

Potential Use of Soluble CD44 in Serum as Indicator of Tumor Burden and Metastasis in Patients with Gastric or Colon Cancer

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ABSTRACT

Soluble CD44 is present in the serum of normal individuals (2.7 ± 1.1 nM). The concentration of soluble CD44 in the serum is elevated in patients with advanced gastric (24.2 ± 9.8 nM) or colon cancer (30.8 ± 11 nM). Serum CD44 concentration correlated with tumor metastasis and tumor burden. Surgical resection of tumors resulted in decreases in serum CD44 levels. By Western blot analysis, monoclonal anti-CD44 antibody reacted with a major protein with molecular weight between 130,000 and 190,000. In addition, two proteins with molecular weights of 72,000 and 80,000 can also be identified. Therefore, different CD44 isoforms may be present in the serum of cancer patients. Serum CD44 concentrations may be an indicator of tumor burden and metastasis in patients with malignant diseases.

INTRODUCTION

CD44 is a glycoprotein present in many cell types. Monoclonal antibodies against CD44 recognize M_r 80,000–90,000 glycoproteins on human lymphoid cells (1–4). The complementary DNA for CD44 has been cloned (5–9). Earlier studies revealed that there were two isoforms of CD44 based on their mRNA sizes and the molecular weights of the proteins. The first form is expressed in hematopoietic cells and has a protein product with a molecular weight of 80,000–90,000 (5–9). A soluble form of this CD44 isoform has been reported to be present in normal serum and in the synovial tissue of patients with rheumatoid arthritis (10, 11). The second isoform is a protein with a molecular weight of 130,000–160,000 expressed weakly in normal epithelium but highly expressed in some carcinomas (8, 9). Most of the carcinomas examined expressed both isoforms of CD44. The M_r 130,000 CD44 isoform contains 120 amino acid more than the M_r 90,000 CD44 in the extracellular portion of the molecule near the transmembrane region (8, 9). Keratinocytes express another CD44 isoform protein with a molecular weight of about 230,000 (12). Recent data suggest that there are at least nine isoforms of CD44 generated by differential splicing of the mRNA (13). The hematopoietic form of CD44 in lymphoid cells represents the basic unit of the CD44 proteins. The other isoforms were created by alternative splicing of the mRNA and the addition of new exons into the extracellular domain near the transmembrane region of the hematopoietic CD44 (13).

The sequence homology between extracellular domains of CD44 and macromolecules of the cellular matrix suggests a general role for CD44 in proteoglycan or collagen mediated matrix adhesion (14, 15). This hypothesis was supported by recent findings that one of the ligands for CD44 is hyaluronic acid (16–18). The receptor for hyaluronic acid has been studied over the last two decades by investigators interested in embryonic development and wound healing (19). Hyal-

uronic acid is a polymer consisting of repeating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid (19). Hyaluronic acid is a major constituent of the extracellular matrix and is believed to create a low resistance matrix, allowing enhanced cell motility. A microenvironment in which cells can migrate is essential for embryonic development and wound repair and is probably important for lymphocyte homing and tumor metastasis (20–22).

CD44 may play an important role in tumor growth and metastasis. Many of the primary carcinoma specimens examined expressed high levels of CD-44 (8). Highly invasive human bladder carcinoma cells express high levels of CD44. The noninvasive bladder carcinoma cell line expresses low levels of CD44 (23). Transformation of fibroblasts with SV40 or Rous sarcoma virus increased the expression of CD44 (24, 25). We established two human melanoma cell lines, SMMU-1 and SMMU-2, from a patient with melanoma. SMMU-1 is CD44⁺ and SMMU-2 is not. SMMU-1 is metastatic in SCID mice when injected s.c. while SMMU-2 is not.² A high expression of CD-44 is associated with aggressive behavior, dissemination, and poor prognosis of human non-Hodgkins lymphomas. Unlike the expression of CD44, expression of two other adhesion molecules, LFA-1 and ICAM-1, was not found to be a significant factor (26).

Introduction of the human hematopoietic form of CD-44 gene into a CD44⁻ tumor cell line, Namalwa, resulted in enhancement of tumor growth and metastasis *in vivo* (27). A rat carcinoma cell line which did not metastasize acquired metastatic properties when transfected with a CD44 gene encoding for a high molecular weight 230,000 CD44 isoform (28, 29). A human homologue of the rat M_r 230,000 CD44 isoform has been shown to be expressed in colorectal carcinomas and adenomatous polyps (30). Analysis of CD44 splice variants in tumor tissues from patients with colon and breast cancer by polymerase chain reaction revealed that there was gross overproduction of the alternatively spliced large molecular weight variants in all tumor tissues examined. In the control samples only the standard product was detected (31, 32). However, using monoclonal antibodies specific for various CD44 isoforms instead of polymerase chain reaction, more recent results revealed that different CD44 isoforms were expressed in many normal tissues, including those from which the tumors arose (33). The reasons for these discrepancies are not known. A colon cancer susceptibility gene in the mouse has been reported to be linked to the CD44 gene on chromosome 2 (34).

In this paper we present preliminary evidence that the concentration of soluble CD44 in the serum is elevated in patients with advanced gastric or colon cancer. Serum CD44 concentration correlated with tumor burden and metastasis of tumors. Surgical resection of tumors resulted in decreases in serum CD44 levels. By Western blot analysis, monoclonal anti-CD44 antibody reacted with a major protein with molecular weight between 130,000 and 190,000. In addition, two less abundant proteins with molecular weights of 72,000 and 80,000 can also be identified. These results suggested the presence of different CD44 isoforms in the serum of cancer patients. Serum CD44 concentrations may be an indicator of tumor burden and metastasis in patients with malignant diseases.

Received 8/9/93; accepted 11/10/93.

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MATERIALS AND METHODS

Monoclonal Antibodies and ELISA³ for Soluble CD44. Murine anti-human CD44 monoclonal antibodies GKW.A3 (IgG2a) and GKW.7.10 (IgG2b) were generated in our laboratory by immunizing normal BALB/c mice with a human melanoma cell line (SMMU) bearing the hematopoietic form of CD44. Fusion and selection of the monoclonal antibody secreting hybridomas were carried out by using standard B-cell hybridoma techniques. GKW.A3 and GKW.7.10 did not react with the human CD44 negative Burkitt's lymphoma Namalwa, but reacted strongly with Namalwa transfected with the human hematopoietic CD44 gene (results not shown). Another monoclonal anti-CD44 antibody, BU52, was obtained from The Binding Site Limited (San Diego, CA). GKW.A3, BU52, and GKW.7.10 recognize different epitopes on CD44 as revealed by competitive binding experiments and by dual color immunofluorescence staining. BU52, GKW.A3, and GKW.7.10 cannot distinguish different CD44 isoforms and bind to all CD44 isoforms (results not shown).

Sera were obtained from normal individuals or cancer patients and kept at -72°C prior to use for determining serum CD44 levels. We used an ELISA assay we have described earlier to detect soluble CD44 in the circulation (35). Briefly, plastic ELISA plates were first coated with $10\ \mu\text{g/ml}$ of protein A purified GKW.7.10 overnight. Plates were washed extensively. Serum from each patient was serially diluted and added to the plates. Plates were washed extensively and a biotin conjugated protein A purified GKW.A3 was used to react with captured soluble CD44 proteins. Avidin conjugated alkaline phosphatase was added to detect bound GKW.A3. In each assay, a genetically engineered recombinant soluble human CD44 chimeric molecule was used to determine the concentration of soluble CD44 present in each serum. Assays were repeated at least twice with each sample with comparable results. The statistical significance of differences among means was determined by using Student's *t* tests.

Purification of Soluble CD44 by Immunosorbent Column and Western Blot Analysis of Purified Proteins. An immunoaffinity column was prepared by conjugating protein A purified GKW.A3 to Sephadex 4B. Serum from 3 patients with metastatic colon cancer or from normal donors were pooled. Thirty-five ml of the serum were applied to the column. After extensive washing, bound materials were eluted with glycine HCl buffer (pH3.5). Eluted materials were neutralized immediately to pH 7.0 and were concentrated by negative pressure dialysis. Equal volumes of the samples from cancer patients and normal controls were separated by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. Proteins were then transferred to nitrocellulose membranes and blotted with monoclonal anti-CD44 antibodies GKW.7.10 or BU52 or control anti-human CD4 antibody.

RESULTS

Serum CD44 Concentration in Patients with Gastric Cancer. We used an ELISA to determine the concentration of soluble CD44 in the sera of normal individuals and 25 patients with various stages of gastric cancer. In 8 patients, tumors were localized in the stomach and no visible metastatic tumors were found in any other organ. Metastatic tumors were found in at least one other site in the other 17 patients. The age, sex, and pathological findings of these patients are listed in Table 1. The concentration of the soluble CD44 in the sera of controls and gastric cancer patients are presented in Fig. 1. The concentration of soluble CD44 in the serum of age and sex matched normal controls was $2.7 \pm 1.1\ \text{nM}$ ($n = 43$). The concentrations of soluble CD44 were elevated in 16 of 17 patients with metastatic gastric cancer. In the 8 patients without metastatic tumors, significantly elevated soluble CD44 concentrations were detected in 5 of the 8 patients. We determined the concentration of soluble CD44 in 15 patients with chronic rheumatic diseases. The concentration of soluble CD44 in these patients were comparable to those found in normal controls ($2.2 \pm 1.6\ \text{nM}$).

Serum CD44 Concentration in Patients with Colon Cancer. We determined the concentration of soluble CD44 in the sera of 25 pa-

Table 1 Patients with gastric cancer

No.	Age	Sex	Tumor (cm)	Metastasis
1	44	M	6	No
2	52	M	6.4	No
3	66	M	6.2	No
4	39	M	3.3	No
5	46	M	5.8	No
6	51	M	4.6	No
7	41	F	4.6	No
8	51	F	3.7	No
9	55	M	7	LN, ^a PR
10	61	M	6	LN, PR
11	39	M	8.5	LN, PR, Liv
12	55	F	5.7	LN
13	41	F	4.1	LN
14	57	M	5.9	LN, PR
15	59	M	9.2	LN
16	66	F	6.7	LN
17	49	M	7.2	LN, PR
18	50	M	6.3	LN, PR
19	57	F	7.1	LN, PR
20	66	M	9.2	LN, PR, Pan
21	71	M	7.2	LN, Pan, Colon
22	77	M	9.7	LN, PR, Colon
23	87	M	7.9	LN, PR, Colon, Spl
24	81	M	6.6	LN, PR, Colon
25	66	M	8.9	LN, PR, Colon, Spl

^a LN, lymph node; PR, peritoneum; Liv, liver; Pan, pancreas; Spl, spleen.

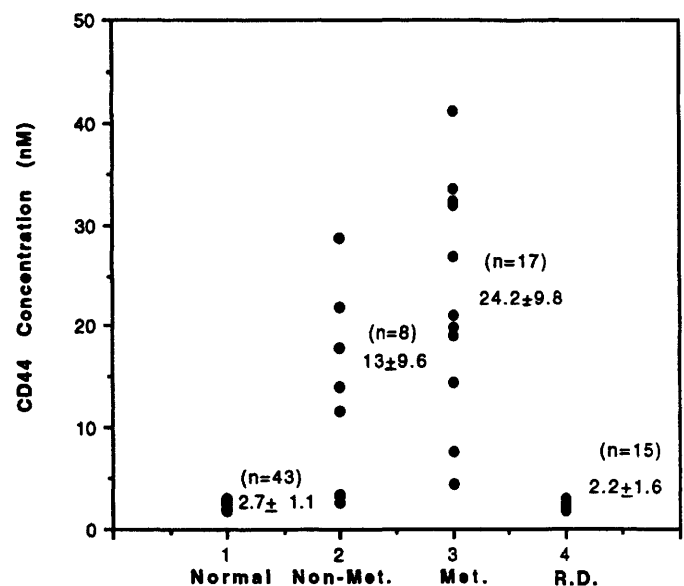


Fig. 1. Soluble CD44 in the serum from normal donors, patients with nonmetastatic gastric cancers, metastatic gastric cancers, and in patients with rheumatic diseases. The numbers represent the average concentration of soluble CD44 in different groups of individuals \pm SD. The *P* value for the differences between CD44 levels in patients with metastatic tumor and normal donor is $P > 0.001$ (Student's *t* test).

tients with different stages of colon cancer. In 10 of these patients with either stage I or stage II adenocarcinoma, tumors were localized in the colon and no metastatic tumors were found in other organs. Metastatic tumors were found in at least one other site in the other 15 patients with stage II, III, or IV adenocarcinoma. The age, sex, and pathological findings of these patients are listed in Table 2. The concentration of the soluble CD44 in the sera of controls and colon cancer patients are presented in Fig. 2. The concentrations of soluble CD44 were significantly elevated in 15 of the patients with metastatic colon cancer. Elevated soluble CD44 concentrations were detected in 4 of the patients with either stage I or stage II adenocarcinoma but without metastasis. Three of these 4 patients had stage II carcinoma with tumor size larger than 4.5 cm. The other patient had stage I carcinoma with a tumor size of less than 3 cm (Table 2).

³ The abbreviation used is: ELISA, enzyme linked immunosorbent assay.

Table 2 Patients with colon cancer

No.	Age	Sex	Tumor (cm)	Histology ^a (Stage) (differentiation)	Metastasis
1	51	M	<3	ADC (II) (M)	No
2	48	F	<3	ADC (II) (M)	No
3	50	M	3.6	ADC (II) (M)	No
4	53	M	<3	ADC (I) (M)	No
5	51	M	<3	ADC (I) (M)	No
6	44	M	<3	UND (I) (P)	No
7	57	F	<3	ADC (I) (H)	No
8	64	M	5.1	ADC (II) (H)	No
9	65	M	5.1	ADC (II) (H)	No
10	49	F	4.7	ADC (II) (H)	No
11	47	M	4.7	ADC (III) (P)	LN, Liv
12	67	M	5.6	ADC (IV) (M)	LN, Liv
13	77	F	7.8	ADC (IV) (H)	PR, LN, Lung, Liv
14	64	M	6.7	ADC (IV) (H)	LN, PR, Liv
15	78	M	7.4	ADC (IV) (M)	Multiple organs
16	54	M	5.7	ADC (III) (H)	LN, PR
17	61	M	4.6	ADC (III) (H)	LN
18	66	F	6.1	ADC (III) (P)	LN, PR, Liv
19	71	M	4.3	ADC (II) (H)	LN
20	44	M	4.9	ADC (III) (P)	LN
21	67	M	7.1	ADC (IV) (M)	LN
22	81	M	>10	ADC (IV) (M)	Multiple organs
23	69	M	>10	ADC (IV) (M)	Multiple organs
24	41	M	4.1	ADC (II) (P)	LN, PR
25	48	M	5.9	ADC (III) (M)	LN, PR

^a ADC, adenocarcinoma; P, poorly differentiated; M, moderately differentiated; H, highly differentiated; LN, lymph node; PR, peritoneum, Liv, liver; UND, undifferentiated.

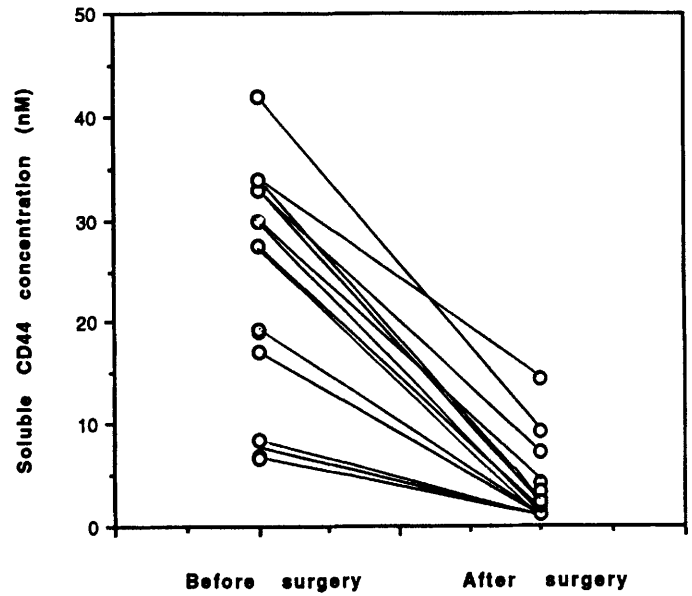


Fig. 3. Soluble CD44 concentration in colon cancer patients before and after surgical resection of tumors. Serum soluble CD44 concentrations were determined in 19 patients with various stages of colon cancers before surgery and after surgical resection of tumors.

soluble CD44 in the sera remain elevated in patients without surgery (results not shown). This observation provides strong evidence that soluble CD44 is an indicator of tumor burden in these patients.

Characterization of Soluble CD44 in Serum of Patients with Gastric or Colon Cancer. Sera from cancer patients and normal controls were first passed through an immunosorbent column, eluted, and separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins with molecular weights of 75,000, 80,000, 100,000, and between 150,000 and 190,000 were identified by Coomassie blue staining of the gel in the materials isolated from the serum of cancer patients. The M_r 100,000 protein is also present in normal serum. In addition, normal serum contained proteins with molecular weights of about 64,000 and 97,000 (results not shown). Western blotting was carried out with two different monoclonal anti-CD44 antibodies or control anti-CD4 antibody (OKT4a). The results of a representative experiment are shown in Fig. 4. Blotting with two different anti-CD44 antibodies (Lanes a and b) but not control anti-CD4 antibody (Lane c), identified one prominent band with molecular weight between 130,000 and 190,000 in the immunoaffinity purified materials from the serum of colon cancer patients. Two less prominent bands with molecular weights of about 72,000 and 80,000 were also identified. These proteins were absent in serum proteins from normal donors blotted with the anti-CD44 antibody (GKWA.3) (Lane d) or with another anti-CD44 monoclonal antibody, BU52, or control anti-CD4 antibody (results not shown). The M_r 100,000 protein visible with Coomassie blue staining of the gel in both the normal serum and serum from cancer patients was absent in Western blot analysis. The nature of this protein is not known.

DISCUSSION

We used an ELISA to demonstrate that the concentrations of soluble CD44 were significantly elevated in patients with gastric cancer and colon cancer. Based on a limited number of patients, there was a significant correlation ($P > 0.001$) between serum CD44 concentration in patients with metastatic cancer and serum CD44 concentration in normal donors. Serum CD44 levels also correlated with tumor burden *in vivo*. Patients with larger tumors had a higher concentration of soluble CD44 in their serum. Soluble CD44 levels in the sera from patients with chronic rheumatic disease were within normal range.

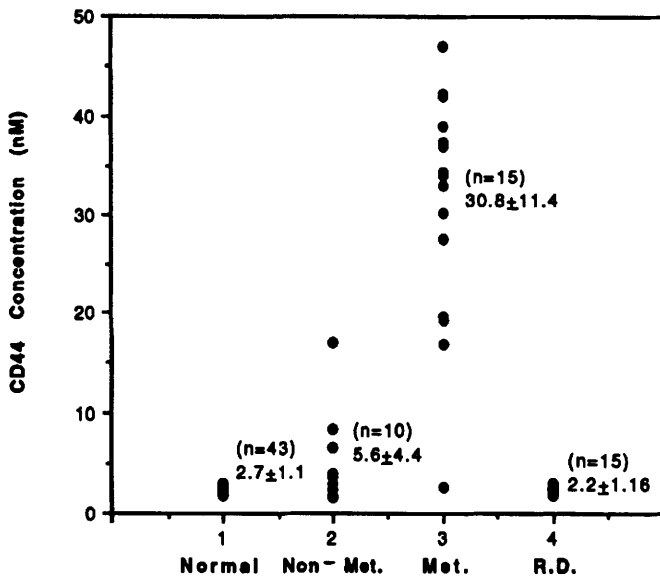


Fig. 2. Soluble CD44 concentration in normal serum, in patients with non-metastatic colon cancers, metastatic colon cancers, and in patients with rheumatic diseases. The numbers represent the average concentration of soluble CD44 in different groups of individuals \pm SD. The P value for the difference between CD44 levels in patients with metastatic tumor and normal donor is $P > 0.001$ (Student's t test).

We also determined the concentration of soluble CD44 in the ascitic fluid of 5 patients with metastatic tumors and ascites. Soluble CD44 concentrations in the ascitic fluids from these patients exceeded 100 nM. The concentration of soluble CD44 in ascites from patients with cirrhosis was 2.2 ± 1.5 nM, $n = 10$ (results not shown).

Reduction of Soluble CD44 Concentration in Serum of Patients after Surgical Resection of Tumor. Tumors from 19 patients with advanced colon cancer were surgically removed. Three weeks after surgery, the concentrations of soluble CD44 were determined. The concentrations of soluble CD44 before and after surgery are presented in Fig. 3. Removal of the tumors resulted in significant decreases in soluble CD44 concentrations in the sera of patients with high concentrations of soluble CD44 prior to surgery. The concentrations of

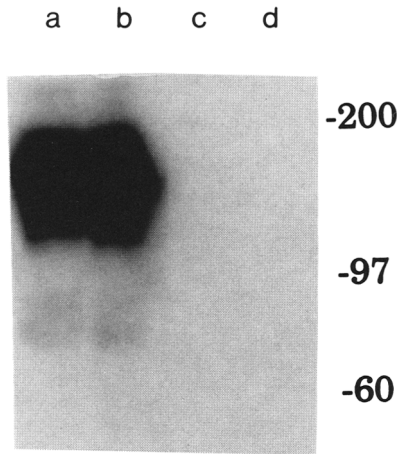


Fig. 4. Western blotting of serum from normal donors and serum from colon cancer patients. Serum from three normal donors or three colon cancer patients were pooled and passed through an anti-CD44 immunosorbent column as described in "Materials and Methods." Bound proteins were eluted and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Separated proteins were transferred to cellulose membrane. Transferred proteins (Lanes a, b, and c) were proteins from the serum of cancer patients were then blotted with two different anti-CD44 monoclonal antibody GKW.7-10 or BU52 (Lanes a and b) or control anti-CD4 monoclonal antibody (Lane c). Lane d, proteins from the serum of normal donors, eluted from anti-CD44 immunosorbent column and blotted with anti-CD44 monoclonal antibody GKW.7-10.

Additional control experiments with serum from patients with inflammatory bowel diseases are in progress.

Soluble CD44 present in the circulation of patients most likely came from tumor cells rather than normal cells. Complete surgical resection of tumor masses from these patients resulted in a significant reduction in their serum CD44 concentration. Surgery or anaesthesia alone did not reduce the concentration of CD44 in the serum (results not shown). Treatment with chemotherapy without surgery also resulted in reductions in soluble CD44 concentrations in the circulation (results not shown). Western blot analysis with two different monoclonal anti-CD44 antibodies revealed the presence of a prominent band with molecular weight between 130,000 and 190,000, and two minor bands with molecular weights of 75,000 and 80,000 in the sera from patients with metastatic tumors. Whether there are multiple proteins present in the M_r 130,000 to 190,000 band remain to be determined. In some cancer serum, we were able to identify two different proteins (M_r 130,000 and 190,000) in this region (results not shown). Only one CD44 protein with a molecular weight of about 82,000 was present in normal serum (10).

Elevated serum CD44 levels in these patients may be due to active shedding of CD44 molecules by the tumor cells. Alternatively, dying tumor cells may release CD44 molecules or CD44 on tumor cells may be released into the circulation by some proteolytic enzymatic mechanisms. Preliminary experiments using human tumor cell lines suggest that shedding of CD44 occurs under *in vitro* conditions (results not shown). The presence of multiple proteins reacting with monoclonal anti-CD44 antibody suggests the presence of different CD44 isoforms in the serum of cancer patients. Alternatively, the smaller molecular weight proteins may be degradation products of a high molecular weight CD44 isoform. Experiments are now in progress to use antibodies specific for each CD44 isoform to determine whether these high molecular weight proteins represent different CD44 isoforms.

We were able to detect low levels of soluble CD44 in sera from normal donors with our ELISA. However, we were unable to detect CD44 in normal serum by Western blot analysis by using our one step purification procedure. In previous studies, a significantly larger volume of normal serum was used for the enrichment of soluble CD44 (10). In addition, multiple purification steps, involving differential sizing and affinity columns, were used in earlier studies (10).

We were unable to detect an elevated soluble CD44 concentration in one patient with metastatic gastric cancer and in one patient with metastatic colon cancer. The reasons why we failed to detect elevated soluble CD44 in these two patients are not known. Since CD44 is not expressed in all human tumor cells, tumor cells from these two patients may not express CD44. The expression of CD44 is known to be controlled by the inhibitor Lutheran *In(Lu)* gene (36). Individuals with the dominant form of $Lu(a^{-}b^{-})$ phenotype express reduced levels of CD44 on their RBC, monocytes, and in their serum (36). The *In(Lu)* gene may also influence the expression of CD44 on tumor cells. These two patients may have the rare dominant $Lu(a^{-}b^{-})$ phenotype, resulting in reduced levels of soluble CD44 in their serum. Immunohistochemical staining of biopsies from these patients with anti-CD44 monoclonal antibodies may confirm this hypothesis.

We were able to detect elevated serum CD44 levels in 4 of 10 patients with nonmetastatic colon cancer and in 5 of 8 patients with nonmetastatic gastric cancer. Of four patients with stage I colon carcinoma, we were able to detect an increase in serum CD44 levels only in one patient. In six patients with nonmetastatic stage II colon cancer, we were able to detect increases in serum CD44 levels in three of these patients. Tumor cells from these three patients were well differentiated adenocarcinoma, suggesting that serum CD44 levels may correlate with the differentiation stages of the tumor cells. However, a well differentiated tumor cell is thought to be less metastatic than a poorly differentiated tumor cell, and therefore the significance of this observation is not known. Experiments are now in progress to analyze more serum samples from patients with stage II adenocarcinoma. In addition we are investigating whether serum from patients with other malignancies also have elevated soluble CD44.

The reasons that we were not able to identify patients with stage I or some of the stage II carcinomas with higher frequency may be related to the monoclonal antibodies we used for our soluble CD44 ELISA. The two anti-CD44 monoclonal antibodies react with the M_r 85,000 CD44 isoform and cannot distinguish different CD44 isoforms. The presence of soluble M_r 82,000 CD44 in normal serum may interfere with our ability to detect small increases in the levels of other tumor associated CD44 isoforms. Using monoclonal antibodies specific for different CD44 isoforms may significantly improve the sensitivity of our ELISA. This approach may eventually allow us to detect a small increase in various CD44 isoform concentrations in patients with early stages of malignant diseases. We may be able to use the ELISA to monitor the levels of soluble CD44 in cancer patients after surgical removal of tumors and/or after chemotherapy as an indicator of the efficacy of the treatments.

ACKNOWLEDGMENTS

We thank Dr. Edward Medof for providing sera from patients with rheumatic diseases. We thank Drs. Michael Bigby, Stan Gerson, and Garison Owens for discussion and suggestions and Ruth Hackett for help with the manuscript.

REFERENCES

- Haynes, B. F., Hale, L. P., Denning, S. M., Le, P. T., and Singer, K. H. The Role of leukocyte adhesion molecules in cellular interactions: Implications for the pathogenesis of inflammatory synovitis. *Springer Semin. Immunopathol.*, *11*: 163-185, 1989.
- 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.
- Trowbridge, I. S., Lesley, J., Schulte, R., and Trotter, J. Biochemical characterization and cellular distribution of a polymorphic, murine cell surface glycoprotein expressed on lymphoid tissues. *Immunogenetic*, *15*: 299-312, 1982.
- Hughes, E. N., Mengod, G., and August, J. T. Murine cell surface glycoproteins. Characterization of a major component of 80,000 daltons as a polymorphic differentiation antigen of mesenchyma cells. *J. Biol. Chem.*, *256*: 7023-7027, 1981.
- Nottenburg, C., Rees, G., and St. John, T. Isolation of mouse CD44 cDNA: structure features are distinct from the primate cDNA. *Proc. Natl. Acad. Sci. USA*, *86*: 8521-8525, 1989.

6. Zhou, D. F., Ding, J. F., Picker, L. J., Bargatze, R. F., Butcher, E. C., and Goeddel, D. V. Molecular cloning and expression of Pgp-1. The mouse homolog of the human H-CAM (Hermes) lymphocyte homing receptor. *J. Immunol.*, *143*: 3390–3395, 1989.
7. Wolffe, E. J., Cause, W. C., Pelfrey, C. M., Holland, S. M., Steinberg, A. D., and August, J. T. The cDNA sequence of mouse Pgp-1 and to human CD44 cell surface antigen and proteoglycan core/link proteins. *J. Biol. Chem.*, *265*: 341–347, 1990.
8. Stamenkovic, I., Aminot, M., Pesando, J. M., and Seed, B. A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. *Cell*, *56*: 1057–1062, 1989.
9. Stamenkovic, I., Aruffo, A., Aminot, 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. Lucas, M. G., Green, A. M., and Telen, M. J. Characterization of the serum In(Lu)-related antigen: identification of a serum protein related to erythrocyte p80. *Blood*, *73*: 596–600, 1989.
11. Haynes, B. F., Hale, L. P., Patton, K. L., Martin, M. E., and McCallum, R. M. Measurement of an adhesion molecule as indicator of inflammatory disease activity. Up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. *Arthritis Rheum.*, *34*: 1434–1441, 1991.
12. 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 heparin sulfate intrinsic membrane proteoglycan with additional exons. *J. Cell Biol.*, *113*: 207–221, 1991.
13. Kahn, P. Adhesion protein studies provide new clue to metastasis. *Science (Washington DC)*, *257*: 614–615, 1992.
14. Rouslahti, E. Structure and biology of proteoglycans. *Annu. Rev. Cell Biol.*, *4*: 229–255, 1988.
15. Hardingham, T. E., and Hosang, A. J. Proteoglycans: many forms and many functions. *FASEB J.*, *6*: 861–870, 1992.
16. Aruffo, A., Stamenkovic, I., Melnick, M., Underhill, C. B., and Seed, B. CD44 is the principal cell surface receptor for hyaluronate. *Cell*, *61*: 1303–1313, 1990.
17. Miyake, K., Underhill, C. B., Lesley, J., and Kincade, P. W. Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. *J. Exp. Med.*, *172*: 69–75, 1989.
18. Lesley, J., Schulte, R., and Hyman, R. Binding of hyaluronic acid to lymphoid cell lines is inhibited by monoclonal antibodies against Pgp-1. *J. Exp. Cell Res.*, *187*: 224–233, 1990.
19. *The Biology of Hyaluronan*. Ciba Foundation Symposium 143. New York: John Wiley & Sons, 1989.
20. Poole, A. R. Proteoglycans in health and disease: structure and functions. *Biochem. J.*, *236*: 1–14, 1986.
21. Reid, T., and Flint, M. H. Changes in glycosaminoglycan content of healing rabbit tendon. *J. Embryol. Exp. Morphol.*, *37*: 489–495, 1974.
22. Underhill, C. B. The interaction of hyaluronate with the cell surface: the hyaluronate receptor and the core protein. *Ciba Found. Symp.*, *143*: 87–99, 1989.
23. Nemet, R. E., Toole, B. P., and Knudson, W. The cell surface hyaluronate binding sites of invasive human bladder carcinoma cells. *Biochem. Biophys. Res. Commun.*, *149*: 249–257, 1987.
24. Underhill, C., and Toole, B. P. Receptors for hyaluronate on the surface of parent and virus-transformed cell lines: binding and aggregation studies. *Exp. Cell Res.*, *131*: 419–423, 1981.
25. Underhill, C., and Toole, B. P. Physical characteristics of hyaluronate binding to the surface of simian virus 40 transformed 3T3 cells. *J. Biol. Chem.*, *155*: 4544–4548, 1980.
26. Horst, E., Meijer, C. J. L. M., Radaszkiewicz, T., Osekopele, G. J., Van Krieken, J. H. J. M., and Pals, S. T. Adhesion molecules in the prognosis of diffuse large cell lymphoma: expression of a lymphocytic homing receptor CD44, LFA-1 and ICAM-1. *Leukemia (Baltimore)*, *4*: 595–599, 1990.
27. 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.
28. Hofmann, U., Rudy, W., Zooler, M., Tolg, C., Ponta, H., Herrlich, P., and Gunthert, U. CD44 splice variants confer metastatic behavior in rats: homologous sequences are expressed in human tumor cell lines. *Cancer Res.*, *51*: 5292–5297, 1991.
29. Gunthert, U., Hofman, M., Rudy, S., Reber, M., Zoller, I., Hausman, S., Matzku, A., 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.
30. Heider, K.-H., Hofmann, M., Hors, E., van den Berg, F., Ponta, H., Herrlich, P., and Pals, S. T. A human homologue of the rat metastasis associated variant of CD44 is expressed in colorectal carcinomas and adenomatous polyps. 1993. *J. Cell Biol.*, *120*: 227–233, 1993.
31. Tanabe, K. K., Ellis, L. M., and Saya, H. Expression of CD44R1 adhesion molecule in colon carcinomas and metastases. *Lancet*, *341*: 725–726, 1993.
32. Matsumura, Y., and Tarin, D. Significance of CD44 gene products for cancer diagnosis and disease evaluation. *Lancet*, *340*: 1053–1058, 1993.
33. Fox, S. B., Gatter, K. C., Jackson, D. G., Screaton, G. R., Bell, M. V., Bell, J. I., Harris, A. L., Simmons, D., and Fawcett, J. *Lancet*, *342*: 548–549, 1993.
34. Moen, C. J., Snoek, M., Augustinus, A., Hart, M., and Demant, P. Sec-1 a novel colon cancer susceptibility gene in the mouse: linkage to CD44 on chromosome 2. *Oncogene*, *7*: 563–566, 1992.
35. Sy, M. S., Guo, Y.-J., and Stamenkovic, I. Inhibition of tumor growth *in vivo* with a chimeric CD44-immunoglobulin molecule. *J. Exp. Med.*, *176*: 623–627, 1992.
36. Telen, M. J., Eisenbarth, G. S., and Haynes, B. F. Human erythrocyte antigens: regulation of expression of a novel erythrocyte surface antigen by the inhibitor Lutheran *In(Lu)* gene. *J. Clin. Invest.*, *71*: 1878–1886, 1983.