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Short Communication

Proteolytic Cleavage of the CD44 Adhesion Molecule in Multiple Human Tumors

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Cell surface adhesion molecules are crucial for the development and/or pathogenesis of various diseases including cancer. CD44 has received much interest as a major adhesion molecule that is involved in tumor progression. We have previously demonstrated that the ectodomain of CD44 undergoes proteolytic cleavage by membrane-associated metalloproteases in various tumor cell lines. The remaining membrane-bound CD44 cleavage product can be detected using antibodies against the cytoplasmic domain of CD44 (anti-CD44cyto antibody). However, the cleavage of CD44 in primary human tumors has not been investigated. Using Western blots with anti-CD44cyto antibody to assay human tumor tissues, we show that the CD44 cleavage product can be detected in 58% (42 of 72) of gliomas but not in normal brain. Enhanced CD44 cleavage was also found in 67% (28 of 42) of breast carcinomas, 45% (5 of 11) of non-small cell lung carcinomas, 90% (9 of 10) of colon carcinomas, and 25% (3 of 12) of ovarian carcinomas. Tumors expressing a CD44 splice variant showed a significantly higher incidence of enhanced CD44 cleavage. The wide prevalence of CD44 cleavage suggests that it plays an important role in the pathogenesis of human tumors. ***(Am J Pathol 2002, 160:441–447)***

CD44 is a widely distributed cell-surface adhesion molecule that is implicated in a diverse range of physiological and pathological processes, including lymphocyte homing and activation, cell-matrix interactions, cell migration, and the regulation of tumor growth and metastasis.[1] The gene encoding the CD44 protein contains 20 exons of which up to 10 variant exons encoding a portion of the ectodomain are alternatively spliced in various combinations, thereby generating numerous CD44 splice variant isoforms (CD44v).[1,2] The standard CD44 (CD44s) lacks all variant exons. All forms of CD44 are heavily glycosylated to varying degrees. The diversity of CD44 functions is compounded by its variable structure.[3–5] The expression of CD44 or its variants has been shown to be associated with tumor progression; however, the data concerning CD44v forms is controversial for some tumors.[1,6–13] Thus, the exact role of CD44 in the progression of human tumors remains obscure and increased interest has been directed at elucidating the possible mechanisms by which CD44 plays a role in human tumors.

The extracellular domain of a number of membrane proteins can be proteolytically cleaved on the extracytoplasmic side.[14] The proteolytic cleavage of membrane proteins has recently emerged as a key mechanism underlying their functional regulation.[15] We have previously demonstrated a proteolysis-based model as one mechanism involved in the regulation of CD44 function.[16–18] Our studies showed that CD44 is proteolytically cleaved at the ectodomain through membrane-associated metalloproteases in various cancer cell lines to produce a membrane-bound cleavage product of ~25 kd.[16] This CD44 ectodomain cleavage was found to play a critical role in CD44-mediated tumor cell migration by regulating the dynamic interaction between CD44 and the extracel-

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lular matrix.[16,19] Transfection studies on NIH3T3 fibroblasts have shown that overexpression of the oncogenic mutant of Ha-Ras can result in enhancement of CD44 cleavage accompanied by the promotion of CD44-mediated cell migration.[18] We have also reported that CD44 cleavage is regulated by multiple signaling pathways, for example, the activation of protein kinase C, the influx of extracellular Ca^{2+}, and members of the Rho family of small GTPases.[17] Based on these observations, CD44 cleavage seems to be a key event for the regulation of CD44 function in human cancers.

Although the literature on CD44 in human tumors has grown in recent years, no previous study has evaluated clinical human tumor specimens for proteolytic cleavage of CD44. We have generated an antibody (Ab) against the intracytoplasmic domain of CD44 (anti-CD44cyto Ab) that can detect the 25-kd cleavage product (Figure 1A).[16] In this study, we have used this Ab to examine whether CD44 cleavage occurs in several types of human tumor tissues. We demonstrate here for the first time that the CD44 cleavage product is frequently detected in brain, breast, lung, colon, and ovarian tumors and the cleavage event may contribute to the oncogenesis of human tumors.

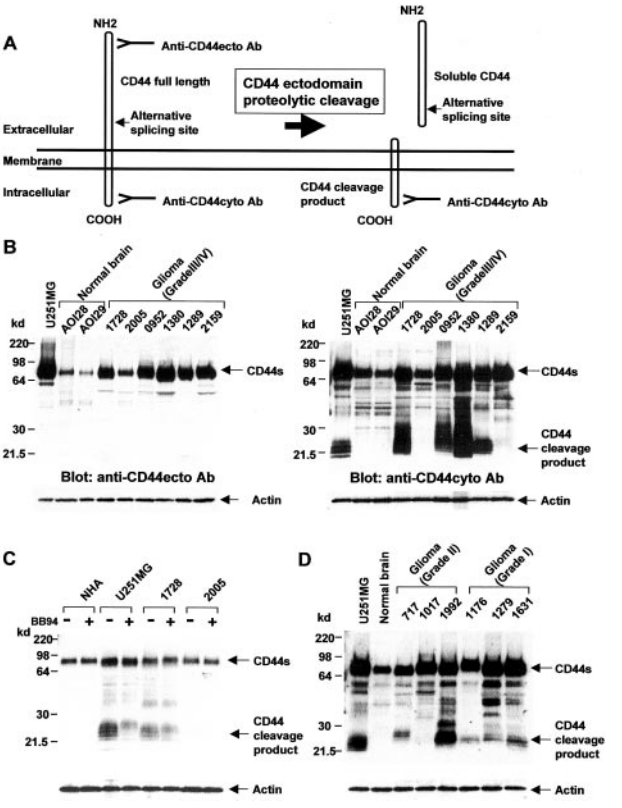

Figure 1. Detection of CD44 cleavage product in gliomas. **A:** Schematic representation of CD44 cleavage. CD44 is proteolytically cleaved in the membrane-proximal region of the extracellular domain by membrane-associated metalloproteases. The CD44 cleavage produces soluble and membrane-tethered fragments of CD44, the latter of which are detectable by Western blot analysis with anti-CD44cyto Ab as a band that migrates at a position corresponding to ~25 kd. **B:** Western blot analysis of U251MG human glioblastoma cell line, normal brain tissue, and WHO grades III and IV glioma tissue specimens. Western blots containing 30 μg of lysates were reacted with anti-CD44ecto mAb (F10-44-2) (**top left**), anti-CD44cyto Ab (**top right**), or anti-actin mAb (**bottom**). Molecular-size standards are shown at the **left** of each panel in kd. The bands corresponding to full-length CD44s, CD44 cleavage products, and actin are indicated by the **arrow**. Actin was used to demonstrate equal loading of protein in each lane. Numbers at the top of lanes refer to patient numbers. **C:** Western blot analysis of normal human astrocytes (NHA), U251MG cells, and brain tumor-derived cell lines (1728 and 2005). The cells were incubated in the absence (minus) or presence (plus) of 100 μmol/L of BB94, a metalloprotease inhibitor, as indicated. Twelve hours before the harvest, all cells received 15 μmol/L of MG132, an intracellular protease inhibitor, to prevent further intracellular proteolytic processing of the CD44 cleavage product. Samples containing equal amounts of protein were separated on 4 to 20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and immunoblots were performed using anti-CD44cyto Ab (**top**). Equal loading of samples was confirmed by blotting with anti-actin mAb (**bottom**). **D:** Western blot analysis of WHO grades I and II glioma tissue specimens. Extracts (30 μg) from grades I and II gliomas were analyzed by Western blot using anti-CD44cyto Ab (**top**). Actin was used to demonstrate equal loading of protein in each lane. Numbers at the top of lanes refer to patient numbers.

Materials and Methods

Tumor Specimens, Cell Lines, and Reagents

Human tumor tissues and corresponding uninvolved normal tissues were obtained under Internal Review Board (IRB)-approved protocols and the diagnoses confirmed by standard histological analysis. All specimens were snap-frozen and stored at −70°C until required. The normal human breast protein was purchased from Clontech (Palo Alto, CA). U251MG cells and brain tumor-derived cell lines were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Normal human astrocytes were purchased from Clonetics (San Diego, CA) and cultured in specialized media (BioWhittaker, Rockland, ME). Carbobenzoxyl-leucinyl-eucinyl-leucinal (MG132) was purchased from Sigma (St. Louis, MO). The hydroxamate-based metalloprotease inhibitor, BB94 (batimastat), was kindly provided by William G. Stetler-Stevenson (National Institutes of Health, Bethesda, MD).

Western Blot Analysis

Tissue specimens and cells were lysed with phosphate-buffered saline (PBS)/TDS buffer (10 mmol/L Na_2HPO_4, 150 mmol/L NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.2% N_aH_3, 0.004% NaF, 1 mmol/L $NaVO_4$, 25 mmol/L β-glycerophosphate, 100 μg/ml phenylmethyl sulfonyl fluoride, and 1 μg/ml each aprotinin and leupeptin) and centrifuged at 15,000 $\times$ *g* to remove insoluble material. Protein concentrations were determined using the BioRad DC protein assay (BioRad, Richmond, CA). Equal amounts of protein were electrophoresed on 4 to 20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and transferred to nitrocellulose filters. The filters were blocked in PBS containing 10% skim milk for 1 hour and then incubated with either a 1:300 dilution of anti-CD44ecto monoclonal antibody (mAb) (F10-44-2; Novocastra, Newcastle, UK), a 1:750 dilution of anti-CD44cyto Ab or a 1:2500 dilution of anti-actin mAb (ICN, Costa Mesa, CA) in PBS containing 0.03% Tween 20 for 50 minutes. The filters were washed three times for 5 minutes each with

PBS containing 0.3% Tween 20 and then incubated for 40 minutes with horseradish peroxidase-conjugated anti-mouse IgG Ab (Amersham, Arlington Heights, IL) for anti-CD44ecto mAb and anti-actin mAb, and with horseradish peroxidase-conjugated anti-rabbit IgG Ab (Amersham) for anti-CD44cyto Ab. Secondary Abs were detected using an enhanced chemiluminescence system (Amersham).

Results

The CD44 Cleavage Is Highly Prevalent in Human Brain Tumors

Initially, the expression of CD44 protein in normal human brain and astrocytic tumors was analyzed by Western blotting using anti-CD44ecto Ab or anti-CD44cyto Ab, which are directed against an epitope in the CD44 ectodomain and CD44 cytoplasmic domain, respectively (Figure 1A). The highly invasive human glioma cell line, U251MG cells, served as a positive control because these cells have previously shown evidence of high CD44 cleavage activity.[16,17] The full-length standard CD44 (CD44s) protein (~85 kd) lacking all variant exons was detected with both anti-CD44ecto Ab and anti-CD44cyto Ab in all samples examined (Figure 1B). Consistent with our previous demonstrations,[16,17] the ~25-kd CD44 cleavage product was detected in U251MG with anti-CD44cyto Ab, but not with the anti-CD44ecto Ab (Figure 1, A and B). Notably, four glioma specimens (specimens 1728 and 1380, both glioblastomas; specimen 0952, a gliosarcoma; and specimen 1289, an anaplastic oligodendroglioma in Figure 1B) exhibited the ~25-kd CD44 cleavage product as well as U251MG cells, whereas the CD44 cleavage product was not observed in normal brain specimens (Figure 1B, right). The anti-CD44ecto Ab failed to detect this fragment (Figure 1B, left), indicating that it is derived from cleavage of the ectodomain. Western blots with Abs against actin, Gab1, a 120-kd cytoplasmic protein, or the epidermal growth factor receptor, a 180-kd transmembrane protein revealed no proteolytic fragments (data not shown). This indicated that the fragments observed with the anti-CD44cyto Ab are not the result of protein degradation.

The CD44 ectodomain cleavage has been previously shown to be mediated through metalloprotease activity.[16,19] To verify that the metalloprotease-mediated CD44 cleavage occurs in these human gliomas, we established cell lines derived from the glioma tumor specimens 1728 and 2005 and analyzed them by Western blotting using anti-CD44cyto Ab. The 2005 cell line was derived from a glioblastoma tumor in which the CD44 cleavage product was not detected (Figure 1B) and this cell line did not show the CD44 cleavage product in culture (Figure 1C). In contrast, the 1728 cell line was derived from a recurrent glioblastoma tumor containing the CD44 cleavage product (Figure 1B) and the cell line also displayed the 25-kd CD44 cleavage product (Figure 1C). Normal human astrocytes did not demonstrate the CD44 cleavage product (Figure 1C). Consistent with earlier observations,[16] the production of CD44 cleavage products in both the 1728 and U251MG cells was markedly reduced in the presence of BB94 (a synthetic hydroxamate metalloprotease inhibitor) (Figure 1C), indicating that the proteolytic cleavage of CD44 in these tumor cells was attributable to metalloprotease activity. Taken together, these results demonstrate that Western blot analysis using anti-CD44cyto Ab allows us to reliably assess the occurrence of CD44 cleavage in human tumor tissues and this method was chosen for ensuing analyses.

Table 1. CD44 Cleavage in Normal Brain and Gliomas

Tissues	No. of cases with CD44 cleavage/ total (%)
Normal brain (cortex)	0/7 (0%)
Gliomas*	
Grade I to II	10/17 (59%)
Grade III to IV	32/55 (58%)
Primary	26/48 (54%)
Recurrent	6/7 (86%)

*Tumors were graded using the WHO designation.

CD44 cleavage was evaluated in 7 normal brain tissue specimens and 72 astrocytic tumors comprised of 17 low-grade [World Health Organization (WHO) grades I and II] and 55 high-grade (WHO grades III and IV) tumors. Representative results of Western blot analysis are shown in Figure 1, B and D. Full-length CD44s (~85 kd) was expressed in all of these tissue specimens, and gliomas generally exhibited higher expression of full-length CD44s than normal brain (Figure 1, B and D). The CD44 cleavage product was detected in 59% (10 of 17) low-grade tumors, 58% (32 of 55) high-grade tumors, and in none of the seven normal brain tissues (Table 1). These results indicate that CD44 cleavage is a tumor-specific phenomenon, although there was no significant association between CD44 cleavage and pathological grade in glioma. Recurrent high-grade tumors were more likely to be positive (6 of 7, 86%) for CD44 cleavage compared to primary high-grade tumor (26 of 48, 54%), but this did not reach statistical significance in this sample size.

CD44 Cleavage in Multiple Human Tumors

CD44 cleavage was next evaluated in primary human carcinomas of the breast, lung, colon, and ovary. We examined 42 breast carcinomas (all invasive ductal carcinomas) by Western blotting using anti-CD44cyto Ab. All of these samples, including the normal breast tissue specimens, showed expression of full-length CD44s protein. The expression level of CD44 cleavage product was much higher in 28 of 42 tumors than in a normal breast specimen obtained from a patient with no evidence of tumor (Figure 2A and Table 2). For 15 tumors, normal tissue adjacent to the tumor was available and representative blots of these paired specimens are shown in Figure 2B. There was enhanced expression of the CD44 cleavage product as compared to the normal tissue (308 and 262 in Figure 2B). CD44 variant isoform (CD44v)

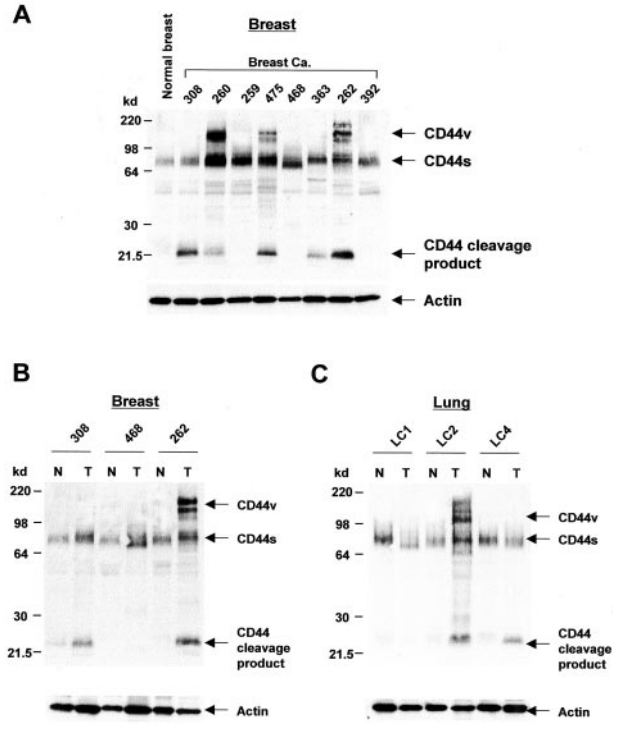

Figure 2. Western blot analysis for the presence of CD44 cleavage product in breast and lung carcinomas. Extracts (30 μg) from breast carcinomas (**A** and **B**), non-small cell lung carcinomas (**C**), and corresponding normal tissue specimens were analyzed by Western blotting as described in Material and Methods. Blots were probed with anti-CD44cyto Ab or anti-actin mAb. Numbers at the top of lanes refer to patient numbers. T and N shown in **B** and **C** denote primary tumors and their matched normal tissues, respectively. Actin was used to demonstrate equal loading of protein in each lane. Molecular-size standards are shown at the **left** in kd. The bands corresponding to full-length CD44s, full-length CD44v cleavage product, and actin are shown by the **arrow**.

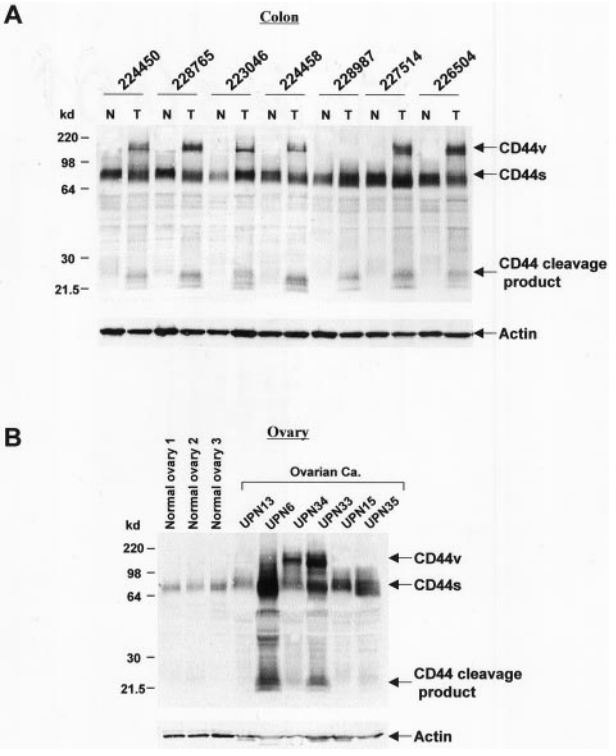

Figure 3. Western blot analysis for the presence of CD44 cleavage product in colon and ovarian carcinomas. The **top** panels of **A** and **B** are Western blot analyses of colon carcinomas (**A**) and ovarian carcinoma (**B**) incubated with anti-CD44cyto Ab. Numbers at the top of lanes refer to patient numbers. T and N denote primary tumors and their matched normal tissues, respectively. Blots with anti-actin mAb (**A** and **B**, **bottom**) were used to demonstrate equal loading of protein in each lane. The bands corresponding to full-length CD44s, full-length CD44v, and CD44 cleavage product are shown by the **arrow**.

expression has been described in multiple human tumors including breast carcinomas.[1,7,20–24] The expression of the higher molecular weight CD44v protein was detected in 11 of 42 (26%) breast carcinomas (Figure 2, A and B, and Table 2). Interestingly, all 11 of the samples expressing CD44v protein also exhibited the CD44 cleavage product.

To investigate the proteolytic cleavage of CD44 in lung carcinomas, Western blot analysis with anti-CD44cyto Ab was performed on 11 cases of non-small cell lung carcinomas and paired normal lung tissues. Small-cell lung carcinomas were excluded in the present study, since we and other groups have previously demonstrated that small-cell lung carcinomas do not express any detectable CD44 protein.[23,25,26] Representative results are shown in Figure 2C. The enhanced CD44 cleavage was observed in 45% (5 of 11) non-small cell lung carcinomas (Table 2), including three of eight adenocarcinomas, one of two squamous cell carcinomas, and one of one adenosquamous cell carcinoma. We found expression of CD44v protein in 27% (3 of 11) non-small cell lung carcinomas (Table 2). Two of the three non-small cell lung carcinomas expressing CD44v protein also exhibited an abundance of CD44 cleavage product (Table 2).

We found a very high incidence of CD44 cleavage in colon carcinomas as compared to their matched normal tissues. The CD44 cleavage product was detected in 90% (9 of 10) colon carcinomas, whereas no reactivity to this product was found in 9 of the 10 paired normal colon specimens (Figure 3A and Table 2). One normal colon specimen displayed a detectable amount of CD44 cleavage product; however, the amount was much lower than

Table 2. CD44 Cleavage and Expression of CD44 Variants in Breast, Lung, Colon, and Ovarian Carcinomas

Tissues	CD44 cleavage	CD44v (+)	CD44 cleavage and CD44v (+)
Breast carcinoma	28/42 (67%)	11/42 (26%)	11/11 (100%)
Lung carcinoma	5/11 (45%)	3/11 (27%)	2/3 (67%)
Colon carcinomas	9/10 (90%)	7/10 (70%)	7/7 (100%)
Ovarian carcinomas	3/12 (25%)	3/12 (25%)	2/3 (67%)
Total	45/75 (60%)	24/75 (32%)	22/24 (92%)

Table 3. Correlation between CD44 Cleavage and CD44v Expression in Breast, Lung, Colon, and Ovarian Carcinomas

Group	No.	CD44 cleavage (%)	*P* value
CD44v protein (+)	24	92% (22/24)	<0.001
CD44v protein (−)	51	45% (23/51)	(CD44v (+) *versus* CD44v (−))

the matched tumor specimen (data not shown). Among the panel of 10 colon carcinomas tested, 7 cases were found to express CD44v protein, and all of the 7 tumors also co-expressed the CD44 cleavage product (Table 2).

Twelve ovarian carcinomas (six serous, three undifferentiated, and three endometrioid adenocarcinoma) were analyzed by Western blots using anti-CD44cyto Ab. CD44s expression in these tumors was higher than in normal ovarian tissues (Figure 3B). We found CD44 cleavage product in 3 of 12 (25%) ovarian carcinomas but not in any of three normal ovaries tested (Figure 3B and Table 2). Expression of CD44v was detected in three tumors and CD44 cleavage product was observed in two of these three tumors (Table 2).

Overall, we found enhanced expression of the CD44 cleavage product in 60% (45 of 75) of these tumors, whereas CD44v expression was observed in 32% (24 of 75) (Table 2). Interestingly, the percentage of CD44 cleavage was significantly higher in tumors expressing CD44v than in tumors expressing only CD44s protein (92% *versus* 45%, $P < 0.001$) (Table 3). We also obtained data on histological grade of these tumors. There seemed to be no direct correlation between the presence of the CD44 cleavage product and the tumor grade (Table 4).[27,28]

Table 4. CD44 Cleavage and Tumor Stage/Histopathological Grade in Breast, Lung, Colon, and Ovarian Carcinomas

	No.	CD44 cleavage (%)
Breast carcinoma*†		
Grade I to II	9	6 (67%)
Grade III	16	11 (69%)
Lung carcinoma		
Stage I to II	3	3 (100%)
Stage III to IV	7	2 (29%)
Colon carcinoma		
Dukes A	2	2 (100%)
Dukes B	4	4 (100%)
Dukes C	4	3 (75%)
Ovarian carcinoma‡		
Stage I to II	4	1 (25%)
Stage III to IV	8	2 (25%)

*Histopathological grade was not available for all tumors.
†Tumors were graded using the modified criteria of Scarff, Bloom, and Richardson.[27]
‡Tumors were staged in accordance with the International Federation of Gynecology and Obstetrics (FIGO) surgical-staging system.[28]

Discussion

There have been many reports studying the expression patterns of CD44 and its variant protein in various human tumors. To the best of our knowledge, these earlier studies uniformly used Abs directed against the ectodomain of CD44. Thus, studies using these Abs would not have detected membrane-tethered CD44 cleavage product yielded by the CD44 ectodomain cleavage (Figure 1A). Because the alternative splicing of CD44 occurs in the ectodomain, CD44 variant exon-specific Abs also suffered from this limitation. We have developed an Ab raised to the COOH-terminal region of the CD44 cytoplasmic domain and demonstrated that it could detect CD44 cleavage products in various cancer cell lines.[16] In the present study, we demonstrate for the first time that the ectodomain cleavage product of CD44 is easily and clearly detectable by Western blot in multiple human tumor tissues using anti-CD44cyto Ab.

We have previously shown that CD44 ectodomain cleavage can be induced in tumor cell lines by several stimuli including Ca^{2+} influx, Ras oncogene expression, and TPA treatment of cells, and that this cleavage can be inhibited by metalloprotease inhibitors.[16–18] Our findings have been supported by more recent reports demonstrating that membrane type 1 matrix metalloprotease (MT1-MMP) cleaves the CD44 ectodomain and promotes cancer cell migration.[19] Our present study demonstrates that this metalloprotease-mediated CD44 cleavage is reproduced in cell lines derived from tumor specimens examined. This fact, and the fact that degradation of other proteins was not observed in the primary tumor samples examined indicate that the ~25-kd CD44 fragment is indeed generated endogenously in these tumors and is not the result of protein degradation.

The CD44 cleavage product was never observed in normal brain and ovary tissue specimens. We observed detectable amounts of CD44 cleavage product on long exposures of normal breast, lung, and colon specimens, although the amount was much lower than that observed in tumor specimens. There are several potential explanations for this observation. The importance of continuous cross talk between tumor cells and surrounding nontumor cells during tumor progression has been described.[29,30] Regarding the regulation of metalloprotease production in the surrounding cells of tumors, tumor cell-derived factors that increase the expression of several metalloproteases in fibroblasts have been reported.[31] Furthermore, a recent report has shown a significantly higher incidence of metalloprotease expression in normal colon tissues of patients with liver metastasis than in those without metastasis.[32] Thus, malignant tumor tissues may alter the expression profile of metalloproteases in their adjacent normal tissues and induce CD44 cleavage. Alternatively, because metalloproteases have been implicated in a variety of normal cellular processes,[33,34] CD44 cleavage may occur at a low level in these normal tissues as part of a normal physiological process that is amplified in tumors. Finally, we cannot completely exclude the possibility that microscopically undetected tumor cells may infiltrate into the adjacent normal tissues.

In our analysis of brain tumors, we found the abundant expression of CD44s protein, yet no detectable CD44v protein was observed in any of these tumor tissues. These results are consistent with earlier studies showing that CD44s protein is the most dominant form of CD44 expressed in brain tumors.[35–38] On the other hand, CD44 cleavage product was clearly detected in 58% (32 of 55) of high-grade gliomas and 59% (10 of 17) of low-grade gliomas, suggesting its potential as a target for new diagnostic and therapeutic approaches. We and others have previously reported that CD44 cleavage promotes tumor cell migration *in vitro*.[16,18,19] However, our present finding that CD44 cleavage was also detected in low-grade (I and II) tumors as well as high-grade (III and IV) gliomas suggest that CD44 cleavage may not necessarily contribute to tumor invasiveness *in vivo*, although WHO grading of gliomas does not depend on invasiveness. On the other hand, this discrepancy may also be attributable to the complicated mechanisms underlying tumor invasion *in vivo*, a highly regulated process requiring the well-orchestrated interplay of various components (organ-specific microenvironment, growth factor signaling, adhesion molecule, proteolytic events, and so forth).

In contrast to brain tumors, 26% of breast carcinomas, 27% of lung carcinomas, 70% of colon carcinomas, and 25% of ovarian carcinoma expressed CD44v protein. With the exception of colon carcinomas, the incidence of CD44v protein expression seems to be lower than what was reported in earlier studies, perhaps because of a difference in methodology.[20–22,39] These studies mainly used reverse transcriptase-polymerase chain reaction to detect expression of the variant forms or Abs specific for the splice variants. In the present study, we demonstrate that CD44 cleavage is more prevalent than CD44v expression in these tumors. Notably, tumors expressing CD44v protein show a significantly higher incidence of the enhanced CD44 cleavage than those expressing only CD44s protein. Because both CD44s and CD44v would generate the same size CD44 cleavage product[16] and all CD44v-expressing tumors co-express the CD44s protein, we cannot conclude the exact origin (CD44s or CD44v) of the CD44 cleavage product observed in these tumors at this point.

The enhanced CD44 proteolytic cleavage was found irrespective of the grade of malignancy in the tumor samples examined, hence CD44 cleavage may define a very early event in tumorigenesis. More recently, we have demonstrated the functional link between CD44 cleavage and consequent signal transduction within cells.[40] It is possible that the CD44 cleavage might impact on a signaling pathway to promote tumor development. The intracellular domain of CD44 has also been shown to bind to merlin and this can send a growth inhibitory signal to cells when the extracellular domain of CD44 is bound to its ligand.[41] The CD44 cleavage would result in loss of the extracellular domain of CD44 and may abolish this inhibitory signal resulting in unregulated cell proliferation. Considering these potential roles for CD44 cleavage, its presence in multiple human tumor types and its high frequency of occurrence indicate that further work is warranted to establish its biological and clinical significance.

Acknowledgments

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