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The glycosphingolipid globotriaosylceramide in the metastatic transformation of colon cancer

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The most devastating aspect of cancer is the emergence of metastases. Thus, identification of potentially metastatic cells among a tumor cell population and the underlying molecular changes that switch cells to a metastatic state are among the most important issues in cancer biology. Here we show that, although normal human colonic epithelial cells lack the glycosphingolipid globotriaosylceramide (Gb3), this molecule is highly expressed in metastatic colon cancer. In addition, a subpopulation of cells that are greatly enriched in Gb3 and have an invasive phenotype was identified in human colon cancer cell lines. In epithelial cells in culture, Gb3 was necessary and sufficient for cell invasiveness. Transfection of Gb3 synthase, resulting in Gb3 expression in noncancerous polarized epithelial cells lacking endogenous Gb3, induced cell invasiveness. Furthermore, Gb3 knockdown by small inhibitory RNA in colon cancer epithelial cells inhibited cell invasiveness. Gb3 is the plasma membrane receptor for Shiga toxin 1. The noncatalytic B subunit of Shiga toxin 1 causes apoptosis of human colon cancer cells expressing Gb3. Injections of the B subunit of Shiga toxin 1 into HT29 human colon cancer cells engraf ted into the flanks of nude mice inhibited tumor growth. These data demonstrate the appearance of a subpopulation of Gb3 containing epithelial cells in the metastatic stage of human colon cancer and suggest their possible role in colon cancer invasiveness.

metastases | invasion | filopodia

Colorectal cancer is the second leading cause of cancer death in the U.S. (1, 2). The high mortality associated with colorectal cancer is related to its ability to spread beyond the large intestine and to invade distant organs. One route to improve understanding of the molecular mechanisms of metastasis is by the identification of genes that are differently expressed during cancer progression and/or are responsible for acquisition of the metastatic phenotype. Although many molecular factors have been identified as contributing to metastases and represent potential targets for treatment (3), much remains to be learned about the biology of the metastatic process. Aberrant glycosylation, which has been observed in essentially all types of human cancers during cancer progression into the metastatic stage, is an example of a metastases-related change that is based, in part, on altered gene expression (4).

Aberrant glycosylation due to tumor transition into an invasive stage is also associated with changes in glycosphingolipid (GSL) composition. GSLs are abundant in the outer leaflet of plasma membranes of nearly all eukaryotic cells and have many diverse functions (5, 6). GSLs serve as receptors for viral and bacterial toxins, and microbial infections may be mediated by interactions of host GSLs with microbial membrane proteins. GSLs at the cell surface modulate transmembrane signal transduction by influencing protein kinases associated with growth factor receptors and PKC. GSL–GSL interactions and formation of so-called glycosynapses appear to provide the basis for a specific cell recognition system independent of the fibronectin/integrin or surface lectin systems (7). Surprisingly large numbers of tumor-associated antigens have been identified as GSLs. Altered GSL structures and cell surface expression patterns are associated with invasive phenotypes of some experimental tumors (7). Thus, it is well established that Gt1b inhibits cell motility and invasiveness in bladder tumors (8). Gangliosides Gt1b, Gd1a, Gm3, and Gm1 inhibit cell proliferation and epidermal growth factor receptor tyrosine phosphorylation (9, 10), whereas depletion of Gt1b and Gm3 by sialidase overexpression facilitates epidermal growth factor receptor phosphorylation and cell migration (10). Oppositely, a different GSL, Gs5, strongly enhances motility of breast cancer cells (11).

We have now identified a form of abnormal glycosylation that correlates with the development of human colon cancer metastasis. Globotriaosylceramide (Gb3) is expressed in metastatic colon cancer and is virtually absent from normal colonic epithelial cells. We found that Gbs-expressing colon cancer cells represent a potentially invasive subpopulation. Molecular manipulation to increase Gb3 expression converts noninvasive epithelial cells into cells with an invasive phenotype, and the molecular alterations that lead to Gb3 up-regulation appear to be sufficient for this transformation. To explore the role of Gb3 in the growth of human colon cancer cell lines established from metastatic lesions, advantage was taken of the fact that Gb3 serves as a receptor (12, 13) for the noncatalytic B subunit of Shiga toxin 1 (Stx1B). As in other cells (14), the selective binding and uptake of the Stx1B by Gbs-positive Caco-2 cells is shown to cause apoptosis. We also show that intratumoral injection of Stx1B inhibited colonic tumor growth in the nude mouse model.

Materials and Methods

Cell Culture and Human Tissue. Caco-2, T84, OK, and HT29 cells were from the American Type Culture Collection. Samples of archived frozen human tissue included normal distal and proximal colon (n = 15), nonmalignant colonic adenomas (n = 3), colon cancer without metastases (n = 3), primary lesions of metastatic colon cancer (n = 5), and colon cancer metastases in liver (n = 2). This study was exempted under the Code of Federal Regulations Title 45 Section 46.101(b) by the Hopkins Institution Review Board.

Fluorescence Microscopy. To evaluate the distribution of Gb3, cells grown on glass coverslips were incubated for 1 h with 0.5 μg/ml Stx1B-Alexa Fluor 488 (Stx1B–488). All experiments, except immunofluorescence, were performed on living cells mounted in a perfusion chamber (Warner Instruments, Hamden, CT) with
Both sides of the membrane were stained, and 0.5-
surface of the filters. After incubation for 24, 48, or 72 h, cells on
formed renal proximal tubule OK cells were seeded on the top

Stx1B–488 (green) and against
(red) and Stx1B–488 (green) to mark Gb3.(

(siRNA) oligonucleotides (17). By using the OLIGOENGINE siRNA
design tool (OligoEngine, Inc., Seattle), five Gb3 synthase cDNA
coding regions suitable for RNA interference were chosen and
DNA oligos were obtained. Caco-2 cells are difficult to transfect
with high efficiency by using lipophilic agents. To overcome this
problem, an adenoviral vector was constructed in collaboration
with the Johns Hopkins Adenoviral Preparation, Purification,
and Titering Core Facility, in which the pSUPER siRNA shuttle vector
with cDNA–GFP (to monitor the efficiency of viral infection) was
ligated. Exposure of \(\approx 50\%\) confluent Caco-2 cells to 1.3 \(\times 10^{10}\)
particles per ml of adenovirus led to 50\% GFP-positive cells, and
this percentage did not increase significantly with higher doses of
the virus. The Gb3 synthase cDNA oligo forward and reverse
sequences gatecccTTTCCGTGTCATATGTGCTCGTcaca-
gagaCGAGCACATGAAACAGGAAGtttt gaaa and agcttttc-
ccaaaaCTTCCGTGTCATATGTGCTCGTctetetgaaCGAGCACATG-
AACAGGAAGggg were the most effective in terms of inhibition
of Gb3 synthesis, which was monitored by both specific Stx1B
expression assay by Western blot.

Gb3 Synthase Knockdown. To affect the silencing of a specific gene,
we used the pSUPER vector ligated with small inhibitory RNA
(siRNA) oligonucleotides (17). By using the OLIGOENGINE siRNA
design tool (OligoEngine, Inc., Seattle), five Gb3 synthase cDNA
coding regions suitable for RNA interference were chosen and
DNA oligos were obtained. Caco-2 cells are difficult to transfect
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ccaaaaCTTCCGTGTCATATGTGCTCGTctetetgaaCGAGCACATG-
AACAGGAAGggg were the most effective in terms of inhibition
of Gb3 synthesis, which was monitored by both specific Stx1B
binding and CD77 mAb. The monolayers were inspected by fluo-
rescence microscopy, and all GFP-positive cells were Gb3-free by
48 h after infection.

For siRNA control experiments, pSUPER vector with scrambled
RNA (scRNA) duplex (catalog no. D-1200-05, Dharmacon Re-
search, Lafayette, CO) was engineered. Caco-2 cells were infected
with \(1 \times 10^{10}\) to \(2 \times 10^{10}\) particles per ml scRNA adenovirus. After
48 h, cells were used for high-performance thin-layer chromatog-
raphy (HPTLC) to assess the changes in Gb3 content or for protein
expression assay by Western blot.

Laser Capture Microdissection (LCM), GSLs Extraction, and HPTLC. To
collect a cancer cell population enriched in epithelial cells, the tissue

Gb3 is expressed in the primary lesions of human metastatic colon cancer and in liver metastases. (A) Normal colonic tissue immunostained against \(\beta\)-catenin
(red) and Stx1B–488 (green) to mark Gb3. (B) Normal colonic tissue labeled with CD77 mAb against Gb3 (red) and cholera toxin B subunit to detect GSLs Gm1 (green).
Note that cells in lamina propria but not epithelial cells are Gb3-positive (n = 54 fields of view). (C) Noncancer colonic adenoma immunolabeled against \(\beta\)-catenin (red)
and Stx1B–488 (green) for Gb3 (n = 12 fields of view). Note that erythrocytes (yellow, do not have nuclei) but not epithelial cells are Gb3-positive. (D) Nonmetastatic colon
adenocarcinoma tissue labeled against pankeratin to detect epithelial cells (red) and Gb3 by Stx1B–488 (green) (n = 12 fields). (E) Metastatic colon cancer tissue
immunostained against \(\beta\)-catenin (green) and Gb3 marked by Stx1B–568 (red). (F) Hematoxylin/eosin staining corresponding to sample in E (n = 29 fields). (G) Colon cancer
metastases into the liver (n = 17 fields) labeled with Stx1B–488 (green) and mAb against \(\beta\)-catenin (red). (H) Normal liver tissue (n = 10 fields) immunostained with
Stx1B–488 (green) and against \(\beta\)-catenin (red). Nuclei are stained with Hoechst dye (blue). (I and J) Tissue sample of hematoylin-stained, nonmetastatic colon cancer
before LCM (I) and after LCM when cancer epithelial cells were collected (J). (K) HPTLC shows the absence of Gb3 in epithelial cells collected by LCM from nonmetastatic
colon cancer (lane 1) as in D, and Gb3 presence in metastatic colon cancer (lane 2) as in E. Lane 3 shows a mixture of purified markers (10 \(\mu\)g/ml Gb3 and 5 \(\mu\)g/ml LacCer).
(L) Relative amount of Gb3 (percentages) in epithelial cells in human tissue normalized to normal colon or liver. Bars: 1, normal colon; 2, noncancer adenomas; 3,
nonmetastatic colon cancer; 4, primary lesion of metastatic colon cancer; 5, colon cancer metastases into liver; 6, normal liver. The relative amount of Gb3 in normal
colon and liver was calculated from analysis of 6-bit fluorescence images as an average of fluorescence intensity per field of view and presented as 100% ± SE.

The amount of Gb3 in cancer tissue was calculated similarly and expressed in percentages relative to control. The relative amount of Gb3 significantly (P < 0.05) increased
in metastatic tissues compared with normal colon and liver, respectively.
samples were hematoxylin stained, vacuum dried, and subjected to LCM (P.A.L.M. Microlaser Technologies, Bernried, Germany) by following manufacturer’s protocols. To detect Gb3 in cancer epithelial cells, the LCM collected cells from two cases of nonmetastatic and two cases of metastatic colon cancers or from Caco-2 cells infected either with small inhibitory Gb3 or scrambled RNA were subjected to HPTLC as described in detail (Supporting Materials and Methods) (18–20).

**Nude Mouse Xenograft Model.** The growth of HT29 human colonic cancer cells injected into the flanks of nude mice was determined with or without intratumor injection of a fixed amount of Stx1B administered every 2 days starting 7 days after tumor injection. Initially, effects of s.c. flank injections of various amounts of Stx1B were determined on nude mouse mortality and on liver, small intestine, and kidney histology at the light microscopy level. All experiments were conducted according to a protocol approved by the Johns Hopkins University School of Medicine Animal Care and Use Committee.

**Statistics.** Data are presented as mean ± SEM. Significance was determined by using Student’s *t* test, and *P* ≤ 0.05 were considered statistically significant.

**Results**

**Gb3 Is Expressed in Colon Cancer Metastases but Not in Normal Colonic Tissue, Noncancer Adenomas, and Nonmetastatic Colon Cancer.** To begin characterizing which types of colon cancer express Gb3, immunofluorescence staining was performed with CD77 Ab against Gb3, or fluorescently labeled Stx1B, which binds specifically to Gb3 (21). Gb3 was not expressed in normal colonic epithelial cells (Fig. 1A and B), nonmalignant colonic adenomas (Fig. 1C), or nonmetastatic primary colon cancers (Fig. 1D). In contrast, as a positive control, another GSL GM1, the cholera toxin B subunit receptor, was readily detectable on the apical surface of colonocytes (Fig. 1B). Gb3-positive cells were present in some cells in the lamina propria (Fig. 1A and B) and in erythrocytes (Fig. 1C). In contrast, high levels of Gb3 were expressed in epithelial cells in primary lesions of metastatic colon cancers and in the colon cancer metastases to the liver (Fig. 1E–I). There was no detectable level of Gb3 in normal hepatocytes (Fig. 1H). To confirm the data gained by immunofluorescence, cancer cells from primary lesions of nonmetastatic colon cancer (Fig. 1J and K) or from metastatic samples were collected by LCM. LCM was done to decrease contamination by erythrocytes, endothelial cells, or inflammatory cells, which are known to express Gb3 (22, 23), and to increase the amount of Gb3 in tissue samples. The relative amount of Gb3 in tissue samples was estimated from immunofluorescence images of tissue double-labeled with Stx1B or CD77 mAb to detect the Gb3 and pankeratin mAb and to ensure that exclusively epithelial cells were included in the analysis. Quantification of Gb3 fluorescence intensity, which corresponds to the relative amount of Gb3 in the sample, showed that Gb3 is up-regulated in primary lesions of metastatic colon cancer tissue (>300% compared with the background autofluorescence of normal colonic epithelial cells, tubular adenomas, and nonmetastatic cancers (Fig. 1L)). Similarly, Gb3 fluorescence intensity in colon cancer metastases to the liver was elevated >300% compared with the normal liver (Fig. 1L). This significant Gb3 elevation in metastatic colon cancer caused us to examine the potential role of Gb3 in development of tumor invasiveness.

**Colon Cancer Cells with Elevated Gb3 Have a Migratory Phenotype.** Human intestinal epithelial Caco-2, T84, and HT29 cells derived from metastatic colon cancer were examined by immunofluorescence microscopy and FACS for the presence of Gb3-expressing cells. When 50–70% confluent, two subpopulations of cells were detected in each cell line: one with or without trace amounts of Gb3 and the other with a high amount of Gb3. The percent of Gb3-expressing cells was different in the three cell types and only ~5% of T84 cells, ~50% of Caco-2 cells, and ~90% of HT29 cells were Gb3-positive. In all three cell types, Gb3-containing cells were mostly concentrated at the leading edge of cell islands. Moreover, cells enriched in Gb3 demonstrated a migratory phenotype and formed filopodia (Fig. 2A, C, and D). In Caco-2 cells (Fig. 2A and D), the filopodia were very thin (~0.2–0.5 μm), variable in number (up to 50 on a single cell), and beaded, and they contained Gb3. The filopodia could exceed 2- to 10-fold the epithelial cell diameter and projected in the direction of monolayer growth and cell spreading. In all three cell lines there was a strong correlation between the amount of Gb3 and appearance of filopodia. Cells that formed filopodia were significantly enriched in Gb3 compared with neighboring cells without filopodia from the same leading edge (Fig. 2A and D). Quantitative analysis of fluorescence intensity of 11 Caco-2 cells with and without filopodia showed that cells with filopodia

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**Fig. 2.** Filopodia-containing cells are present at the leading edge of a living colon carcinoma monolayer detected by staining of the GSL Gb3 in HT29 cells. (A) x–y plane from 3D reconstruction of fluorescent confocal optical sections of representative Caco-2 cells with Gb3-containing filopodia marked by Stx1B–488. Filopodia and filopodia-containing cells are significantly enriched in Gb3-positive cells compared with the rest of the cells from the leading edge. (Scale bar, 10 μm.) (B) x–z projection from 3D reconstruction of confocal optical sections of representative Caco-2 cell with Gb3-containing filopodia, which is significantly taller than adjacent cells in the monolayer. (Vertical scale bar, 25 μm.) (C) Gb3-enriched filopodia-containing HT29 cells (blue, nuclei stained by Hoechst). (D) Gb3-positive cells with filopodia on the leading edge of T84 monolayer (red, Cyto7 dye). (E) Relative amount of Gb3 normalized per pixel in Caco-2 cells. Bars: 1, without filopodia; 2, with filopodia (*P* < 0.05). (F) The heights of Caco-2 cells. Bars: 1, non-filopodia-containing cells; 2, Gb3-up-regulating, filopodia-containing cells (*P* < 0.05 compared with bar 1).
Cells Containing Gb3 and Filopodia Are Invasive. The appearance of cells with filopodia is consistent with their involvement in cell spreading, migration, or invasiveness. Because Gb3-enriched filopodia-containing cells were detected in colonic cancer cell lines and because epithelial cells with higher amounts of Gb3 were an exclusive feature of metastatic colonic cancer, we hypothesized that these cells might represent an invasive pool. To test this hypothesis, we applied a standardized chemoinvasive assay (16) that correlates with metastatic ability in vivo (24). In this assay, the movement of Caco-2 cells across a filter treated from the bottom side with laminin, a chemoattractant, was monitored. This assay demonstrated that only filopodia-containing Gb3-enriched cells migrate to the bottom surface of the filter and do so by the initial movement of filopodia through the filter pores (Fig. 3 A–D).

Filopodia-containing cells that migrated through the filter created a confluent monolayer of cells. Attempts to clone Gb3-enriched Caco-2 cells resulted in formation of cell monolayer with only ~50% Gb3-containing cells, similar to that in wild-type Caco-2 monolayers. Analysis of the expression of the Gb3 synthase mRNA, an enzyme responsible for Gb3 synthesis (22, 25), in the subpopulation of Gb3-negative vs. Gb3-up-regulating Caco-2 cells (Fig. 6 A and B, which is published as supporting information on the PNAS web site) showed the presence of Gb3 synthase mRNA in both cell types, with only a nonsignificant 1.4-fold elevation in Gb3-enriched cells (Supporting Materials and Methods). This result indicates that the invasive subpopulation of cells does not occur because of the differential expression of the Gb3 synthase gene but, rather, because of the regulation of protein and/or lipid expression by uncharacterized signal transduction mechanisms.

Gb3 Is Necessary for Colon Cancer Cell Invasiveness. One way to further explore the role of Gb3 in invasiveness is to inhibit Gb3 synthesis in colon cancer cells and determine the impact on cell invasiveness. There are no specific inhibitors of Gb3 synthesis (26). Thus, to address this question, we used siRNA (17) to knock down the Gb3 synthase gene (siGb3). For these experiments, Caco-2 cells were infected with adenovirus containing siGb3 to silence the Gb3 synthase gene and thus prevent Gb3 synthesis (Fig. 3E). To ensure
Gb3 Is Sufficient for Epithelial Cell Invasiveness. To test whether Gb3 expression is sufficient for epithelial cells to invade, we studied OK cells, which do not endogenously express Gb3, do not bind Stx1B (Fig. 4A), and are not invasive based on the fact they do not migrate through the membrane pores (Fig. 4B). These cells were transiently transfected with a Gb3 synthase cDNA (Supporting Materials and Methods) (22), shown to express Gb3 synthase (Fig. 4E), and then tested by chemoinvasive assay. Gb3-expressing OK cells formed Gb3-positive filopodia on the leading edge of growing monolayers (Fig. 4C) similar to that in T84, Caco-2, and HT29 cells. Moreover, as shown by the chemoinvasive assay (Fig. 4D), OK cells now penetrated through the filter. Importantly, only Gb3-positive OK cells penetrated through the filter, whereas cells without Gb3 from the same monolayer (Fig. 4D) and control cells transfected only with empty vector did not migrate through the filter at all and behaved similarly to the nontransfected cells (Fig. 4B).

B Subunit of Shiga Toxin Selectively Kills Gb3-Positive Colon Cancer Cells in Cell Culture Models and Mouse Xenographs. It has been shown that the noncatalytic Stx1B triggers apoptosis of Gb3-expressing Burkitt’s lymphoma cells (14). To test whether Stx1B selectively causes apoptosis in Gb3-positive colonic cells, T84 cells were exposed to Stx1B for up to 168 h. Mitochondria were monitored as a viability control by using tetramethylrhodamine (7, 27). After 24 h of exposure to Stx1B, virtually all T84 cells that lacked Gb3 and, thus, did not internalize Stx1B had active mitochondria (Fig. 8A, which is published as supporting information on the PNAS web site). However, cells that accumulated Stx1B had inactive mitochondria. Incubation of T84 monolayers with Stx1B for longer times led to elimination of Gb3-positive cells (Fig. 8B). In contrast, in the same monolayer, cells that did not take up Stx1B had active mitochondria and survived. In addition, OK cells, which do not express Gb3, were resistant to Stx1B-mediated apoptosis (Fig. 8C and D). Stx1B-mediated apoptosis of Caco-2 cells was also confirmed by the DNA-laddering assay (Fig. 8E) (28). We conclude that Stx1B selectively kills Gb3-expressing cells.

To test whether Stx1B affects growth of colon tumors that contain Gb3, we used the nude mouse xenograph model (29). The nontoxic dose of Stx1B was first determined in mice (two each) injected s.c. with Stx1B 10 ng/μg/kg, 50 ng/μg/kg, 100 ng/μg/kg, and 200 ng/μg/kg. By day 20 after the Stx1B injection, none of the animals died or appeared ill. These mice were euthanized by CO2 inhalation. The liver, small intestine, and kidney were shown to be histologically normal (data not shown). Then nude mice (n = 10 animals) were injected s.c. in their flanks with 1 × 10⁷ HT29 human colon cancer cells. When tumors reached 0.3 cm in diameter (~7 days after injection), mice were divided into two groups: the control group (n = 5 mice), in which tumors were injected with PBS only, and a test group, in which tumors on one flank were injected every other day with a nontoxic dose of Stx1B (50 ng/μg/kg, n = 6 mice) and tumors on the other flank were injected with an equal volume of PBS. Animals were monitored over 7 weeks, by which time some tumors reached ~1.5 cm size, and the animals had to be euthanized according to our animal protocol. As shown in Fig. 5A, Stx1B injections significantly inhibited tumor growth in nude mice. Examination of tumors for the presence of apoptotic cells using the TUNEL assay (28) showed that there were many apoptotic cells in Stx1B-injected tissue (Fig. 5B), whereas only a few apoptotic cells per tumor were detected in tumors injected with PBS (Fig. 5C). These data show that the B subunit of Stx1 alone causes apoptotic death of Gb3-positive human colon cancer cells.

Discussion

We have identified a subpopulation of invasive colon cancer cells based on molecular and phenotypic characteristics. Analysis of metastatic human colon cancer tissue samples showed that significant up-regulation of the GSL Gb3 was associated with development of metastasis. A similar subpopulation of cells with high levels
of Gb3 expression and a phenotype found was in three colon cancer cell lines established from metastatic human colon cancers. These cell lines may serve as a model for invasiveness development in a cancer cell population. It has been shown that the epithelial cells in human colon lack detectable Gb3 (23, 30). This finding is in good agreement with our data that Gb3 is virtually absent from normal colonic epithelial cells but is present in colon cancer in its metastatic stage.

Gb3 has been associated with other human cancers and was identified as the Burkitt’s lymphoma antigen (31). Elevated expression of Gb3 was also detected in astrocytoma cell lines (32) and in several highly metastatic types of human tumors, including ovarian and breast cancers (33). Significant Gb3 accumulation was also previously reported in testicular seminoma, which correlated with the metastatic potential (34, 35). These data support our finding that Gb3 expression is a marker for colon epithelial tumor cell transformation from primary cancer into the metastatic stage and indicate that several types of invasive tumors may have common mechanisms for metastasis.

Mechanistic studies of the role of Gb3 in metastases were performed on cell culture models. Our data demonstrate that Gb3 overexpression is necessary and sufficient for the appearance of cells with an invasive phenotype. In colon carcinoma epithelial cells, the presence of Gb3-containing filopodia correlates with cell invasiveness. Filopodia are used to penetrate through the permeable support in a chemoinvasive assay. Importantly, elimination of Gb3 from colon cancer cells by Gb3-synthase siRNA knockdown, which was verified by the Stx1B binding assay, completely inhibited cell migration. This result shows that Gb3 is necessary for cell invasiveness. Introduction of human Gb3-synthase into nontransformed, noninvasive OK cells that do not synthesize endogenous Gb3 induced Gb3 and converted noninvasive epithelial cells into invasive cells. Thus, the presence of Gb3 was also sufficient to induce the invasive phenotype in nontransformed cells.

Failure of Caco-2 cells to maintain the migratory phenotype when Gb3-enriched cells separated by FACs were cultured shows that Gb3-enriched invasive cells do not represent separate clones. This result indicates that changes in signal transduction pathways, which lead to Gb3 expression due to some as yet unknown mechanisms, rather than genetic alterations alone, are responsible for appearance of the invasive pool among the population of colon cancer cells. Gb3 expression can be driven by several molecular mechanisms that do not exclude but rather complement each other. One possibility is that the enzyme Gb3 synthase (a1,4-galactosyltransferase), which catalyzes the transfer of galactose to LacCer, and produces Gb3, is significantly up-regulated during cancer progression from the noninvasive stage into the invasive stage. Human Gb3 synthase has recently been cloned (22, 25). Although expression of Gb3 synthase has not yet been reported in metastatic vs. normal colonic epithelial tissue using gene arrays (Oncomine Cancer array data; www.oncomine.org), significant differences in the amount of Gb3 synthase mRNA between Caco-2 with up-regulated Gb3 and Caco-2 without a detectable amount of Gb3, make this mechanism very unlikely to be responsible for the appearance of Gb3-positive cancer epithelial cells. Other mechanisms, such as differences in the abundance of Gb3 synthase enzyme or its activity, may be responsible for the transition of noninvasive colon cancer cells into invasive and/or metastatic cells.

Gb3 is best known as the receptor for the B subunit of Shiga toxin 1. Our data show that, similarly to that in Gb3-positive Burkitt’s lymphoma cells (14), Stx1B selectively kills the subpopulation of Gb3-up-regulating colonic epithelial cells, which possess invasive potential. Next, we tested whether the ability of Stx1B to cause apoptosis might be used against colon cancer tumors grown in nude mice. Treatment of HT29 cell tumors in nude mice with Stx1B substantially inhibited tumor growth compared with nontreated control tumors, and, after 10 days, Stx1B treatment stabilized the tumor size, whereas nontreated tumors continued to increase in size. Thus, application of Stx1B or Stx1B linked to other more potent antitumor agents might be an approach to target the specific Gb3-expressing subpopulation of cells in human metastatic colon cancers.

In summary, the expression of the GSL Gb3 strongly correlates with the metastatic potential of human colon cancer. Gb3-enriched colon cancer cells represent an invasive subpopulation, and Gb3 expression is necessary and sufficient for this invasiveness in cell culture models. The overexpression of Gb3 makes it a possible marker for detection of a potentially invasive cell subpopulation in colon cancer.

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