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CD44 Variant Isoform Expression and Breast Cancer Prognosis

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We examined the expression of CD44 isoforms in samples of breast cancer tissues from 95 patients by reverse transcription-polymerase chain reaction and immunohistochemistry, and tried to correlate the results with survival period. At the RNA level, expression of exon v2 was observed in 33 (35%) and that of v6 in 69 (73%) of the 95 specimens. Patients with CD44v2 mRNA expression had significantly shorter survival times than those with v2-negative tumors (P=0.05), but there was only a weak correlation, if any, between v6 mRNA expression and overall survival (P=0.06). Tumor tissue from 22 (23%) and 72 (76%) patients showed positive immunoreactivity with monoclonal antibody (mAb) M23.6.1. (CD44v2) and mAb 2F10 (CD44v6), respectively. Immunohistochemical evidence of CD44v2 peptide expression correlated with overall survival (P=0.02), but there was no such association with CD44v6 expression in these tumors (P=0.67). There were significant correlations between v2 immunoreactivity and higher histological grade and lower levels of estrogen and progesterone receptor. There was no significant correlation between v6 immunoreactivity and such clinicopathological characteristics. Although the expression of v2 was significantly associated with reduced overall survival, it was not an independent prognostic factor because it also correlated with progesterone receptor status. These findings suggest that v2 isoform expression might have more value than v6 expression for clinical use.

Key words: CD44 variant — Breast cancer — Prognostic factor — RT-PCR — Immunohistochemistry

The term CD44 denotes a large family of trans-membrane glycoproteins, expressed as a number of isoforms, assembled by alternative splicing of a number of variably expressed exons. Ten of the exons of the gene are consistently expressed in all cell types as a predominant isoform called the standard form (CD44s). Peptides encoded by the variably expressed exons of the gene can be incorporated into the polypeptide backbone corresponding to the standard form at a specific insertion site in the proximal extra-membrane domain. Post-translational modifications, including glycosylation, then result in a diverse range of isoforms (CD44v) expressed in a cell-type-specific manner according to prevailing circumstances. These proteins are known to be involved in a wide variety of cell functions.[1)] Since Gunthert *et al*.[2)] reported that overexpression of exon 11(v6) of the CD44 gene may cause tumor metastasis in rat pancreatic tumor cells, several clinicopathological studies have indicated that elevated expression of CD44v isoforms containing this component correlates with faster progression of neoplastic disease, metastasis and reduced survival period. Thus, for example, most studies are in agreement that strong and abundant v6 expression is correlated with higher grading and poorer prognosis of non-Hodgkin's lymphoma.[3–6)] However, for breast cancer, there are contradictory reports concerning the relation between v6 expression and prognosis. Kaufmann *et al*.[7)] and Dall *et al*.[8)] reported that the CD44 epitope encoded by exon v6 was an independent prognostic factor, but Friedrichs *et al*.[9)] failed to find a significant correlation between expression of the isoform and short survival. The current study was undertaken to resolve this discrepancy and also to evaluate whether a new CD44v mAb which we have recently raised against an epitope encoded by exon 7 (v2) could be a better prognostic marker. We have previously reported that expression of this v2-encoded epitope is elevated in many tumors including breast cancer.[10–13)]

MATERIALS AND METHODS

Tissue samples This study was conducted on samples of snap-frozen tissue from 95 primary breast cancers, diagnosed between December 1989 and November 1992 in the National Cancer Center Hospital, Tokyo.

RT-PCR and Southern hybridization mRNA was isolated from a single frozen section (6 μm) of each sample of tumor tissue with a Micro-FastTrack Kit (Invitrogen, San Diego, CA), followed by cDNA synthesis using a

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The abbreviations used are: CD44s and CD44v, standard and variant isoforms of CD44, respectively; RT-PCR, reverse transcription-polymerase chain reaction.

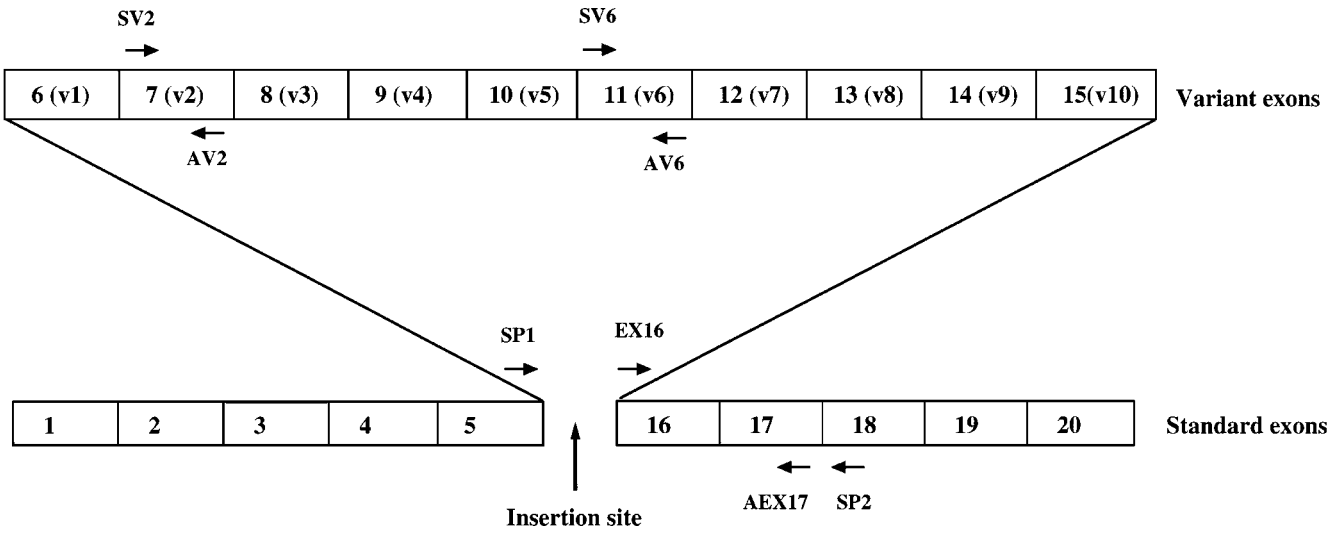

Fig. 1. Structure of the CD44 gene showing the positions to which the primers anneal. The synthesized cDNA was amplified by PCR using the primers SP1 and SP2, with which all the inserted variant forms as well as the standard portion (194 bp) of CD44 exons can be amplified. The probe sizes used were: v2, 123 bp; v6, 129 bp and standard probe, 121 bp.

cDNA Cycle Kit (Invitrogen). The synthesized cDNA was amplified by 35 cycles of PCR. The cycle conditions were: 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min. The primers used were: SP1, 5′-TGGATCACCGACAG-CACAGACAGA-3′; SP2, 5′-GATGCCAAGATGATCAG-CCATTCTGGA-3′. With these, all the inserted variant exons as well as the standard portion of CD44 exons can be amplified (Fig. 1). CD44 PCR products were resolved on a 1.2% agarose gel, blotted onto Hybond N+nylon membrane (Amersham, Buckinghamshire, UK), and subsequently hybridized with CD44 variant exon-specific probe. Each probe was made by amplifying the TA plasmid clones containing variant exons or standard exons,[13] using related primers (Fig. 1). Each PCR product for probe was then extracted and directly labeled with peroxidase to produce the chemiluminescence probe used in the ECL system (Fig. 1). The condition used for hybridization, highly stringent washing of the filters and signal detection were those recommended in the manufacturer's protocol for the ECL system (Amersham). The primers used for making probes were: Ex16, 5′-AGACCAAGACA-CATTCCA-3′; AEx17, 5′-TGTCCTTATAGGACCAGA-3′; Sv2, 5′-TTGATGAGCACTAGTGCTACAGCA-3′; Av2, 5′-CATTTGTGTTGTTGTGTGAAGATG-3′; Sv6, 5′-GGCAA-CTCCTAGTAGTACAACG-3′; Av6, 5′-CAGCTGTCCCT-GTTGTCGAATG-3′.

Immunohistochemistry Cryostat tissue sections (6 μm) were fixed in 100% ice-cold methanol for 20 min, washed in Tris-buffered saline (TBS) and preincubated with 20% normal rabbit serum in TBS. After having been washed with TBS, separate sections were incubated with the primary antibodies F10-44-2 (3.3 μg/ml), 2F10 (10 μg/ml) or M23.6.1. (20 μg/ml), which recognize CD44s, CD44v6 and CD44v2, respectively, in 1% normal rabbit serum diluted with TBS at 4°C overnight, followed by 10 min at 37°C next morning. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol and the sections were incubated with a 1:400 dilution of biotinylated anti-mouse immunoglobulin (Dako Corp., Santa Barbara, CA) at 37°C for 60 min, followed by incubation with horseradish peroxidase-conjugated ABComplex (Dako Corp.) at room temperature for 60 min. The peroxidase activity was developed with 3, 3-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) for 20 min, and the reaction was stopped in water. All sections were counterstained with Mayer's haemalum. Staining was defined as positive if more than 5% of tumor cells were stained and the accompanying control, without primary antibody, was negative. The histological samples were assigned a numerical code, and CD44 staining was assessed blind, without knowledge of survival data or other patient-related information.

Statistical methods The Kaplan-Meier model was used for disease-free and overall survival curves.[14] Differences in survival between subgroups of patients were compared by using the log rank test. The Cox proportional hazards model was used in multivariate regression analysis of survival data.[15] The relationship between CD44 expression and well-known risk factors was analyzed by using the χ^2 test and the Mantel-Haenszel test.

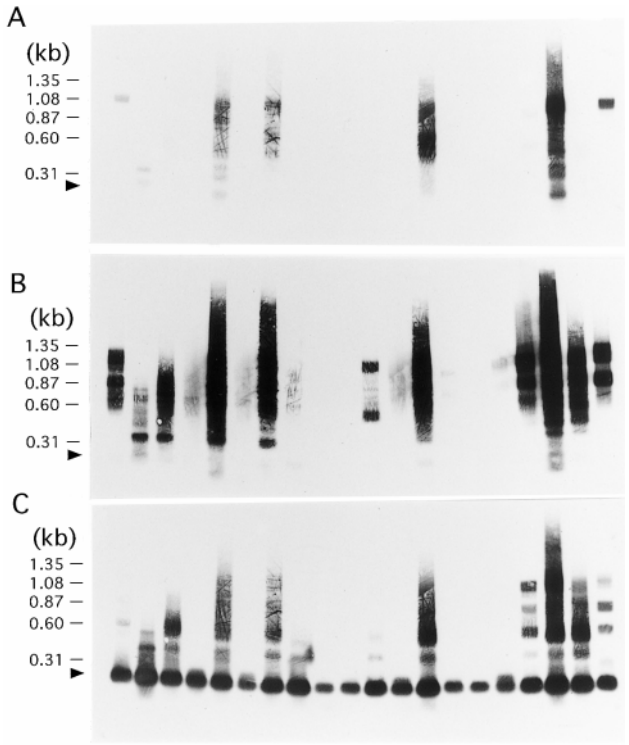

Fig. 2. RT-PCR/Southern hybridization. CD44 PCR products were hybridized with CD44 variant exon specific probes: Probes for CD44s, v2 and v6 were generated by amplifying the TA plasmid clones containing standard exons or variant exons using related primers. Each PCR product was then extracted and directly labeled with peroxidase to produce the chemiluminescence probe used in the ECL system. Panels A, B and C show the same filter hybridized with probes for v2, v6 and the standard form of CD44, respectively. In panel A and B, some samples showed long intense smears indicating that overabundant transcripts produced by the CD44 gene in tumor cells contain immature mRNA species of a wide range of sizes. Others showed occasional and discrete bands, or no signal at all. In panel C, the dense 194 bp band (arrowhead) at the base of all the tracks represents the constitutively expressed standard form CD44. All 95 samples including the 20 in this figure showed the dense 194 bp band, confirming comparable loading of the tracks and quality of starting RNA.

RESULTS

RT-PCR and Southern blotting (Fig. 2) The standard form was expressed in all 95 samples tested. Hybridization with v2 and v6 probes revealed several expression patterns. Some samples showed long, intense smears indicating that overabundant transcripts produced by the CD44 gene in tumor cells contain immature mRNA species with a wide range of sizes. Others showed occasional and discrete bands, or no signal at all. A positive signal was observed in 33 (35%) of the samples with v2 probe and in 69 (73%) with the v6 probe.

Immunohistochemical analysis (Fig. 3) Reactivity with all the CD44-specific antibodies was localized mainly on the cell membranes, and some cells showed positive staining of the cytoplasm. All tumor tissue samples tested were positively stained with F10-44-2(CD44s). Twenty-two (23%) showed staining with mAb M23.6.1. (CD44v2) and 72 (76%) with 2F10 (CD44v6). All of the CD44v2-positive samples were also CD44v6 positive.

Statistical analysis The correlation between CD44v2 and v6 expression and clinicopathological characteristics is summarized in Table I. CD44v2 protein expression was significantly associated with the histological grade of breast cancer tissue specimens studied ($P<0.05$), but v6 protein was not. Reduced expression of estrogen receptor and progesterone receptor was also correlated with v2 protein expression and v6 mRNA expression ($P<0.05$). No association was found between expression of the CD44 variants studied here and menopausal status, node status, tumor size or staging. The relationship between clinicopathological characteristics, including CD44 expression status, and prognosis is shown in Table II. Comparison of the length of disease-free survival with expression of CD44v6 and v2-containing isoforms did not reveal statistically significant associations. Fig. 4 shows the Kaplan-Meier curves for the overall survival of the patients. Patients with CD44v2-positivity (at either the mRNA level or the protein level) had significantly shorter survival times than those with v2-negative tumors ($P=0.05$ at the mRNA level, $P=0.02$ at the protein level) (Table II, Fig. 4, B and C). On the other hand, positivity of CD44v6 at either the mRNA or the protein level did not correlate with overall survival (Table II, Fig. 4, B and D). In the Cox proportional hazard regression model, selected by the score criterion, only progesterone receptor status (<15 fmol/mg; $P<0.001$) and lymph node status (>10; $P<0.01$) independently influenced on survival.

DISCUSSION

Many studies have linked the expression of CD44 isoforms to tumor progression and metastatic capability in human malignant diseases, such as gastrointestinal cancer,[16–19] cervical cancer,[20] renal cancer,[21] non-Hodgkin's lymphoma,[5, 6] neuroblastoma,[22, 23] and breast cancer.[7, 8] There is also some direct experimental support for this hypothesis. A highly metastatic clone derived from a single cell of a rat pancreatic adenocarcinoma cell line expressed CD44v6-containing isoforms, but a non-metastatic sister clone from the same cell line did not.[2] Direct transfection of CD44 cDNA containing v6 into non-metastatic rat pancreatic cell clone induced it to become metastatic.[2] Also, co-injection of an antibody against v6-

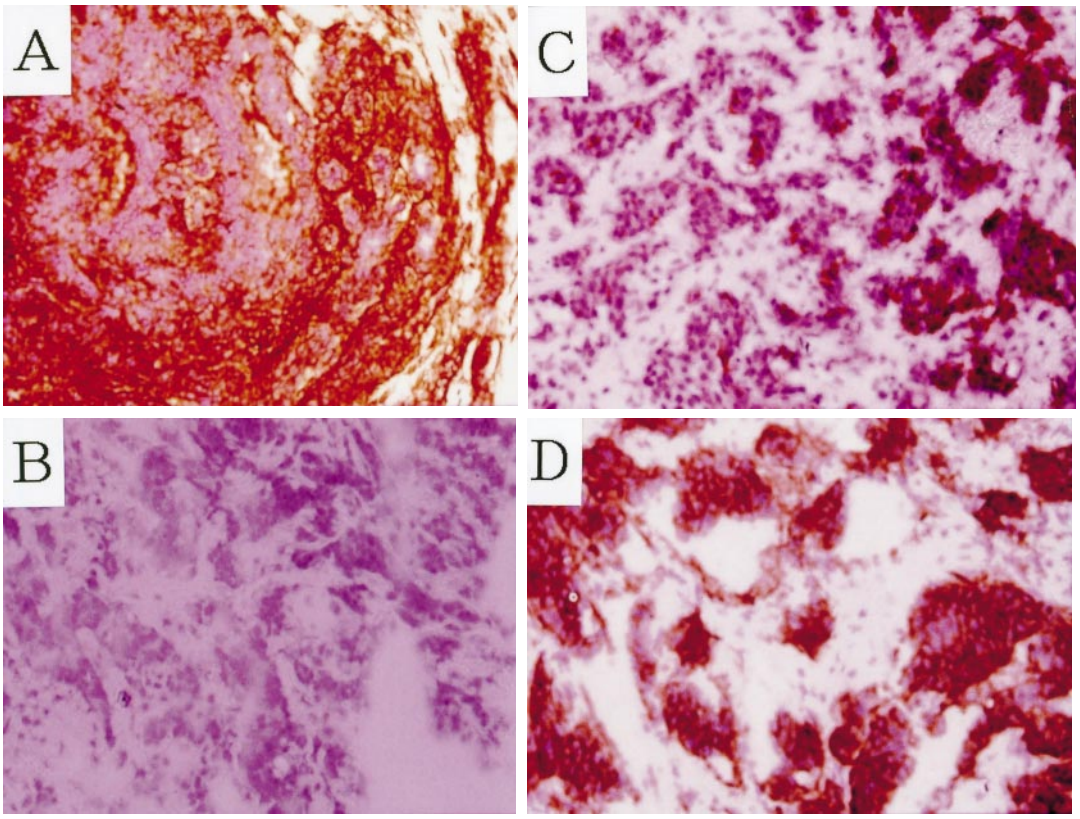

Fig. 3. Immunohistochemistry of breast cancer tissue: A, Both cancer and normal interstitial parts were stained strongly with mAb F10-44-2 to CD44s; C, Both positive and negative part were stained with mAb M23.6.1. to CD44v2 in the same cancerous lesions. On the other hand, the normal interstitial site was negative. D, The staining pattern with mAb 2F10 to CD44v6 showed a mosaic pattern like that with M23.6.1. in cancerous lesions, but CD44v6 was not detectable in normal interstitial parts. B, Negative control. (A–D, same tissue sample; ×200)

encoded peptide with the metastatic cells suppressed their metastatic behavior.[24] However, it is also relevant to mention that knockout of both copies of the CD44 gene in another metastatic tumor cell line was reported to have no effect on the metastatic capability.[25]

In the present study, we could not find a statistically significant correlation between the expression of v6-containing proteins and shorter survival period in breast cancer. This concurs with the results obtained by Friedrichs *et al*.[9] However, using the new monoclonal antibody M23.6.1. directed against a v2-encoded epitope, it was observed that the presence of v2-positive cells in breast cancers is associated with decreased survival period of the patients (P=0.02, Fig. 4A). The correlations of v6 and v2 mRNA expression with survival confirmed those observed at the protein level (P=0.67 for v6, P=0.05 for v2). These findings suggested that v2 isoform expression might have more value than v6 expression as a guide to the prognosis of patients with breast cancer. However, multivariate analysis demonstrated that, in this study of 95 patients, none of the indices of CD44 gene expression which we studied, including v2 proteins, was an independent prognostic factor. Reduced estrogen and progesterone receptor levels in carcinoma tissues showed a statistically significant correlation with v2 expression, but not with v6 at the protein level (Table I). These results suggest that v2 antigen expression may be associated with cellular dedifferentiation. On the other hand, in the case of mRNA expression detected by RT-PCR, reduced levels of the hormone receptors were correlated with v6, but not with v2. At present, we can not interpret this inconsistency of v2 and v6 expression using immunohistochemistry and RT-PCR. However, we have shown that most of the diverse mRNA isoforms of CD44 do not detectably translate into proteins.[26] Therefore, we think that expression at the protein level may be more significant than that at the mRNA level. Care is necessary in interpreting the clinical significance of v2-containing CD44 proteins in breast cancer,

Table I. Correlation between CD44 v2 and v6 Expression and Clinicopathological Characteristics

	No. of patients	Immunohistochemistry		RT-PCR	
		v2	v6	v2	v6
		(% positive cases)			
Menopausal status					
pre-	49	10 (20.4)	39 (79.6)	14 (28.6)	33 (67.3)
post-	46	12 (26.1)	33 (71.7)	19 (41.3)	36 (78.3)
Node status					
positive	62	13 (21.0)	45 (72.6)	12 (19.4)	43 (69.4)
negative	32	9 (28.1)	27 (84.4)	21 (65.6)	26 (81.3)
unknown	1				
Tumor size					
pT1	14	3 (21.4)	12 (85.7)	6 (42.9)	10 (71.4)
pT2	54	13 (24.1)	39 (72.2)	19 (35.2)	42 (77.8)
pT3	18	4 (22.2)	14 (77.8)	5 (27.8)	11 (61.1)
pT4	8	2 (25.0)	7 (87.5)	3 (37.5)	6 (75.0)
unknown	1				
Stage					
I	12	3 (25)	10 (83.3)	5 (41.7)	8 (66.7)
II	53	13 (24.5)	40 (75.5)	19 (35.9)	42 (79.3)
III	26	3 (11.5)	18 (69.2)	5 (19.2)	18 (34.0)
IV	3	2 (66.7)	3 (100)	1 (33.3)	1 (33.3)
Estrogen receptor[c)]					
≥20	48	7 (14.6)	38 (79.2)	16 (33.3)	31 (64.6)
<20	46	15[a)] (32.6)	34 (73.9)	17 (37.0)	38[a)] (82.6)
unknown	1				
Progesterone receptor[c)]					
≥16	47	6 (12.8)	35 (74.5)	14 (29.8)	30 (63.8)
<16	47	16[a)] (34.0)	37 (78.7)	19 (40.4)	39[a)] (82.9)
unknown	1				
Histological grade					
G1	11	0 (0)	8 (72.7)	2 (18.2)	7 (63.6)
G2	44	9 (20.5)	31 (70.5)	16 (36.4)	34 (77.3)
G3	39	12[b)] (30.8)	32 (82.1)	14 (35.9)	27 (69.2)
unknown	1				

a) Significantly different by χ^2 test ($P<0.05$).
b) Significantly different by Mantel-Haenszel test ($P<0.05$).
c) fmol/mg protein.

Table II. Prognostic Value of Clinicopathological Characteristics and Expression of CD44 Variants

	P value	
	Disease-free survival	Overall survival
Menopausal status	0.858	0.702
Node status	0.107	0.265
Tumor size	0.017	0.097
Estrogen receptor	0.260	0.032
Progesterone receptor	0.018	<0.001
Histological grade	0.192	0.197
v2 protein	0.253	0.018
v6 protein	0.825	0.673
v2 mRNA	0.320	0.047
v6 mRNA	0.354	0.063

P value was determined by using the log rank test.

although our earlier work on the expression of v2[10, 26)] still indicates that it may have some clinical utility.

There are now numerous publications recording unusual or disorderly CD44 gene expression in many different types of human cancer. Several of these suggest a link between cancer and the degree of expression of certain exons: for example v6 in non-Hodgkin's lymphoma,[5, 6)] v6 or v8 to v10 in colonic cancer,[18, 19)] v6 or v7 to v8 in cervical cancer,[20)] and v9 inz stomach cancer.[16)] On the other hand, several reports have indicated that CD44s expression is associated with longer survival in neuroblastoma.[22, 23)] This large body of information is difficult to interpret because there has been great variation in the methods used to observe and measure expression of the many components of this large gene. There is also rel-

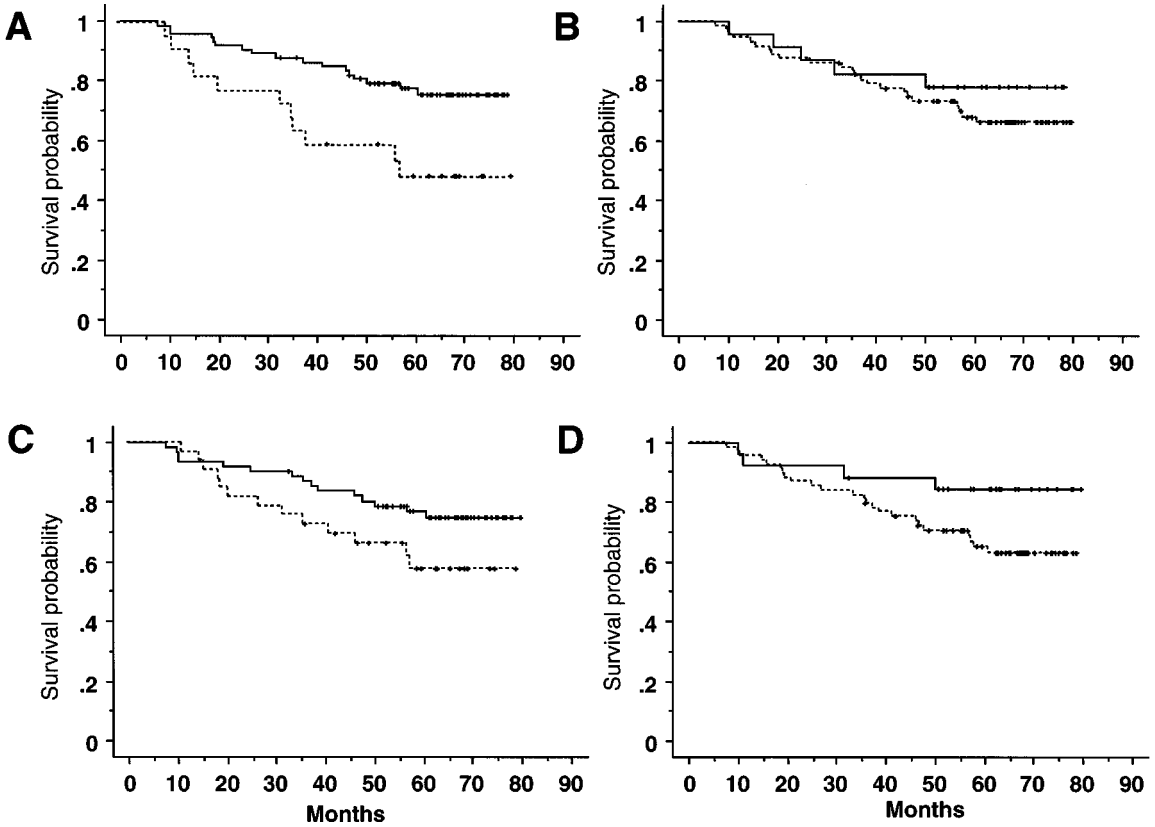

Fig. 4. Overall survival probability of breast cancer patients categorized according to expression of CD44 isoforms. A: v2 epitope, —— M23.6.1. negative, ····· M23.6.1. positive, *P*=0.02, B: v6 epitope, —— 2F10 negative, ····· 2F10 positive, *P*=0.67, C: v2 mRNA, —— v2 mRNA negative, ····· cv2 mRNA positive, *P*=0.05 and D: v6 mRNA, —— v6 mRNA negative, ····· v6 mRNA positive, *P*=0.06.

atively little information on the state of CD44 expression at different stages in the neoplastic process or in different parts of an invading neoplasm. Furthermore, there are distinct differences in the patterns of CD44 expression in normal cells and tissues according to their anatomical location and cell lineage; for example, abundant expression of many CD44 isoforms is seen in normal skin and in squamous epithelia, while the expression of CD44v is often restricted to cells in the basal layer and most v forms are not found in cells on the mucosal surface.[27–29] Hence, the changes in expression in tumors derived from the corresponding cell types could be related to the natural patterns of expression of this gene.

It is becoming increasingly clear that profound disturbances in the regulation of expression of this complex gene and in the splicing of its many exons are commonly seen in neoplastic cells. It is still unknown whether these abnormalities are causal or whether they are merely incidental side effects of tumor pathogenesis. Much further work is needed before these issues will be unraveled, but it is already evident that unusual CD44 gene expression is worthy of investigation because of its potential to provide us with powerful new diagnostic or prognostic markers. Deeper analysis might also provide new insights into the mechanisms of malignancy.

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REFERENCES

1) Haynes, B. F., Telen, M. J., Kale, L. P. and Denning, S. M. CD44 — a molecule involved in leukocyte adherence and T-cell activation. *Immunol. Today*, **10**, 423–428 (1989).
2) Gunthert, U., Hofmann, M., Rudy, W., Reber, S., Zoller, M., Haussmann, 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).
3) Koopman, G., Heider, K. H., Horst, E., Adolf, G. R., van den Berg, F., Ponta, H., Herrlich, P. and Pals, S. T. Activated human lymphocytes and aggressive non-Hodgkin's lymphomas express a homologue of the rat metastasis-associated variant of CD44. *J. Exp. Med*., **177**, 897–904 (1993).
4) Terpe, H. J., Koopmann, R., Imhof, B. A. and Gunthert, U. Expression of integrins and CD44 isoforms in non-Hodgkin's lymphomas: CD44 variant isoforms are preferentially expressed in high-grade malignant lymphomas. *J. Pathol*., **174**, 89–100 (1994).
5) Stauder, R., Eisterer, W., Thaler, J. and Gunthert, U. CD44 variant isoforms in non-Hodgkin's lymphoma: a new independent prognostic factor. *Blood*, **85**, 2885–2899 (1995).
6) Ristamaki, R., Joensuu, H., Soderstrom, K. O. and Jalkanen, S. CD44v6 expression in non-Hodgkin's lymphoma: an association with low histological grade and poor prognosis. *J. Pathol*., **176**, 259–267 (1995).
7) Kaufmann, M., Heider, K. H., Sinn, H. P., von Minckwitz, G., Ponta, H. and Herrlich, P. CD44 variant exon epitopes in primary breast cancer and length of survival. *Lancet*, **345**, 615–619 (1995).
8) Dall, P., Heider, K. H., Sinn, H. P., Skroch-Angel, P., Adolf, G., Kaufmann, M., Herrlich, P. and Ponta, H. Comparison of immunohistochemistry and RT-PCR for detection of CD44v-expression, a new prognostic factor in human breast cancer. *Int. J. Cancer*, **60**, 471–477 (1995).
9) Friedrichs, K., Franke, F., Lisboa, B. W., Kugler, G., Gille, I., Terpe, H. J., Holzel, F., Maass, H. and Gunthert, U. CD44 isoforms correlate with cellular differentiation but not with prognosis in human breast cancer. *Cancer Res*., **55**, 5424–5433 (1995).
10) Borgya, A., Woodman, A., Sugiyama, M., Donie, F., Kopetzki, E., Matsumura, Y. and Tarin, D. Isolation and characterisation of antibodies which specifically recognise the peptide encoded by exon 7 (v2) of the human CD44 gene. *J. Clin. Pathol. Mol. Pathol*., **48**, M241–M250 (1995).
11) Matsumura, Y. and Tarin, D. Significance of CD44 gene products for cancer diagnosis and disease evaluation. *Lancet*, **340**, 1053–1058 (1992).
12) Matsumura, Y., Hanbury, D., Smith, J. and Tarin, D. Non-invasive detection of malignancy by identification of unusual CD44 gene activity in exfoliated cancer cells. *Br. Med. J*., **308**, 619–624 (1994).
13) Matsumura, Y., Sugiyama, M., Matsumura, S., Hayle, A. J., Robinson, P., Smith, J. C. and Tarin, D. Unusual retention of introns in CD44 gene transcripts in bladder cancer provides new diagnostic and clinical oncological opportunities. *J. Pathol*., **177**, 11–20 (1995).
14) Kaplan, E. L. and Meier, P. Non parametric estimation from incomplete distributions. *J. Am. Stat. Assoc*., **53**, 457–481 (1958).
15) Cox, D. R. Regression models and life table method in analysing survival. *J. R. Stat. Soc*., **34**, 187–220 (1972).
16) Mayer, B., Jauch, K. W., Günthert, U., Figdor, C. G., Schildberg, F. W., Funke, I. and Johnson, J. P. De-novo expression of CD44 and survival in gastric cancer. *Lancet*, **342**, 1019–1022 (1993).
17) 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).
18) Mulder, J. W., Kruyt, P. M., Sewnath, M., Oosting, J., Seldenrijk, C. A., Weidema, W. F., Offerhaus, G. J. and Pals, S. T. Colorectal cancer prognosis and expression of exon-v6-containing CD44 proteins [see comments]. *Lancet*, **344**, 1470–1472 (1994).
19) Yamaguchi, A., Urano, T., Goi, T., Saito, M., Takeuchi, K., Hirose, K., Nakagawara, G., Shiku, H. and Furukawa, K. Expression of a CD44 variant containing exons 8 to 10 is a useful independent factor for the prediction of prognosis in colorectal cancer patients. *J. Clin. Oncol*., **14**, 1122–1127 (1996).
20) Kainz, C., Kohlberger, P., Tempfer, C., Sliutz, G., Gitsch, G., Reinthaller, A. and Breitenecker, G. Prognostic value of CD44 splice variants in human stage III cervical cancer. *Eur. J. Cancer*, **31A**, 1706–1709 (1995).
21) Terpe, H. J., Storkel, S., Zimmer, U., Anquez, V., Fischer, C., Pantel, K. and Gunthert, U. Expression of CD44 isoforms in renal cell tumors. Positive correlation to tumor differentiation. *Am. J. Pathol*., **148**, 453–463 (1996).
22) Favrot, M. C., Combaret, V. and Lasset, C. CD44—a new prognostic marker for neuroblastoma. *N. Engl. J. Med*., **329**, 1965 (1993).
23) Combaret, V., Gross, N., Lasset, C., Frappaz, D., Peruisseau, G., Philip, T., Beck, D. and Favrot, M. C. Clinical relevance of CD44 cell-surface expression and N-*myc* gene amplification in a multicentric analysis of 121 pediatric neuroblastomas. *J. Clin. Oncol*., **14**, 25–34 (1996).
24) Seiter, S., Arch, R., Reber, S., Komitowski, D., Hofmann, M., Ponta, H., Herrlich, P., Matzku, S. and Zoller, M. Prevention of tumor metastasis formation by anti-variant CD44. *J. Exp. Med*., **177**, 443–455 (1993).
25) Driessens, M. H., Stroeken, P. J., Rodriguez Erena, N. F., van der Valk, M. A., van Rijthoven, E. A. and Roos, E. Targeted disruption of CD44 in MDAY-D2 lymphosar-

coma cells has no effect on subcutaneous growth or metastatic capacity. *J. Cell Biol.*, **131**, 1849–1855 (1995).
26) Woodman, A. C., Sugiyama, M., Yoshida, K., Sugino, T., Borgya, A., Goodison, S., Matsumura, Y. and Tarin, D. Analysis of anomalous CD44 gene expression in human breast, bladder, and colon cancer and correlation of observed mRNA and protein isoforms. *Am. J. Pathol.*, **149**, 1519–1530 (1996).
27) Higashikawa, K., Yokozaki, H., Ue, T., Taniyama, K., Ishikawa, T., Tarin, D. and Tahara, E. Evaluation of CD44 transcription variants in human digestive tract carcinomas and normal tissues. *Int. J. Cancer*, **66**, 11–17 (1996).
28) Sugiyama, M., Woodman, A., Sugino, T., Crowley, S., Ho, K., Smith, J., Matsumura, Y. and Tarin, D. Non-invasive detection of bladder cancer by identification of abnormal CD44 proteins in exfoliated cancer cells in urine. *J. Clin. Pathol. Mol. Pathol.*, **48**, M142–M147 (1995).
29) Abbasi, A. M., Chester, K. A., Talbot, I. C., Macpherson, A. S., Boxer, G., Forbes, A., Malcolm, A. D. and Begent, R. H. CD44 is associated with proliferation in normal and neoplastic human colorectal epithelial cells. *Eur. J. Cancer*, **29A**, 1995–2002 (1993).