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Increased Content of Chondroitin Sulfate Proteoglycan in Human Colorectal Carcinoma Metastases Compared With the Primary Tumor as Determined by an Anti-Chondroitin-Sulfate Monoclonal Antibody

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To determine if the amount of chondroitin sulfate proteoglycan (CSPG) in human colorectal tumor tissue correlates with the tumor's aggressiveness we immunochemically determined the CSPG levels in colorectal carcinomas at different stages. A total of 50 specimens—4 polyps, 15 stage B tumors, 9 stage C tumors, 12 stage D tumors, 7 liver metastases, and 3 lymph node metastases—were examined. Tumor tissues were extracted with 4 M guanidine hydrochloride containing protease inhibitors. The extracts were serially diluted and blotted onto nitrocellulose membranes. Reactivity of a chondroitin sulfate-specific mouse monoclonal antibody (CS-56) was determined by biotinylated goat antimouse Ig and avidin-biotin-peroxidase complex. After comparing tissues from tumors at different stages (classified by the presence or absence of metastasis), we could not find a positive or negative correlation between the amount of CSPG in primary colorectal carcinoma tissues and the tumor's metastatic potential. However, the metastatic foci in the liver or lymph node contained higher amounts of CSPG than the primary tumors did. Immunohistochemical staining of colon carcinoma tissue with CS-56 revealed that CSPG is predominantly localized in fibrotic portions in the tumor tissues. Two-year follow-up studies indicated that a high level of CSPG in primary tumors was not predictive of recurrence.

Key words: human colorectal cancer, metastasis, chondroitin sulfate proteoglycan, dot-blot analysis

The prognosis for cancer is often directly related to the presence or absence of metastases. This is particularly true of colorectal cancer, in which the five-year survival rate is greater than 60% for patients with localized disease (Dukes' stage B)

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and smaller than 5% for those with disseminated metastases (Dukes' stage D) [1,2]. Dukes' classification system is a good indicator of survival [3,4]; however, stage of disease is not sufficient for prediction of recurrence or development of metastasis in individual patients, especially in those without apparent metastases at the time of surgical removal of primary colorectal tumors. The search for a specific marker for those tumor cells most likely to metastasize may lead to the development of a new prognostic indicator.

Recent work with experimental animals has shown that highly metastatic tumor cells have a variety of specific biochemical properties different from those of normal cells, e.g., cell surface glycoproteins [4–6], cell adhesiveness [7,8], specific enzymes for the degradation of basement membranes, and extracellular constituents [8–10]. However, little is known about biochemical properties associated specifically with human colorectal carcinoma cells having higher metastatic potentials. We were interested in examining if biochemical changes known to occur on cell surface and extracellular molecules during malignant transformation also influence the metastatic potential of colorectal cancer. We have already shown that the expression of high molecular weight fucosylated glycoproteins in the distal colon and rectum [11] and sulfated glycoproteins [12] tends to decrease upon progression of carcinomas. On the other hand, we have found that collagenolytic activity of human colorectal carcinoma is not related to the stage of disease [13].

Proteoglycans are major constituents of the connective tissue stroma of most organs and are involved in tissue organization and other biological processes, such as cell proliferation and migration [14–16]. Altered levels of proteoglycan production and release and changes in the structure of glycosaminoglycan chains have been reported in a variety of transformed cells and tumor tissues [17,18]. Increased production of chondroitin sulfate proteoglycan (CSPG) has been reported in transformed fibroblasts [19] and hepatocytes [20] as well as in more tumorigenic melanoma and mammary carcinoma [21,22]. Iozzo et al. recently demonstrated an increased amount of CSPG in human colon carcinoma tissues compared with normal colonic epithelium [23]. Iozzo has also shown that colon carcinoma cells produce factors that stimulate the production of CSPG by colonic fibroblast cells in culture [24]. Such a tumor cell–host interaction comparable with desmoplastic response may influence the metastatic propensity of tumors [25]. However, correlation of proteoglycan alterations in colorectal carcinoma tissues with the tumor's metastatic potential has not been shown.

We have demonstrated by metabolic ^{35}S labeling and biochemical analysis of sulfated glycoconjugates that the production of sulfomucin by colorectal carcinoma decreases during the tumor's progression and metastasis [12]. The amount of sulfated proteoglycans slightly increases at the same time. Since this proteoglycan consists mainly of CSPG, we focused on CSPG as a potential marker of human colorectal carcinoma metastasis. We measured the amount of CSPG in the extracts of colorectal tumors at different stages by using an anti-chondroitin-sulfate monoclonal antibody (CS-56) [26]. After examining the amount of CSPG in colorectal primary carcinomas that did or did not metastasize and comparing the amount of CSPG in primary carcinoma with that in metastasis, we could not find a correlation between the content of CSPG in the bulk of primary colorectal carcinoma and its aggressiveness or metastatic potential.

MATERIALS AND METHODS

Selection of Patients and Staging

Patients with histologically proven polyps and adenocarcinoma of the colon and rectum were selected for this study. Those who had undergone previous radiotherapy or chemotherapy or who had had colon carcinoma previously were excluded. Staging was based on the Dukes' classification system [3].

Specimen Processing

Tumor specimens of approximately 0.5–1.0 g were obtained from the intraluminal edge of colorectal tumors. Specimens of liver and lymph node metastases from colorectal carcinoma were obtained when available. The surface portion of the metastasis was removed to eliminate the possible influence of fibrotic tissues on the analytical data. Necrotic portions of the tumors also were excluded. Tumor tissues were immediately frozen in liquid nitrogen and stored at −70°C until use. Thirty milligrams of the tissue was minced and mixed with 300 μl of proteoglycan extracting buffer (PG buffer) containing 4 M guanidine-HCl, 4% Zwittergent 3–12 (Calbiochem Behring, La Jolla, CA), 0.1 M sodium acetate buffer (pH 6.0), and protease inhibitors [10 mM ethylenediaminetetraacetate, 10 mM benzamidine (Sigma Chemical Co., St. Louis, MO), 25 mM ε-aminocaproic acid (Sigma), 5 mM phenylmethylsulfonylfluoride (Sigma), 10 μg/ml N-tosyl-L-phenylalanine chloromethyl ketone, 10 μg/ml Nα-P-tosyl-L-lysine chloromethyl ketone (Sigma), 20 mU/ml aprotinin (Sigma), and 2 mM N-ethylmaleimide (Sigma)]. After ultrasonication on ice for 10 sec (Ultrasonic Cell Disrupter, Heat System Ultrasonics, Inc., Farmingdale, NY), the mixture was incubated on ice for 18 hr with occasional gentle mixing. Supernatant was collected by centrifugation at 13,000g for 10 min. Protein concentration was determined by the method of Lowry et al. [27].

Preparation of ^{35}S-sulfated Proteoglycans

A ^{35}S-sulfated proteoglycan fraction was prepared from colon carcinoma tissue as described below and used to assess whether dot-blot analysis could be employed for the measurement of proteoglycans. One hundred milligrams of fresh tumor obtained from a 73-yr-old man with stage C sigmoid colon adenocarcinoma was rinsed with Dulbecco's phosphate-buffered saline (DPBS) containing 50 units/ml of penicillin, 50 μg streptomycin, and 1.25 μg/ml of amphotericin B; minced with scalpel blade into small pieces; and incubated in 1 ml of a one-to-one mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium containing 10% fetal bovine serum, 50 units/ml of penicillin, 50 μg/ml streptomycin, 1.25 μg/ml of amphotericin B, and 50 μCi/ml of [^{35}S]Na_2SO_4 under humidified conditions in 5% CO_2 at 37°C for 48 hr. The tissue was removed by centrifugation, and ^{35}S-sulfated materials were extracted with 1 ml of PG buffer as described above. The extract was applied to a Sephadex PD-10 column (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with 50 mM sodium acetate buffer (pH 6.0) containing 50 mM NaCl, 8 M urea, 1 mM phenylmethylsulfonylfluoride, and 0.02 U/ml of aprotinin and eluted with the same buffer. Each 0.1-ml fraction was collected, and radioactivity was measured after small aliquots of each fraction were mixed with Liquiscint (National Diagnostics, Somerville, NJ). Radioactive materials eluted at the void volume fractions were pooled and diluted with nine parts per volume of 8 M urea containing

buffer A [50 mM sodium acetate buffer (pH 6.0), 50 mM NaCl, and 0.2% CHAPS] and then applied to a DEAE-Sephacel column (1.2 × 3 cm) equilibrated with the same buffer. The column was washed with about 20 ml of buffer A, and the ^{35}S-sulfated molecules were eluted first with 10 ml buffer B of [0.1 M acetate buffer (pH 6.0), 0.2 M NaCl in 8 M urea, and 0.2% CHAPS] and second with buffer C [15 ml of 0.23 M acetate buffer (pH 6.0), 0.5 M NaCl in 8 M urea, and 0.2% CHAPS]. Radioactivity in each 1-ml effluent fraction was measured. The fractions eluted with buffers B (peak I) and C (peak II) were respectively pooled, dialyzed against distilled water, and lyophilized. Peak I was mostly sulfated mucin, and peak II was proteoglycans including chondroitin sulfate and heparan sulfate as major components [12].

Recovery of Proteoglycans After Transfer Onto Nitrocellulose Membrane Under Various Conditions

Proteoglycans labeled with ^{35}S from 100-mg tumor tissue samples were dissolved in 0.5 ml of distilled water and used for testing recovery of these molecules after blotting onto nitrocellulose membranes. Five-microliter aliquots of ^{35}S-labeled proteoglycans were mixed with 95 μl of 6 M urea, 1% CHAPS, and 4 M guanidine-HCl containing 0.1 M sodium acetate (pH 6.0), 2% sodium dodecyl sulfate (SDS), or 90% ethanol and blotted onto a nitrocellulose membrane on Hybri Dot (Bethesda Research Laboratories, Inc., Gaithersburg, MD) under moderate negative pressure generated by water aspirator. After being rinsed in DPBS each dot was separated and incubated in 0.5 ml of NCS (Amersham Corp., Arlington Heights, IL) at 37°C for 18 hr. The samples were then mixed with 10 ml of Liquiscint (National Diagnostics) and their radioactivity was measured by a 1214 RACKBETA liquid scintillation counter (LKB Instruments, Gaithersburg, MD).

In order to test the effect of the protein concentration of the samples on the recovery of proteoglycans on nitrocellulose membranes, an extract prepared from tumor tissue derived from a 60-yr-old woman with Duke's stage D sigmoid colon adenocarcinoma was diluted with 4 M guanidine-HCl containing 0.1 M sodium acetate (pH 6.0) into different concentrations (7.4–0.12 mg protein/ml). A fixed amount of radiolabeled proteoglycans (2,000 cpm) was added to each diluted extract (100 μl), which was then transferred onto a nitrocellulose membrane and processed as described above.

Chondroitinase ABC Treatment of Tumor Tissue

To confirm the specificity of the antibody in our experimental system, an extract from a chrondroitinase avidin-biotin-peroxidase complex (ABC)-treated tissue was prepared. Thirty milligrams of fresh liver metastasis tissue obtained from a 58-yr-old woman with colon carcinoma was rinsed with DPBS, minced, and incubated at 37°C for 18 hr with 2 U/ml of chondroitinase ABC (ICN Radiochemicals, Irvine, CA) in 1 ml of 50 mM Tris-HCl (pH 8.0) containing 60 mM sodium acetate, 50 mM NaCl, 0.01% bovine serum albumin (BSA), 0.02% sodium azide, and 2 mM phenylmethylsulfonylfluoride. Control samples were incubated without chondroitinase ABC. After incubation, tumor tissues were removed by centrifugation at 13,000g for 10 min and extracted with 300 μl of PG buffer as described above. The extracts were diluted, transferred to a nitrocellulose membrane, and allowed to react with anti-chondroitin-sulfate monoclonal antibody as described below.

Semiquantitative Determination of CSPG in Tissue Extracts by Monoclonal Antibody CS-56

The anti-chondroitin-sulfate IgM monoclonal antibody CS-56 was kindly supplied by Dr. Benjamin Geiger (Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel). The nature of this antibody has been described by Avnur and Geiger in detail elsewhere [26]. The tumor extracts were serially diluted twice with 4 M guanidine-HCl containing 0.1 M sodium acetate (pH 6.0) and blotted onto nitrocellulose membranes. The membranes were rinsed in phosphate-buffered saline (PBS), incubated in PBS containing 2% normal goat serum at 25°C for 18 hr, and allowed to react with 200 μg/ml of CS-56 at 25°C for 1 hr. The membranes were washed in PBS for 1 hr, allowed to react with biotinylated antimouse IgM (Vector Laboratories, Burlingame, CA), diluted in PBS containing 1% BSA, washed in PBS for 1 hr, and allowed to react first with ABC-peroxidase reagent (Vector Labs) and then with 4-chloro-1-naphthol as peroxidase substrates. The maximum dilution of the antibody to give visible reactivity (i.e., Fig. 1, row a, dot 6) was recorded as titration score.

Immunohistochemical Localization of CSPG

Immunohistochemical localization of CSPG was studied with a primary adenocarcinoma of left colon and liver metastasis from a 52-yr-old man. Four-micron sections were made from a formalin-fixed and paraffin-embedded permanent pathological specimen. The sections were treated with 0.2% hydrogen peroxide to destroy endogeneous peroxidase, rehydrated, presoaked with 5% BSA (RIA grade) in PBS overnight at 4°C, and reacted with CS-56 (200 μg/ml diluted in 1% BSA) for 1 hr. The sections were then processed under the same conditions as dot-blot assay described above, except that 3,3′-diaminobenzidine were used for a peroxidase substrate.

RESULTS

Optimal Conditions for Dot-Blot Analysis of Proteoglycans

We have recently shown that colon tumor tissue synthesizes two different classes of sulfated macromolecules (slightly acidic peak I and highly acidic peak II) in vitro, which were separated by DEAE-Sephacel ion-exchange chromatography [12]. The less acidic peak I was sulfated mucin, and highly acidic peak II was proteoglycans

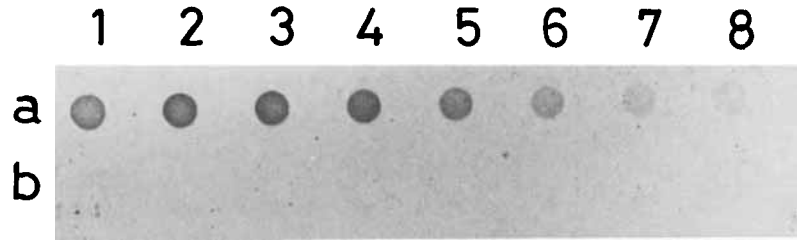

Fig. 1. Effect of the absence (**a**) or presence (**b**) of chondroitinase ABC treatment on the reactivity of CS-56 to tissue extract. Thirty milligrams of fresh tumor tissue obtained from colon carcinoma metastasis was incubated with or without chondroitinase ABC and extracted with 10 volumes of PG buffer, blotted onto a nitrocellulose membrane, and allowed to react with CS-56 as described in Materials and Methods. The starting concentration of the extracts in the twofold dilution was 0.25 mg protein/ml.

containing chondroitin sulfate and heparan sulfate [12]; [^{35}S]-sulfate-labeled proteoglycans (2,000 cpm/ml) were used to compare their binding to nitrocellulose membrane under the different conditions described below. Radiolabeled proteoglycans were dissolved or uniformly suspended in 6 M urea, 1% CHAPS, 4 M guanidine-HCl, and 2% SDS or 90% ethanol, and 100 μl of each sample was blotted onto nitrocellulose membranes. The 4 M guanidine-HCl containing 0.1 M sodium acetate (pH 6.0) allowed maximum binding of proteoglycans to nitrocellulose membranes (60% of input radioactivity). Other allowed less than 20% binding efficiency, so 4 M guanidine-HCl was used thereafter for the dot-blot analysis of proteoglycans. To examine the effect of protein concentration on the efficiency of dot-blot analysis of proteoglycans, we diluted the extract to different concentrations (7.4–0.12 mg protein/ml) with 4 M guanidine-HCl. Radiolabeled proteoglycans were added to the various dilutions of the tumor extracts and blotted. The bound radioactivities were 5, 26, 44, and 47% of total at concentrations of 7.4, 0.93, 0.46, and 0.12 mg protein/ml, respectively. The maximum binding of radiolabeled proteoglycans was observed at a protein concentration of 0.12 mg/ml. The binding efficiency was extremely low at protein concentrations more than 1 mg/ml. As protein concentration decreased, the binding efficiency of proteoglycans appeared to increase, reaching a plateau at protein concentrations ranging between 0.12 and 0.42 mg/ml. Therefore, the dot-blot analyses of proteoglycans described below were done with protein concentrations of less than 0.25 mg/ml.

Specificity of CS-56

The monoclonal antibody CS-56 used in the present study was directed to chondroitin sulfate [26]. Its specificity was confirmed by the results shown in Figure 1. The CS-56 reacted to colon tumor extract blotted onto a nitrocellulose membrane (Fig. 1, row a), but the reactivity was completely eliminated when the tumor tissue had been treated with chondroitinase ABC, which degrades chondroitin 4- and 6-sulfates, prior to extraction (Fig. 1, row b).

Semiquantitative Determination of CSPG in Colorectal Carcinoma Tissues at Different Stages

Extracts were prepared from four polyps, 36 primary colorectal carcinoma tissues, and ten metastases at a ratio of 100 mg wet tissue/10 ml buffer. The protein concentration of these extracts ranged between 12.0 and 20.4 mg/ml and thus we diluted them with the same buffer to 0.25 mg/ml. The extracts were serially diluted twofold and blotted onto nitrocellulose membranes. The samples were allowed to react with CS-56 and were immunochemically stained and the titration score was recorded (Fig. 2). The reactivity of primary cancer tissue extracts to this antibody varied from one specimen to another and seemed slightly higher in stage C and D tumors than in stage B disease. However, there was no statistically significant correlation between antibody reactivity and the stage of colorectal carcinomas. The reactivity of metastases to this antibody was compared with that of the corresponding primary carcinoma tissues (Fig. 3); the metastases appeared to contain slightly greater amounts of CSPG than the primary carcinomas from which they were derived ($P < .027$).

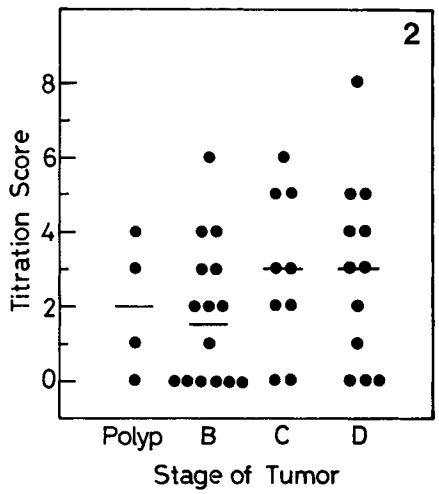

Fig. 2. Amount of CSPG in the extracts of tumors at different stages as determined by CS-56. Tumor extracts were prepared, blotted onto nitrocellulose membranes, allowed to react with CS-56, and scored as described in Materials and Methods. The starting concentration of the extracts in the twofold dilution was 0.25 mg protein/ml. Staging was based on the Dukes' classification system [3]. In case the first well showed no reactivity, the titration score of the extract was defined as 0. Bars show the mean volumes of the scores.

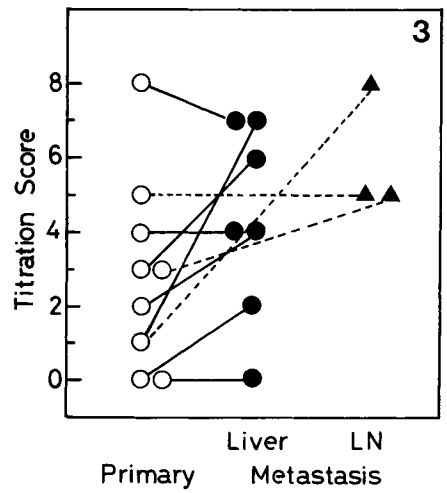

Fig. 3. Differences between CSPG content in primary tumor and in metastasis. The CSPG content in extracts from metastases was compared to those from the corresponding primary tumors. Experimental conditions were the same as those described in the legend for Figure 2. ○, primary tumor; ●, liver metastasis; and ▲, lymph node (LN) metastasis. The corresponding primary tumor and metastasis were tied with a solid line (liver metastasis) or a dotted line (lymph node metastasis) ($P < .027$ by Wilcoxon signed-rank test).

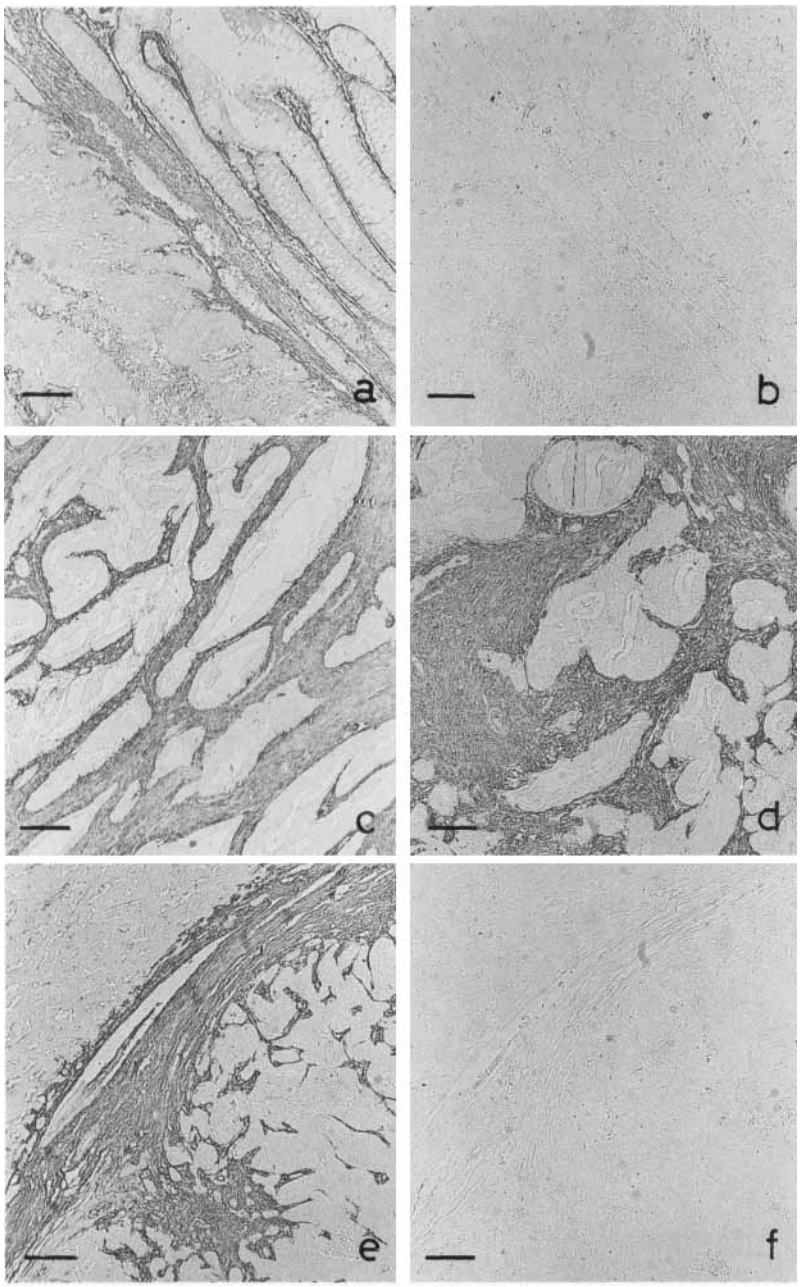

Fig. 4. Immunohistochemical localization of CSPG. Adenocarcinoma of left colon and liver metastasis from a 52-yr-old man was used. **a**: Superficial portion of primary carcinoma stained with CS-56 followed by biotinylated goat antimouse IgG and avidin-biotinyl peroxidase complex; 3,3′-diaminobenzidine was used as a substrate. Positive staining is seen associated with fibrotic tissues in carcinoma and adjacent colonic mucosa. **b**: Another serial section stained under the same condition as a except that CS-56 was omitted. **c**: Deep portion of the same tumor stained with CS-56. This antibody binds predominantly to desmoplastic portions in carcinoma tissue. CSPG is apparently produced by host-derived cells in the fibrotic tissues. **d**: Hepatic metastasis from the same patient. CS-56 bound to fibrotic tissues, which appeared more extensively than in the primary tumor. **e**: Border of liver metastatic tumor and adjacent liver tissue (top left). **f**: The same field in another serial section processed without CS-56. Bars indicate 100 μm.

Immunohistochemical Localization of CSPG in Colorectal Carcinoma Tissues Using CS-56

Immunohistochemical localization of CS-56 was studied with tissue sections of primary adenocarcinoma of left colon and hepatic metastasis. CSPG localized in lamina propria in colonic mucosa adjacent to carcinoma tissues (top right, Fig. 4a). In carcinoma tissues adjacent to normal mucosa desmoplastic response was not extensive, and weak CS-56 reactivity was seen (bottom left, Fig. 4a). On the other hand, at the deep portion of the same tumor, extensive desmoplasia was observed, and CS-56 bound to these fibrotic tissues (Fig. 4c). Extensive desmoplasia was also seen associated with hepatic metastasis from the same patient (Fig. 4d,e). These results clearly indicated that CSPG is predominantly localized in fibrotic tissues in colorectal carcinomas and that there was a intratumoral heterogeneity in CSPG contents.

Patient Follow-Up

Recurrence of colorectal cancer within 2 yr after the initial surgery was followed up for those patients classified as stage B or C. The status was available for 19 patients. Figure 5 shows the amount of CSPG in primary tumors using CS-56. Three patients died of recurrence with liver metastasis. The CSPG contents of the primary tumors of these patients having recurrence were not significantly different from those who were disease-free for 2 yr.

DISCUSSION

Proteoglycans are considered to be dynamic components that influence fundamental biological processes including cellular proliferation, recognition, and differentiation. Changes in proteoglycan production by tumor cells have been suggested to be related to tumorigenicity, disease progression, and metastasis [18,28,29]. In-

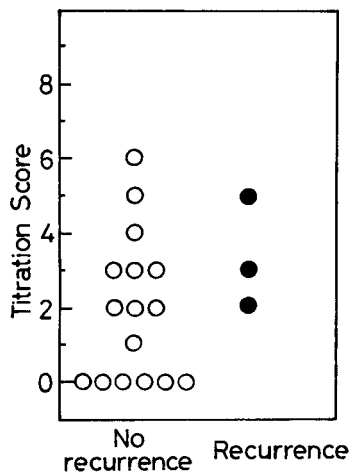

Fig. 5. Two-year follow up of those patients diagnosed as stage B or C at the time of surgery of the primary tumors. The titration scores with CS-56 were plotted according to the clinical status of the patients.

creased amounts of CSPG have been reported in carcinomas of the lung [30,31], liver [32,33], prostate [34], and colon [23,35,36]. Chondroitin sulfate is also known to stimulate the growth of mammary carcinoma cells in vitro [37], of Ehrlich ascites tumor cells in vivo [38], and of chondroitinase to inhibit the growth of Ehrlich ascites tumor cells [39]. This evidence indicates significant involvement of CSPG in malignant transformation and progression. However, little is known regarding the importance of CSPG production in determining the metastatic potential of human tumors in vivo or its potential use as a prognostic marker.

We have been interested in elucidating the determinants of colorectal cancer metastases. We have previously shown that there is no correlation between size of the primary colorectal carcinoma and the stage of disease as classified by the presence of metastasis [40]. Therefore, primary colorectal carcinomas at different stages should contain tumor cells that express cellular or extracellular phenotypes related to different metastatic potentials. Production of CSPG might be one of these phenotypes.

We recently analyzed metabolically labeled [^{35}S]-sulfated macromolecules of human colorectal carcinoma and showed that metastases produce slightly higher amounts of [^{35}-S]-labeled proteoglycans than the primary tumors do when CSPG accounted for approximately 50% of the radioactivity in the primary carcinomas [12]. In this study the amount of CSPG detected by CS-56 in tumor tissue extracts did not differ with the stages of the tumor. Although the amount of CSPG tended to be slightly higher in stage C and D tumors than in stage B tumors, the differences were not statistically significant. On the other hand, we observed higher amounts of chondroitin sulfate detected by CS-56 in metastases than in primary carcinoma tissues. It is not known whether these differences were due to the selective colonization of colorectal carcinoma cells having higher capacity to stimulate CSPG production by the host tissue or to the stimulation of CSPG production by the different host tissue microenvironment. Also, it remains possible that the portion of primary tumors responsible for producing metastases was a small fraction, and the analysis of randomly taken tumor tissues from the superficial edges did not reflect the phenotypes related to the formation of metastases. In such an event, even if there had been a higher content of CSPG in the portion of tumors responsible for the metastasis formation, that content would have been diluted by the noncontributing population. Therefore, we have studied histochemical localization of CS-56 using peroxidase methods (Fig. 4). In carcinoma tissues, CS-56–positive sites were seen associated with extracellular fibrotic portions which was more extensively developed in the invasive edge of the primary tumors and metastasis (Fig. 4). As an additional method, we also used high iron diamine (HID) staining [41] in combination with chondroitinase ABC treatment to localize CSPG in histological sections of formalin-fixed tumor tissues, which gave a similar result. The results clearly show that there was an intratumoral heterogeneity in CSPG contents. Whether they reflect heterogeneous tumor cell populations, and whether one of these subpopulations is responsible for the formation of metastasis, remain to be answered.

Our study is based on semiquantitative dot-blot assay of CSPG in guanidine-HCl extracts. Preliminary attempts to use other methods in immunochemical measurement of CSPG using CS-56 were not successful. For example, binding assay with enzyme-linked second antibody or radiolabeled second antibody could not be applied because we could not quantitatively immobilize CSPG extracted from tumor tissues on plastic plates. Guanidine-HCl extraction which was suitable for quantitative solu-

bilization of CSPG from tissues followed by dot-blot analysis in the same solution gave highly reproducible and quantitative recovery of ^{35}S-labeled materials from colorectal carcinoma tissues.

In conclusion, we compared the amount of CSPG measured using CS-56 extracts from primary colorectal carcinoma tissue at different Dukes' stages. The amount of CSPG in the primary carcinoma did not correlate with presence or absence of metastasis. On the other hand, the amount in metastases was significantly higher than the amount in primary tumor tissues. Although CSPG appeared to be an extracellular component of host origin as shown in Figure 4 and as previously described [18,24], this molecule may play a functionally important role in determining metastasis. As an example, histological observation indicated that vascular invasion as well as liver colonization of colon carcinoma were accomplished not by a single cell but as aggregates possibly including host-derived stromal constituents [42].

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