

Published in final edited form as:

J Invest Dermatol. 2009 June ; 129(6): 1321–1324. doi:10.1038/jid.2009.13.

Shedding Light on Proteolytic Cleavage of CD44: The Responsible Sheddase and Functional Significance of Shedding

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Abstract

CD44 is the major cell-surface receptor for hyaluronan, which is implicated in cell–cell and cell–matrix adhesion, cell migration, and signaling. Studies have shown that CD44-dependent migration requires CD44 to be shed from the cell surface and that matrix metalloproteinase–mediated cleavage may provide an underlying mechanism. However, the full spectrum of proteases that may participate in CD44 shedding has yet to be defined. In this issue, Anderegg *et al.* demonstrate that ADAM10, but not ADAM17 or MMP14, mediates constitutive shedding of CD44 in human melanoma cells and that knockdown of ADAM10 blocks the antiproliferative activity of the soluble proteolytic cleavage product of CD44.

Roles of CD44 and ADAM10 in melanoma progression

Studies have suggested an important role for CD44 in melanoma growth and progression. Thus, increased expression of CD44 and CD44–hyaluronan (HA) interaction correlate, respectively, with melanoma progression and metastatic proclivity of melanoma cells (De Wit *et al.*, 1996). CD44 mediates HA-induced melanoma cell proliferation (Ahrens *et al.*, 2001a), and CD44–HA interaction is required for melanoma development in mouse models (Bartolazzi *et al.*, 1994). Functional blocking anti-CD44 antibody inhibits growth and metastasis of human melanoma cells (Guo *et al.*, 1994), and soluble CD44 inhibits melanoma tumor growth by blocking HA binding to cell-surface CD44 (Ahrens *et al.*, 2001b). In addition, hepatocyte growth factor engagement of its receptor, c-Met, upregulates expression of CD44v6 in murine melanoma cells (Recio and Merlino, 2003), and HA-mediated interaction between CD44 and epidermal growth factor receptor promotes melanoma cell motility by activating protein kinase C signaling (Kim *et al.*, 2008).

ADAM (a disintegrin and metalloproteinase) is a family of cell-surface proteases that are related to matrix metalloproteinases (MMPs) and contain several defined functional domains, including metalloproteinase, disintegrin, cysteine-rich, and transmembrane domains, as well as a cytoplasmic tail. ADAMs serve as the major class of sheddases for many important cell-surface receptors, and they play an essential role in regulating interactions between tumor cells and their microenvironment and in initiating the activation of key signaling pathways.

Expression levels of many ADAM family members are elevated in human cancers, and studies

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CONFLICT OF INTEREST

The authors state no conflict of interest.

have suggested important roles for ADAMs in cancer growth and progression (reviewed by Mochizuki and Okada, 2007). Currently, little is known about the role of ADAM10 and the other ADAM family members in melanoma initiation and progression. However, ADAM15 inhibits B16F10 pulmonary metastasis, and the disintegrin domain of ADAM15 displays an antiproliferative effect on melanoma cells (Murphy, 2008). Additional studies are required to determine the level of functional involvement of ADAM family proteases in the pathogenesis of human melanoma.

CD44 shedding and soluble CD44

Shedding of cell-surface adhesion receptors plays an important role in modulating cell–cell and cell–matrix adhesion, as well as in signaling initiated by interactions of adhesion receptors and their ligands in the microenvironment. CD44 shedding is observed in a variety of human tumors *in vivo* (Okamoto *et al.*, 2002). Elevated soluble CD44 (solCD44) levels in serum correlate with tumor burden and metastatic potential of gastric and colon cancer, as well as unfavorable outcomes in non-Hodgkin's lymphoma (reviewed by Nagano and Saya, 2004). In addition, increased levels of soluble CD44 variant v6 (solCD44v6) are associated with increased tumor size, lymph node metastasis (Mayer *et al.*, 2008), and resistance to chemotherapy (Kopp *et al.*, 2001) in breast cancer patients.

CD44 shedding is triggered and regulated by multiple signaling pathways, including activation of protein kinase C, Ca²⁺ influx, and activity of small GTPases, including that of Ras (reviewed by Nagano and Saya, 2004). Epidermal growth factor and heregulin can induce CD44 shedding, whereas blocking the activity of ErbB-2 by trastuzumab inhibits shedding (Pályi-Krekk *et al.*, 2008), which may contribute to the mechanisms underlying the antitumor efficacy of monoclonal antibodies that target ErbB receptor tyrosine kinases. In addition, oligosaccharides of HA and chondroitin sulfate E trigger shedding of CD44 from the tumor-cell surface through the activity of unidentified proteases (Sugahara *et al.*, 2008).

CD44 is composed of an extracellular (ecto-) domain that contains an HA-binding cartilage link protein–related domain and a membrane-proximal region, a transmembrane domain, and a COOH-terminal cytoplasmic tail. The cytoplasmic tail interacts with a number of cytoskeletal proteins, including the Band 4.1 superfamily members: the ezrin-radixin-moesin family proteins and merlin. CD44 is sequentially cleaved proteolytically, first within the ectodomain, which results in the formation of a soluble extracellular fragment of CD44 (solCD44), and then within the intracellular domain (ICD), which produces an intracellular fragment (CD44_{ICD}). CD44_{ICD} can translocate into the nucleus to regulate transcription of target genes, including *CD44* itself, which provides a positive feedback regulatory mechanism for CD44 expression (Nagano and Saya, 2004). CD44 shedding is thought to be a functionally important process that triggers signaling pathways and regulates CD44-mediated functions.

Cleavage of the ICD of CD44 is mediated by presenilin-dependent γ -secretase, whereas the shedding of CD44 is mediated by membrane-associated metalloproteases, including membrane type 1 MMP (MT1-MMP or MMP14), ADAM10, and ADAM17 (Nagano and Saya, 2004). CD44 shedding is thought to result in enhanced cell motility. This notion is supported by the observation that migration of highly aggressive melanoma cells on HA is associated with increased shedding and turnover of CD44 (Goebeler *et al.*, 1996). However, the exact identity of the sheddase of CD44 in different cell types has not yet been established. Anderegg *et al.* (2009, this issue) show that although ADAM10, ADAM17, and MMP14 are expressed in human melanoma biopsies and CD44-positive human melanoma cells, ADAM10, but not MMP14 or ADAM17, is involved in constitutive shedding of CD44 from melanoma cells. In addition, Anderegg *et al.* show that inhibition of CD44 shedding and the corresponding

generation of solCD44 by ADAM10 knockdown augments HA-induced proliferation of melanoma cells in a CD44-dependent manner.

It is important to distinguish between solCD44 generated through proteolytic shedding, which results in a fragment presumably lacking the binding domains for its sheddases, and recombinant soluble CD44 generated through molecular cloning, which includes the entire extracellular domain (referred to hereafter as CD44_{ECD}) that should contain the binding domains for its sheddases and ligands. CD44_{ECD} may therefore serve as a dominant negative regulator of CD44 shedding and interactions between cell-surface CD44 and its ligands. Studies have consistently shown that CD44_{ECD} and CD44_{ECD}-Fc fusion proteins antagonize CD44 receptor function and display potent antitumor activity (Bartolazzi *et al.*, 1994; Yu and Stamenkovic, 2000). On the other hand, elevated levels of solCD44 in patient serum correlate with tumor burden, metastatic potential, and resistance to chemotherapy (Kopp *et al.*, 2001; Nagano and Saya, 2004; Mayer *et al.*, 2008). A potentially attractive view is that the solCD44 level is a surrogate marker for the intracellular level of CD44_{ICD} and that CD44_{ICD} exerts protumor activity, whereas solCD44 is a by-product of the proteolytic cleavage of CD44 that may primarily regulate CD44-dependent cell-surface interactions. Additional studies are required to fully understand whether and how CD44_{ICD} activates signaling pathways that impact tumorigenesis and tumor progression and/or serve as a transcriptional cofactor that regulates the expression of relevant target genes. The identification of ADAM10 as the sheddase of CD44 in human melanoma cells by Anderegg *et al.* (2009) may help in the development of target-based anticancer therapy aimed at modulating CD44 shedding.

CD44 and cancer stem cells

There is increasing evidence to suggest the existence of a small population of specialized cancer cells that display stem-cell properties, commonly referred to as cancer stem cells (CSCs) or cancer initiating cells. CSCs are characterized by their ability to self-renew, differentiate into various lineages, and reconstitute the cellular hierarchy of the tumor from which they are derived in serial xenotransplant assays. These cells are highly resistant to chemo- and radiotherapy and are believed to be responsible for tumor recurrence following therapeutic intervention (Reya *et al.*, 2001). CD44 has been identified as one of the most consistent markers of CSCs from a variety of malignancies, including leukemia, breast, colon, ovarian, prostate, pancreatic, and head and neck cancers (Reya *et al.*, 2001; Croker and Allan, 2008). Thus, CD44⁺ CD24^{-low} breast cancer cells display CSC characteristics, with as few as 100 being able to form tumors in nude mice, whereas a hundred- to a thousand-fold more CD44⁻ cells fail to do so (Al-Hajj *et al.*, 2003). Similarly, a CD44⁺/CD24⁺/ESA⁺/CD133⁺ population of pancreatic cancer cells has been shown to be highly tumorigenic; CD44⁺/CD117⁺ ovarian cancer-initiating cells overexpress ABCG2 and are more resistant to cisplatin and paclitaxel than their CD44⁻ counterparts, and CD44⁺ cells of squamous cell carcinoma of the head and neck display CSC-like characteristics, including the expression of higher levels of genes related to chemoresistance compared with CD44⁻ cells. Furthermore, CD44 has been shown to play an essential role in engraftment of leukemia stem/initiating cells in the bone marrow, an event that is a key precursor to leukemia development (see review by Croker and Allan, 2008). A recent study showed that CD44 is also functionally important for colorectal cancer stem cells (Du *et al.*, 2008). Together, these results indicate a potentially important role for CD44 in the formation, maintenance, and/or function of CSCs.

Perspectives and future directions

The recent definition of CSCs and the suggestion that they may play a central role in tumor development and resistance to anticancer therapies require the identification of selective markers that could be used as therapeutic targets. Expression of CD44 by CSCs of a variety of

malignancies and evidence that CD44 may play a functionally relevant role in the maintenance of these cells warrant investigation of targeting CD44 as a means of inhibiting tumor formation, progression, and relapse. Given that CD44 shedding correlates with tumor burden and progression, a better understanding of the underlying molecular and biochemical basis of the shedding may lead to the development of novel strategies and more effective therapeutic agents that target the bulk of cancer cells and CSCs.

There are many unanswered questions related to CD44 shedding. Does ADAM10 serve as the major sheddase of CD44 in other cancer types? If not, which other ADAMs might play this role? How is CD44 shedding regulated, and which domains of CD44 are responsible for binding to the sheddases? What is the molecular basis for distinguishing constitutive from induced shedding of CD44? Is CD44 shed in CSCs, and what might be the functional consequence of CD44 shedding in these cells? How do the products of CD44 shedding—solCD44 and CD44_{ICD}—contribute to tumor progression and initiation/maintenance of CSCs? What are the mechanisms underlying the functions of solCD44 and CD44_{ICD}? Answers to these questions will set the foundation for future CD44-based therapeutic interventions in cancer.

ACKNOWLEDGMENTS

We are grateful for support from the Swiss National Scientific Research Foundation and the Molecular Oncology National Center of Competence in Research (to IS) and from the Department of Defense–US Army Medical Research (W81XWH-05-1-0191 to QY) and the National Cancer Institute, National Institutes of Health (R01CA116510 to QY).

REFERENCES

- Ahrens T, Assmann V, Fieber C, Termeer C, Herrlich P, Hofmann M, et al. CD44 is the principal mediator of hyaluronic-acid-induced melanoma cell proliferation. *J Invest Dermatol* 2001a;116:93–101. [PubMed: 11168803]
- Ahrens T, Sleeman JP, Schempp CM, Howells N, Hofmann M, Ponta H, et al. Soluble CD44 inhibits melanoma tumor growth by blocking cell surface CD44 binding to hyaluronic acid. *Oncogene* 2001b;20:3399–3408. [PubMed: 11423990]
- Al-Hajj M, Wicha MS, Ito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;100:3983–3988. [PubMed: 12629218]
- Andereg U, Eichenberg T, Parthaune T, Haiduk C, Saalbach A, Milkova L, et al. ADAM10 is the constitutive functional sheddase of CD44 in human melanoma cells. *J Invest Dermatol* 2009;129:1471–1482. [PubMed: 18971959]
- Bartolazzi A, Peach R, Aruffo A, Stamenkovic I. Interaction between CD44 and hyaluronate is directly implicated in the regulation of tumor development. *J Exp Med* 1994;180:53–66. [PubMed: 7516417]
- Crocker AK, Allan AL. Cancer stem cells: implications for the progression and treatment of metastatic disease. *J Cell Mol Med* 2008;12:374–390. [PubMed: 18182063]
- De Wit PE, Van Muijen GN, De Waal RM, Ruiter DJ. Pathology of malignant melanoma, including new markers and techniques in diagnosis and prognosis. *Curr Opin Oncol* 1996;8:143–151. [PubMed: 8727307]
- Du L, Wang H, He L, Zhang J, Ni B, Wang X, et al. CD44 is of functional importance for colorectal cancer stem cells. *Clin Cancer Res* 2008;4:6751–6760. [PubMed: 18980968]
- Goebeler M, Kaufmann D, Bröcker EB, Klein CE. Migration of highly aggressive melanoma cells on hyaluronic acid is associated with functional changes, increased turnover and shedding of CD44 receptors. *J Cell Sci* 1996;109:1957–1964. [PubMed: 8832418]
- Guo Y, Ma J, Wang J, Che X, Narula J, Bigby M, et al. Inhibition of human melanoma growth and metastasis in vivo by anti-CD44 monoclonal antibody. *Cancer Res* 1994;54:1561–1565. [PubMed: 7511044]
- Kim Y, Lee YS, Choe J, Lee H, Kim YM, Jeoung D. CD44–epidermal growth factor receptor interaction mediates hyaluronic acid-promoted cell motility by activating protein kinase C signaling involving

- Akt, Rac1, Phox, reactive oxygen species, focal adhesion kinase, and MMP-2. *J Biol Chem* 2008;283:22513–22528. [PubMed: 18577517]
- Kopp R, Classen S, Wolf H, Gholam P, Possinger K, Wilmanns W. Predictive relevance of soluble CD44v6 serum levels for the responsiveness to second line hormone or chemotherapy in patients with metastatic breast cancer. *Anticancer Res* 2001;21:2995–3000. [PubMed: 11712800]
- Mayer S, Zur Hausen A, Watermann DO, Stamm S, Jäger M, Gitsch G, et al. Increased soluble CD44 concentrations are associated with larger tumor size and lymph node metastasis in breast cancer patients. *J Cancer Res Clin Oncol* 2008;134:1229–1235. [PubMed: 18438684]
- Mochizuki S, Okada Y. ADAMs in cancer cell proliferation and progression. *Cancer Sci* 2007;98:621–628. [PubMed: 17355265]
- Murphy G. The ADAMs: signalling scissors in the tumour microenvironment. *Nat Rev Cancer* 2008;8:929–941. [PubMed: 19005493]
- Nagano O, Saya H. Mechanism and biological significance of CD44 cleavage. *Cancer Sci* 2004;95:930–935. [PubMed: 15596040]
- Okamoto I, Tsuiki H, Kenyon LC, Godwin AK, Emler DR, Holgado-Madruga M, et al. Proteolytic cleavage of the CD44 adhesion molecule in multiple human tumors. *Am J Pathol* 2002;160:441–447. [PubMed: 11839564]
- Pályi-Krek Z, Barok M, Kovács T, Saya H, Nagano O, Szöllosi J, et al. EGFR and ErbB2 are functionally coupled to CD44 and regulate shedding, internalization and motogenic effect of CD44. *Cancer Lett* 2008;263:231–242. [PubMed: 18276068]
- Recio JA, Merlino G. Hepatocyte growth factor/scatter factor induces feedback up-regulation of CD44v6 in melanoma cells through Egr-1. *Cancer Res* 2003;63:1576–1582. [PubMed: 12670907]
- Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414:105–111. [PubMed: 11689955]
- Sugahara KN, Hirata T, Tanaka T, Ogino S, Takeda M, Terasawa H, et al. Chondroitin sulfate E fragments enhance CD44 cleavage and CD44-dependent motility in tumor cells. *Cancer Res* 2008;68:7191–7199. [PubMed: 18757435]
- Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev* 2000;14:163–176. [PubMed: 10652271]