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 oligo-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 growing in the liver of a nude mouse following intrasplenic injection. Analyses of human colorectal carcinoma tissues and liver metastases revealed a polydisperse WGA-reactive high-molecular-weight component similar to that seen in tumors growing in nude mice. The mean value of WGA binding to high-molecular-weight glycoproteins in the primary tumors of stage B1 patients was smaller than that of all other primary tumors. Comparison of primary tumors with liver metastases from the same patients indicated that the level of SGP=900-like high-molecular-weight glycoproteins in metastases was not always higher than those in primary tumors.

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 immunochemical 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 MDAY 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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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 m M CaCl2, 100 mM phenylethylmalonimidohydriode; NaDodSO4, sodium dodecyl sulfate; RCA1, Ricinus communis agglutinin-I; TBS, 25 mm Tris-HCl buffer, pH 7.2, containing 0.12 m sodium chloride.

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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 (10⁵/0.1 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 [6⁵²H]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, 10⁷ 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 by 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 x g for 5 min, mixed with 0.25 ml of 3 x 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 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 x g for 5 min, were processed with electrophoresis sample buffer as described before (5, 6, 13).

Extraction of Colorectal Tumors. 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 carcinoma tissues. Portions of polyacrylamide gels, 1 cm long, corresponding to SGP-900 (between 5 mm above and 5 mm below) were cut and counted by a γ counter. 125I-WGA binding to the M, ~900,000 region of a total of 55 carcinoma tissue specimens were compared after normalizing 125I-WGA binding according to the binding of SGP-900 from HT-29 LMM cells on individual slab gels.

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 M₁, 190,000 component migrated as a broad band, suggesting that this component has a polydispersed nature. The molecular weights of the two other 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 M₁, 115,000 component was slightly weaker 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 ovalbumin, 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 sialoglycoproteins 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), M₁, 115,000, 145,000, 190,000, and metastasis-specific large components acquired WGA reactivity, which suggested that N-acetylglucosamine residues resistant to peridate oxidation were exposed after removal of sialic acid followed by Smith degradation (19). Direct Smith degradation

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Footnote:
were sialoglycoproteins and that the differential binding of
These results confirmed that $M_\text{r}$ 115,000, 145,000, 190,000,
atively high-molecular-weight glycoproteins and to estimate their
Density Polyacrylamide Gel Electrophoresis. To separate rela-
internal structures. Metabolic labeling of these cells with $[^3H]$glucosamine re-
differential terminal sialylation but to the difference in the
WGA to the high-molecular-weight component was not due to
components unless sialic acid had been removed. An additional
high-molecular-weight component was also identified by RCA\textsubscript{1} 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 $M_\text{r}$ 115,000, 145,000, 190,000,
450,000, 740,000, and differentially expressed components were sialoglycoproteins and that the differential binding of
WGA to the high-molecular-weight component was not due to
terminal sialylation but to the difference in the internal structures.

Metabolic labeling of these cells with $[^3H]$glucosamine revealed numerous constituents. Some of them appeared to coincide with the WGA-binding sialoglycoproteins. One band migrated at $M_\text{r}$ $\sim$190,000 and another band at $M_\text{r}$ $\sim$900,000. The highest expression of this component with HT-29 LMM indicated that the incorporation of $[^3H]$glucosamine was in the high-molecular-weight WGA-binding sialoglycoprotein (Fig. 1c).

Resolution of High-Molecular-Weight Glycoprotein by Low-Density Polyacrylamide Gel Electrophoresis. To separate relatively high-molecular-weight glycoproteins and to estimate their
without prior removal of sialic acid eliminated WGA reactivity of all the glycoproteins except the $M_\text{r}$ 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 RCA\textsubscript{1} after removal of sialic acid from the glycoproteins, using mild acid hydrolysis (Fig. 1b). RCA\textsubscript{1} bound none of these components unless sialic acid had been removed. An additional high-molecular-weight component was also identified by RCA\textsubscript{1} 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 $M_\text{r}$ 115,000, 145,000, 190,000, 350,000, 450,000, 740,000, and 900,000 were clearly separated. The differentially expressed glycoprotein was shown to have an apparent $M_\text{r}$ $\sim$900,000 calibrated from the positions of laminin ($M_\text{r}$ $\sim$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 $M_\text{r}$ 900,000 sialoglycoprotein was much higher than the binding to $M_\text{r}$ 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 $M_\text{r}$ 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
SIALOGLYCOPROTEINS IN COLON CANCER METASTASIS

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 $6.7 \times 10^6$ 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 ($OV$), $M_r \approx 45,000$ is indicated. Arrow indicates the position of the $M_r \approx 900,000$ sialoglycoprotein.

Growing after injections at different sites. Lane 5 shows that HT-29 cells injected directly to the liver and growing in the liver expressed very high level of sialoglycoproteins compared with those cells injected s.c. These results clearly indicated that HT-29 colon carcinoma cells growing in the liver expressed higher level of sialoglycoproteins regardless of the route of injection. Whether this was due to the selective growth of a tumor cell subpopulation which expressed higher sialoglycoproteins or to the reversible physiological effects of organ microenvironmental factors is unknown at this time.

Detection of SGP-900 in Human Colorectal Carcinoma and Metastasis. The profiles of WGA-stained human colorectal primary carcinoma and liver metastases are shown in Fig. 5. Although the resolution of the glycoproteins was not clear, a broad band could be seen at the approximate migration distance corresponding to $M_r \approx 900,000$. The diffuse appearance was very similar to that of the $M_r \approx 900,000$ sialoglycoprotein observed with HT-29 cells growing in situ in nude mice. WGA binding did not occur when the gels were treated with mild acid, confirming that these are sialoglycoproteins. The $M_r \approx 900,000$ sialoglycoprotein-like molecule was present in most of human colorectal tissues including primary carcinoma, adjacent mucosa tissues, and liver metastasis of colorectal carcinoma. SGP-900-like sialoglycoproteins in colorectal primary carcinoma tissues from 49 patients, including 6 liver metastases, were compared (see "Materials and Methods" for the procedure). Statistical analyses based on these data are summarized 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). Colonie 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 sialylated glycoproteins that are increasingly produced by carcinoma cells.

There is evidence to indicate that cell surface sialoglycopro-
SIALOGLYCOPROTEINS IN COLON CANCER METASTASIS

Fig. 4. Glycoprotein profiles of detergent extracts of HT-29 human colon carcinoma cells grown in vivo in nude mice. Three % polyacrylamide gels were used for the separation. The gels were stained with 123I-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 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 (I), M, 980,000; large subunit of laminin (LS), M, -220,000. The arrow indicates the position of the M, 900,000 sialoglycoprotein.

teins 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 123I-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 of transverse carcinoma of transverse colon and three liver metastases. Lane 9, adjacent mucosa; Lane 8 was from the liver metastasis. Lanes 9-14 were derived from multiple liver metastases (HT-29 LMM) in nude mice. The arrow indicates the position of the M, 900,000 sialoglycoprotein.

In LMM cells, whereas an additional high-molecular-weight glycoprotein was seen with these two cell lines established 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.

the tumor at the primary inoculation site (HT-29 SP1) and 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 from liver metastases, especially HT-29 LMM which showed more stable expression of this high-molecular-weight glycoprotein as well as metastatic potential.

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 glycosylation 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 SGP-900 influenced the adhesive interaction of these cells with human lymphocytes (data not shown).

From the analysis of HT-29 cells grown in nude mice, the following conclusions were drawn: (a) there seemed to be an enhancement of the production of M, 900,000 sialoglycoprotein by the HT-29 cells growing in situ; (b) the difference between

Table 1 Comparisons of levels of SGP-900-like WGA-binding sialoglycoproteins in colorectal carcinoma

<table>
<thead>
<tr>
<th>Comparison according to:</th>
<th>Category</th>
<th>No. of cases</th>
<th>Mean log (l + x̄)</th>
<th>SE</th>
<th>Difference</th>
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<tbody>
<tr>
<td>Stage (20, 21) (primaries)</td>
<td>B1</td>
<td>17</td>
<td>0.306</td>
<td>0.057</td>
<td>B1 &lt; other</td>
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<tr>
<td></td>
<td>B2</td>
<td>8</td>
<td>0.485</td>
<td>0.052</td>
<td>P = 0.0234</td>
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<tr>
<td></td>
<td>C</td>
<td>13</td>
<td>0.483</td>
<td>0.073</td>
<td>NS*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>11</td>
<td>0.489</td>
<td>0.074</td>
<td>NS*</td>
</tr>
<tr>
<td>Grade (22) (primaries)</td>
<td>Well differentiated</td>
<td>9</td>
<td>0.516</td>
<td>0.120</td>
<td>NS*</td>
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<tr>
<td></td>
<td>Moderately differentiated</td>
<td>24</td>
<td>0.374</td>
<td>0.044</td>
<td>NS*</td>
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<tr>
<td></td>
<td>Poorly differentiated</td>
<td>1</td>
<td>0.486</td>
<td></td>
<td></td>
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<tr>
<td>Recurrence within 2 yrs (primaries of stage B1, B2, and C)</td>
<td>Yes</td>
<td>5</td>
<td>0.508</td>
<td>0.056</td>
<td>NS*</td>
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<tr>
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<td>No</td>
<td>12</td>
<td>0.491</td>
<td>0.090</td>
<td>NS*</td>
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<td>Primaries or metastases</td>
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<td>49</td>
<td>0.412</td>
<td>0.034</td>
<td>NS*</td>
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<tr>
<td></td>
<td>Metastasis</td>
<td>6</td>
<td>0.612</td>
<td>0.169</td>
<td>NS*</td>
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</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 (\( M \), ~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 *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.

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t\( \times 10^6 \)

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**Table 2** 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>HT-29 sialoglycoproteins</th>
<th>Laminin</th>
<th>Fetuin</th>
<th>Ovalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>900*</td>
<td>740</td>
<td>450</td>
<td>190</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mild acid hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mild acid hydrolysis and</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Smith degradation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Intensity is based on the density of autoradiogram.

* M, \( \times 10^6 \).
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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