical expression of CD44v8–10, not CD44s, is closely linked to enhanced tumor-initiation ability of gastric cancer cells.[64] CD44v8–10 could rescue the attenuated tumor-initiating potential caused by silencing of total CD44.[64]

In addition, CD44 displayed its effect on cancer tumorigenesis by association with other molecules. Sorted CD29^{high}/CD44^{high} A431 cells showed higher proliferating ability in vitro and in NOD/SCID mice compared with CD29^{low}/CD44^{low} cells.[55] CD24^{–}/CD44^{+} subpopulation displayed enhanced ability of tumorigenesis.[34,65–67] Using mammary fat pad injection in C57BL/6 SCID, CD24^{–}/CD44^{+} cells showed much more enhanced ability of forming solid tumor than that of unmanipulated BT-20 cells; these findings indicate that CD24^{–}/CD44^{+} subpopulation had stronger tumorigenicity.[65] Therefore, the expression of CD44 plays a key role in self-renewal and tumorigenesis in certain cell types.

CD44 on adhesion, invasion, and metastasis

CD44 plays pivotal roles in promoting tumor invasion and metastasis by contributing to adhesion of tumors cells to endothelium and fibronectin-enriched matrices.[68] CD44v possesses E-selectin ligand activity; expression of CD44 in both breast and colon cancer cell enhances adhesion to endothelial cell and correlates with metastasis potential.[69,70] CD44 potentiated the adhesion of basal-like breast cancer cell to endothelium and fibronectin in an alpha5B1-integrin-dependent manner, while CD44 knockdown attenuated adhesion ability.[68] Silencing CD44 expression attenuated adhesion to endothelial cells and reduced invasion; however, no effect on cancer cell proliferation was observed. In vivo study demonstrated that elevated CD44 expression enhanced post-intravasation events and distant metastasis in mouse model.[71] In glioblastoma multiforme, a highly invasive brain tumor, decreased CD44 expression reduced cell adhesion to HA, and CD44/HA association contributed to the mechanosensing and invasive ability.[72] The low-density LRP-1 regulates the adhesion and deadhesion balance in cancer cell. LRP-1-mediated internalization of CD44

determines the adhesive function of cancer cell.[73] Besides, tumor suppressor gene *FOXP3* repressed CD44 protein expressions to suppress adhesion, resulting in reduced invasion and metastasis of human breast cancer cells.[74] Recent study showed that aggressive cancer cell acquired the ability to transdifferentiate into endothelial features and form vasculogenic networks. CD44/c-Met signaling plays a critical role in this plasticity.[75]

Increasing evidence indicates that CD44 promotes tumor invasion and metastasis in multiple cancer types, including bladder cancer,[17] breast cancer,[76–79] prostate cancer,[80] pancreatic cancer,[48,50] and ovarian cancer.[81] Cho et al found that CD44 expression was positively correlated with the invasion and metastasis ability of colon cancer SW480 cells.[46] CD44 overexpression conferred cells with increased cell invasion, whereas knockdown of *CD44* by shRNA attenuated cell invasion in 24 hours after cell plating,[46] as evaluated by matrigel invasion assay. The mechanism involved in the regulation of migration and invasion in colon cancer cells may depend on the inhibition of the association of the membrane-located E-cadherin and β-catenin by CD44.[46] Silencing of *CD44* in human bladder cancer 5637 and T24 cells inhibited angiogenesis, migration, and invasion of these cells.[17] CD44 upregulation and nuclear β-catenin conveyed the enhanced invasion ability of MCF-7-14 breast cancer cells and their invasive clone CL6 cells. CD44 also promotes invasion and metastasis of breast cancer cells by modulating c-Src transcription[78] or upregulating serine protease and collagen-degrading enzymatic expression and activity.[79] Klarmann et al found that only the DU145 and LNCaP cells with CD44(+) phenotype demonstrated invasive activity on matrigel, while CD44(+) and CD44(−) cells showed equal migration across the control membrane in response to serum.[80] Gao et al found that the level of CD44 is much higher in synchronous metastasis than primary ovarian cancer tissue, and downregulation of CD44 inhibited the migration and invasion capabilities of ovarian cancer cells (SKOV-3TR and OVCAR8TR cells).[81]

CD44 isoforms also affect invasive function in several tumor types. In liver cancer, high expression of CD44s was significantly and positively correlated with HCC invasive macroscopic appearance, intrahepatic dissemination, and frequent vascular invasion.[52] The knockdown of CD44v6 in human colon carcinoma LoVo and HCT116 cells decreased function with HGF-induced cell migration.[54]

CD44 is also combined with some other molecules to promote invasion and metastasis. MCF-7-14 cells, which had enhanced migratory and invasive ability compared with MCF-7 cells, displayed increased CD44 expression and decreased CD24 expression compared with MCF-7 cells.[16] CD44–podoplanin interaction promotes directional migration in SCC cells and plays a role in driving tumor cell migration during malignancy.[82]

CD44 and cancer therapy

CD44 expression correlates with resistance to chemotherapy and radiotherapy. Many reports support that functional inhibition of CD44 at gene or protein level reverses some malignant behaviors and sensitizes to therapy. For example, knockdown of CD44 in liver tumor HLE cells sensitized these cells to sorafenib-induced cell death, accompanied with decreased levels of anti-apoptotic proteins (MCL-1 and Survivin). Further analysis demonstrated that this effect depended on TGF-β signaling.[28] CD44 level was also found to be higher in the lower chamber cells which display significantly more gemcitabine resistance ability, compared with the upper chamber cells and the bulk Panc-1 cells.[49] CD44 also accounts for resistance to doxorubicin in patients with breast cancer[18] and to sunitinib in clear cell renal cell carcinomas.[83] Gao et al found that only paclitaxel-resistant ovarian cancer cells SKOV-3TR and OVCAR8TR exhibited strong expression of CD44, while the paclitaxel-sensitive ovarian cancer cells (SKOV-3 and OVCAR8) exhibit normal level of CD44 expression.[81]

In addition, it was found that the subset of CSCs with CD44^{high}/CD24^{low} cell-surface antigen was more resistant to cancer chemotherapy, radiotherapy, and endocrine therapy than the major population of cancer cells which were more differentiated in human breast tumors.[84] Therefore, CD44 may be a potential target for cancer treatment. Recent studies showed that CD44 knockdown suppressed spheroid colony formation and attenuated cancer progression in bladder cancer T24-L cells (lung-metastatic T24).[15] Besides, CD44 isoforms are also capable of modifying therapeutic effects.[85] Knockdown of CD44v6 in multiple prostate cancer cell lines reduced sphere formation, inhibited invasive abilities, and enhanced chemo-/radiosensitivity. Molecular analyses revealed the downregulation of PI3K/Akt/mTOR and Wnt/β-catenin signaling pathway.[86]

Anti-CD44 mAb significantly inhibited cell migration and invasion of breast cancer MCF-7 cells by inducing CD44 degradation from the cell surface,[16] indicating that CD44 may be a novel molecular target. Also, it was found that CD44 was a crucial regulator of acute myeloid leukemia stem cells, and antibody to CD44 could dramatically reduce leukemic repopulation in NOD/SCID mice transplanted with human acute myeloid leukemia.[87]

Clinical significance of CD44

Accumulating evidence demonstrates that CD44 is closely linked to clinical features of various cancer types, including prostate cancer,[22] gastric cancer,[23] malignant glioma,[25] colon cancer,[54] kidney cancer,[83] and breast cancer.[16,88] Correlation analyses of CD44 expression in prostate cancer tissues indicated that the high CD44 expression was significantly associated with biochemical recurrence and distant metastasis. Thus, CD44 may be a poor prognostic marker of prostate cancer.[22] High expressions of CSC marker CD44 in gastric cancer patients with curative resection were prominent in early recurrence.[23] Upregulation of CD44 may also be a potential predictive and therapeutic target for breast cancer metastasis.[16] It is noteworthy that sunitinib treatment of metastatic clear cell carcinoma induced CD44 expression in tumor tissues and high CD44 expression was associated with poor treatment outcome.[83]

Isoforms of CD44 predict clinical outcome. High CD44s expression was detected in the locally recurrent HCCs after local ablation therapy (LAT) compared to initial HCCs. In addition, high CD44s expression was associated with the intrahepatic dissemination of HCC after LAT. These observations suggested that high CD44s expression was an aggressive factor for recurrence after LAT for HCC.[52] Similarly, CD44s expression was upregulated in high-grade human breast tumors.[21] However, a contrary observation was also reported. Immunohistochemical analysis of 60 breast cancer tissues showed that CD44s negatively correlated with tumor diameter and tumor-node-metastasis TNM stage, but CD44v6 positively correlated with tumor diameter and tumor-node-metastasis stage.[89] In addition, multivariate analysis demonstrated that high CD44v6 expression was an independent poor prognostic factor for disease-free survival and overall survival (OS) in colorectal cancer.[54] Saito et al found that a high level of CD44v6 expression was inversely correlated with histological differentiation of the tumor in colon cancer cell line LoVo and HCT116 and it could independently predict a poor prognosis in disease-free survival and OS.[54] Analysis of immunohistochemical staining for CD44v9 in 333 gastric cancer tissues found that the positive expression rates of CD44v9 in tumor were higher than those in nontumor tissues. Moreover, CD44v9 expression level correlated with progression. Pathological analyses indicated that intestinal subtype or well-differentiated gastric cancer showed higher CD44v9 in comparison with diffuse-type or poorly differentiated gastric cancer. Importantly, the strong positive expression in early gastric cancer indicated poor prognosis and appeared to be associated with lymph node metastasis.[90]

However, some studies found that CD24^{+}/CD44^{−} are indicator for poor prognosis in early invasive breast cancer,[91] or CD44 predicts a better OS, which is opposite to most results of CD44. Generally speaking, CD44 is indicated to be a promising biomarker for diagnosis[92] and prognosis.[93,94]

Conclusion

Above all, extensive studies of CD44 have provided new insights into the role of CD44 in cancer. CD44 plays an important role in cancer development, partly through regulating EMT and other pathways (Figure 3), and it could be a useful prognostic marker for various cancer types. However, opposite results were also reported.[88] It remains a challenge to determine which isoforms are more important in cancer development or which molecules associate with CD44. Specific antibody targeting to CD44 has acquired promising effect in some preclinical studies, but further analyses are still required before translation to clinic trial.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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