CD44 s expression in human colon carcinomas influences growth of liver metastases. Int J Cancer 85: 523–526

ARTICLE *in* INTERNATIONAL JOURNAL OF CANCER · MARCH 2000

Impact Factor: 5.09 · DOI: 10.1002/(SICI)1097-0215(20000215)85:4<523::AID-IJC13>3.0.CO;2-6 · Source: PubMed

5 AUTHORS, INCLUDING:

Kazuhisa Takahashi
Juntendo University
325 PUBLICATIONS **2,412** CITATIONS

SEE PROFILE

Kenneth K Tanabe
Massachusetts General Hospital
209 PUBLICATIONS **7,657** CITATIONS

SEE PROFILE

Int. J. Cancer: **85,** 523–526 (2000)

Publication of the International Union Against Cancer

CD44S EXPRESSION IN HUMAN COLON CARCINOMAS INFLUENCES GROWTH OF LIVER METASTASES

Seung Ho CHOI, Kazuhisa TAKAHASHI, Hiroshi ETO, Sam S. YOON and Kenneth K. TANABE*

Division of Surgical Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

CD44 is a family of cell-surface adhesion molecules which exist in several isoforms arising from mRNA alternative. Malignant transformation of colonic mucosa is associated with alterations in CD44 expression, which result in up-regulation of high-molecular-weight CD44 isoforms and down-regulation of CD44s. We have demonstrated that stable transfection of CD44s into colon-carcinoma cell lines reduces their tumorigenicity. To understand the influence of CD44s expression on the metastatic potential of human colon carcinomas, we measured the ability of several different CD44s-transfected colon carcinomas to establish experimental liver metastases following splenic inoculation in mice. We observed that introduction of CD44s into 2 different human colon carcinoma cell lines, HT29 and KM12C6, resulted in reduced growth of liver metastases by as much as 75%. To explore the relationship between hyaluronate adhesion and metastasis, we transfected HT29 cells with cDNA encoding a mutant CD44s that does not bind to hyaluronate. HT29 transfectants expressing this mutant CD44s demonstrate an 84% reduction in growth of liver metastases, despite minimal binding to hyaluronate by the mutant CD44s. In concert, these results indicate that CD44s down-regulation, which occurs with malignant transformation of colonic mucosa, is associated with enhanced growth of experimental liver metastases. Consequently, the functional consequences of CD44s down-regulation in colon carcinomas may be just as significant as the consequences of up-regulation of other CD44 isoforms. *Int. J. Cancer* 85:523–526, 2000.

CD44 is a family of cell-surface adhesion molecules encoded by a single gene. Several CD44 isoforms exist as a result of alternative splicing, thereby providing significant heterogeneity in CD44 expression among different tissues. The breadth of CD44 post-translational modifications that exist adds to the diversity of CD44 molecules that may be expressed on cell surfaces (Brown *et al.,* 1991). The most common CD44 isoform is present on cells of hematopoetic origin and does not contain any of the alternatively spliced exons that encode a portion of the extracellular domain. The transcript for this standard isoform, referred to as CD44s (and also known as CD44H), contains exon 5 spliced directly to exon 16. In contrast, inclusion of different combinations of variant exons between exon 5 and exon 16 results in seemingly countless higher-molecular-weight CD44 isoforms. CD44 is broadly distributed in normal and malignant tissues; however, malignant transformation is frequently associated with alterations in CD44 alternative splicing (Dall *et al.,* 1994; Tanabe *et al.,* 1993). Several investigators have examined the correlation between CD44-isoform-expression patterns and tumor stage in patients with lymphoma, gastric carcinoma, breast cancer, and colon carcinoma (Friedrichs *et al.,* 1995; Jalkanen *et al.,* 1991; Miwa *et al.,* 1996; Wielenga *et al.,* 1993). The mechanisms that lead to these associations have yet to be determined.

CD44s is expressed in normal colon mucosa, but this expression is either lost or down-regulated as a result of malignant transformation (Tanabe *et al.,* 1995). To study the phenotypic consequences of CD44s down-regulation in colon carcinoma, we transfected colon-carcinoma cell lines with CD44s cDNA to reintroduce expression of this adhesion molecule. Introduction of CD44s cDNA into colon-cancer cell lines restores cell-surface expression of CD44s and reduces their tumorigenicity (Takahashi *et al.,* 1995; Tanabe *et al.,* 1995). The ability of CD44s expression to reduce tumorigenicity may correlate with its ability to bind to hyaluronate (Takahashi *et al.,* 1995). These observations prompted us to test whether the introduction of CD44s into colon carcinomas might also reduce growth of experimental liver metastases. We now demonstrate that cell-surface expression of the CD44s adhesion molecule reduces tumor growth in the liver after splenic inoculation of human colon-carcinoma cells. By using a mutant form of CD44s that is incapable of adhesion to hyaluronate, we determined that this reduction in formation and growth of liver metastases is independent of its ability to bind to hyaluronate. Our earlier findings indicate that transformation of normal colonic mucosa to carcinoma is associated with alterations in CD44 alternative splicing that result in down-regulation of CD44s. Together, our findings suggest that modulation of CD44 alternative splicing is one mechanism that may enhance tumor metastases.

MATERIAL AND METHODS

Cell lines, transfectants, and antibodies

The human colon-carcinoma cell line HT29 was obtained from the ATCC (Rockville, MD). The human colon-carcinoma clones KM12C6 and KM12L4 are related (Morikawa *et al.,* 1988*a*) and were kindly provided by Dr I. Fidler (MD Anderson Cancer Center, Houston, TX). All cell lines were maintained in DMEM/F12 with glutamine (GIBCO BRL, Gaithersburg, MD) supplemented with 8% (v/v) FCS (GIBCO BRL) with an appropriate concentration of G418 (Sigma, St. Louis, MO). HT29Δneo, HT29ΔCD44s, KM12C6Δneo, KM12C6ΔCD44s, KM12L4Δneo and KM12L4ΔCD44s transfectants have been described (Takahashi *et al.,* 1995). The MAbs F10-44-2 (Biodesign International, Kennebunk, ME) and BU52 (Binding Site, San Diego, CA) are directed against epitopes common to all CD44 isoforms. The construct containing cDNA for mutant CD44s with arginine 41 mutated to alanine has been described (Takahashi *et al.,* 1996) and was introduced into HT29 cells by electroporation. Cell-surface CD44 expression was examined on all G418-resistant clones.

Grant sponsor: National Institutes of Health; Grant numbers: CA64454, DK43351, and CA71345; Grant sponsors: Claude E. Welch Research Fellowship; American Cancer Society.

S.H. Choi's present address is: Department of Surgery, Yongdong Severance Hospital, Yonsei University College of Medicine, Yongdong P.O. Box 1217, Seoul, Republic of Korea.

K. Takahashi's present address is: Department of Respiratory Medicine, Juntendo University, School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo, 113 Japan.

H. Eto's present address is: Department of Urology, Kobe University School of Medicine, Kobe 650-0017, Japan.

*Correspondence to: Division of Surgical Oncology, Massachusetts General Hospital, Cox 626, Boston, MA 02114, USA. Fax: (617) 724-3895.

Received 18 May 1999; Revised 4 September 1999

Confirmation of cell-surface expression of CD44

Immunoprecipitation of cell-surface labeled proteins to confirm expression of CD44 on stable transfectants was performed as described (Takahashi *et al.,* 1995).

FACS analysis

Cells were harvested with 5 mM EDTA in PBS, and single-cell suspensions were incubated with MAb BU52 on ice for 30 min. After washing, cells were incubated with fluorescein-labeled anti-mouse MAb (Sigma) on ice for 30 min. Immunofluorescence was detected with FACScan (Becton Dickinson, Mountain View, CA).

Adhesion assay

Cell-surface CD44 binding to hyaluronate was examined in 96-well flat-bottomed plates coated with hyaluronate, as described (Takahashi *et al.,* 1999).

Experimental-liver-metastasis assay

5 week old male BALB/c nude mice (Steele Laboratory, Massachusetts General Hospital, Boston, MA) were allowed to acclimatize for one week. Transfectants were harvested using only 5 mM EDTA in PBS without trypsin and re-suspended as a single-cell suspension in Hanks's balanced salt solution, free of Mg^{2+} and Ca^{2+}. After induction of general anesthesia with methoxyflurane, a left subcostal incision was performed on the mice, the spleen was exposed, and cells were inoculated intrasplenically. The mice were observed for the indicated time period, at which point their livers were harvested through a midline incision. Experiments were performed in duplicate.

Statistical analyses

The number of liver lobes containing metastases were compared using Fisher's exact test (InStat; Graphpad Software, New York, NY).

RESULTS

CD44s expression reduces metastatic tumor growth in the liver

To examine the influence of CD44s expression on metastasis, we established an experimental-liver-metastasis assay for each of 3 human colon-carcinoma cell lines. We have established stable transfectants that express CD44s (designated with the suffix ΔCD44s) using HT29, KM12C6 and KM12L4 human colon-carcinoma cell lines (Takahashi *et al.,* 1995). We confirmed cell-surface expression of CD44s by immunoprecipitation of cell-surface labeled proteins using CD44 MAb F10-44-2 (data not shown). Pilot experiments were performed to establish the optimal technique for establishing experimental liver metastases with these cell lines. We performed a number experiments, varying several conditions, including the number of cells implanted into the spleen; trypsin *vs.* no trypsin to harvest cells; splenectomy *vs.* no splenectomy following tumor-cell inoculation; and number of weeks until animals were killed. Liver metastases were typically too numerous to count, and their distribution among lobes and throughout any given lobe were too heterogeneous to permit evaluation of the percentage volume replacement of the liver by tumor. When we decreased the number of cells implanted into the spleen, the standard deviation for the number of liver metastases became unacceptably high. The liver surface area occupied by tumor was taken as a reproducible endpoint. For HT29 transfectants, 5×10^6 cells were inoculated intrasplenically and mice were killed 20 days later. All mice inoculated with HT29 transfectants developed liver metastases; however, the extent of metastases differed significantly (Table I). Mice inoculated with HT29Δneo cells developed more liver metastases, with 24 of 40 lobes (60%) demonstrating tumor replacement of at least one quarter of the lobe. In mice inoculated with HT29ΔCD44s cells, only 6 of 40 lobes (15%) demonstrated tumor replacement of at least one quarter of the lobe ($p < 0.0001$). The metastatic deposits in the liver were too numerous to count. Also, the metastatic foci were not evenly distributed throughout each lobe, but rather were concentrated near the periphery of each lobe in the subcapsular region.

TABLE I – EXPERIMENTAL LIVER METASTASES

Transfectant	Mice with liver metastases (%)	Hepatic lobes with >25 covered with metastases (%)
HT29Δneo	10/10 (100.0)	24/40 (60.0)
HT29ΔCD44s	10/10 (100.0)	6/40 (15.0)
HT29Δ41R/A	8/8 (100.0)	3/32 (9.4)
KM12C6Δneo	5/8 (62.5)	0/32 (0.0)
KM12C6ΔCD44s	2/8 (25.0)	0/32 (0.0)
KM12L4Δneo	2/8 (25.0)	0/32 (0.0)
KM12L4ΔCD44s	2/7 (28.6)	0/28 (0.0)

In a similar experimental-liver-metastasis assay, 1×10^6 KM12C6 or KM12L4 transfectants were inoculated intrasplenically, and mice were killed 30 days later. Out of 8 mice inoculated with KM12C6Δneo cells, 5 developed liver metastases, as compared with only 2 of 8 mice inoculated with KM12C6ΔCD44s cells. When KM12L4 transfectants were compared, 2 of 8 mice inoculated with KM12L4Δneo cells and 2 of 7 mice inoculated with KM12L4ΔCD44s cells developed liver metastases of similar magnitude. Because of the lower experimental metastatic potential of KM12L4 cells in our assay, compared with the KM12C6 and HT29 transfectants, we were not able to determine with statistical significance whether CD44s expression on KM12L4 cells reduces their metastatic potential as it does when expressed on KM12C6 or HT29 cells. Presumably, differences in our experimental-metastasis assay compared with that used originally to develop the KM12L4 cells (Morikawa *et al.,* 1988*b*) accounts for our finding of a lower metastatic potential of KM12L4 cells compared to KM12C6 cells. For example, we did not use trypsin to harvest the cells, since it cleaves cell-surface CD44 (data not shown). Nonetheless, the reduction in HT29 and KM12C6 metastatic tumor growth in the liver as a result of cell-surface CD44s expression was quite apparent.

Relationship between CD44s hyaluronate adhesion and growth of liver metastases

The relationship between CD44s adhesion to hyaluronate and growth of liver metastases is interesting, especially when the above results are viewed with the knowledge that CD44s expressed on HT29 cells and KM12C6 cells effectively binds to hyaluronate, while CD44s expressed on KM12L4 cells does not effectively bind hyaluronate (Takahashi *et al.,* 1995). To better explore the relationship between hyaluronate binding and growth of liver metastases we used a mutant form of CD44s with a single amino-acid substitution in the B loop (hyaluronate-binding) domain that reduces its adhesion to hyaluronate. This mutation involves a change from arginine at residue 41 to alanine (Peach *et al.,* 1993). We transfected HT29 cells with cDNA encoding this mutant CD44s and isolated G418-resistant clones (designated with the suffix Δ41R/A). We confirmed the presence of cell-surface mutant CD44s by immunoprecipitation (Fig. 1*a*) and confirmed that this mutant CD44s does not bind to hyaluronate as well as wild-type CD44s (Fig. 1*b*). HT29Δ41R/A transfectants (5×10^6) were inoculated intrasplenically into nude mice, which were killed 20 days later. All mice inoculated with HT29Δ41R/A transfectants developed liver metastases (as did all mice inoculated with either HT29Δneo or HT29ΔCD44s transfectants). However, in mice inoculated with HT29Δ41R/A transfectants, only 3 of 32 lobes (9.4%) showed tumor replacement of at least one quarter of the lobe, which is essentially identical to the results obtained using HT29ΔCD44s (Table I). All our results combined suggest that CD44s expression on human colon-carcinoma cells reduces the growth of experimental liver metastases, and that this reduction is not dependent on the hyaluronate-binding function of CD44.

DISCUSSION

Multiple steps are required for successful hematogenous metastasis, including detachment from the primary tumor, degradation

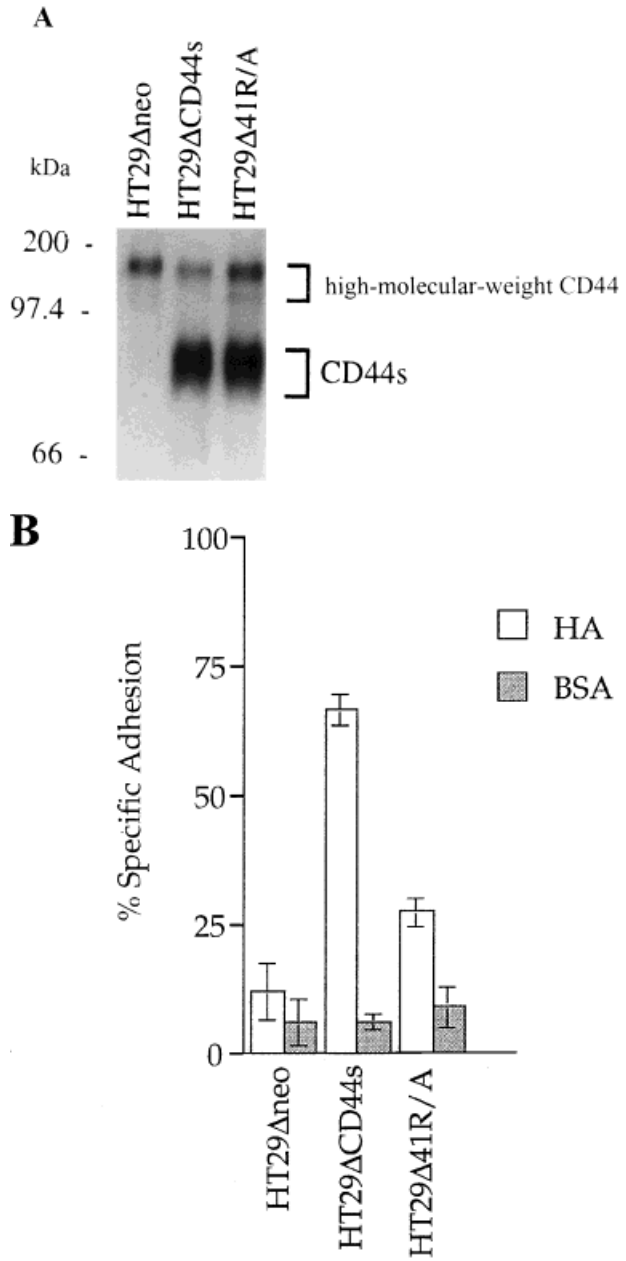

FIGURE 1 – CD44s expression on HT29 cells and hyaluronate adhesion. (*a*) Cell lysates from HT29Δneo, HT29ΔCD44s, or CD44Δ41R/A cells were subjected to immunoprecipitation, the MAb F10-44-2 being used after labeling of cell-surface proteins with biotin. (*b*) HT29 transfectants were examined for their adhesion to solid-phase hyaluronate.

of extracellular matrix, migration, invasion into blood vessels, survival from host defense mechanisms, adhesion to the endothelium in distant organs, and growth in the new organ environment. The process of metastasis is modulated by communication between tumor-cell-adhesion molecules and the extracellular environment. CD44 appears to be involved in several steps of the metastatic cascade. Experimental data from *in vitro* models of invasion and metastases suggest that CD44 expression influences tumor-cell migration (Thomas *et al.,* 1992), MMP-2 production (Takahashi *et al.,* 1999), invasion (Yu and Stamenkovic, 1999), presentation of heparin-binding growth factors (Bennett *et al.,* 1995*a*), adhesion to peritoneal mesothelium (Cannistra *et al.,* 1993), and hyaluronate degradation (Culty *et al.,* 1992).

A number of investigators have examined the role of CD44 in metastases, using *in vivo* assays. Most notably, Günthert *et al.* (1991) demonstrated that over-expression of a v6-containing CD44 isoform by rat pancreatic-carcinoma cells enhances their metastatic potential. Of note, the rat pancreatic-carcinoma clone with low metastatic potential that was used in this study expressed CD44s. In contrast, using a different animal model, Sy *et al.* (1991) demonstrated that Namalwa lymphoma cells transfected to over-express CD44s show greater metastatic potential than parental cells. Birch *et al.* (1991) demonstrated that melanoma cells selected for high expression of CD44s demonstrate a greater metastatic potential than cells selected for low expression of CD44s. At first glance, results of these published experiments appear to be at odds with the results presented in the current study. We observed that expression of CD44s by different human colon-carcinoma cell lines reduces their metastatic potential. However, it should be recognized that significant differences exist between the models used in each of these studies, including differences in the tumor cell lines and host animals. Many traits are required of metastatic cells, and individual tumor cell lines presumably differ in the array of necessary metastatic traits that they lack. Consequently, a specific CD44 isoform may be necessary to complement the inherent metastatic traits of one cell line, and a different CD44 isoform may be necessary to complement those of another cell line. In addition, specific CD44 post-translational modifications (*e.g.,* glycosylation and glycosaminoglycan substitution) directly modulate CD44 function (Bennett *et al.,* 1995*b;* Dasgupta *et al.,* 1996; Takahashi *et al.,* 1996). Significant heterogeneity exists in the spectrum of post-translational machinery existing in different types of tumors. Consequently, it follows that any correlation between CD44 expression and metastatic potential will depend on CD44 post-translational modification in the particular tumor cell line.

The association between CD44s expression and down-regulation of metastases has been reported by Gao *et al.* (1997) using a different experimental-metastasis model. These investigators demonstrated that experimental down-regulation of CD44s in Dunning rat prostate-cancer cells enhances their metastatic potential, and that up-regulation of CD44s in the highly metastatic Dunning AT3.1 clone greatly suppresses its metastatic potential. This observed down-regulation of prostate-cancer metastases with CD44s expression is very similar in concept to our findings that CD44s expression reduces growth of experimental liver metastases. In addition, Souka *et al.* (1997) demonstrated that down-regulation or complete loss of CD44 expression is associated with enhanced cell proliferation, while Jalkanen *et al.* (1991) reported that rapidly proliferating, highly aggressive non-Hodgkin's lymphomas often do not express CD44.

CD44 is the main cell-surface receptor for hyaluronate and contains sequences homologous with the B domain of cartilage link protein, which is responsible for hyaluronate binding (Peach *et al.,* 1993). Now, experimental results demonstrate that enhanced hyaluronate binding is not required for the suppression of metastatic potential induced by over-expression of CD44s in Dunning AT3.1 cells (Gao *et al.,* 1998). The absence of a consistent association between CD44s adhesion to hyaluronate and down-regulation of metastases reported by these authors is in line with our own findings in human colon-carcinoma cells.

Several high-molecular-weight CD44 isoforms have been identified in human tumors, and many investigators have been interested in the phenotypic consequences of over-expression of these variant CD44 isoforms. In contrast, we observed that in addition to variant CD44-isoform over-expression, transformation of colonic mucosa to carcinoma is associated with down-regulation of CD44s. We have focused many of our investigations on the consequences of this CD44s down-regulation. We demonstrated earlier that reintroduction of CD44s into colon-carcinoma cell lines is associated with a 50% reduction in their tumorigenicity and growth rate (Takahashi *et al.,* 1995). Consequently, the reduction in bulk of HT29ΔCD44s liver metastases observed in the present

study could in theory be partially attributable simply to a reduction in tumor-cell growth rate. However, the finding that HT29Δ41R/A transfectants demonstrate *in vitro* growth rates similar to those of HT29Δneo transfectants, yet demonstrate a reduction in liver metastases, argues against this possibility, as does the reduction in the number of mice developing metastases following injection with KM12C6ΔCD44s transfectants, as compared with controls.

The finding that CD44s expression results in down-regulation of metastatic tumor growth in the liver in 2 unrelated human colon-carcinoma cell lines makes it unlikely that our results may be explained solely on the basis of clonal variation. Moreover, introduction of mutant CD44s into HT29 cells produced similar observations. Nonetheless, at this point it is premature to speculate on the mechanisms by which reintroduction of CD44 into colon carcinomas reduces their tumorigenicity and metastatic potential. The mechanism does not appear to be related to CD44 adhesion to hyaluronate.

In summary, alterations in CD44 alternative splicing associated with transformation of colonic mucosa to carcinoma result in up-regulation of variant CD44 isoforms that have been implicated in tumor metastases. In addition, malignant transformation is associated with down-regulation of CD44s. Introduction of CD44s into colon-carcinoma cells reduces their tumorigenicity and the growth of experimental liver metastases. Accordingly, the functional consequences of CD44s down-regulation in colon carcinomas may be just as significant as the consequences of up-regulation of other CD44 isoforms in tumor progression.

REFERENCES

BENNETT, K.L., JACKSON, D.G., SIMON, J.C., TANCZOS, E., PEACH, R., MODRELL, B., STAMENKOVIC, I., PLOWMAN, G. and ARUFFO, A., CD44 isoforms containing exon V3 are responsible for the presentation of heparin-binding growth factor. *J. Cell Biol.,* **128,** 687–698 (1995*a*).

BENNETT, K.L., MODRELL, B., GREENFIELD, B., BARTOLAZZI, A., STAMENKOVIC, I., PEACH, R., JACKSON, D.G., SPRING, F. and ARUFFO, A., Regulation of CD44 binding to hyaluronan by glycosylation of variably spliced exons. *J. Cell Biol.,* **131,** 1623–1633 (1995*b*).

BIRCH, M., MITCHELL, S. and HART, I.R., Isolation and characterization of human melanoma-cell variants expressing high and low levels of CD44. *Cancer Res.,* **51,** 6660–6667 (1991).

BROWN, T.A., BOUCHARD, T., ST. JOHN, T., WAYNER, E. and CARTER, W.G., Human keratinocytes express a new CD44 core protein (CD44E) as a heparan-sulfate intrinsic membrane proteoglycan with additional exons. *J. Cell Biol.,* **113,** 207–221 (1991).

CANNISTRA, S.A., KANSAS, G.S., NILOFF, J., DEFRANZO, B., KIM, Y. and OTTENSMEIER, C., Binding of ovarian-cancer cells to peritoneal mesothelium *in vitro* is partly mediated by CD44H. *Cancer Res.,* **53,** 3830–3838 (1993).

CULTY, M., NGUYEN, H.A. and UNDERHILL, C.B., The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. *J. Cell Biol.,* **116,** 1055–1062 (1992).

DALL, P., HEIDER, K.H., HEKELE, A., VON MINCKWITZ, G., KAUFMANN, M., PONTA, H. and HERRLICH, P., Surface protein expression and messenger-RNA-splicing analysis of CD44 in uterine cervical cancer and normal cervical epithelium. *Cancer Res.,* **54,** 3337–3341 (1994).

DASGUPTA, A., TAKAHASHI, K., CUTLER, M. and TANABE, K.K., O-linked glycosylation modifies CD44 adhesion to hyaluronate in colon-carcinoma cells. *Biochem. biophys. Res. Commun.,* **227,** 110–117 (1996).

FRIEDRICHS, K., FRANKE, F., LIBOA, B.W., KÜGLER, G., GILLE, I., TERPE, H.J., HÖLZEL, F., MAAS, N. and GÜNTHERT, U., CD44 isoforms correlate with cellular differentiation but not with prognosis in human breast cancer. *Cancer Res.,* **55,** 5424–5433 (1995).

GAO, A.C., LOU, W., DONG, J.T. and ISAACS, J.T., CD44 is a metastasis-suppressor gene for prostatic cancer located on human chromosome 11p13. *Cancer Res.,* **57,** 846–849 (1997).

GAO, A.C., LOU, W., SLEEMAN, J.P. and ISAACS, J.T., Metastasis suppression by the standard CD44 isoform does not require the binding of prostate-cancer cells to hyaluronate. *Cancer Res.,* **58,** 2350–2352 (1998).

GÜNTHERT, U., HOFMANN, M., RUDY, W., REBER, S., ZÖLLER, M., HAUBMANN, I., MATZKU, S., WENZEL, A., PONTA, H. and HERRLICH, P., A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell,* **65,** 13–24 (1991).

JALKANEN, S., JOENSUU, H., SODERSTROM, K.O. and KLEMI, P., Lymphocyte homing and clinical behavior of non-Hodgkin's lymphoma. *J. clin. Invest.,* **87,** 1835–1840 (1991).

MIWA, T., WATANABE, A., YAMADA, Y., SHINO, Y., YAMADA, T., YAMASHITA, J., MATSUDA, M. and NAKANO, H., Progression in gastric carcinoma relative to the ratio of CD44 epithelial variant transcript to CD44 hematopoietic variant transcript. *Cancer,* **77,** 25–29 (1996).

MORIKAWA, K., WALKER, S.M., JESSUP, J.M. and FIDLER, I.J., *In vivo* selection of highly metastatic cells from surgical specimens of different primary human colon carcinomas implanted into nude mice. *Cancer Res.,* **48,** 1943–1948 (1988*a*).

MORIKAWA, K., WALKER, S.M., NAKAJIMA, M., PATHAK, S., JESSUP, J.M. and FIDLER, I.J., Influence of organ environment on the growth, selection, and metastasis of human colon-carcinoma cells in nude mice. *Cancer Res.,* **48,** 6863–6871 (1988*b*).

PEACH, R.J., HOLLENBAUSH, I., STAMENKOVIC, I. and ARRUFO, A., Identification of hyaluronic-acid-binding sites in the extracellular domain of CD44. *J. Cell Biol.,* **122,** 257 (1993).

SOUKKA, T., SALMI, M., JOENSUU, H., HAKKINEN, L., SOINTU, P., KOULU, L., KALIMO, K., KLEMI, P., GRENMAN, R. and JALKANEN, S., Regulation of CD44v6-containing isoforms during proliferation of normal and malignant epithelial cells. *Cancer Res.,* **57,** 2281–2289 (1997).

SY, M.S., GUO, Y.J. and STAMENKOVIC, I., Distinct effects of two CD44 isoforms on tumor growth *in vivo. J. exp. Med.,* **174,** 859–866 (1991).

TAKAHASHI, K., ETO, H. and TANABE, K.K., Involvement of CD44 in matrix metalloproteinase-2 regulation in human melanoma cells. *Int. J. Cancer,* **80,** 387–395 (1999).

TAKAHASHI, K., STAMENKOVIC, I., CUTLER, M., DASGUPTA, A. and TANABE, K.K., Keratan-sulfate modification of CD44 modulates adhesion to hyaluronate. *J. biol. Chem.,* **271,** 9490–9496 (1996).

TAKAHASHI, K., STAMENKOVIC, I., CUTLER, M., SAYA, H. and TANABE, K.K., CD44 hyaluronate binding influences growth kinetics and tumorigenicity of human colon carcinomas. *Oncogene,* **11,** 2223–2232 (1995).

TANABE, K.K., ELLIS, L.M. and SAYA, H., Expression of the CD44R1 adhesion molecule is increased in human colon carcinomas and metastases. *Lancet,* **341,** 725–726 (1993).

TANABE, K.K., STAMENKOVIC, I., CUTLER, M., and TAKAHASHI, K., Restoration of CD44H expression in colon carcinomas reduces tumorigenicity. *Ann. Surg.,* **222,** 493–501 (1995).

THOMAS, L., BYERS, R., VINK, J. and STAMENKOVIC, I., CD44H regulates tumor-cell migration on hyaluronate-coated substrate. *J. Cell Biol.,* **113,** 971–977 (1992).

WIELENGA, V.J.M., HEIDER, K.-H., OFFERHAUS, J.A., ADOLF, G.R., VAN DEN BERG, F.M., PONTA, H., HERRLICH, P. and PALS, S.T., Expression of CD44 variant proteins in human colorectal cancer is related to tumor progression. *Cancer Res.,* **53,** 4754–4756 (1993).

YU, Q. and STAMENKOVIC, I., Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev.,* **13,** 35–48 (1999).