Differential Expression of a Sialoglycoprotein with an Approximate Molecular Weight of 900,000 on Metastatic Human Colon Carcinoma Cells Growing in Culture and in Tumor Tissues

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ABSTRACT

Wheat germ agglutinin (WGA)-binding cellular glycoproteins produced by HT-29 human colon carcinoma and its variant cells established from liver metastases in nude mice after intrasplenic injection were analyzed by polyacrylamide gel electrophoresis. On 5.5% polyacrylamide gels five major sialoglycoproteins (approximate M, 115,000, 145,000, 190,000, 450,000, and 740,000) reactive with WGA were common to the parental and metastatic sublines. There was an additional component of M, ~900,000 that was prominent in cells established from liver metastases. Specific removal of sialic acid from the glycoproteins eliminated WGA binding, indicating that all the WGA-binding glycoproteins including the M, 900,000 component were sialoglycoproteins. Smith degradation following mild acid hydrolysis resulted in formation of WGA-binding carbohydrate chains on M, 115,000, 145,000, 190,000, and 900,000 components, but not on M, 450,000 and 740,000 components, which indicated that these two sialoglycoproteins bore different oligosaccharides from the other sialoglycoproteins. The M, 900,000 component was more prominent with HT-29 cells growing in nude mice than those growing in vitro. WGA binding to the M, 900,000 component of metastasis-derived HT-29 cells growing in a nude mouse was higher than that of parental cells growing in nude mice. The expression in liver metastases derived from parental as well as metastatic cells was higher than the primary tumor growing in the spleen of the same mouse, indicating that the levels of M, 900,000 sialoglycoprotein (SGP=900) were regulated by intrinsic and environmental factors. The influence of organ microenvironmental factors was confirmed by analyzing sialoglycoproteins of HT-29 cells grown in vivo in nude mice that received intrasplenic injection and having higher liver metastatic potential expressed a larger amount of a high-molecular-weight Sialoglycoprotein. The molecular weight of this glycoprotein was estimated as 900,000 by gel electrophoresis in 3% polyacrylamide. A similar difference was observed with HT-29 variant-derived tumors growing in nude mice. Furthermore, WGA binding to the M, 500,000–1,000,000 region of electrophoretically separated extracts of nonmetastatic primary human colorectal carcinoma tissues (stage B1) was significantly lower than that of the other primary carcinomas.

INTRODUCTION

Tumor metastasis results from a series of specific interactions between tumor cells and host cells and tissues that are mediated by cell surface molecules (1). In a variety of experimental metastasis models in mice and rats, differential expression of cell surface glycoproteins between tumor cells with high and low metastatic potentials was revealed by use of immunohistochemical probes such as lectins (2–6). These cell surface glycoproteins play a variety of roles in determining metastatic potentials of the tumor cells. We have shown by use of an inhibitor of glycoprotein formation that melanoma cell surface sialoglycoproteins played a central role in determining blood-borne lung colonization, acting as an adhesive receptor for endothelial cells (7, 8). We have also shown that mouse RAW 117 lymphoma variant cells selected for liver colonization express larger amounts of sialoglycoproteins on their surfaces (6). Differential adhesion of these cells to liver parenchymal cells (9, 10), as well as to liver sinusoidal endothelial cells (11), may be explained by their cell surface molecules. Another important aspect of the functional significance of tumor cell surface sialoglycoproteins is their influence in host immune recognition and elimination of the tumor cells. For example, mouse MDA-MB-231 tumor cell mutants with defects in sialoglycoconjugate synthesis were more sensitive to natural killer cells and thus possessed lower metastatic potential (12).

Little information, however, is available on the differences in the cell surface properties of metastatic tumor cells in humans. We have been attempting to find markers of progression and metastasis by analyzing tumor tissue specimens taken during surgery. We have already shown that the expression of high-molecular-weight fucosylated glycoprotein detected by Ulex europaeus agglutinin I increased in carcinoma compared to normal mucosa but was low in the primary tumors that had already produced metastasis (13). Production of a sulfated glycoprotein decreased upon colon carcinogenesis and decreased further after progression and metastasis (14). In contrast to the decrease of sulfated mucin, increased expression of sialylated mucin in colorectal carcinoma compared with normal mucosa has been histochemically detected (15). However, studies have not been conducted to elucidate the molecular basis and functional significance of this phenomenon. Furthermore, preliminary studies on colorectal primary carcinoma and metastasis indicated that there were at least several major sialoglycoproteins with different molecular weights detectable in the tumor tissues (16).

In this study we identified at least six major sialoglycoproteins bound to WGA3 from HT-29 human colon carcinoma cells. HT-29 variant cells were established from the tumors grown in vivo in nude mice that received intrasplenic injections of HT-29 cells. A cell line established from liver metastatic foci and having higher liver metastatic potential expressed a larger amount of a high-molecular-weight sialoglycoprotein. The molecular weight of this glycoprotein was estimated as 900,000 by gel electrophoresis in 3% polyacrylamide. A similar difference was observed with HT-29 variant-derived tumors growing in nude mice. Furthermore, WGA binding to the M, 500,000–1,000,000 region of electrophoretically separated extracts of nonmetastatic primary human colorectal carcinoma tissues (stage B1) was significantly lower than that of the other primary carcinomas.

MATERIALS AND METHODS

Cells. Human HT-29 colon carcinoma cells originally derived from a well-differentiated adenocarcinoma of colon were grown on plastic

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2 To whom requests for reprints should be addressed.

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3 The abbreviations used are: WGA, wheat germ agglutinin; DPBS, Dulbecco's phosphate-buffered saline; buffer D, 5 mM Tris-HCl buffer, pH 7.3, containing 0.25 M sucrose, 50 μM CaCl2, 100 μM phenylmethylsulfonyl fluoride; NaDodSO4, sodium dodecyl sulfate; RCA1, Ricasius communis agglutinin-I; TBS, 25 mM Tris-HCl buffer, pH 7.2, containing 0.12 M sodium chloride.
tissue culture dishes in a 1:1 mixture of Dulbecco’s modified minimum essential medium and Ham’s F-12 medium (GIBCO, Grand Island, NY), supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT) under humidified conditions in the presence of 5% CO₂. This cell line is designated HT-29 P. These cells (10⁶/0.1 ml) were injected into the spleens of nude mice and the mice were killed 60 days later. Tumors growing in the spleens and livers were harvested. HT-29 variant cell lines were established from a tumor grown in the spleen (SP1) and multiple tumors grown in the liver (LMM). Metabolic radiolabeling of the glycoproteins was achieved by incubating these cells in the presence of 10 μCi/ml of [6-³H]glucosamine (35 Ci/mmol) for 48 h. The density of the cells reached subconfluency after a 48-h incubation.

Metastasis Formation in Nude Mice. Formation of metastasis of colon carcinoma cells HT-29 P and HT-29 LMM in nude mice was assayed and the tumor tissues were obtained as follows. Tumor cells grown in tissue culture were harvested by brief treatment with 0.25% trypsin and 2 mM EDTA and suspended in DPBS at a concentration of 10⁶/ml. Each 0.1-ml aliquot was injected into the spleens of nude mice (7–10 mice/group) by making a small opening on the left flanks. The mice were killed 60 days later and visually detectable tumor foci on the liver and on the other organs were counted. A similar technique was applied for intrahepatic injection of 10⁵/0.1 ml cells.

Extraction of Cellular Glycoproteins. Extracts of cultured cells were prepared as previously described (2, 3). Briefly, 10⁶ HT-29 cells were detached from culture dishes by brief treatment with 0.25% trypsin and 2 mM EDTA and washed with DPBS and then with buffer D. The cells were lysed with 0.5 ml of buffer D supplemented with 0.5% Nonidet P-40 with occasional mixing at 4°C (on ice) for 1 h. The supernatant was collected by centrifugation at 13,000 × g for 5 min, mixed with 0.25 ml of 3 × concentrated electrophoresis sample buffer, and heated at 100°C for 5 min. Tumor tissues obtained from the primary injection site and liver metastases were extracted by similar methods. Fifty mg of tumor tissues were rinsed with DPBS and then with buffer D. The tissues were minced into pieces less than 0.5 mm and with extracted with 0.5 ml of buffer D supplemented with 0.5% Nonidet P-40 on ice for 18 h. The supernatants, after centrifugation at 13,000 × g for 5 min, were processed with electrophoresis sample buffer as described before (5, 6, 13).

Extraction of Colorectal Carcinoma Tissue. Colorectal primary tumor specimens of approximately 0.5–1.0 g were obtained from the luminal edge of colorectal tumors. In this study, 90 specimens, including primary carcinoma and adjacent normal mucosa from 43 patients, were analyzed. Analytical data on liver biopsies taken of metastatic disease at the time of the surgical removal of primary tumor were also included in some cases. The surface portion of the liver metastasis were removed to eliminate the possible influence of fibrotic tissues on the analytical data. Necrotic portions of the tumors were also excluded. Normal mucosa was obtained from an area approximately 5 cm from the primary carcinoma. The tissues were extracted with buffer D supplemented with 0.5% Nonidet P-40, as described above, and processed for NaDodSO₄ polyacrylamide gel electrophoresis.

Electrophoresis. Polyacrylamide gel electrophoresis in the presence of NaDodSO₄ was performed in Laemmli’s buffer system, as previously described (5, 6, 14). In this study, we used 5.5 or 6% running gels with 3.3% stacking gels and 3% running gels with 2.5% stacking gels. In the latter case, Gelbond PAG (FM Corp., Rockland, ME) was used in order to see the gels. Electrophoresis was performed under constant current (35 mA/1-5 mm thick, 16-cm wide slab gel) for 4 h. Molecular weight markers used for 6% gels and 3% gels were a mixture of dye-conjugated myosin, phosphorylase b, bovine serum albumin, ovalbumin and α-chymotrypsinogen (BRL, Bethesda, MD), and laminin purified from Engelbreth-Holm-Swarm sarcoma grown in C57BL/6 mice (17), respectively. Gels were either stained with 0.25% Coomassie brilliant blue in 10% acetic acid:25% isopropanol or fixed in 10% acetic acid:25% isopropanol before processing, chemical treatment, or lectin staining.

Detection of Lectin Reactivity of Glycoproteins before and after Chemical Modification. In situ chemical modification of glycoproteins after electrophoretic separation was performed as described previously (5, 6, 18). Mild acid hydrolysis was applied in certain experiments to remove sialic acid. Untreated or treated gels were stained with 125I-labeled WGA (5 μg/ml) or biotinylated WGA (5 μg/ml). 125I-Labeled WGA-stained 5.5% or 6% gels were washed, dried, and processed for autoradiography as previously described (5, 6, 18). 125I-Labeled WGA-stained 3% gels bound to Gelbond PAG were repeatedly washed with TBS containing 0.05% sodium azide until the radioactivity of the wash fell below 100 cpm/ml. The gels were air dried after soaking in 2.5% glycerol and were processed for autoradiography. Biotinylated WGA-treated 3% gels were washed with TBS for more than 18 h, with several solution changes, treated with a complex of avidin and biotinylated horseradish peroxidase (ABC reagent; Vector Laboratories, Burlingame, CA) for 4 h, and washed with TBS for 2 h, with three solution changes and then with water with five consecutive immediate changes. 4-Chloro-1-naphthol (2 mM) was used to visualize bound peroxidase.

RESULTS

Differential Expression of WGA-binding Glycoproteins. Fig. 1a shows WGA-binding glycoprotein profiles in 6% polyacrylamide gels of HT-29 P, SP1, and LMM cells. At least five different glycoproteins were observed. The approximate molecular weight of relatively small ones was estimated as 115,000, 145,000, and 190,000 from their migration distances. The M₉, 190,000 component migrated as a broad band, suggesting that this component has a polydispersed nature. The molecular weights of the other two components were estimated to be 450,000 and 740,000 as described below. Another larger WGA-binding glycoprotein was seen only with cell lines established from metastases, i.e., HT-29 LMM. The amount of 125I-WGA bound to this component was highest with HT-29 LMM (Fig. 1a, arrow). The intensity of the M₉, 115,000 component was slightly weakly with cells established from metastases.

Biochemical Nature of the WGA-binding Glycoproteins. To study the biochemical characteristics of the carbohydrate chains of the WGA-binding glycoproteins, the glycoproteins separated by NaDodSO₄ polyacrylamide gel electrophoresis were chemically modified in situ prior to staining with 125I-WGA. Removal of terminal sialic acid from the carbohydrate chains completely eliminated the WGA-binding capacity of all the glycoproteins (Fig. 2). These changes were not due to nonspecific degradation or release of the glycoproteins from the gels because ovomucin, used as a molecular weight marker, maintained its reactivity with WGA after the same treatment. The loss of WGA reactivity after mild acid treatment clearly indicated that all the WGA-binding glycoproteins of HT-29 colon carcinoma cells were glycopolypeptides and that terminal sialic acid was responsible for the binding of WGA to these glycoproteins. When desialylated glycoproteins were further processed for Smith degradation (Fig. 2), M₉, 115,000, 145,000, 190,000, and metastasis-specific large components acquired WGA reactivity, which suggested that N-acetylgalactosamine residues resistant to periodate oxidation were exposed after removal of sialic acid followed by Smith degradation (19). Direct Smith degradation

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These results confirmed that M, 115,000, 145,000, 190,000, respectively, high-molecular-weight glycoproteins and to estimate their metabolic [3H]-glucosamine labeling followed by polyacrylamide gel electrophoresis on 5.5% gels. On each panel, Lanes 1–3 were loaded with detergent lysates of 6.7 × 10⁶ HT-29P, HT-29 SP1, and HT-29 LMM cells, respectively. In a and b lysates were from non-radiolabeled cells. In c lysates were made from the same number of cells previously incubated with 10 μCi/ml [3H]glucosamine for 24 h. Fig. 1a is an autoradiogram taken after the gels were stained with [125I]-WGA, as described in the text. Fig. 1b is an autoradiogram taken after the gels were treated with mild acid and stained with [125I]-RCA. Fig. 1c is an autoradiogram taken after the gels were processed with Enhance. Molecular weight markers are myosin (MY), Mₐ ~200,000; phosphorylase b (PH), Mₐ ~93,000; and bovine serum albumin, (BA), Mₐ ~66,000. Arrow indicates the position of the differentially expressed high-molecular-weight component.

Fig. 1. Glycoprotein profiles of HT-29 human colon carcinoma cells revealed by lectin bindings after polyacrylamide gel electrophoresis, or metabolic [3H]-glucosamine labeling followed by polyacrylamide gel electrophoresis on 5.5% gels. On each panel, Lanes 1–3 were loaded with detergent lysates of 6.7 × 10⁶ HT-29P, HT-29 SP1, and HT-29 LMM cells, respectively. In a and b lysates were from non-radiolabeled cells. In c lysates were made from the same number of cells previously incubated with 10 μCi/ml [3H]glucosamine for 24 h. Fig. 1a is an autoradiogram taken after the gels were stained with [125I]-WGA, as described in the text. Fig. 1b is an autoradiogram taken after the gels were treated with mild acid and stained with [125I]-RCA. Fig. 1c is an autoradiogram taken after the gels were processed with Enhance. Molecular weight markers are myosin (MY), Mₐ ~200,000; phosphorylase b (PH), Mₐ ~93,000; and bovine serum albumin, (BA), Mₐ ~66,000. Arrow indicates the position of the differentially expressed high-molecular-weight component.

without prior removal of sialic acid eliminated WGA reactivity of all the glycoproteins except the Mₐ 115,000 component (Fig. 2), indicating that the carbohydrate structures of each of the sialoglycoproteins were different. Also, the results demonstrated that the differential WGA binding to the high-molecular-weight region was not simply due to differences in sialylation.

Profiles of Sialoglycoproteins Revealed by Other Methods. Glycoprotein profiles very similar to those seen with WGA staining were observed when polyacrylamide gels were stained with RCA₁ after removal of sialic acid from the glycoproteins, using mild acid hydrolysis (Fig. 1b). RCA₁ bound none of these components unless sialic acid had been removed. An additional high-molecular-weight component was also identified by RCA₁ binding after mild acid hydrolysis of the cells established from metastasis, at approximately the same migration distance as the WGA-binding high-molecular-weight sialoglycoprotein. These results confirmed that Mₐ 115,000, 145,000, 190,000, 450,000, 740,000, and 900,000 were clearly separated. The differentially expressed glycoprotein was shown to have an apparent Mₐ ~900,000 calibrated from the positions of myosin (Mₐ ~880,000).

Sialoglycoprotein Expression of Tumor Cells Growing in Vivo. Fig. 4a shows the profile of WGA-binding glycoproteins of HT-29 cells growing in vivo. The sites of tumor growth include spleen, which is the primary injection site, and three metastatic lesions at the peritoneal wall, liver, and lymph nodes. The relative intensity of WGA-binding to Mₐ 900,000 sialoglycoprotein was much higher than the binding to Mₐ 740,000, 450,000, or 190,000 components, in contrast to the cases of the cells grown in vitro. Among the tumors derived from HT-29 parental cells, the primary growth at the spleen showed less WGA binding to this component. The intensity increased in the following order: splenic primary < peritoneal wall invasion < lymph node metastasis < liver metastasis. Among the tumors derived from HT-29 LMM cells, the splenic primary, peritoneal invasion, and lymph node metastasis were approximately the same intensity, while liver metastasis from LMM cells were the highest among all the tumors. The WGA-binding components were sialoglycoproteins because there was no WGA binding to the glycoprotein previously treated with mild acid. These results clearly indicated that the level of Mₐ 900,000 sialoglycoprotein production was regulated both by intrinsic and environmental factors. Fig. 4b is the result of another experiment to analyze WGA-binding sialoglycoproteins of HT-29-derived tumor...
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Fig. 2. Glycoprotein profiles of HT-29 LMM cells after polyacrylamide gel electrophoresis revealed by 125I-WGA binding before and after chemical modification of the glycoproteins. The electrophoresis was on 6% gels. Lanes 1 and 2 were loaded with lysates of 6.7 x 10^6 HT-29 LMM cells and a mixture of molecular weight markers. Fig. 2a, untreated; Fig. 2b, gels were previously treated with 50 mM sulfuric acid to remove sialic acid from the glycoproteins; Fig. 2c, gels were first treated with 50 mM sulfuric acid and then processed for Smith degradation (see text); Fig. 2d, gels were directly processed for Smith degradation. Molecular weight markers are the same as in Fig. 1, except that the position of ovalbumin (OV), M, ~45,000 is indicated. Arrow indicates the position of the M, ~900,000 sialoglycoprotein.

Fig. 3. Electrophoretic separation of high-molecular-weight glycoproteins by using 3% polyacrylamide gels. Fig. 3a, autoradiograph after 125I-WGA staining. Fig. 3b, the gels were fixed and stained with biotinylated WGA followed by avidin-biotinyl peroxidase complex staining (see text). Fig. 3c, the cell lysates were derived from [3H]glucosamine-labeled cells. Lanes 1 and 2 contain lysates of HT-29 P and HT-29 LMM cells. The bars indicate the position of intact laminin (LI), M, ~880,000; large subunit of laminin (LL), M, ~440,000; and small subunit of laminin (LS), M, ~220,000. Arrow indicates the position of the M, 900,000 sialoglycoprotein.

in Table 1. Comparisons based on the staging according to Dukes (20) with Astler and Coller modification (21) indicated that stage B1 primaries contain significantly lower levels of SGP-900-like sialoglycoproteins that all other primaries (P = 0.0234). Metastases contain higher levels of SGP-900-like molecules than corresponding primaries in 4 of 6 cases, but the sample number was not large enough to draw a conclusion. Two-year follow-up of the stage B and C patients did not show correlation between the levels of SGP-900-like glycoproteins and patients' survival.

DISCUSSION

Alterations of sialoglycoproteins in colorectal carcinoma were first reported as a histochemical observation showing that sialomucin level exceeds that of sulfomucin in carcinoma tissue compared with normal mucosa (15). Similar changes were also seen in normal mucosa adjacent to carcinoma and they were thought to represent premalignant changes in colonic epithelium (23). Colonic mucoproteins were a mixture of heterogeneous glycoproteins, were isolated from normal mucosa and carcinoma, and biochemically characterized by Gold and Miller (24). The mucin-like glycoprotein from carcinoma contained more sialic acid than that from normal mucosa, whereas the contents of other carbohydrate moieties were very similar. Carcinoma mucin also showed a higher aspartate:threonine ratio (24). However, little is known regarding biological functions of sulfated glycoproteins that are increasingly produced by carcinoma cells.

There is evidence to indicate that cell surface sialoglycopro-
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Fig. 4. Glycoprotein profiles of detergent extracts of HT-29 human colon carcinoma cells grown in vivo in nude mice. Three % polycrylamide gels were used for the separation. The gels were stained with 125I-WGA and autoradiographed. Fig. 4a, Lane 1, a lysate of 6.7 x 10^6 HT-29 P cells; Lane 2, HT-29 P cells grown at the primary injection site (spleen); Lane 3, HT-29 P cells invading peritoneal wall; Lane 4, HT-29 P cells metastasized to the liver; Lane 5, HT-29 P cells metastasized to the lymph nodes; Lane 6, HT-29 LMM cells grown at the primary injection site (spleen); Lane 7, HT-29 LMM cells invading peritoneal wall; Lane 8, HT-29 LMM cells metastasized to the liver; Lane 9, HT-29 LMM cells metastasized to the lymph nodes. All tissue lysates are 5 mg wet tissue equivalent. Fig. 4b, Lane 1, a lysate of 6.7 x 10^6 HT-29 P cells; Lane 2, HT-29 P cells metastasized to the lymph nodes (same as Fig. 4a, Lane 5); Lane 3, HT-29 cells invading peritoneal body wall (same as panel a-3); Lane 4, HT-29 cells metastasized to the liver (same as Fig. 4a, Lane 4); Lane 5, HT-29 cells growing at the primary injection site in the liver; Lane 6, HT-29 P cells invading to body wall from a liver primary; Lane 7, HT-29 cells growing at the s.c. primary injection sites. All the lysates loaded in the lanes are equivalent to 5 mg wet tissues. Molecular weight markers used are intact laminin (LI), M, ~880,000; large subunit of laminin (LL), M, ~440,000; and small subunit of laminin (LS), M, ~220,000. The arrow indicates the position of the M, 900,000 sialoglycoprotein.

Proteins are associated with their metastatic potentials, at least in experimental animals. We have shown in the mouse B16 melanoma system that a group of sialoglycoproteins detectable by the binding of peanut agglutinin was increasingly expressed in variants highly metastatic to the lung (5). We have also shown that sialoglycoproteins seen by the binding of 125I-WGA were increased on large cell lymphoma highly metastatic to the liver (6). The significance of sialoglycoproteins was reported in many other experimental systems of metastasis such as the Eb/ESb and MDAY cell lines (12, 25-27). There is a positive correlation between cell surface sialyltransferase activity and metastatic potential of B16 melanoma (28). An inhibitor of sialyltransferase was effective in blocking lung metastasis of mouse colon carcinoma in vivo (29). Therefore, we hypothesized that increased sialoglycoprotein production by colorectal carcinoma in humans was associated with the ability of the tumor cell to metastasize. Bresalier et al. (30) have already shown histochemically that liver metastases of colorectal carcinoma express lower level of peanut agglutinin-binding sites, which indicated increased sialylation.

Furthermore, recent work on metastases strongly suggested that the primary tumor consists of cell populations with different metastatic potentials and that metastases result from the selective growth of subpopulations with highly metastatic potentials (31, 32). For colon cancer, considerable evidence now exists that the primary colon tumors in mice (33-35) and humans (36, 37) consist of heterogeneous cell populations with different metastatic potentials. Our previous findings (38) indicated that there was no correlation between the size of the primary lesion, i.e., tumor burden, and development of metastasis among patients with colorectal carcinoma. This suggested that certain biological and biochemical characteristics of particular colorectal carcinoma cells were related to their metastatic potential. So far, biochemical characteristics of human tumor cells have not been found to be associated with metastatic potentials, except for our previous observation that decreased expression of both Ulex europeus agglutinin I-reactive high-molecular-weight fucosylated glycoproteins and high-molecular-weight sulfomucin were associated with increased metastatic potentials (13, 14). These previous conclusions were made based on the analytical results from a large number of human colorectal carcinoma specimens available from surgical resections.

In this study, we analyzed human colon carcinoma cells established from the primary inoculation site and metastases. The HT-29 LMM cells, which were established from liver metastases, produced a greater number of liver metastases than the parental cells. HT-29 SP1 cells did not give consistent in vivo assay data, probably because of the instability of the metastatic phenotypes. As expected from our previous studies on metastatic murine melanoma and lymphoma cells, there were several major sialoglycoproteins revealed by the binding of WGA to the electrophoretically separated colorectal carcinoma cell lysates. These WGA-binding components were sialoglycoproteins because WGA failed to bind to them if the entire slab gels were previously treated with mild acid to hydrolyze sialic acid of the glycoproteins (17). Most of the sialoglycoproteins were well preserved among different cell lines established from...
The expression of M, 115,000 glycoprotein was slightly lower in Lane 10, specimen from a primary transverse colon carcinoma; Lane 11, specimen from another portion of the same colon carcinoma; and Lane S was from the liver metastasis. Lanes 9-14 were derived from the differential WGA binding to the component of this high-molecular-weight sialoglycoprotein was seen with these two cell lines established in LMM cells, whereas an additional high-molecular-weight sialoglycoprotein was found from multiple liver métastases (HT-29 LMM) in nude mice. The tumor at the primary inoculation site (HT-29 SP1) and metastases which were derived from liver metastasis. Lanes 6-8 were from a 63-year-old woman who had two well-differentiated adenocarcinomas of the rectum and a solitary liver metastasis. The specimen on Lane 3 was derived from mucosa adjacent to the tumor. The specimen on Lane 4 was derived from primary carcinoma. The specimen on Lane 5 was derived from liver metastasis. Lanes 6-8 were from a 71-year-old man who had two well-differentiated adenocarcinomas of the rectum and a solitary liver metastasis. Lane 6 was from the adjacent mucosa; Lane 7 was from the primary rectal carcinoma; and Lane 8 was from the liver metastasis. Lanes 9-14 were derived from a 63-year-old woman who developed one moderately differentiated adenocarcinoma of the sigmoid colon and a solitary liver metastasis. Lane 9, adjacent mucosa; Lane 10, specimen from a primary transverse colon carcinoma; Lane 11, specimen from another portion of the same colon carcinoma; Lanes 12-14 were derived from three separate metastatic liver foci. Intact laminin (LI), M, ~880,000; large subunit of laminin (LL), M, ~440,000; small subunit of laminin (LS), M, ~220,000. The arrow indicates the position of the M, 900,000 sialoglycoprotein.

We were interested in finding the molecular background for the differential WGA binding to the component of this high-molecular-weight range, since differential sialylation or glyco-sylation might be responsible for this change, as described above. However, by comparing WGA reactivity to this component after chemical modification reactions of the carbohydrate chains, such as Smith degradation in combination with desialylation, we concluded that the difference could not be attributed to sialylation changes or changes in the carbohydrate structure. Namely, the unique increased expression of the high-molecular-weight sialoglycoprotein is seen after a desialylation-Smith degradation sequence. Therefore, it is very likely that the difference is due to the increased expression of whole glycoprotein copies (6, 18). Alternatively, it could also be due to the increased number of the same carbohydrate chains attached to the polypeptide backbone. Another very interesting conclusion from these experiments is that the structures of the internal portions of the carbohydrate chains on the different sialoglycoproteins are not identical. Table 2 summarizes the changes in WGA reactivities of these sialoglycoproteins before and after chemical modification reactions. If the carbohydrate portions of different glycoproteins have identical structures, then the reactivity with WGA after these treatments must have been the same among different glycoproteins. Using [3H]glucosamine-labeled preparation of a mixture of M, 900,000, 740,000 and 450,000 sialoglycoproteins, we have shown that most of the carbohydrate chains are labile to alkaline treatment, therefore, similar carbohydrate chains to the ones isolated and characterized from normal colon by Podolsky (39), might be present. Many of these carbohydrate chains were estimated to have terminal N-acetylglucosamine after Smith degradation.

Since we found that the high-molecular-weight sialoglycoprotein might be a molecule associated with metastatic potential of colon carcinoma, we next attempted to electrophoretically resolve this high-molecular-weight region by lowering the concentration of the polyacrylamide gels. By using 3% polyacrylamide gels bound to GelBond PAG, we successfully separated these high-molecular-weight sialoglycoproteins. The molecular weight of the sialoglycoprotein expressed by the cells from metastases was estimated as 900,000, using laminin and its subunits as molecular weight markers. Therefore, we tentatively named it SGP-900. At least a portion of SGP-900 appeared to be located on the cell surface because increased expression of the high-molecular-weight sialoglycoprotein was found from multiple liver metastases (HT-29 LMM) in nude mice. The expression of M, 115,000 glycoprotein was slightly lower in LMM cells, whereas an additional high-molecular-weight sialoglycoprotein was seen with these two cell lines established in LMM cells, whereas an additional high-molecular-weight sialoglycoprotein was seen with these two cell lines established in LMM cells.

<table>
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<th>Comparison according to:</th>
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<td>Primaries or metastases</td>
<td>Primary</td>
<td>49</td>
<td>0.412</td>
<td>0.034</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Metastasis</td>
<td>6</td>
<td>0.612</td>
<td>0.169</td>
<td></td>
</tr>
</tbody>
</table>

* WGA binding to SGP-900 region relative to HT-29 LMM cell lysates.
* NS, differences not significant.
parental cells and LMM cells was maintained even after the enhancement of the production of the \( M, 900,000 \) component; and (c) among different organ sites, liver metastasis of HT-29 cells produced the greatest amount of the \( M, 900,000 \) sialoglycoprotein based on the same weight of wet tumor tissues. Increased expression of SGP-900 is also seen with tumors growing in the liver of a nude mouse following intrahepatic injection. Such analyses of SGP-900 in the tumors derived from HT-29 cells inoculated into nude mice revealed that the cells growing in situ and in the metastasis might be selected populations. Alternatively, the apparent increased expression of SGP-900 might be influenced by the organ microenvironment. A similar observation was reported with a WGA-reactive sialoglycoprotein (\( M, \sim 150,000 \)) of hamster lymphosarcoma (40). According to Chan et al. (40), the production of this glycoprotein was enhanced when the lymphosarcoma was growing in the liver. It was also known that the sialoglycoprotein production by the primary explant of rat mammary adenocarcinoma was higher than production by the established cell line, but it rapidly regressed upon passages in vitro (41). However, the SGP-900 production by HT-29 cells was not entirely determined by the organ environment. There was a difference in SGP-900 between intrasplenic primary tumors derived from HT-29 P and HT-29 LMM and also between liver metastasis derived from these two cell lines. Therefore, the level of SGP-900 was apparently influenced by both cellular origin and environment.

An SGP-900-like molecule was also found in human colorectal carcinoma tissues. There were WGA-binding components at the approximate range of \( M, 900,000 \) in the extracts of primary colorectal carcinoma and liver metastases as well as in adjacent colonic mucosa. The profiles of WGA-binding glycoproteins after electrophoretic separation in the presence of NaDodSO\(_4\) on 3% polyacrylamide gels of the tumor extracts were similar to those from the HT-29 cell-derived tumors grown in nude mice. The expression of the SGP-900-like WGA-binding sialoglycoprotein was expressed at a higher level in 4 of 6 liver metastases than the primaries of the same patient. However, we could not find a statistically significant difference. There was no correlation between the level of SGP-900-like molecules and \textit{Ulex europeus} agglutinin reactivity to high-molecular-weight glycoproteins. We do not know why the WGA-binding components in the tumor extracts, both HT-29-derived and human tumors, have diffuse appearances. The tumors consist of not only carcinoma cells but also of a variety of host-derived cells. Therefore, we could not eliminate the possibility that a portion of the WGA-binding high-molecular-weight glycoproteins in the tumor tissues were produced by the host cells.

Since increased sialoglycoprotein production seems common with many liver metastatic tumor cells, sialoglycoproteins may be responsible for organ-specific colonization processes. Alternatively, high-molecular-weight sialoglycoproteins may conceal recognition sites on the tumor cells responsible for specific or nonspecific immunological modulation of tumor growth. We are currently investigating purification and characterization of SGP-900 and monoclonal antibody production against it.

**ACKNOWLEDGMENTS**

We thank Dr. I. J. Fidler for providing HT-29 human colon carcinoma cells with defined metastatic potential in nude mice, and Dr. G. L. Nicolson for his advice and discussions. We also thank Lisa Daniel and Carolyn P. Cooke for their technical assistance, Eleanor Felonia and Susan Lyman for preparation of the manuscript, and Tania Busch for illustrations.

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**Table 2** \textit{\(^{131}\)I-WGA binding to sialoglycoproteins produced by HT-29 LMM human colon carcinoma cells before and after chemical modification of the carbohydrate chains**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intensity*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HT-29 sialoglycoproteins</strong></td>
<td></td>
</tr>
<tr>
<td>900+</td>
<td>740+</td>
</tr>
<tr>
<td>450+</td>
<td>190+</td>
</tr>
<tr>
<td>145+</td>
<td>115+</td>
</tr>
<tr>
<td>Laminn</td>
<td>440+</td>
</tr>
<tr>
<td>Fetuin</td>
<td>220+</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>68+</td>
</tr>
<tr>
<td></td>
<td>45+</td>
</tr>
</tbody>
</table>

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* Intensity is based on the density of autoradiogram.

* \( M, \times 10^9 \).
Differential Expression of a Sialoglycoprotein with an Approximate Molecular Weight of 900,000 on Metastatic Human Colon Carcinoma Cells Growing in Culture and in Tumor Tissues

Tatsuro Irimura, Debora A. Carlson, Janet Price, et al.


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