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# Up-regulation of CD44 Variant Exon Expression in Endometrial Carcinomas: Analysis of mRNA and Protein Isoforms, and Relation to Clinicopathological Factors

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**In order to clarify the relation between expression of individual CD44 variant exons and tumor progression, 34 endometrial carcinomas (endometrioid type) were investigated, as well as 27 samples of normal endometrium, using a combination of reverse transcription-polymerase chain reaction (RT-PCR) and Southern blot hybridization (SBH). Western blotting was also performed for comparison of protein levels with the results of the RT-PCR/SBH methods. Analysis of gross CD44 splicing patterns demonstrated high-level expression of variant isoforms in endometrial carcinomas as compared with normal endometrium. Exon-specific RT-PCR/SBH assays revealed large, abundant transcripts of individual variant exons, in particular v3, v4, and v5, in tumors, but these isoforms were also expressed in normal endometria, suggesting a lack of tumor-specificity. No individual CD44 variant transcripts were associated with any of the prognostic factors investigated. Parallel observations showed variant CD44 transcripts to be more readily detectable than protein isoforms in the same samples. These findings indicate that in endometrial carcinomas, expression of individual variant CD44 exons is markedly up-regulated, but this molecule may not be useful as a consistent indicator of tumor progression.**

Key words: CD44 — Endometrial carcinoma — RT-PCR — Southern blot — Western blot

CD44 forms constitute a polymorphic family of immunologically related integral membrane proteoglycans and glycoproteins which play important roles in lymphocyte homing, lymphocyte activation, cell-cell adhesion, cell-matrix adhesion, cell migration, and tumor metastasis.[1–4] Genomic analysis has revealed that the CD44 gene is 60 to 80 kbp in size, located on human chromosome 11p13, and composed of at least 20 exons.[5, 6] The standard form of CD44 (CD44s) with a molecular weight of 80–90 kDa lacks all variant exons through 6 to 15 and is widely distributed in lymphoid and non-lymphoid tissues, while the larger variant isoforms (CD44v) contain sequences encoded by these variant exons in different combinations, being normally expressed on a variety of epithelial cells.[7, 8] Some of the variant isoforms have been indicated to produce functional changes. For example, the presence of the 396 bp insert in the epithelial variant of CD44 reduces the affinity for hyaluronic acid, although CD44s is known to be the principal receptor for this molecule.[9]

It has been documented that transfection of cDNA coding for CD44 into a non-metastasizing rat pancreatic carcinoma cell line conferred metastatic properties, while pretreatment with anti-variant CD44 prevented metastasis formation.[10, 11] Deregulation of alternative splicing of the CD44 gene is typical of a number of human malignant tumors, suggesting a close correlation with progression or dedifferentiation.[12–14] Moreover, it has been asserted that abnormal expression of CD44 is a useful molecular marker for early detection of urinary bladder and colorectal carcinomas.[15, 16]

In endometrial carcinoma, although Fujita *et al.*[17] demonstrated an association between decreased CD44 expression and lymph-vascular space involvement of tumor cells, it is still unclear whether specific alternative splicing variants play a role in tumor development or progression. In the present study, we therefore investigated endometrial carcinomas, using a combination of the reverse transcription-polymerase chain reaction (RT-PCR), Southern blotting hybridization and western blotting assays, and compared the results with several clinicopathological factors.

## MATERIALS AND METHODS

**Cases** A total of 34 cases of endometrial carcinomas (endometrioid type), surgically resected at the Kitasato University Hospital from 1995 to 1997, were investigated. Twenty-seven samples (13 proliferative, 4 secretory and 10 in the atrophic phase) of normal endometrium obtained from patients undergoing total hysterectomy for non-neo-

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plastic diseases were also examined. Tissues were snap-frozen immediately after surgical excision and stored at –80°C until use. Simultaneously, specimens were routinely fixed in 10% formalin and processed for embedding in paraffin wax. Histological diagnosis was performed according to the criteria of the International Federation of Gynecology and Obstetrics.[18] The carcinoma cases comprised 20 grade (G) 1, 11 G2, and 3 G3 lesions. Clinicopathological factors, including clinical stage, degree of myometrial invasion, lymph node status and histological grade, were also assessed for comparison with the results for variant CD44 mRNA expression.

**RT-PCR** Total cellular RNA was extracted from tumor and normal tissues using Isogen (Nippon Gene Co., Tokyo) in accordance with the manufacturer's instructions. cDNA was synthesized from 5 μg of total RNA using RAV-2 reverse transcriptase (Takara, Shiga) in the presence of random primers (Takara) and a ribonuclease inhibitor (Takara) in a 20 μl reaction volume at 42°C for 60 min. Three microliters of cDNA solution was amplified by Taq polymerase (Takara) in a volume of 25 μl with the P1 (5′-GACACATATTGCTTCAATGCTTCAGC-3′) and P2 (5′-GATGCCAAGATGATCAGCCATTCTG-GAAT-3′) primers described by Matsumura and Tarin.[19] These anneal to exon 3/exon 4-linked and exon 16/exon 17-linked regions of the CD44 gene, respectively (Fig. 1). For detection of each variant exon, forward primers derived from the 5′ sequences of exon 6b (v2,5′-GAT-GAGCACTAGTGCTACAGCAAC-3′), exon 7 (v3,5′-GTACGTCTTCAAATACCATCTCAG-3′), exon 8 (v4,5′-CAACCACACCACGGGCTTTTGACC-3′), exon 9 (v5,5′-ATGTAGACAGAAATGGCACCACTG-3′), exon 10 (v6,5′-TCCAGGCAACTCCTAGTAGTACAA-3′), exon 11 (v7,5′-CAGCCTCAGCTCATACCAGCCATC-3′), and exon 12 (v8,5′-ATATGGACTCCAGTCATAGTACAA-3′), as described by Gotley *et al.*,[20] were each used with a common reverse P2 primer (Fig. 1). The PCR procedure was performed with 30 cycles of denaturation at 94°C for 0.5 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, with a pre-denaturing time of 2 min and a final extension time of 7 min. As a negative control, water was used instead of template cDNA for each examination. To examine the quality and quantity of the synthesized cDNA, the β-actin gene[21] (sense, 5′-TGCTATCCAGGCTGTGCTAT-3′ and anti-sense, 5′-GATGGAGTTGAAGGTAGTTT-3′) was also amplified.

**Southern blot hybridization (SBH)** A 10 μl aliquot of each PCR reaction mixture was electrophoresed in a 2% agarose gel and transferred to a Hybond N nylon membrane (Amersham, Tokyo) with 10× SCC solution overnight. After prehybridization using DIG Easy Hyb (Boehringer Mannheim, Tokyo) solution, filters were hybridized overnight with digoxigenin-labeled exon-specific probes. The sequences of the oligonucleotide probes for CD44s, CD44v3, CD44v6, CD44v10, and β-actin were as follows: probe S (5′-CCTGAAGAAGATTGTA-CATCAGTCACAGAC-3′), probe v3 (5′-GTACGTCTTC-AAATACCATCTCAG-3′), probe v6 (5′-TCCAGGCAA-CTCCTAGTAGTACAA-3′), probe v10 (5′-GATGTCAC-AGGTGGAAGAAGAGACCCA-3′) (Fig. 1), and probe β-actin (5′-ACTGACTACCTCATGAAGATCCTCACCG-AG-3′). Hybridization signals were detected with a DIG Luminescent Detection kit (Boehringer Mannheim). The conditions used for hybridization, washing, and detection

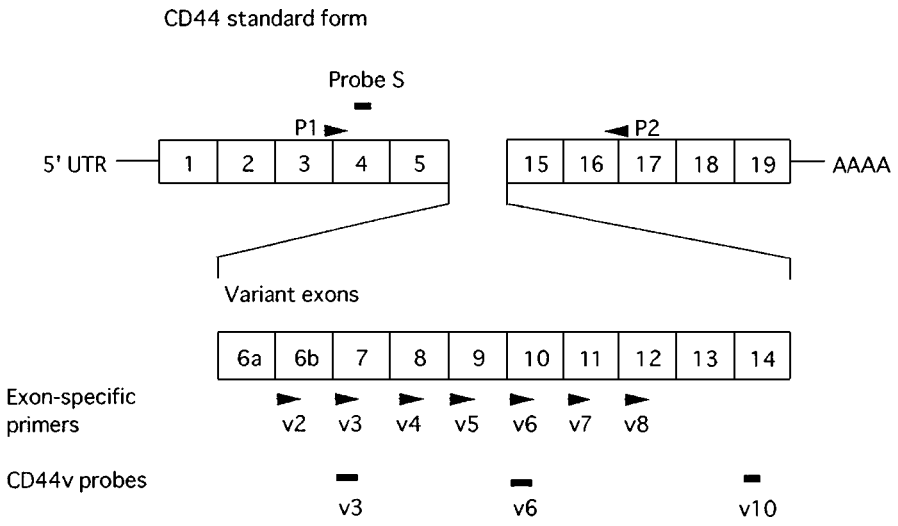


Fig. 1. Schematic illustration of the CD44 gene, demonstrating the positions of the primers and probes used. UTR, untranslated region.

were in line with the manufacturer's recommendations. Between each hybridization the filter was stripped before being rehybridized with another probe.

The blots were scored to calculate the percentage positivity for each CD44 variant exon. For the exon-specific RT-PCR/SBH assay, the hybridization signals were subdivided into two categories: large transcripts showing the expected molecular weights, and small transcripts demonstrating shorter fragments than those expected.

**Western blot analysis** Tissue samples were homogenized in 0.1 *M* phosphate-buffered saline (PBS) solution and clarified by centrifugation (12000 rpm, 30 min). The supernatants were mixed 1:1 (v/v) with 2× sample buffer (Laemmli sample buffer[22]), and then the concentration of protein was determined with a Bio-Rad protein assay kit and adjusted to 10 $\mu$g/lane by dilution with 2× sample buffer. Protein samples were boiled for 5 min before separation by 6% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The separated proteins were electroblotted (50 mA for 2 h) onto Immobilon-P (Millipore, Tokyo) using blotting buffer (48 m*M* tris, 39 m*M* glycine, 20% methanol, 0.1% SDS). The membranes were blocked with Block Ace (Dainihon Seiyaku Co., Osaka) to avoid non-specific reactions and then incubated overnight at 4°C with optimum dilutions of primary antibodies. The antibodies used were anti-human phagocytic glycoprotein-1 mouse monoclonal antibody for CD44s (×100 dilution, Dako, Copenhagen, Denmark), and anti-CD44v10 mouse monoclonal antibody (BMS119, mAb VFF-14, ×15 dilution, Bender MedSystem, Vienna, Austria) binding to the variant exons 8 to 10. Reactivity was detected by incubation with peroxidase-conjugated anti-mouse Ig G (×1000 dilution, Dako) for 1 h at room temperature and signals were visualized using Western Blot Chemiluminescence Reagent ("NEN" Life Science Products, Boston, MA). As a positive control, normal uterine cervical epithelium was used, since it has abundant expression of CD44 standard and variant forms.[23, 24]

**Statistics** Comparative data were analyzed using the Mann-Whitney U test. The criterion for statistical significance was defined as $P<0.05$.

## RESULTS

**CD44 mRNA expression in normal and malignant endometrium** RNAs obtained from 27 normal endometrial and 34 endometrial carcinoma samples could all be successfully amplified using primer sets for the β-actin gene, which provided a fragment with a molecular size of 446 bp (Fig. 2).

In ethidium-bromide-stained gels, RT-PCR products amplified with primers P1 and P2 were detected as two major bands, approximately 500 bp (standard framework) and 900 bp [combination of standard and epithelial (v8–10) forms], along with several minor bands. In addition, weak bands over 1500 bp in size were also amplified in tumor but not normal samples (data not shown).

Three probes annealing to the variant 3, 6, and 10 regions predominantly reacted with multiple transcripts having high molecular weights. The expression of CD44v containing these regions was markedly increased in all tumor samples, the hybridization signals being weak or undetectable in normal tissues (Fig. 2).

The probe S hybridized with multiple alternatively spliced CD44 mRNA transcripts containing sequences from exons in the standard and variant regions; strong signals were also obtained for the CD44s (Fig. 2).

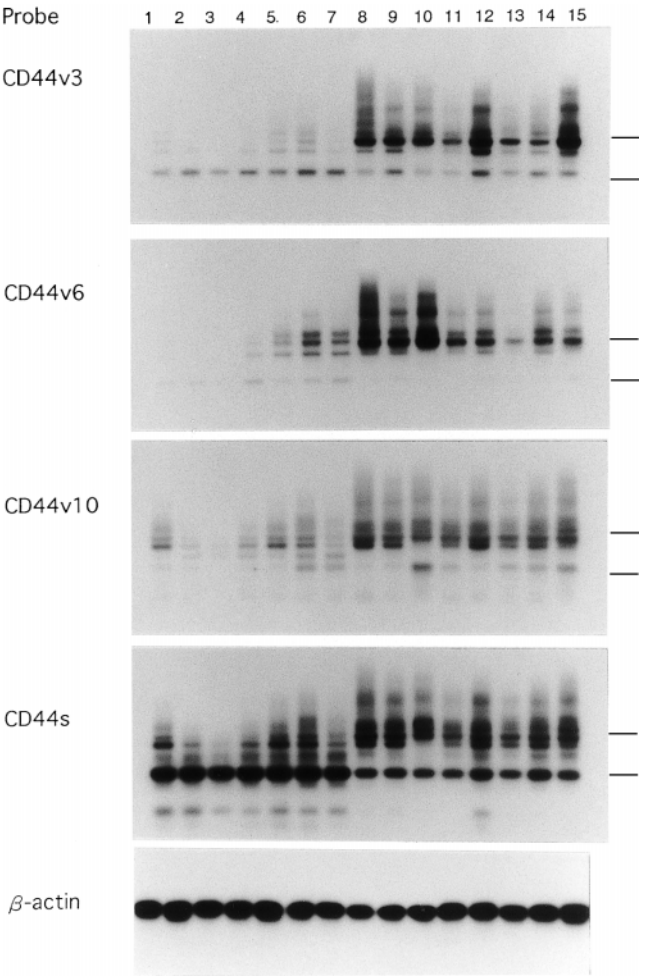


Fig. 2. A combination of RT-PCR with primers P1-P2 and Southern blot hybridization using probes CD44v3, CD44v6, CD44v10, CD44s, and β-actin. Strong signals for variant exons are apparent for endometrial carcinomas (lanes 8 to 15) as compared with normal samples (lanes 1 to 7). Note hybridization of probe S with the CD44s. Upper and lower bars indicate 1000 bp and 500 bp, respectively.

**Exon-specific RT-PCR/SBH assay** To examine in more detail the range of CD44 variant exons, RT-PCR using exon-specific forward primers together with a common reverse primer P2 was performed. In ethidium-bromide-stained gels, PCR products amplified with primers v8 and P2 (epithelial transcript with a standard frame) were detected as a 549 bp band, and the sizes of other amplicons showed a stepwise increase from CD44v8 through to CD44v2 (1287 bp). In addition, minor PCR products of 600 bp to 1000 bp were also found with some primer sets (data not shown).

Fig. 3 shows results from a combination of exon-specific RT-PCR and SBH for tumor and normal samples. To examine the specificity of this assay, hybridizations using probes v3, v6, and S were performed. As expected, probes v3 or v6 only hybridized to the PCR products containing their sequences, while probe S reacted to amplicons of the CD44 standard framework, but not variant isoforms. Although smear patterns with combinations of primers v3-P2 and probe v3 (v3, lane 3), or primers v6-P2 and probe v6 (v6, lane 6), respectively, were observed, this was due to identical sequences between forward primers and oligonucleotide probes. In contrast, probe v10 was able to detect the full range of alternatively spliced CD44 variant exons (v2–v8) with the expected molecular weight range (large transcripts), the signals sequentially decreasing from v8 through to v2, particularly in normal samples.

Several combinations of small transcripts, in particular v2- or v3-containing isoforms, were also observed in both normal and tumor tissues, when probes v3, v6, and v10, respectively, were used.

**Range of variant CD44 expression in normal and malignant endometrium** Based on the results of exon-specific RT-PCR/SBH assay using probe v10, positivity for large transcripts containing CD44v3, v4, and v5 exons in tumors was significantly higher than in normal tissues, while a similar heterogeneity in the patterns for CD44 variant 6, 7, and 8 exons was observed (Fig. 4A).

A high frequency of CD44v3-containing small transcripts was observed in both normal and tumor tissues, but positivity for CD44v2-containing isoforms in the latter was significantly higher than in the former (Fig. 4B).

**Correlation between CD44 expression and prognostic factors in endometrial carcinomas** As shown in Fig. 5, positivity of individual CD44 large transcripts through v2 to v8 did not correlate with any of the clinicopathological parameters, such as clinical stage, degree of myometrial invasion, lymph node status, and grade of histological differentiation (data not shown).

**Western blot analysis of normal epithelium and tumors** With a monoclonal antibody for CD44s, protein isoforms were observed with molecular weights ranging from approximately 70 kDa to 180 kDa. In the normal group, bands were only detected in the 70 to 120 kDa range with relatively weak intensity. In tumor samples, however, additional major bands showing a molecular weight of about 160 kDa were also detected in some tumor samples (Fig. 6). When the presence of a band at 160 kDa was defined as positive, according to the criteria described by Sugiyama *et al.*,[25] positive signals were observed for 10 (30.3%) of 33 tumors, but none of the normal cases. With a monoclonal antibody against the CD44v8 to v10 regions, exhibiting a molecular weight range of approximately 90 to 250 kDa, along with a

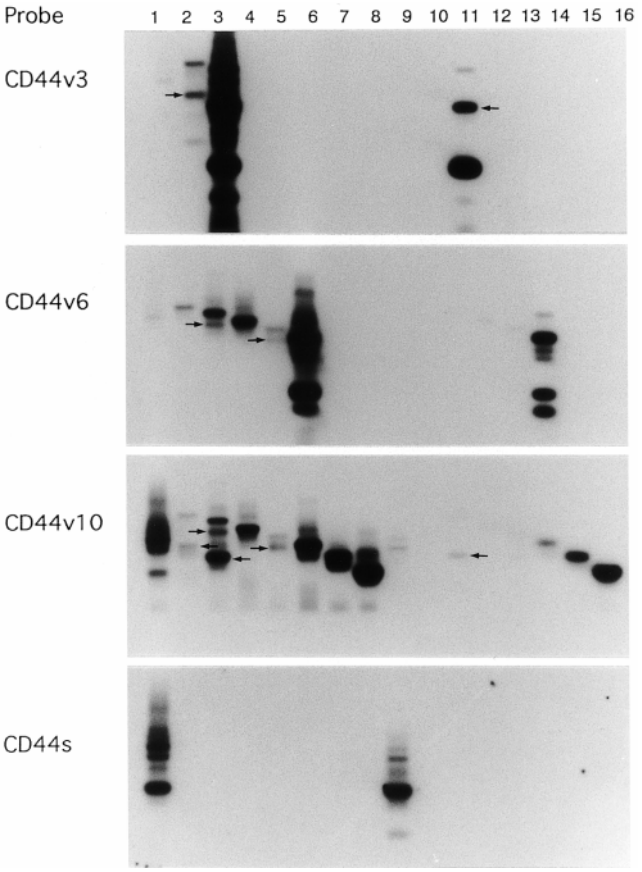


Fig. 3. A combination of exon-specific RT-PCR and Southern blot hybridization using probes CD44v3, CD44v6, CD44v10, and CD44s for an endometrial carcinoma (lanes 1–8) and normal endometrium (lanes 9–16). Different combinations of signals for large transcripts are apparent with the probes. Small transcripts, in particular CD44v2- and v3-containing isoforms, are also evident (indicated by arrows). Primer sets; lanes 1 and 9, P1-P2; lanes 2 and 10, v2-P2; lanes 3 and 11, v3-P2; lanes 4 and 12, v4-P2; lanes 5 and 13, v5-P2; lanes 6 and 14, v6-P2; lanes 7 and 15, v7-P2; lanes 8 and 16, v8-P2. The smear pattern in v3 (lane 3) and v6 (lane 6) is due to identical sequences between forward primers and probes.

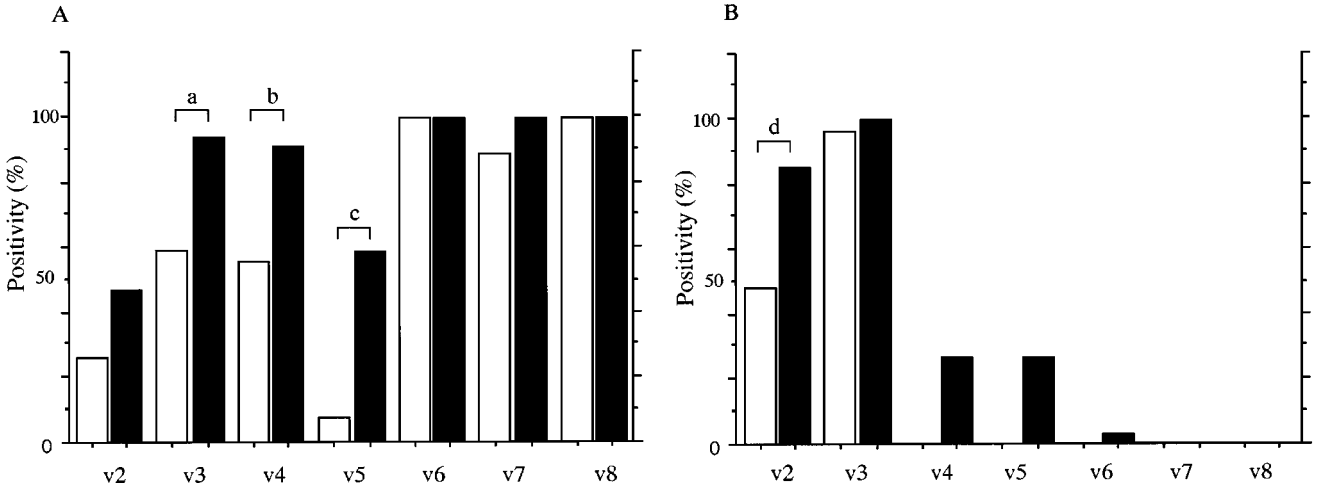


Fig. 4. Comparison of positivity for individual variant exons between normal and malignant endometrial samples. A, large transcripts; B, small transcripts; ☐ normal endometrium, *n*=27; ■ endometrial carcinoma, *n*=34; *n*, number of cases. a, *P*=0.02; b, *P*=0.018; c, *P*=0.0006; d, *P*=0.0013.

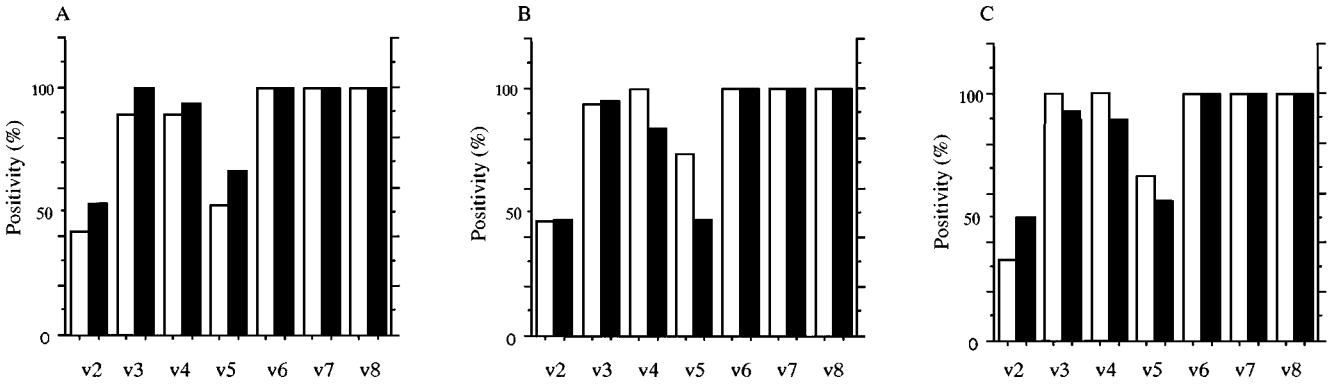


Fig. 5. Relation between variant CD44 expression and clinicopathological factors for endometrial carcinomas. *n*, number of cases. A, Clinical stage, ☐ stage I, *n*=19, ■ stage II/III/IV, *n*=15; B, Myometrial invasion (MI), ☐ MI<1/2, *n*=14, ■ MI>1/2, *n*=20; C, lymph node metastasis, ☐ positive, *n*=6, ■ negative, *n*=28.

smear pattern (Fig. 6), positivity was observed for 13 (39.4%) of 33 tumors and 10 (50 %) of 20 normal tissues.

## DISCUSSION

Quantitative up-regulation of CD44 mRNA transcripts containing variant 8 to 10 exons has been demonstrated for a variety of normal and tumor tissues,[9, 20, 26] CD44v8–10 (epithelial forms) being usually co-expressed in epithelium.[24] In our RT-PCR results with primers P1-P2/SBH assay (Fig. 2), probe v10 always hybridized to the PCR products obtained from normal and tumor samples, with varying intensity, even when signals were undetectable with probes v3 or v6. These findings were confirmed by the exon-specific RT-PCR/SBH assay (Fig. 3), indicating that CD44v containing v8 to v10 exons are widely expressed in normal to malignant endometrium. In this study, we therefore analyzed the positivity for individual variant exons on the basis of the results for exon-specific assays using probe v10.

Detection of individual alternative splicing variants of CD44 mRNAs is of interest, since it is relevant to the question of whether a specific CD44 isoform has a promoting or a suppressing effect on tumor development and progression. Some studies, however, have indicated no overexpression of any individual variant exons specific to tumors.[19, 20] In our results, large transcripts containing individual variant exons, in particular v3, v4, and v5

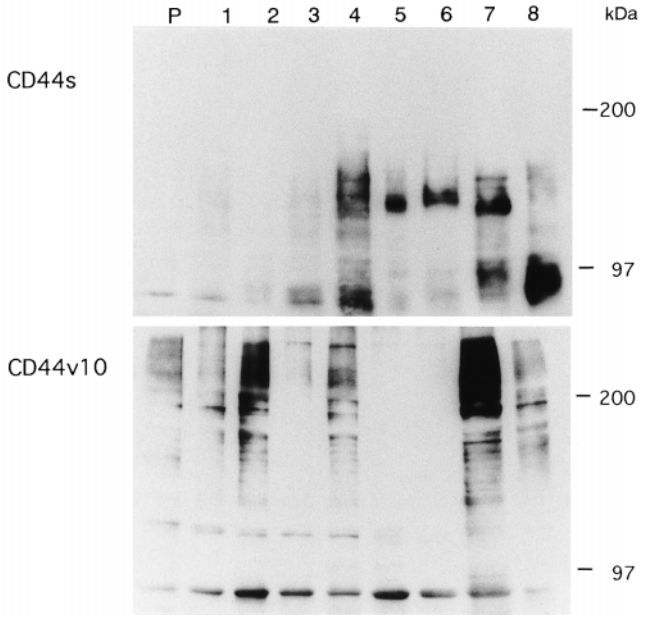


Fig. 6. Western blot analysis of normal endometria (lanes 1–3) and endometrial carcinomas (lanes 4–9). With the CD44s antibody (upper), major bands of about 160 kDa are apparent for tumor samples, while minor bands of approximately 70 to 120 kDa are evident in both normal and tumor cases. With the CD44v10 antibody (lower), major bands of approximately 180 to 250 kDa are found in both samples, along with a smear pattern. P, positive control (uterine cervical squamous epithelium).

exons, were observed in tumors, but these isoforms were also expressed in some normal endometrial samples, similarly suggesting the absence of tumor-specific variant exons. Conversely, tumor cells may simply produce large transcripts containing additional exons more abundantly, maintaining a similar CD44 alternative splicing pattern to that of the normal endometrial glandular cells.

A close association between aberrant CD44 expression and tumor progression has been demonstrated for a variety of human malignancies. Overexpression of CD44 variant exons has been reported to indicate a more aggressive phenotype in non-Hodgkin's lymphomas, and colorectal and gastric carcinomas,[28–30] while down-regulation has been documented to be related to invasive features in neuroblastomas, and prostatic and bladder carcinomas.[31–33] Gotley *et al.*[20] recently indicated that no individual CD44 variant exon was clearly associated with colorectal carcinoma progression. In endometrial carcinomas, it has been reported that reduced CD44 expression may be related to metastasis of endometrial cancer cells through the lymphvascular space.[17] Our data, however, showed no correlation between individual variant exons and known prognostic factors. Although the reason for this discrepancy is unclear, we conclude from the present results that CD44 may not be useful as a consistent prognostic indicator for endometrial carcinomas.

In this study, positivity for CD44v2-containing small isoforms in tumors was significantly higher than for normal samples, and CD44v4-, v5-, and v6-containing small transcripts were also observed in tumors but not normal cases, while a similar frequency of detection of CD44v3-containing small transcripts was observed for both. Although we did not perform DNA sequencing, these small isoforms may be due to a lack of some variant exons in comparison with large transcripts. Recently, Goodison *et al.*[34] demonstrated that in normal colonic mucosa, the standard exon 5 was characteristically adjacent to variant exon 3, but in tumors, transcripts in which exon 5 abutted onto variant exon 2 were common. Given the presence of an alternative splice acceptor site on variant exon 3,[35] CD44v3-containing small transcripts appear to be a common feature during CD44 alternative splicing in normal and malignant endometria. In contrast, increases in other small isoforms in tumors, in particular those containing CD44v2 isoforms, may be closely associated with up-regulation of expression of large CD44 transcripts.

Woodman *et al.*[36] recently reported some striking and unexpected discrepancies in the proportions of the prevalent CD44 mRNA and protein isoforms expressed. Thus, comparative studies of transcription and translation in tumor cells suggested that many of the CD44 transcripts detected by RT-PCR may not be translated. Similar findings have been demonstrated for DCC genes in normal osteocytes and osteosarcomas.[21] While Dall *et al.*[37] reported a good correlation between immunohistochemistry and RT-PCR data, our parallel observations indicated that many CD44 transcripts containing a variety of exon combinations are more readily detectable than protein isoforms in the same samples. The possible reasons for the relative lack of CD44 proteins in our study include: 1) protein products may be degraded during sample preparation and processing due to protease activity; 2) alteration of protein structure resulting from alternative splicing of mRNA may be important, since it has been reported that insertion of products of some variant exons in the variably expressed region may induce conformational changes in the peptide backbone, resulting in the masking of epitopes; 3) the sensitivity of western blotting may be below the threshold of detection for the protein isoforms.

In conclusion, the present study demonstrated that expression of individual variant CD44 exons is markedly up-regulated in endometrial carcinomas, but this molecule may not be useful as a consistent indicator of tumor progression.

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REFERENCES


1) Haynes, B. F., Telen, M. J., Hale, L. P. and Denning, S. M. CD44: a molecule involved in leukocyte adherence and T-cell activation. *Immunol. Today*, **10**, 423–428 (1989).
2) Underhill, C. CD44: the hyaluronan receptor. *J. Cell Sci.*, **103**, 293–298 (1992).
3) Gunthert, U. CD44: a multitude of isoforms with diverse functions. *Curr. Top. Microbiol. Immunol.*, **184**, 47–63 (1993).
4) Lesley, J., Hyman, R. and Kincade, P. W. CD44 and its interaction with extracellular matrix. *Adv. Immunol.*, **54**, 271–335 (1993).
5) Screaton, G. R., Bell, M. V., Bell, J. I. and Jackson, D. G. The identification of a new alternative exon with highly restricted tissue expression in transcripts encoding the mouse Pgp-1 (CD44) homing receptor. *J. Biol. Chem.*, **268**, 12235–12238 (1993).
6) Tolg, C., Hofman, M., Herrlich, P. and Ponta, H. Splicing choice from ten variant exons establishes CD44 variability. *Nucleic Acids Res.*, **21**, 1225–1229 (1993).
7) Fox, S. B., Fawcett, J., Jackson, D. G., Collins, I., Gatter, K. C., Harris, A. L., Gearing, A. and Simmons, D. L. Normal human tissues, in addition to some tumors, express multiple different CD44 isoforms. *Cancer Res.*, **54**, 4539–4546 (1994).
8) Mackay, C. R., Terpe, H.-J., Stauder, R., Marston, W. L., Stark, H. and Gunthert, U. Expression and modulation of CD44 variant isoforms in humans. *J. Cell Biol.*, **124**, 71–82 (1994).
9) Stamenkovic, I., Aruffo, A., Amiot, M. and Seed, B. The hematopoietic and epithelial forms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate-bearing cells. *EMBO J.*, **10**, 343–348 (1991).
10) Rudy, W., Hofmann, M., Schwartz-Albeiz, R., Zoller, M., Heider, K. M., Ponta, H. and Herrlich, P. Two major CD44 proteins expressed on a metastatic rat tumor cell line are derived from different splice variants: each one individually suffices to confer metastatic behaviour. *Cancer Res.*, **53**, 1262–1268 (1993).
11) Reber, S., Matzku, S., Gunthert, H., Ponta, P., Herrlich, P. and Zoller, M. Retardation of metastatic tumor growth after immunisation with metastasis-specific monoclonal antibodies. *Int. J. Cancer*, **46**, 919–927 (1990).
12) 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).
13) 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).
14) Tanabe, K. K., Ellis, L. M. and Saya, H. Expression of CD44R1 adhesion molecule in colon carcinomas and metastases. *Lancet*, **341**, 725–726 (1993).
15) 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).
16) Yoshida, K., Bolodeoku, J., Sugino, T., Goodison, S., Matsumura, Y., Warren, B. F., Toge, T., Tahara, E. and Tarin, D. Abnormal retention of intron 9 in CD44 gene transcripts in human gastrointestinal tumors. *Cancer Res.*, **55**, 4273–4277 (1995).
17) Fujita, N., Yaegashi, N., Ide, Y., Sato, S., Nakamura, M., Ishiwata, I. and Yajima, A. Expression of CD44 in normal human *versus* tumor endometrial tissues: possible implication of reduced expression of CD44 in lymph-vascular space involvement of cancer cells. *Cancer Res.*, **54**, 3922–3928 (1994).
18) Creasman, W. T. Announcement, FIGO stages: 1988 revisions. *Gynecol. Oncol.*, **35**, 125–127 (1989).
19) Matsumura, Y. and Tarin, D. Significance of CD44 gene products for cancer diagnosis and disease evaluation. *Lancet*, **340**, 1053–1058 (1992).
20) Gotley, D. C., Fawcett, J., Walsh, M. D., Reeder, J. A., Simmons, D. L. and Antalis, T. M. Alternatively spliced variants of the cell adhesion molecule CD44 and tumour progression in colorectal cancer. *Br. J. Cancer*, **74**, 342–351 (1996).
21) Horstmann, M. A., Posl, M., Scholz, R. B., Anderegg, B., Simon. P., Baumgaertl, K., Delling, G. and Kabisch, H. Frequent reduction or loss of *DCC* gene expression in human osteosarcoma. *Br. J. Cancer*, **75**, 1309–1317 (1997).
22) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).
23) Herold-Mende, C., Seiter, S., Born, A., Patzelt, E., Schupp, M., Zoller, J., Bosch F. X. and Zoller, M. Expression of CD44 splice variants in squamous epithelia and squamous cell carcinomas of the head and neck. *J. Pathol.*, **179**, 66–73 (1996).
24) Seiter, S., Tilgen, W., Herrmann, K., Schadendorf, D., Patzelt., E., Moller, P. and Zoller, M. Expression of CD44 splice variants in human skin and epidermal tumours. *Virchows Arch.*, **428**, 141–149 (1996).
25) 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).
26) Behzad, F., Seif, M. W., Campbell, S. and Aplin, J. D. Expression of two isoforms of CD44 in human endometrium. *Biol. Reprod.*, **51**, 739–747 (1994).
27) Terpe, H. J., Stark, H., Prehm, P. and Günthert, U. CD44 variant isoforms are preferentially expressed in basal epithelia of non-malignant human fetal and adult tissues. *Histochemistry*, **101**, 79–89 (1994).

28) Terpe, H.-J., Koopmann, R., Imhof, B. A. and Günthert, 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).
29) Wielenga, V. J. M., Heider, K.-H., Offerhaus, G. 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).
30) Washington, M. K., Gottfried, M. R. and Telen, M. J. Expression of the cell adhesion molecule CD44 in gastric adenocarcinoma. *Lab. Invest.*, **68**, 52A (1993).
31) Gross, N., Beretta, C., Peruisseau, G., Jackson, D., Simmons, D. and Beck, D. CD44H expression by human neuroblastoma cells: relation to MYCN amplification and lineage differentiation. *Cancer Res.*, **54**, 4238–4242 (1994).
32) Kallakury, B. V. S., Yang, F., Figge, J., Smith, K. E., Kausik, S. J., Tacy, N. J., Fisher, H. A. G., Kaufman, R., Figge, H. and Ross, J. S. Decreased levels of CD44 protein and mRNA in prostate carcinoma: correlation with tumor grade and ploidy. *Cancer*, **78**, 1461–1469 (1996).
33) Sugino, T., Gorham, H., Yoshida, K., Bolodeoku, J., Nargund, V., Cranston, D., Goodison, S. and Tarin, D. Progressive loss of CD44 gene expression in invasive bladder cancer. *Am. J. Pathol.*, **149**, 873–882 (1996).
34) Goodison, S., Yoshida, K., Sugino, T., Woodman, A., Gorham, H., Bolodeoku, J., Kaufmann, M. and Tarin, D. Rapid analysis of distinctive CD44 RNA splicing preferences that characterize colonic tumors. *Cancer Res.*, **57**, 3140–3144 (1997).
35) Screaton, G. R., Bell, M. V., Jackson, D. G., Cornelis, F. B., Gerth, U. and Bell, J. I. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. *Proc. Natl. Acad. Sci. USA*, **89**, 12160–12164 (1992).
36) 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).
37) 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)