CD44 Expression in the Stromal Matrix of Colorectal Cancer: Association with Prognosis

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ABSTRACT

CD44, a cell hyaluronate receptor, is implicated in the metastatic behavior of some cancer cells. This study analyzed CD44 expression in topographic tissue sites of colorectal cancers to determine its association with patient survival and clinicopathological characteristics.

Immunohistochemical localization of the core CD44 and the v6 splice variant domains was examined by use of paraffin-fixed sections from 133 stage II or III colorectal cancers that previously had been evaluated for other diagnostic markers. Expression in malignant epithelium, stromal matrix, and stromal cells was compared to patient survival by univariate, multivariate, and bootstrap (reproducibility) analysis.

Core CD44 staining was present in the malignant epithelium of 85% of tumors, the stromal matrix of 90%, and the stromal cells of 98%. The v6 splice variant domain was present in the epithelium of 77% of tumors but was less frequent in the stromal matrix (12%; \( P < 0.001 \)) and stromal cells (17%; \( P < 0.001 \)). Absence of core CD44 immunoreactivity in the stromal matrix was associated with increased death rate (hazard ratio, 2.4; 95% confidence interval, 1.2-4.8; \( P = 0.02 \)), making this one of the most significant adverse prognostic variables, along with an age of 60 years or older, poor differentiation of the cancer, extramural venous invasion, chromosome 18q allelic loss, and nonwhite race.

This study shows that core CD44 and v6 splice variant antigens are differentially expressed in the epithelium and stroma of colorectal cancers. A model that includes core CD44 immunoreactivity in stromal matrix along with other prognostic factors may improve identification of high-risk and low-risk patients.

INTRODUCTION

Survival rates after curative resection of patients with colorectal cancer are strongly related to stage. Patients with stage II colorectal cancer (Dukes' B: tumor extending through the bowel wall without lymph node or distant metastasis) have a five-year survival rate of about 70%. Those with stage III disease (Dukes' C: regional lymph node metastasis without distant metastasis) have a 5-year survival rate of about 50% (1). Thus, the clinical outcome of patients with these intermediate stages of colorectal cancer varies considerably, and in individual cases, it is often difficult to predict what the prognosis will be. Additional prognostic factors are needed to identify high-risk and low-risk prognostic groups and thereby contribute to selecting patients for adjuvant chemotherapy and radiation therapy.

The possible prognostic value in human cancer of CD44, a hyaluronate receptor expressed on some epithelial, hematopoietic, and mesenchymal cells (2), has been studied extensively. The protein is highly polymorphic, with alternative splicing of 20 exons and 19 introns, and differential expression of N- and O-linked oligosaccharides and chondroitin sulfate (3, 4). Studies of its biological properties have implicated this molecule in a variety of cellular functions, including aggregation (5), motility (6), and the metastatic behavior of some cancer cells (7).

The reported relationships of CD44 to human cancer vary markedly. In some types of cancer, CD44 may have little, if any, role. CD44 was rarely detected in small cell carcinoma of the lung (8) or in pancreatic endocrine tumors (9). Tumors of squamous differentiation show down-regulation of CD44 products containing an epitope in variant exon v3 (exon 7; Refs. 10 and 11) or v6 (exon 10; Refs. 10 and 12). In other cancers, specifically neuroblastomas, the presence of CD44 has been associated with improved prognosis. Neuroblastoma patients with CD44-positive tumors had longer progression-free survival than those with CD44-negative tumors, and advanced tumors, as compared to the more differentiated or less advanced, were found to lack CD44 (13). Many other reports have described the overexpression of CD44 or of one or more of its isoforms as a feature of a variety of human cancers, including carcinoma of the colorectum (14-28), stomach (29), pancreas (30), breast (31), and cervix (32), and lymphoma (33). Reports of an association between the expression of the molecule and higher patient mortality rates or more aggressive metastasis of colorectal, breast, and stomach cancers have included the core CD44 (19), splice variant forms of CD44 (18, 23, 28, 29), and the specific CD44 isoforms v6 (exon 10; Refs. 10, 17, 20, 27, 31, and 33). The application of CD44 expression or that of its various isoforms to clinical management or to estimates of prognosis remains questionable, however, due to relatively
small numbers of patients and/or short follow-up in represented studies (16, 19, 20, 27) or to unresolved differences between the results of studies on the relationship of CD44 and its isoforms to the progression of carcinoma (17, 34).

We have previously observed immunolocalization of CD44 in the stroma, as well as the malignant epithelia, of colorectal cancers (35). The importance of tumor stroma as a specialized tissue containing many molecules that could possibly act as regulators of neoplastic cell behavior is well recognized, as are the complex interactions between tumor cells and molecules of the surrounding matrix (36). CD44, as a cell hyaluronate receptor, putatively acts as one of these molecules. This report describes a topographic analysis of CD44 expression in colorectal cancer, including a novel evaluation of the significance of CD44 immunoreactivity detected in the stromal cells and matrix of the cancer tissue. The studies were performed with a cohort of 133 stage II or III sporadic colorectal cancers that has been analyzed extensively for prognostic factors (37). Immunohistochemistry of formalin-fixed, paraffin-embedded specimens was conducted both for an amino-terminal domain present in all described CD44 molecules (core CD44) and for the splice variant exon v6 (CD44v6) that occurs in a specific subset of the CD44 molecules implicated as a factor in cancer progression. Additionally, the results of this study were developed into a prognostic model incorporating other previously obtained clinical and pathological data (37).

PATIENTS AND METHODS

Patient Cohort and Specimens. One hundred thirty-three routine formalin-fixed, paraffin-embedded specimens of stage II or III sporadic colorectal cancer were evaluated for CD44 expression. The specimens were obtained from 145 consecutive curative surgical resections of primary colorectal tumor performed at The Johns Hopkins Hospital between July 1986 and December 1990 and enrolled in a study of prognostic markers (37). This period was studied because postoperative adjuvant chemotherapy was not administered routinely to patients at this institution until 1991. Patients were not enrolled in the cohort if they had any of the following: clinical evidence of hereditary nonpolyposis colorectal cancer syndrome, malignant tumor outside the colon within the previous 5 years, synchronous adenocarcinoma of the large bowel, colorectal cancer associated with idiopathic inflammatory bowel disease, or preoperative radiation or chemotherapy. Twelve cases had insufficient tumor for CD44 immunohistochemistry and were excluded, leaving 133 specimens for analysis. Stage at diagnosis (based on pathological and clinical evaluation), tumor site, tumor differentiation, and patients’ age and sex were reported in a previous study of these patients (37). Follow-up was updated from our previous study through the Johns Hopkins Tumor Registry and was based on chart reviews and yearly contacts with the physician or patient. Two cases had no clinical follow-up data. Death occurred in 57 patients. Median follow-up after resection in survivors was 62 months.

Antibodies. The anti-CD44 mouse monoclonal antibody U9M2, a gift from Dr. James Hildreth (Johns Hopkins University School of Medicine, Baltimore, MD) (38), was used at a concentration of 0.625 μg/ml. This antibody was prepared from spleen cells of a mouse immunized with human peripheral blood-adherent cells, and the protein epitope of this antibody is located in the amino-terminal domain of the CD44 molecule (38, 39). This monoclonal antibody detects all known forms of CD44, including the splice variant forms, because all CD44 molecules contain exons 1 through 5. The anti human CD44 variant 6 mouse monoclonal antibody BBA 13 (2F10, R&D Systems, Minneapolis, MN) was used at 50 μg/ml. This antibody was prepared from a mouse immunized with human chimeric fusion protein CD44v3–10-Fc.

Immunohistochemistry. Five-μm-thick tissue sections on silane-coated slides were stained by capillary action immunoperoxidase technology (Tech Mate 1000, Bio-Tek Solutions, Inc., Santa Barbara, CA; Ref. 40) using the mouse monoclonal antibodies U9M2 and BBA13. A negative control was included in each run.

The sections were treated with xylene for 8 min to remove paraffin; rehydrated for 4 min with 100% ethanol, for 2 min with 95% ethanol, and for 15 s with 70% ethanol; treated for 20 min in a microwave oven (41) with glycine buffer (42) for antigen retrieval; and then treated for 15 min with endogenous peroxidase activity blocking solution (Secondary Detection Kit Peroxidase/diaminobenzidine, SDK605, Bio-Tek Solutions, Inc.). The sections were incubated with blocking serum from the kit for 15 min to eliminate nonspecific background immunostaining. After these procedures, sections were incubated overnight at room temperature with the U9M2 antibody or BBA13 antibody diluted in kit dilution buffer. The sections were next treated for 30 min at room temperature with kit secondary antibody, followed by incubation with kit tertiary antibody complex for 30 min at room temperature. The reaction product was visualized by incubating with kit diaminobenzidine chromogen. A brown reaction product appeared within 20 min, at which time the reaction was terminated by transferring the sections to water. After 10 s of counterstaining with methyl green (Sigma Chemical Co., St. Louis, MO), sections were dehydrated in graded ethanols and coverslipped with Baxter Accu-Mount permanent mounting medium (Baxter Healthcare Corporation, McGaw Park, IL).

Immunohistochemical Evaluation Procedures. All slides were coded for review. Topographic expression of CD44 in each tumor was evaluated as malignant epithelium, stromal matrix composed of connective tissue, and stromal cells represented by fibroblasts, lymphocytes, macrophages, plasma cells, and, very rarely, single tumor cells. Most neoplastic epithelium and stroma showed marked heterogeneity and focal distribution of staining with both anti-CD44 antibodies. Levels of protein expression were graded separately into four groups: (a) no detectable expression; (b) questionable or faint expression detected in less than one-third of cells in each compartment or the area of the stromal matrix; (c) strong, heterogeneous, or localized expression in one-third to two-thirds of cells in each compartment or the area of the stromal matrix, termed “positive” (+); (d) strong and homogeneous expression in more than two thirds of cells in each compartment or the area of the stromal matrix, termed “strongly positive” (++). For statistical purposes, subgroups a and b were combined into the “negative” (−) group.

The accuracy of the positive and strongly positive catego-
ries was further tested and confirmed by ranking each slide from the lowest to highest in intensity and extent of staining for each marker type and location. For those samples in which the intensity of staining seemed similar, the samples were ranked according to the estimated percentages of positive area. The rank values were then used in a logistic regression model to predict staining category. Significant associations of the rank values with the staining categories (P < 0.001) were found for all markers tested: U9M2 staining of epithelium, U9M2 staining of stromal cells, U9M2 staining of stromal matrix, and BBA13 staining of epithelium. For all markers, increasing rank (i.e., darker and/or more extensive staining) increased the probability of being in the + or ++ categories.

**Statistical Analysis.** The major statistical end point of this study was survival after curative resection. Event time distributions for this end point were estimated with the method of Kaplan-Meier (43) and compared using log-rank statistics (44) or the Cox proportional hazards regression model (45). Factors tested for prognostic value were CD44 expression in the tumor epithelium, stromal matrix, and stromal cells as indicated by staining with monoclonal antibodies to core CD44 or the variant 6 domain. For each of the combinations of marker type and location, the patients whose tumors had positive staining were compared to the reference group (negative staining). The HR for the reference group was assigned as 1.0. Other possible prognostic factors included in the analysis were age, chromosome 18q allelic loss, extramural venous invasion, race, sex, stage, tumor differentiation, tumor site, and tumor size (37). A HR greater than 1.0 from the Cox regression models indicated shorter survival times than the reference group (i.e., greater hazard of death). The 95% CIs give the range of true values for the HRs that are statistically compatible with the estimated HR.

Multivariate Cox proportional hazards models were used for simultaneous control of the effects on survival of more than one prognostic factor. The HRs in these models indicate the effect of a particular factor on survival with the other factors in the model held constant. In this analysis, factors that were potentially prognostic when considered alone (i.e., P less than 0.15) were entered into a multiple regression model. Nonsignificant factors were then removed from the model in a stepwise process with reestimation of HRs at each step. Factors previously determined to be prognostic factors for colorectal cancer that were included in multivariate regression models were chromosome 18q allelic loss, extramural venous invasion, race, stage, and tumor differentiation (37).

The statistical technique of bootstrapping (46) was applied to these data to determine the reproducibility of the results of the multivariate survival model. This technique uses repeated random sampling with replacement from the original data set to generate a large number of new bootstrap samples. Each bootstrap sample is of the same sample size as the original data set, but it may contain some patients more than once and/or some patients not at all. The purpose of this resampling is to generate data approximating the entire population of patients and thereby calculate improved estimates of the variability and stability of a

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**Table 1**  Frequency and intensity of core CD44 or v6 isoform expression in three compartments of 133 colorectal cancers and univariate HRs and 95% CIs for survival based on CD44 expression

The HRs indicate the effect of a particular factor on survival. The HR for the reference group is 1.0. HRs from the Cox regression models less than 1.0 indicate longer survival durations (lower hazard of death). The total number of observed cases was 133.

<table>
<thead>
<tr>
<th>Core CD44</th>
<th>%</th>
<th>n</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
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<tr>
<td>Epithelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>15</td>
<td>20</td>
<td>1.00</td>
<td>(0.29-1.43)</td>
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<td>+</td>
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<td>0.65</td>
<td>(0.33-1.29)</td>
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<tr>
<td>+ +</td>
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<td>80</td>
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<tr>
<td>-</td>
<td>2</td>
<td>3</td>
<td>1.00</td>
<td>(0.12-9.69)</td>
<td>0.95</td>
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<td>11</td>
<td>1.08</td>
<td>(0.18-9.50)</td>
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<tr>
<td>+ +</td>
<td>90</td>
<td>119</td>
<td>1.31</td>
<td>(0.14-0.62)</td>
<td>0.002</td>
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<td>-</td>
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<td>+ +</td>
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<td>57</td>
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<tr>
<td>-</td>
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<td>+</td>
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<td>73</td>
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<td>(0.18-9.50)</td>
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<td>-</td>
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<td>1.00</td>
<td>(0.12-9.69)</td>
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<td>5</td>
<td>1.91</td>
<td>(0.33-1.29)</td>
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3 The abbreviations used are: HR, hazard ratio; CI, confidence interval; PFS, prognostic factor score.
Fig. 1 CD44 immunohistochemistry. A, core CD44 staining in malignant epithelium of colorectal cancer. Core CD44 was highly expressed in the malignant epithelium (▲) of this example. In the stromal area, a few cells stained for core CD44 ( Asterisk), and weak stromal matrix staining is also evident. U9M2 anti-CD44 monoclonal antibody, ×250 magnification. Counterstained with methyl green. B, core CD44 staining predominantly in stromal matrix of colorectal cancer. Core CD44 was highly expressed in the stromal matrix (▲) of this example, but only a few cancer cells (▲) were stained. U9M2 anti-CD44 monoclonal antibody, ×250 magnification. Counterstained with methyl green. C, CD44 splice variant 6 staining predominantly in epithelium of colorectal cancer. CD44 variant 6 was highly expressed in malignant epithelium (▲), whereas few stromal cells (▲) were stained, and the stromal matrix was negative. BBA13 anti-CD44 variant 6 monoclonal antibody, ×250 magnification. Counterstained with methyl green.

Fig. 2 Core CD44 staining and survival of patients with stage III colorectal cancer. The survival rate of stage III patients with core CD44 staining in stromal matrix was significantly better than that of patients whose tumors lacked stromal staining (P = 0.02). The number of patients in each group is shown.

RESULTS

CD44 Localization in Malignant Tissue. Core CD44 immunoreactivity in colorectal cancer was commonly found in the malignant epithelium (85%; 113 of 133) and nonneoplastic stromal cells (98%; 130 of 133; Table 1 and Fig. 1A). However, there was a surprisingly high prevalence of cases with staining by anti-core CD44 in the stromal matrix (90%; 119 of 133), including a small subset of cases with core CD44 staining in stromal cells and the stromal matrix but no or faint CD44 expression of malignant epithelium (10%; 13 of 133; Fig. 1B). In contrast to the localization of core CD44 in stromal matrix and stromal cells, CD44v6 immunoreactivity was present predominantly in the malignant epithelium with infrequent stromal staining (Fig. 1C). The fractions of tumors that were posi-
tumors positive for stromal matrix core CD44 were stage III patients.

The vast majority (79%; Table 1) were negative for core CD44 in stromal matrix and stromal cells = (Table 1). In contrast, only 47% (56 of 119) of patients with negative CD44 staining at the two sites with anti-core CD44 monoclonal antibody (113 of 119; Table 1). These findings suggest a functional difference in the expression of core CD44 molecules in the stroma as compared to the v6 form.

**Comparison of CD44 Expression and Patient Survival.**

The novel finding of this study was that the absence of core CD44 immunoreactivity in any of the tissue compartments, either the malignant epithelial cells, stromal cells, or stromal matrix, as determined by grading or rank order of staining, was not related to survival. Likewise, CD44v6 domain immunoreactivity in any of the tissue compartments showed no significant relationship with survival. The frequency of positive staining in both the stromal matrix and stromal cells was very low [n = 16 (12%) and n = 23 (17%), respectively; Table 1].

**Clinical Characteristics of the Cohort.** Survival data and univariate estimates for clinical factors associated with this cohort are given in Table 2. The absence of core CD44 staining in the stroma was comparable to the other factors that are prominently associated with increased risk of death from colorectal cancer, i.e., extramural venous invasion, loss of one allele of chromosome 18q, poor differentiation of the cancer, age over 60, nonwhite race, and stage III disease as compared to stage II.

**Stromal CD44 and Other Prognostic Factors.** Multivariate analyses comparing stromal core CD44 staining and the other prognostic factors in Table 2 were performed with the presence of stromal core CD44 recorded as being either negative (−) or positive (+ and ++). Tumor size, sex, tumor site, and tumor stage were sequentially stepped out of the regression equation, and estimates from the resulting model are given in Table 3. Age 60 years or older, poor differentiation of the cancer, extramural venous invasion, negative stromal core CD44, chromosome 18q allelic loss, and nonwhite race were the strongest prognostic factors. After adjustment for the other factors in this model, negative stromal core CD44 increased risk by 2.4 times (95% CI, 1.2-4.8).

**PFS.** The clinically relevant subsets of prognostic factors based on this multivariate model were calculated for each patient as a PFS. Each prognostic factor in the model was given a score of 1; otherwise, the score assigned was 0. The number of prognostic factors present per patient was then added to determine the final score for that patient. For example, a patient with poor differentiation of the cancer, extramural venous invasion, negative stromal core CD44, chromosome 18q allelic loss, and nonwhite race would have PFS = 4, with four factors in the high-risk category. Kaplan-Meier curves for the scores are shown in Fig. 3 for demonstration purposes. The patient in the example, falling into the highest risk group (PFS = 4), would be expected to have approximately a 23% chance of surviving 3 years.

**Bootstrap Assessment of the Stability of the Multivariate Cox Proportional Hazards Model.** The prognostic factors demonstrating the most significant multivariate HR were selected by bootstrap analysis (Table 3). Selection frequency was greater than 50% for each of the following factors: age, 62%; tumor differentiation, 70%; extramural venous invasion, 84%; stromal matrix core CD44, 62%; chromosome 18q allelic loss, 58%; and race, 82%. These percentages give an indication of how often these factors would be identified as important prognostic factors in studies of similar size with a similar method of variable selection.

The stability of the variable selection process to small subsets of patients is reflected in the differences in selection frequencies between factors of similar significance levels. For
A multivariate Cox proportional hazards regression model was used to simultaneously control the effects of more than one prognostic factor on survival. The HR for the reference group is 1.0. HRs from the Cox regression models less than 1.0 indicate longer survival durations. The total number of observed deaths was 56.

<table>
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<tr>
<th></th>
<th>n</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
<th>% of bootstrap samples in which factor was significant</th>
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<td>Age</td>
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<tr>
<td>&lt;60</td>
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<td>≥60</td>
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<td>(1.23–7.04)</td>
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<td>Differentiation</td>
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<td>Good or moderate</td>
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<td>(1.23–7.04)</td>
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<td>Poor</td>
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<td>Positive</td>
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<td>Loss</td>
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<td>Race</td>
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<td>2.15</td>
<td>(1.23–7.04)</td>
<td>0.015</td>
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Fig. 3 Survival by PFS. The survival rate of patients with a lower number of prognostic factors was significantly better than that of patients with a higher number of prognostic factors. The number of patients in each group is shown.

example, the Ps for age and race were very similar. The HR for age was 2.9, indicating almost a 3-fold increase in risk for patients 60 years old and older compared to those younger than 60, whereas the HR for races other than white was 2.2. Although age appears to be the stronger predictor, it was only selected in 82% of the samples. This is an indication that the impact of age in our study may have been due to one or possibly a few particularly influential patients. This was confirmed by an analysis of residuals in which it was found that the removal of one particularly influential patient did in fact decrease the magnitude of the effect for age from HR = 2.9 to HR = 2.2.

DISCUSSION

This study of stromal CD44 stemmed from our earlier observation that colorectal cancer was associated with enhanced staining of core CD44 not only in the neoplastic epithelial cells but also in stromal cells and matrix (35). The basic observation is a significant correlation between patient survival and the absence of stromal staining with antibodies directed against the core epitope of CD44. Although the predominant evidence points to a form of CD44 as the source of the stromal staining, antibody binding to some other stromal matrix molecule (e.g., to proteoglycan link protein, a cell matrix component with sequence homology to CD44) may also be considered. However, it is not evident why such a component would be limited to a subset of tumor tissues, and CD44 cross-reactivity with link protein or other stromal molecules has not been reported. Although the origin and identity of the stromal antigen remain to be proven, current evidence points to two possible sources of stromal CD44. (a) Extracellular CD44 has been detected in a variety of studies of serum (50), plasma (50), efferent lymph (51), and synovial fluid (52), and has been recovered from the culture medium of keratinocytes and carcinoma cells (53, 54). A suggested source of the soluble CD44 in these studies is enzymatic cleavage of CD44 and shedding from cells. This hypothesis was supported by the observation that the protein isolated from mouse or human serum or lymph, or released from leukocytes induced by cell activation or treated with anti-CD44 monoclonal antibody, had a molecular mass lower than that of membrane-bound C4D4. Moreover, the CD44 in mouse serum failed to react with antibodies to the cytoplasmic domain of CD44 (55). The concept of shedding due to proteolysis is also supported by the observation that cell surface CD44 of murine...
lymphocytes or macrophages is highly sensitive to trypsin and releases a Mr 65,000 fragment (56, 57). (b) Alternatively, our finding of large amounts of stromal core CD44 in some malignant tissues despite the absence of staining with epithelial core CD44 suggests a mechanism other than cell surface proteolysis to explain the presence of stromal core CD44. From other studies, we have evidence of a soluble and secreted form of CD44 lacking exons 6–20, Mr 55,000. The corresponding mRNA, found in both normal and neoplastic cells, appears to be a major form of CD44 mRNA in colorectal tumor tissues. Lacking a transmembrane domain, a protein product derived from this mRNA would be secreted from the cell as a soluble molecule. This molecule, lacking the variant domains, would then be shed into the extracellular matrix. This soluble form of CD44 would not be detected by routine formalin-fixed, paraffin-embedded sections, whereas the proteolytically generated soluble core CD44 may be detected.

Several previous studies have reported that the expression of core CD44 or CD44 variant 6 (CD44v6) is associated with reduced patient survival (17, 19, 20, 22–24, 26–28, 58–61). A consistent finding in all studies of colorectal cancer is overexpression of CD44, particularly the variant forms of the molecule (CD44v) in many, but not all, neoplasms. However, this increased expression of CD44v, including v6, varies greatly, with increased expression of CD44v, including v6, occurs in many, but not all, neoplasms. However, this increased expression of CD44v, including v6, varies greatly, with some neoplasms containing low levels of CD44v or none at all. CD44v is not restricted to malignant cells but also occurs in colorectal adenomas that are not invasive, and in many normal tissues as well (61–64). Association of CD44 expression with cancer prognosis must be made on the basis of detailed statistical analysis, which was not described in several of the reports (19, 20, 27, 28, 59).

This study shows for the first time that staining of the stromal matrix of colorectal neoplasms by an anti-core CD44 monoclonal antibody may be an important diagnostic factor in patients with stage III colorectal cancer. Univariate analysis showed that the absence of stromal matrix CD44 staining was associated with an over 2-fold risk of death of patients with core CD44 staining in the tumor stromal matrix. Comparison with other recognized risk factors by univariate analysis indicates that stromal matrix core CD44 reactivity is among the six most significant prognostic variables, along with age 60 years or older, poor differentiation, extramural vein invasion, chromosome 18q allelic loss, and nonwhite race. Multivariate analysis

\(* X-L. Yang, unpublished observation.

\( a \) F, frozen tissue; P, paraffin-embedded tissue; PC, polyclonal sera.

(b) This study.
by the Cox regression model and bootstrap estimates of these data showed that the absence or faint expression of core CD44 in the stromal matrix increased the risk of death approximately 2.5-fold. This multivariate analysis formed the basis of a PFS in which the sum of these risk factors in individual patients produced a prognostic score that divided the patient population into four distinct subsets with very different expected survival probabilities, ranging from greater than 97% survival rate at 33 months with zero or one risk factor to only about 23% chance of surviving 3 years with four or more adverse prognostic factors. This model needs to be validated in an independent group of patients, and the predictive discrimination of this model for new subjects has not been quantified. Additional studies of this prognostic score may identify high-risk and low-risk patients and contribute to patient selection for therapeutic regimens.

An explanation of the less favorable prognosis associated with absence of core CD44 staining in the stromal matrix could relate to interaction between colorectal cancer cells and stroma. The extracellular molecule that retains the hyaluronate binding domains and occupies the sites to which the cancer cells would attach could block attachment. Therefore, absence of CD44 staining in the matrix could permit cancer cell attachment and motility and contribute to the process of metastasis. Haynes et al. (52) have reported an inverse relationship between soluble CD44 levels in synovial fluid and the numbers of immigrating blood cells in arthritic joints. Also, a soluble CD44-immunoglobulin fusion protein has been reported to inhibit the growth and dissemination of a CD44 positive human lymphoma in nude mice (65). The identification and characterization of CD44 molecules present in the stroma of colorectal neoplasms and elucidation of their origin, fate, and association with the malignant epithelium are needed.

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CD44 expression in the stromal matrix of colorectal cancer: association with prognosis.

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