

# **Cancer Research**

## **Cancer Metastasis Cancer Cell Lines Selected in an Animal Model for Colon Cell Surface Sialoprotein Alterations in Metastatic Murine Colon**

Robert S. Bresalier, Richard W. Rockwell, Rajvir Dahiya, et al.

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## <sub>|CANCER RESEARCH 50. 1299–1307. February 15. 1990|</sub><br>Cell Surface Sialoprotein Alterations in Metastatic Murine Colon Cancer Cell<br>Lines Selected in an Animal Model for Colon Cancer Metastasis<sup>1</sup> CANCER RESEARCH 50, 1299–1307, February 15, 1990|<br>Cell Surface Sialoprotein Alterations in Metastatic Murine Colon Cancer<br>Lines Selected in an Animal Model for Colon Cancer Metastasis<sup>1</sup><br>Robert S. Bresalier,<sup>2</sup> Richard W. Cell Surface Sialoprotein Alterations in Metastatic Murine Colon Cancer Cell<br>Lines Selected in an Animal Model for Colon Cancer Metastasis<sup>1</sup><br>Robert S. Bresalier,<sup>2</sup> Richard W. Rockwell, Rajvir Dahiya, Quan-Yang Duh, and Y **Lines Selected in an Animal Model for Colon Cancer Metastasis<sup>1</sup><br>Robert S. Bresalier,<sup>2</sup> Richard W. Rockwell, Rajvir Dahiya, Quan-Yang Duh, and Young S. Kim<br>Gastrointestinal Research Laboratory, Veterans Administration Me**

## ABSTRACT

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Alterations in cell surface proteins and glycoproteins may play a key<br>
in determining the metastatic behavior of tumor cells. The cell FORSITY THE MANUTE ALLOWING THE MANUTE CONSTRANT CHARGED ABSTRACT<br>
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Surface proteins of a series of related murine colon ABSTRACT<br>
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surface proteins of a series of related murine colon cancer cells sele Alterations in cell surface proteins and glycoproteins may play a key<br>role in determining the metastatic behavior of tumor cells. The cell<br>surface proteins of a series of related murine colon cancer cells selected<br>in an a Alterations in cell surface proteins and glycoproteins may play a key<br>role in determining the metastatic behavior of tumor cells. The cell<br>surface proteins of a series of related murine colon cancer cells selected<br>in an a role in determining the metastatic behavior of tumor cells. The cell<br>surface proteins of a series of related murine colon cancer cells selected<br>in an animal model for colon cancer metastasis (R. S. Bresalier *et al.*,<br>Canc surface proteins of a series of related murine colon cancer cells selected<br>in an animal model for colon cancer metastasis (R. S. Bresalier *et al.*,<br>Cancer Res., 47: 1398–1406, 1987) were therefore compared by a variety<br>o in an animal model for colon cancer metastasis (R. S. Bresalier *et al.*,<br>Cancer Res.,  $47:1398-1406, 1987$ ) were therefore compared by a variety<br>of biochemical methods. Lactoperoxidase-catalyzed iodination of cell<br>surfac Cancer Res., 47: 1398–1406, 1987) were therefore compared by a variety<br>of biochemical methods. Lactoperoxidase-catalyzed iodination of cell<br>surface proteins followed by sodium dodecyl sulfate-polyacrylamide gel<br>electropho of biochemical methods. Lactoperoxidase-catalyzed iodination of cell<br>surface proteins followed by sodium dodecyl sulfate-polyacrylamide gel<br>electrophoresis demonstrated quantitative and qualitative differences in<br>the cell surface proteins followed by sodium dodecyl sulfate-polyacrylamide gel<br>electrophoresis demonstrated quantitative and qualitative differences in<br>the cell surface protein profiles of parental cell line 51B (low metastatic<br>p electrophoresis demonstrated quantitative and qualitative differences in 191<br>the cell surface protein profiles of parental cell line 51B (low metastatic (9-<br>potential) and its metastatic derivatives 51B LiM 5 and 51B LiM potential) and its metastatic derivatives 51B LiM 5 and 51B LiM 6.<br>Labeling of sialic acid-containing proteins suggested that, in the case of<br>at least four of these proteins  $(M, 170,000, 120,000, 95,000,$  and 55,000),<br>thi Labeling of sialic acid-containing proteins suggested that, in the case of<br>at least four of these proteins  $(M, 170,000, 120,000, 95,000,$  and 55,000),<br>this represented an increase in radioactive labeling of sialoglycoprot at least four of these proteins  $(M, 170,000, 120,000, 95,000,$  and 55,000),<br>this represented an increase in radioactive labeling of sialoglycoproteins<br>from the metastatic lines. Affinity chromatography of solubilized  $^{12$ this represented an increase in radioactive labeling of sialoglycoproteins strom<br>from the metastatic lines. Affinity chromatography of solubilized <sup>125</sup>I-<br>labeled cell membrane proteins revealed a 2- to 3-fold increase in this represented an increase in radioactive iabeling or statogycoproteins<br>from the metastatic lines. Affinity chromatography of solubilized  $125$ <br>labeled cell membrane proteins revealed a 2-to 3-fold increase in wheat<br>lab labeled cell membrane proteins revealed a 2- to 3-fold increase in wheat<br>germ agglutinin and *Sambucus nigra* lectin binding associated with the<br>inter<br>metastatic lines, compared to the poorly metastatic parent. Sodium<br>dode germ agglutinin and *Sambucus nigra* lectin binding associated with the<br>metastatic lines, compared to the poorly metastatic parent. Sodium<br>dodecyl sulfate-polyacrylamide gel electrophoresis of material eluted gates have<br>fr metastatic lines, compared to the poorly metastatic parent. Sodium<br>dodecyl sulfate-polyacrylamide gel electrophoresis of material eluted<br>from these columns demonstrated enhancement of proteins from the<br>dentified major sial dodecyl sulfate-polyacrylamide gel electrophoresis of material eluted<br>from these columns demonstrated enhancement of proteins from the<br>metastatic cells corresponding in molecular weight to the previously<br>identified major s From these columns demonstrated enhancement of proteins from the  $(17)$ , metastatic cells corresponding in molecular weight to the previously metal<br>identified major sialoglycoproteins. Neuraminidase-releasable mem-<br>cell s metastatic cells corresponding in molecular weight to the previously met<br>
identified major sialoglycoproteins. Neuraminidase-releasable mem-<br>
cell<br>
brane-associated sialic acid and sialyltransferase activities were 2- to 3 identified major sialoglycoproteins. Neuraminidase-releasable mem-<br>
old higher in the metastatic cell lines compared to the parental line.<br>
Liver colonization after intrasplenic injection of the various lines into<br>
syngene brane-associated sialic acid and sialyltransferase activities were 2- to 3-<br>fold higher in the metastatic cell lines compared to the parental line.<br>Liver colonization after intrasplenic injection of the various lines into<br> fold higher in the metastatic cell lines compared to the parental line.<br>
Liver colonization after intrasplenic injection of the various lines into<br>
syngeneic mice was dramatically reduced by prior removal of cell surface<br> Liver colonization after intrasplenic injection of the various lines into<br>syngeneic mice was dramatically reduced by prior removal of cell surface<br>sialic acid. Immunohistochemical staining of primary and metastatic<br>tumors syngeneic mice was dramatically reduced by prior removal<br>sialic acid. Immunohistochemical staining of primary at<br>tumors formed after cecal injection of parental 51B sugge<br>metastasis by wheat germ agglutinin-binding tumor c

## INTRODUCTION

ther support the concept that cell membrane sialylation is important<br>
determining the metastatic potential of cancer cells.<br>
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Alterations in cell surface proteins and glycoproteins are<br>
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common during carcinogenesis and may play a key role in<br>
determining the metastatic behavior of tumor cells (1–4). The<br>
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cell surface is thought to play a role in several stages of metas-<br>
tasis includi Alterations in cell surface proteins and glycoproteins are<br>common during carcinogenesis and may play a key role in<br>determining the metastatic behavior of tumor cells  $(1-4)$ . The<br>cell surface is thought to play a role in Alterations in cell surface proteins and glycoproteins are<br>common during carcinogenesis and may play a key role in<br>determining the metastatic behavior of tumor cells  $(1-4)$ . The<br>cell surface is thought to play a role in common during carcinogenesis and may play a key role in<br>determining the metastatic behavior of tumor cells  $(1-4)$ . The<br>cell surface is thought to play a role in several stages of metas-<br>tasis including cell growth regula determining the metastatic behavior of tumor cells  $(1-4)$ . The<br>cell surface is thought to play a role in several stages of metas-<br>tasis including cell growth regulation, motility during invasion,<br>homotypic and heterotypi tasis including cell growth regulation, motility during invasion,<br>homotypic and heterotypic interactions with other tumor cells,<br>platelets, and the immune system, and the adherence of met-<br>astatic cells to endothelia and t homotypic and heterotypic interactions with other tumor cells,<br>platelets, and the immune system, and the adherence of met-<br>sialyltran<br>astatic cells to endothelia and the extracellular matrix. Al-<br>though a universal "metast platelets, and the immune system, and the adherence of met-<br>astatic cells to endothelia and the extracellular matrix. Al-<br>though a universal "metastasis-related cell surface phenotype"<br>has yet to be identified, differences astatic cells to endothelia and the extracellular matrix. Al-<br>though a universal "metastasis-related cell surface phenotype" MA<br>has yet to be identified, differences in the sialylation of cell<br>surface glycoproteins and gly though a universal "metastasis-related cell surface phenotype" MA'<br>has yet to be identified, differences in the sialylation of cell<br>surface glycoproteins and glycolipids are common when com-<br>paring cells of low and high m has yet to be identified, differences in the sialylation of cell<br>surface glycoproteins and glycolipids are common when com-<br>paring cells of low and high metastatic potential (5–20). Cell<br>surface sialylation has been impli surface glycoproteins and glycolipids are common when com<br>paring cells of low and high metastatic potential  $(5-20)$ . Cel<br>surface sialylation has been implicated in affecting such diverse<br>characteristics as invasive poten characteristics as invasive potential (15), tumor cell-mediated<br>platelet aggregation (18, 21), resistance to T-cell-mediated im-<br>mune destruction (11, 22), and alterations in tumor cell adher-<br>mune destruction (11, 22), a

**Puan-Yang Duh, and Young S. Kim**<br> *Expartment of Medicine, University of California, San Francisco, California 94121*<br>
ence to basement membrane components and target cells (2, 9,<br>
10, 12). Increased cell surface sialic a 10, 12). Increased cell surface sialic acid has been shown to epartment of Medicine, University of California, San Francisco, California 94121<br>ence to basement membrane components and target cells (2, 9,<br>10, 12). Increased cell surface sialic acid has been shown to<br>correlate with met epariment of meaicine, University of Catifornia, San Francisco, Catifornia 94121<br>ence to basement membrane components and target cells (2, 9,<br>10, 12). Increased cell surface sialic acid has been shown to<br>correlate with met ence to basement membrane components and target cells (2, 9, 10, 12). Increased cell surface sialic acid has been shown to correlate with metastatic potential in some studies (5, 8, 15, 17), while in others the position of ence to basement membrane components and target cells  $(2, 9, 10, 12)$ . Increased cell surface sialic acid has been shown to correlate with metastatic potential in some studies  $(5, 8, 15, 17)$ , while in others the positi correlate with metastatic potential in some studies  $(5, 8, 15, 17)$ , while in others the position of sialic acid on terminal carbohydrate structures appears more important in determining correlate with metastatic potential in some studies (5, 8, 15, 17), while in others the position of sialic acid on terminal carbohydrate structures appears more important in determining the extent of metastasis (9, 13, 16, 17), while in others the position of sialic acid on terminal carbohydrate structures appears more important in determining the extent of metastasis  $(9, 13, 16, 19)$ . The majority of cultured cell lines used in these stud carbohydrate structe<br>the extent of meta<br>cell lines used in t<br>thelial tumors su<br>lymphomas (9, 11<br>(9–11, 13, 23).<br>Colorectal canc cell lines used in these studies have been derived from nonepi-<br>the ial tumors such as murine melanomas  $(5-8, 14)$ , T-cell<br>lymphomas  $(9, 11-13, 15, 16, 19)$ , and leukemia-like tumors<br> $(9-11, 13, 23)$ .<br>Colorectal cancer thelial tumors such as murine melanomas  $(5-8, 14)$ , T-cell lymphomas  $(9, 11-13, 15, 16, 19)$ , and leukemia-like tumors

lymphomas  $(9, 11-13, 15, 16, 19)$ , and leukemia-like tumors  $(9-11, 13, 23)$ .<br>Colorectal cancer represents the second most common epi-<br>the lial cancer in North America and the third most common<br>cancer world-wide. Since c  $(9-11, 13, 23)$ .<br>Colorectal cancer represents the second most common epi-<br>the lial cancer in North America and the third most common<br>cancer world-wide. Since colon cancer-related mortality is<br>strongly correlated with ext Colorectal cancer represents the second most common epi-<br>thelial cancer in North America and the third most common<br>cancer world-wide. Since colon cancer-related mortality is<br>strongly correlated with extent of tumor cell in cancer world-wide. Since colon cancer-related mortality is  $(17, 18, 25-36)$ , including differences between primary and strongly correlated with extent of tumor cell invasion and<br>metastasis at diagnosis (24), there has been a great deal of<br>interest in identifying cellular determinants of colon cancer<br>metastasis. Alterations in cell surface metastasis at diagnosis (24), there has been a great deal of<br>interest in identifying cellular determinants of colon cancer<br>metastasis. Alterations in cell surface and secreted glycoconju-<br>gates have been described in colon interest in identifying cellular determinants of colon cancer<br>metastasis. Alterations in cell surface and secreted glycoconju-<br>gates have been described in colon cancer tissues and cell lines<br>(17, 18, 25–36), including dif metastasis. Alterations in cell surface and secreted glycoconju-<br>gates have been described in colon cancer tissues and cell lines<br>(17, 18, 25–36), including differences between primary and<br>metastatic tumors (17, 29, 32–34) gates have been described in colon cancer tissues and cell lines<br>(17, 18, 25–36), including differences between primary and<br>metastatic tumors (17, 29, 32–34) and between intratumoral<br>cell subpopulations with different biol  $(17, 18, 25-36)$ , including differences betw<br>metastatic tumors  $(17, 29, 32-34)$  and betw<br>cell subpopulations with different biological pi<br>In the case of at least one murine colon can<br>which differ in their metastatic pot extractive tumors (17, 29, 32–34) and between intratumoral<br>Il subpopulations with different biological properties (28, 31).<br>the case of at least one murine colon carcinoma, sublines<br>inch differ in their metastatic potenti cell subpopulations with different biological properties (28, 31).<br>In the case of at least one murine colon carcinoma, sublines<br>which differ in their metastatic potential also differ in their<br>degree of cell surface sialyla

In the case of at least one murine colon carcinoma, sublines<br>which differ in their metastatic potential also differ in their<br>degree of cell surface sialylation  $(17, 18)$ .<br>We have recently described the development of an which differ in their metastatic potential also differ in their<br>degree of cell surface sialylation (17, 18).<br>We have recently described the development of an animal<br>model for colon cancer metastasis, which was used to esta degree of cell surface sialylation (17, 18).<br>We have recently described the development of an animal<br>model for colon cancer metastasis, which was used to establish<br>and characterize murine colon cancer cell lines which diff We have recently described the development of an animal<br>model for colon cancer metastasis, which was used to establish<br>and characterize murine colon cancer cell lines which differ in<br>their liver-metastasizing abilities dur model for colon cancer metastasis, which was used to establish<br>and characterize murine colon cancer cell lines which differ in<br>their liver-metastasizing abilities during cecal growth (37). It<br>was demonstrated that the cell and characterize murine colon cancer cell lines which differ in<br>their liver-metastasizing abilities during cecal growth (37). It<br>was demonstrated that the cells selected for enhanced met-<br>astatic potential are more invasiv their liver-metastasizing abilities during cecal growth (37). It<br>was demonstrated that the cells selected for enhanced met-<br>astatic potential are more invasive *in vivo* and *in vitro* and that<br>they secrete a basement memb was demonstrated that the cells selected for enhanced met-<br>astatic potential are more invasive *in vivo* and *in vitro* and that<br>they secrete a basement membrane-degrading protease (type IV<br>collagenase) in proportion to th astatic potential are more invasive *in vivo* and *in vitro* and that<br>they secrete a basement membrane-degrading protease (type IV<br>collagenase) in proportion to their invasive and metastatic<br>abilities. These tumor cells ha they secrete a basement membrane-degrading protease (type IV collagenase) in proportion to their invasive and metastatic abilities. These tumor cells have also been shown to adhere rapidly to pure cultures of hepatic sinus collagenase) in proportion to their invasive and metastatic abilities. These tumor cells have also been shown to adhere rapidly to pure cultures of hepatic sinusoidal endothelial cells *in vitro*. The present study was und abilities. These tumor cells have also been shown to adhere<br>rapidly to pure cultures of hepatic sinusoidal endothelial cells<br>*in vitro*. The present study was undertaken to compare the cell<br>surface proteins and glycoprotei rapidly to pure cultures of hepatic sinusoida<br>in vitro. The present study was undertaken to<br>surface proteins and glycoproteins of these re<br>cell lines by a variety of biochemical means.<br>surface sialylation, cell membrane si cell lines by a variety of biochemical mean<br>surface sialylation, cell membrane sialop<br>sialyltransferase levels are also compared.<br>MATERIALS AND METHODS<br>Regents Lactoneroxidase clucose oxidase surface sialylation, cell membrane sialoprotein profiles, and<br>sialyltransferase levels are also compared.<br>MATERIALS AND METHODS<br>Reagents. Lactoperoxidase, glucose oxidase, galactose oxidase, N-<br>acetylneuraminic acid (siali

MATERIALS AND METHODS<br>Reagents. Lactoperoxidase, glucose oxidase, galactose oxidase, N<br>acetylneuraminic acid (sialic acid), N-acetyl glucosamine, neuramini-<br>dase type X from *Clostridium perfringens*, and NP40<sup>3</sup> were purc MATERIALS AND METHODS<br>Reagents. Lactoperoxidase, glucose oxidase, galactose oxidase, N-<br>acetylneuraminic acid (sialic acid), N-acetyl glucosamine, neuramini-<br>dase type X from *Clostridium perfringens*, and NP40<sup>3</sup> were pur MATERIALS AND METHODS<br>Reagents. Lactoperoxidase, glucose oxidase, galactose oxidase, N-<br>acetylneuraminic acid (sialic acid), N-acetyl glucosamine, neuramini-<br>dase type X from *Clostridium perfringens*, and NP40<sup>3</sup> were pur Reagents. Lactoperoxidase, glucose oxidase, galactose oxidase, N-<br>acetylneuraminic acid (sialic acid), N-acetyl glucosamine, neuramini-<br>dase type X from *Clostridium perfringens*, and NP40<sup>3</sup> were purchased<br>from Sigma Chem acetylneuraminic acid (sialic acid), *N*-acetyl glucosamine, neuramini-<br>dase type X from *Clostridium perfringens*, and NP40<sup>3</sup> were purchased<br>from Sigma Chemical Co. (St. Louis, MO); sodium dodecyl sulfate,<br>acrylamide, an dase type X from *Clostridium perfringens*, and NP40<sup>3</sup> were purchased<br>from Sigma Chemical Co. (St. Louis, MO); sodium dodecyl sulfate,<br>acrylamide, and bisacrylamide from U.S. Biochemicals (Cleveland,<br>OH); albumin from Cal from Sigma Chemical Co. (St. Louis, MO); sodium dodecyl sulfate,<br>acrylamide, and bisacrylamide from U.S. Biochemicals (Cleveland,<br>OH); albumin from Calbiochem (La Jolla, CA); sodium [<sup>3</sup>H]borohy-<br>dride (52 Ci/mmol) and car 1); albumin from Calbiochem (La Jolla, CA); sodium ['H]borohy-<br>de (52 Ci/mmol) and carrier-free Na<sup>125</sup>I from ICN (Irvine, CA);<br>tofluor from National Diagnostics (Manville, NJ); biotinylated<br>GA from EY Laboratories (San M

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<sup>2</sup> Supported by the Research Service of the Veterans Administ <sup>1</sup> Supported by the Research Service of the Veterans Administrative VA Research Associate (R. S. B.), Merit Review (R. S. B., Y. S. K.), a<br>Investigator (Y. S. K.) awards, and National Cancer Institute Grant C<br>S. K.).<br><sup>2</sup>

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accordance with 18 dride (52 Ci/mmol) and carrier-free Na<sup>125</sup>I from ICN (Irvine, CA);<br>Autofluor from National Diagnostics (Manville, NJ); biotinylated<br>WGA from EY Laboratories (San Mateo, CA); streptavidin-peroxidase<br><sup>3</sup>The abbreviations us WGA from EY Laboratories (San Mateo, CA); streptavidin-peroxidase<br>
<sup>3</sup> The abbreviations used are: NP40, Nonidet P-40; NeuAc, N-acetylneuraminic<br>
acid; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosa <sup>3</sup> The abbreviations used are: NP40, Nonidet P-40, NeuAc, N-acetylneuraminic<br>acid; Gal, galactose; GalNAc, N-acetylgalactosamine; GIcNAc, N-acetylglucosa-<br>mine; HBSS, Hanks' buffered saline solution; IdUR, iododeoxyuridin <sup>3</sup> The abbreviations used are: NP40, Nonidet P-40; NeuAc, N-acetylneuraminic acid; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosa-mine; HBSS, Hanks' buffered saline solution; 1dUR, iododeoxyuridin PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; SNA, Sambucus nigra lectin; WGA, wheat germ agglutinin.

ANIMAL MODEL FOR COLON CANCER METASTASIS<br>Conjugate from Zymed Laboratories (South San Francisco, CA); aga- ml of 0.1 M solution in PBS) was added for 10 min. The cells were rose-bound WGA and SNA from Vector Laboratories (Burlingame, ANIMAL MODEL FOR COLO<br>conjugate from Zymed Laboratories (South San Francisco, CA); aga-<br>rose-bound WGA and SNA from Vector Laboratories (Burlingame,<br>CA); and UDP-[U-<sup>3</sup>H]galactose (specific activity, 289 mCi/mmol) and ANIMAL MODEL FOR COLON CA<br>conjugate from Zymed Laboratories (South San Francisco, CA); aga- ml of<br>rose-bound WGA and SNA from Vector Laboratories (Burlingame, wash<br>CA); and UDP-[U-<sup>3</sup>H]galactose (specific activity, 289 mCi ANIMAL MODEL FOR COLON CA<br>
conjugate from Zymed Laboratories (South San Francisco, CA); aga- ml o<br>
rose-bound WGA and SNA from Vector Laboratories (Burlingame, wash<br>
CA); and UDP-[U-<sup>3</sup>H]galactose (specific activity, 289 m conjugate from Zymed Laboratories (South San Francisco, CA); aga-<br>rose-bound WGA and SNA from Vector Laboratories (Burlingame, wash<br>CA); and UDP-[U-<sup>3</sup>H]galactose (specific activity, 289 mCi/mmol) and tritia<br>CMP-[4,5,6,7,8 conjugate from Zymed Laboratories (South San Francisco, CA); aga-<br>rose-bound WGA and SNA from Vector Laboratories (Burlingame, wash<br>CA); and UDP-[U-<sup>3</sup>H]galactose (specific activity, 289 mCi/mmol) and tritia<br>CMP-[4,5,6,7,8 rose-bound WGA and SNA from Vector Laboratories (Burlingame, wash CA); and UDP-[U-<sup>3</sup>H]galactose (specific activity, 289 mCi/mmol) and tritia CMP-[4,5,6,7,8,9-<sup>14</sup>C]sialic acid (173 mCi/mmol) from New England were Nuclear  $CMP$ -[4,5,6,7,8,9-<sup>14</sup>C]sialic acid (173 mCi/mmol) from New England were washed with PBS-PMSF and membrane-enriched fractions were Nuclear (Boston, MA). OSM was purified from ovine submaxillary prepared for SDS-PAGE as ab Nuclear (Boston, MA). OSM was purified from ovine submaxillary preparal proparation of Sigman (38), and transferring SD was obtained from Sigma. Sialic acid was removed from OSM and gelel transferrin by heating with  $0.1 \$ used as acceptors for the sialyltransferase assays.<br>Cell Lines. Parental murine colon cancer cell line 51B and its s obtained from Sigma. Sialic acid was removed from OSM and gel<br>
insferrin by heating with  $0.1 \text{ N}$  HCl at  $80^{\circ}$ C for  $90$  min. The samples (43)<br>
re neutralized with  $0.5 \text{ N}$  NaOH and dialyzed against 5 to 6 volume transferrin by heating with  $0.1 \text{ N HCl}$  at  $80^{\circ}\text{C}$  for  $90 \text{ min}$ . The samples (43)<br>were neutralized with  $0.5 \text{ N NaOH}$  and dialyzed against 5 to 6 volumes run<br>of distilled water for 48 h. The dialyzed samples were ly

metastatic derivatives 51B LiM 5 and 51B LiM 6 were established as<br>previously described (37). 51B consists of a heterogeneous population<br>of cells and forms poorly to moderately differentiated carcinomas with<br>limited metast limited metastatic ability when grown in the cecal line 51B and its<br>metastatic derivatives 51B LiM 5 and 51B LiM 6 were established as<br>Kod<br>previously described (37). 51B consists of a heterogeneous population<br>of cells and Cell Lines. Parental murine colon cancer cell line 51B and its<br>metastatic derivatives 51B LiM 5 and 51B LiM 6 were established as<br>previously described (37). 51B consists of a heterogeneous population<br>of cells and forms poo metastatic derivatives 51B LiM 5 and 51B LiM 6 were established as Koda<br>previously described (37). 51B consists of a heterogeneous population dried<br>of cells and forms poorly to moderately differentiated carcinomas with<br>lim previously described (37). 51B consists of a heterogeneous population director of cells and forms poorly to moderately differentiated carcinomas with Lelimited metastatic ability when grown in the cecal walls of syngeneic of cells and forms poorly to moderately differentiated carcinomas with<br>limited metastatic ability when grown in the cecal walls of syngeneic<br>BALB/c mice. 51B LiM 5 and 51B LiM 6, cell lines with high liver-<br>incentastasizin limited metastatic ability when grown in the cecal walls of syngeneic by la BALB/c mice. 51B LiM 5 and 51B LiM 6, cell lines with high liver-<br>metastasizing ability during cecal growth, were established by serially sonisele BALB/c mice. 51B LiM 5 and 51B LiM 6, cell lines with high liver-<br>metastasizing ability during cecal growth, were established by serially sonic<br>selecting cells which metastasized from cecum to liver in the previously for : selecting cells which metastasized from cecum to liver in the previously<br>described model. These cell lines metastasize to the liver in 100% of<br>animals after cecal injection and have enhanced liver-metastasizing<br>potential described model. These cell lines metastasize to the liver in 100% of super<br>animals after cecal injection and have enhanced liver-metastasizing Labor<br>potential compared to parental 51B when injected s.c. or i.v. (37). Cel animals after cecal injection and have enhanced liver-metastasizing Lipotential compared to parental 51B when injected s.c. or i.v. (37). Cell Wines were grown and maintained in Dulbecco's modified Eagle's min-<br>imal essen potential compared to paren<br>lines were grown and maints<br>imal essential medium supp<br>penicillin (100 units/ml), an<br>environment. Early passage<br>were used for all studies.<br>Histological Examination es were grown and maintained in Duibecco's modified Eagle's min-<br>al essential medium supplemented with 10% fetal bovine serum, acety<br>nicillin (100 units/ml), and streptomycin (100  $\mu$ g/ml), in a 7% CO<sub>2</sub> in 1<br>vironment. imal essential medium supplemented with 10% fetal bovine serum, acety<br>penicillin (100 units/ml), and streptomycin (100  $\mu$ g/ml), in a 7% CO<sub>2</sub> in F<br>environment. Early passage cell lines (passaged fewer than 15 times) thy

environment. Early passage cell lines (passaged fewer than 15 times)<br>were used for all studies.<br>Histological Examination and Histochemistry. Specimens for histo-<br>logical examination were fixed in 10% formalin for 24 h and environment. Early passage cell lines (passaged fewer than 15 times) thylam<br>were used for all studies. dialy:<br>Histological Examination and Histochemistry. Specimens for histo-<br>tion,<br>logical examination were fixed in 10% fo were used for all studies. dialy<br>
Histological Examination and Histochemistry. Specimens for histo-<br>
logical examination were fixed in 10% formalin for 24 h and embedded Si<br>
in paraffin. Serial 5- $\mu$ m sections were then Histological Examination and Histochemistry. Specimens for histo-<br>logical examination were fixed in 10% formalin for 24 h and embedded Si<br>in paraffin. Serial 5- $\mu$ m sections were then cut and stained with hema-<br>sociatory logical examination were fixed in 10% formalin for 24 h and embedded<br>in paraffin. Serial 5- $\mu$ m sections were then cut and stained with hema-<br>toxylin and eosin or processed for histochemistry. WGA binding in fract<br>fixed in paraffin. Serial 5- $\mu$ m sections were then cut and stained with hema-<br>toxylin and eosin or processed for histochemistry. WGA binding in fracti<br>fixed tissues was determined using the streptavidin-peroxidase tech-<br>nique toxylin and eosin or processed for histochemistry. WGA binding in fractive dissues was determined using the streptavidin-peroxidase technique, as previously described (39). Biotinylated WGA was used at a  $\mu$ l a working c tions. que, as previously described (39). Biotinylated WGA was used at <br>rking concentration of  $2 \mu g/ml$ . The lectin specificity was confirme<br>incubating 50  $\mu$ l of biotinylated lectin with 50  $\mu$ l of 0.5 M hapter<br>gar (N-acetylgl working concentration of 2  $\mu$ g/ml. The lectin specificity was confirmed was<br>by incubating 50  $\mu$ l of biotinylated lectin with 50  $\mu$ l of 0.5 M hapten (100<br>sugar (N-acetylglucosamine) before application to duplicate ti

by incubating 50  $\mu$ I of biotinylated lectin with 50  $\mu$ I of 0.5 M hapten (100 sugar (*N*-acetylglucosamine) before application to duplicate tissue sec-<br>tions.<br>**Labeling of Cell Surface Proteins and Glycoconjugates.** La tions.<br> **Labeling of Cell Surface Proteins and Glycoconjugates.** Lactoperoxidase-catalyzed iodination of cell surface proteins was performed by a modification of a previously described method (40). Cells were grown to conf **Labeling of Cell Surface Proteins and Glycoconjugates.** Lactoperoxi-<br>dase-catalyzed iodination of cell surface proteins was performed by a<br>modification of a previously described method (40). Cells were grown lines<br>to con modification of a previously described method (40). Cells were grown linear portion of the curve.<br>to confluency in 75-cm<sup>2</sup> tissue culture flasks and washed 4 times with Glycosyltransferase Assays. Cultured cells (75% con to confluency in 75-cm<sup>2</sup> tissue culture flasks and washed 4 times with<br>
PBS (pH 7.4) and then once with 3 mm glucose in PBS. The tyrosine-<br>
containing residues of cell surface proteins were labeled *in situ* by the 8 ml containing residues of cell surface proteins were labeled *in situ* by the 8 n<br>addition of 2 ml of 3 mm glucose in PBS, 10  $\mu$ l (5 units) of lactoper-<br>pelloxidase, and 250  $\mu$ Ci of carrier-free Na<sup>125</sup>I. The reaction wa addition of 2 ml of 3 mm glucose in PBS, 10  $\mu$ l (5 units) of lactoper-<br>oxidase, and 250  $\mu$ Ci of carrier-free Na<sup>125</sup>I. The reaction was initiated w<br>by the addition of 10  $\mu$ l (15 units) of glucose oxidase and continu oxidase, and 250  $\mu$ Ci of carrier-free Na<sup>125</sup>I. The reaction was initiated w<br>by the addition of 10  $\mu$ l (15 units) of glucose oxidase and continued for<br>30 min at 25°C. Cells were washed 4 times in PBS containing 1 mM [ by the addition of 10  $\mu$ l (15 units) of glucose oxidase and continued for 50 min at 25°C. Cells were washed 4 times in PBS containing 1 mM [(G. NaI and 5 mM PMSF and scraped into 4 ml PBS-PMSF. Labeled cells OSs were th 30 min at 25°C. Cells were washed 4 times in PBS containing 1 mM [(Gal Nal and 5 mM PMSF and scraped into 4 ml PBS-PMSF. Labeled cells OSer were then centrifuged at 600  $\times$  g for 10 min, resuspended in 500  $\mu$ l (pH PBS-Nal and 5 mm PMSF and scraped into 4 ml PBS-PMSF. Labeled covere then centrifuged at  $600 \times g$  for 10 min, resuspended in 500 PBS-PMSF, sonicated twice for 20 s, and then ultracentrifuged  $100,000 \times g$  for 1 h. The membrane

PBS-PMSF, sonicated twice for 20 s, and then ultracentrituged at  $1.75-100,000 \times g$  for 1 h. The membrane-enriched pellet thus obtained was cellul resuspended in 500  $\mu$  PBS-PMSF, and aliquots were taken for protein (45). resuspended in 500  $\mu$ l PBS-PMSF, and aliquots were taken for protein (45).<br>determination (41) and SDS-polyacrylamide gel electrophoresis. conce<br>Labeling of cell surface proteins with galactose oxidase and tritiated (unl determination (41) and SDS-polyacrylamide gel electrophoresis. concellable concellated with galactose oxidase and tritiated (unlasodium borohydride was performed as previously described (40), with acid modifications. Cell sodium borohydride was performed as previously described (40), with acid modifications. Cells cultured in 75-cm<sup>2</sup> tissue culture flasks were labeled min, *in situ* after being washed 4 times with isotonic PBS (pH 7.4). W in situ after being washed 4 times with isotonic PBS (pH 7.4). Washed<br>cells were treated with 25 units  $(45 \mu g)$  of galactose oxidase in 2.5 ml<br>Hanks' balanced salt solution (pH 7.0) for 60 min at 37°C. Cells were<br>then wa cells were treated with 25 units  $(45 \mu g)$  of galactose oxidase in 2.5 ml<br>Hanks' balanced salt solution (pH 7.0) for 60 min at 37°C. Cells were<br>then washed with PBS (pH 8.3) and incubated with 2.5 mCi of tritiated<br>sodium Hanks' balanced salt solution (pH 7.0) for 60 min at 37°C. Cells were<br>then washed with PBS (pH 8.3) and incubated with 2.5 mCi of tritiated<br>sodium borohydride for 30 min at 25°C in 2 ml HBSS (pH 8.0). The<br>labeled cells wer labeling. beled cells were then washed 4 times in PBS-PMSF and membrane-<br>
riched fractions were prepared for SDS-PAGE as above. Parallel after<br>
sks were incubated with 0.2 units of neuraminidase type X (C. acce<br> *celf stringens*) in

flasks were incubated with 0.2 units of neuraminidase type X (C. accep<br>perfringens) in 2.5 ml HBSS (pH 6.0), for 30 min at 37°C, prior to Ga<br>labeling.<br>mine-<br>cell surface sialoproteins were labeled according to a modificat

washed with PBS (pH 8.0) and then incubated for 30 min with 2.5 mCi N CANCER METASTASIS<br>ml of 0.1 M solution in PBS) was added for 10 min. The cells were<br>washed with PBS (pH 8.0) and then incubated for 30 min with 2.5 mCi<br>tritiated sodium borohydride in 2 ml HBSS (pH 8.0). Labeled cells N CANCER METASTASIS<br>ml of 0.1 M solution in PBS) was added for 10 min. The cells were<br>washed with PBS (pH 8.0) and then incubated for 30 min with 2.5 mCi<br>tritiated sodium borohydride in 2 ml HBSS (pH 8.0). Labeled cells<br>we N CANCER METASTASIS<br>ml of 0.1 M solution in PBS) was added for 10 min. The cells were<br>washed with PBS (pH 8.0) and then incubated for 30 min with 2.5 mCi<br>tritiated sodium borohydride in 2 ml HBSS (pH 8.0). Labeled cells<br>we ml of 0.1 M solution in PBS) was add<br>washed with PBS (pH 8.0) and then inc<br>tritiated sodium borohydride in 2 ml<br>were washed with PBS-PMSF and mei<br>prepared for SDS-PAGE as above.<br>SDS-Polyacrylamide Gel Electropho of 0.1 M solution in PBS) was added for 10 min. The cells were<br>shed with PBS (pH 8.0) and then incubated for 30 min with 2.5 mCi<br>tiated sodium borohydride in 2 ml HBSS (pH 8.0). Labeled cells<br>re washed with PBS-PMSF and me washed with PBS (pH 8.0) and then incubated for 30 min with 2.5 mCi<br>tritiated sodium borohydride in 2 ml HBSS (pH 8.0). Labeled cells<br>were washed with PBS-PMSF and membrane-enriched fractions were<br>prepared for SDS-PAGE as

of distilled water for 48 h. The dialyzed samples were lyophilized and<br>
used and stained vith Coomassie brilliant blue,<br>
Cell Lines. Parental murine colon cancer cell line 51B and its<br>
Cell Lines. Parental murine colon can tritiated sodium borohydride in 2 ml HBSS (pH 8.0). Labeled cells<br>were washed with PBS-PMSF and membrane-enriched fractions were<br>prepared for SDS-PAGE as above.<br>SDS-Polyacrylamide Gel Electrophoresis. SDS-polyacrylamide sl were washed with PBS-PMSF and membrane-enriched fractions were<br>prepared for SDS-PAGE as above.<br>SDS-Polyacrylamide Gel Electrophoresis. SDS-polyacrylamide slab<br>gel electrophoresis was performed according to the method of La SDS-Polyacrylamide Gel Electrophoresis. SDS-polyacrylamide slab<br>gel electrophoresis was performed according to the method of Laemmli<br>(43), using a discontinuous buffer system and 3% stacking and 7%<br>running gels. Equal amou gel electrophoresis was performed according to the method of Laemmli<br>(43), using a discontinuous buffer system and 3% stacking and 7%<br>running gels. Equal amounts of protein or equal amounts of radioactiv-<br>ity were applied running gels. Equal amounts of protein or equal<br>ity were applied to parallel lanes for each cell lin<br>labeled proteins were fixed and stained with Co<br>soaked in Autofluor (National Diagnostics), dri<br>Kodak X-O-Mat AR film. Ge were applied to parallel lanes for each cell line. Gels containing <sup>3</sup>H-<br>eled proteins were fixed and stained with Coomassie brilliant blue,<br>aked in Autofluor (National Diagnostics), dried, and used to expose<br>dak X-O-Mat A labeled proteins were fixed and stained with Coomassie brilliant blue,<br>soaked in Autofluor (National Diagnostics), dried, and used to expose<br>Kodak X-O-Mat AR film. Gels containing <sup>125</sup>I-labeled proteins were<br>dried and X-r

sonicating briefly in PBS-PMSF containing 1% NP40 and extracting Kodak X-O-Mat AR film. Gels containing <sup>125</sup>I-labeled proteins were<br>dried and X-ray film was similarly exposed.<br>Lectin Affinity Chromatography. Cell surface proteins were labeled<br>by lactoperoxidase-catalyzed iodination, a Lectin Affinity Chromatography. Cell surface proteins were labeled<br>by lactoperoxidase-catalyzed iodination, and membrane-enriched frac-<br>tions were prepared as described above. Proteins were solubilized by<br>sonicating brief by lactoperoxidase-catalyzed iodination, and membrane-enriched fractions were prepared as described above. Proteins were solubilized by sonicating briefly in PBS-PMSF containing 1% NP40 and extracting for 2 h at 4°C. Afte tions were prepared as described above. Proteins were solubilized by<br>sonicating briefly in PBS-PMSF containing 1% NP40 and extracting<br>for 2 h at 4°C. After centrifugation at 100,000  $\times$  g for 1 h, the<br>supernatant was app sonicating briefly in PBS-PMSF containing  $1\%$  NP40 and extracting<br>for 2 h at 4°C. After centrifugation at  $100,000 \times g$  for 1 h, the<br>supernatant was applied to a column of agarose-bound WGA (Vector<br>Laboratories), succiny for 2 h at 4°C. After centrifugation at 100,000  $\times$  g for 1 h, the<br>supernatant was applied to a column of agarose-bound WGA (Vector<br>Laboratories), succinylated WGA or SNA. The WGA and succinylated<br>WGA columns were washed supernatant was applied to a column of agarose-bound WGA (Vect<br>Laboratories), succinylated WGA or SNA. The WGA and succinylate<br>WGA columns were washed with 3 volumes of PBS (pH 7.1) containin<br>0.1% NP40 and eluted in a step Laboratories), succinylated WGA or SNA. The WGA and succinylated<br>WGA columns were washed with 3 volumes of PBS (pH 7.1) containing<br>0.1% NP40 and eluted in a stepwise fashion with 0.2 and 0.5  $\text{M}$  N-<br>acetylglucosamine. WGA columns were washed with 3 volumes of PBS (pH 7.1) containing 0.1% NP40 and eluted in a stepwise fashion with 0.2 and 0.5 M Nacetylglucosamine. The SNA column was eluted with 0.5 M lactose in PBS (pH 7.1) containing  $0.1\%$  NP40 and eluted in a stepwise facetylglucosamine. The SNA column v<br>in PBS (pH 7.1) containing  $0.1\%$ <br>thylammonio]-1-propane sulfonate. Mat<br>dialyzed against 4 liters of distilled wat<br>tion, and submitted for SDS-PA etylglucosamine. The SNA column was eluted with 0.5 M lactose<br>PBS (pH 7.1) containing 0.1% 3-[(3-cholamidopropyl)-dime-<br>plammonio]-1-propane sulfonate. Material from the eluted peaks was<br>lyzed against 4 liters of distilled in PBS (pH 7.1) containing  $0.1\%$  3-[(3-cholamidopropyl)-dime-<br>thylammonio]-1-propane sulfonate. Material from the eluted peaks was<br>dialyzed against 4 liters of distilled water, concentrated by lyophiliza-<br>tion, and subm

thylammonio]-1-propane sulfonate. Material from the eluted peaks was<br>dialyzed against 4 liters of distilled water, concentrated by lyophiliza-<br>tion, and submitted for SDS-PAGE.<br>Sialic Acid Determination. Neuraminidase-rel tion, and submitted for SDS-PAGE.<br>
Sialic Acid Determination. Neuraminidase-releasable membrane-as-<br>
sociated sialic acid was determined as follows. Cell membrane-enriched<br>
fractions were prepared from approximately 10<sup>7</sup> tion, and submitted for SDS-PAGE.<br>
Sialic Acid Determination. Neuraminidase-releasable membrane-as-<br>
sociated sialic acid was determined as follows. Cell membrane-enriched<br>
fractions were prepared from approximately 10<sup>7</sup> sociated sialic acid was determined as follows. Cell membrane-enriched<br>fractions were prepared from approximately 10<sup>7</sup> cells, as described<br>above, and resuspended by sonicating in 500  $\mu$ l HBSS, pH 6.0. A 50-<br> $\mu$ l aliqu fractions were prepared from approximately 10' cells, as described<br>above, and resuspended by sonicating in 500  $\mu$ l HBSS, pH 6.0. A 50-<br> $\mu$ l aliquot was removed for protein determination, and the remainder<br>was incubated above, and resuspended by sonicating in 500  $\mu$ l HBSS, pH 6.0. A 50-<br> $\mu$ l aliquot was removed for protein determination, and the remainder<br>was incubated at 37°C for 1 h, in a shaking water bath, with 0.2 units<br>(100  $\mu$  $\mu$ l aliquot was removed for protein determination, and the remainder<br>was incubated at 37°C for 1 h, in a shaking water bath, with 0.2 units<br>(100  $\mu$ l) affinity-purified neuraminidase type X from *C. perfringens*.<br>The m was incubated at 37°C for 1 h, in a shaking water bath, with 0.2 units (100  $\mu$ ) affinity-purified neuraminidase type X from *C. perfringens*.<br>The mixture was centrifuged at 100,000 × *g* for 1 h and the supernatant was (100  $\mu$ ) attinity-puritied neural<br>The mixture was centrifuged at<br>was assayed for free sialic ac<br>Standard curves were construction of sialic acid,<br>linear portion of the curve.<br>Clycosyltransferase Assays. is emixture was centrifuged at  $100,000 \times g$  for 1 h and the supernatant s assayed for free sialic acid by the thiobarbituric method (44), and ard curves were constructed for each experiment with known ncentrations of sial was assayed for free sialic acid by the thiobarbituric method (44).<br>Standard curves were constructed for each experiment with known<br>concentrations of sialic acid, and determinations were made in the<br>linear portion of the c

Standard curves were constructed for each experiment with known<br>concentrations of sialic acid, and determinations were made in the<br>linear portion of the curve.<br>Glycosyltransferase Assays. Cultured cells (75% confluent mono concentrations of sialic acid, and determinations were made in the<br>linear portion of the curve.<br>Glycosyltransferase Assays. Cultured cells (75% confluent mono-<br>layers) were washed 3 times with PBS and harvested by scrapin Glycosyltransferase Assays. Cultured cells (75% confluent mono-<br>ers) were washed 3 times with PBS and harvested by scraping into<br>ml of PBS with a cell scraper. Cells were centrifuged, and the cell<br>llet was suspended and s layers) were washed 3 times with PBS and harvested by scraping in 8 ml of PBS with a cell scraper. Cells were centrifuged, and the coellet was suspended and sonicated in 1 ml of PBS (pH 7.1). Aliquos were used directly fo

were then centrifuged at 600 × g for 10 min, resuspended in 500  $\mu$ l (pH 6.8), 20 mM MnCl<sub>2</sub>, 200  $\mu$ M ATP, 400  $\mu$ g of appropriate acceptor,<br>PBS-PMSF, sonicated twice for 20 s, and then ultracentrifuged at 1.75–2.0  $\$ 8 ml of PBS with a cell scraper. Cells were centrifuged, and the cell<br>pellet was suspended and sonicated in 1 ml of PBS (pH 7.1). Aliquots<br>were used directly for glycosyltransferase assays or frozen at  $-70^{\circ}$ C.<br>Sialylt pellet was suspended and sonicated in 1 ml of PBS (pH 7.1). Aliquots<br>were used directly for glycosyltransferase assays or frozen at  $-70$ °C.<br>Sialyltransferase activities were determined using asialotransferrin<br>[(Gal $\beta$ 1were used directly for glycosyltransferase assays or frozen at  $-70^{\circ}$ C.<br>
Sialyltransferase activities were determined using asialotransferrin<br>
(Gal $\beta$ 1-4GlcNAc<sub>2</sub>)Man<sub>3</sub>GalNAc<sub>2</sub>] and asialo-OSM (GalNAc $\alpha$ 1-<br>
OSer/Th Sialyltransferase activities were determined using asialotransferrin<br>[(Gal $\beta$ 1-4GlcNAc<sub>2</sub>}Man<sub>3</sub>GalNAc<sub>2</sub>] and asialo-OSM (GalNAc $\alpha$ 1-<br>OSer/Thr) as acceptors. Assay mixtures consisted of cacodylate buffer<br>(pH 6.8), 20 m [(Gal $\beta$ 1-4GlcNAc<sub>2</sub>]Man<sub>3</sub>GalNAc<sub>2</sub>] and asialo-OSM (GalNAc $\alpha$ 1-<br>OSer/Thr) as acceptors. Assay mixtures consisted of cacodylate buffer<br>(pH 6.8), 20 mM MnCl<sub>2</sub>, 200  $\mu$ M ATP, 400  $\mu$ g of appropriate acceptor,<br>1.75-2.0 OSer/Thr) as acceptors. Assay mixtures consisted of cacodylate buffer (pH 6.8), 20 mM MnCl<sub>2</sub>, 200  $\mu$ M ATP, 400  $\mu$ g of appropriate acceptor, 1.75–2.0  $\mu$ M (70,000–80,000 cpm) CMP-sialic acid, 50–100  $\mu$ g of cellular (pH 6.8), 20 mM MnCl<sub>2</sub>, 200  $\mu$ M ATP, 400  $\mu$ g of appropriate acceptor, 1.75–2.0  $\mu$ M (70,000–80,000 cpm) CMP-sialic acid, 50–100  $\mu$ g of cellular protein, and 0.1% Triton X-100, in a total volume of 100  $\mu$ l (45). (45). All assays were performed within the linear range of both enzyme concentration and incubation time. The amount of CMP-sialic acid (unlabeled and labeled) used yielded optimum transfer of labeled sialic acid to the a (45). All assays were performed within the linear range of both enzyme concentration and incubation time. The amount of CMP-sialic acid (unlabeled and labeled) used yielded optimum transfer of labeled sialic acid to the a concentration and incubation time. The amount of CMP-sialic acid (unlabeled and labeled) used yielded optimum transfer of labeled sialic acid to the acceptors. Reaction mixtures were incubated at 37°C for 90 min, and the (unlabeled and labeled) used yielded optimum transfer of labeled sialic<br>acid to the acceptors. Reaction mixtures were incubated at  $37^{\circ}$ C for 90<br>min, and the reaction was terminated by the addition of 2 ml ice-cold<br>1% acid to the acceptors. Reaction mixtures were incubated at 37°C for 90 min, and the reaction was terminated by the addition of 2 ml ice-cold 1% phosphotungstic acid in 0.5 m HCl. Precipitates were washed 3 times with cold min, and the reaction was terminated by the addition of 2 ml ice-cold 1% phosphotungstic acid in 0.5 m HCl. Precipitates were washed 3 times with cold phosphotungstic acid and dissolved in 500  $\mu$ l of NCS tissue solubili 1% phosphotungstic acid in 0.5 M HCl. Precipitates were washed 3 times with cold phosphotungstic acid and dissolved in 500  $\mu$ l of NCS tissue solubilizer (Amersham/Searle, Arlington Heights, IL). The solubilized precipit times with cold phosphotungstic acid and dissolved in 500  $\mu$ l of NCS<br>tissue solubilizer (Amersham/Searle, Arlington Heights, IL). The sol-<br>ubilized precipitates were mixed with Omnifluor (New England Nu-<br>clear) toluene ubilized precipitates were mixed with Omnifluor (New England Nuclear) toluene cocktail and counted in a liquid scintillation counter. Net radioactivity transferred to exogenously added acceptor was determined after subtra

enriched fractions were prepared for SDS-PAGE as above. Parallel after subtracting the amount of radioactivity transferred to endogenous<br>flasks were incubated with 0.2 units of neuraminidase type X (C. acceptors.<br>perfring clear) toluene cocktail and counted in a liquid scintillation counter. Net<br>radioactivity transferred to exogenously added acceptor was determined<br>after subtracting the amount of radioactivity transferred to endogenous<br>acc after subtracting the amount of radioactivity transferred to endogenous<br>acceptors.<br>Galactosyltransferase activity ( $\beta$ 1–4-galactosyltransferase) was deter-<br>mined using a reaction mixture consisting of 0.1 M PBS (pH 7.1), Galactosyltransterase activity ( $\beta$ 1-4-galactosyltransterase) was determined using a reaction mixture consisting of 0.1 M PBS (pH 7.1), 20 mM MnCl<sub>2</sub>, 200  $\mu$ M ATP, 10 mM GlcNAc, 17-20 nM UDP-[<sup>3</sup>H]-galactose (50,000-60

ANIMAL MODEL FOR COLON CANCER METASTASIS<br>was then applied to a 0.5 × 2 cm Dowex 1 × 8 formate (100–200 mesh) galactose oxidase-sod ANIMAL MODEL FOR COLOI<br>was then applied to a  $0.5 \times 2$  cm Dowex  $1 \times 8$  formate (100–200 mesh) g:<br>column (Bio-Rad, Richmond, CA), and the products were separated by **Example ANIMAL MODEL FOR COLON C**<br>
was then applied to a  $0.5 \times 2$  cm Dowex  $1 \times 8$  formate (100–200 mesh) galae<br>
column (Bio-Rad, Richmond, CA), and the products were separated by N-ac<br>
washing each column with 2 ml dis ANIMAL MODEL FOR COLON C<br>was then applied to a  $0.5 \times 2$  cm Dowex  $1 \times 8$  formate (100–200 mesh) gala<br>column (Bio-Rad, Richmond, CA), and the products were separated by N-ac<br>washing each column with 2 ml distilled water ( ANIMAL MODEL FOR COLON (ANIMAL MODEL FOR COLON ON ANIMAL MODEL FOR COLON (SAMPLE FOR COLON ACCLON ACCLON ACCLON AND COLON THE COLON MUSLEM Washing each column with 2 ml distilled water (46). An aliquot of each or a cluate was then applied to a  $0.5 \times 2$  cm Dowex  $1 \times 8$  formate (100–200 mesh) ga<br>column (Bio-Rad, Richmond, CA), and the products were separated by<br>washing each column with 2 ml distilled water (46). An aliquot of each<br>eluate w was then applied to a  $0.5 \times 2$  cm Dowex  $1 \times 8$  formate (100–200 mesn) galacolumn (Bio-Rad, Richmond, CA), and the products were separated by  $N$ -a washing each column with 2 ml distilled water (46). An aliquot of each o column (Bio-Rad, Richmond, CA), and the products were separated by<br>washing each column with 2 ml distilled water (46). An aliquot of each<br>eluate was mixed with ACS II scintillation fluid (Amersham, Arlington<br>of the eluate eluate was mixed with ACS II scintillation fluid (Amersham, Arlington<br>Heights, IL) and counted in a liquid scintillation counter. Values<br>obtained without added acceptor were taken as radioactivity transferred<br>to endogenous obtained without added acceptor were taken as radioactivity transferred

with PBS, and incubated in situ for 30 min with 0.2 units neuraminidase type X and C. *perfringens* in 2 ml PBS (pH 7.0). These conditions were transferred to the exogenously added acceptor.<br>
Liver Colonization after Neuraminidase Treatment. Tumor cells were<br>
grown to confluency in 75-cm<sup>2</sup> tissue culture flasks, washed 3 times<br>
with PBS, and incubated in situ fo transferred to the exogenously added acceptor.<br>
Liver Colonization after Neuraminidase Treatment. Tumor cells were<br>
grown to confluency in 75-cm<sup>2</sup> tissue culture flasks, washed 3 times<br>
with PBS, and incubated *in situ* Liver Colonization arter Neuraminidase Treatment. Tumor cells were<br>grown to confluency in 75-cm<sup>2</sup> tissue culture flasks, washed 3 times<br>with PBS, and incubated in situ for 30 min with 0.2 units neuraminidase<br>type X and C grown to confluency in 75-cm<sup>-</sup> tissue culture flasks, washed 3 times<br>with PBS, and incubated in situ for 30 min with 0.2 units neuraminidase<br>type X and C. perfringens in 2 ml PBS (pH 7.0). These conditions were<br>found to r WHIT PISS, and included *in stid* for 50 film white 0.2 dims neuralinimates speed<br>type X and *C. perfringens* in 2 ml PBS (pH 7.0). These conditions were<br>found to remove greater than 95% of cell membrane-associated sialic by pe  $\lambda$  and c. perfingens in 2 mi FBS (pH 7.0). These conditions were costated followed to remove greater than 95% of cell membrane-associated sialic <sup>125</sup>I-<br>acid while maintaining cell viability. Control cells were tr Example manufanting cent via only. Control cents were treated identi-<br>
really except that neuraminidase was omitted during incubation. Greater<br>
then 90% of cells remained viable during the procedure, as determined<br>
by tryp Easy except that heurannimidase was omitted during included in. Oreater<br>than 90% of cells remained viable during the procedure, as determined<br>by trypan blue exclusion. Cells were then washed 5 times with PBS,<br>harvested, r than 90% of cells remained viable during the procedure, as determined<br>by trypan blue exclusion. Cells were then washed 5 times with PBS,<br>harvested, resuspended in serum-free Dulbecco's modified Eagle's min-<br>of 1<br>a modific by trypan blue exclusion. Cens were then washed 3 times with FBS,<br>harvested, resuspended in serum-free Dulbecco's modified Eagle's min-<br>imal essential medium at a concentration of 10' cells/ml, and used in<br>anodification of mal essential medium at a concentration of 10<sup>7</sup> cells/ml, and used in<br>a modification of a splenic injection model for liver colonization (47).<br>BALB/c mice (15-g females; Simonsen Laboratories, Gilroy, CA) were<br>anesthetiz mal essential medium at a concentration of 10' cells/ml, and used in<br>a modification of a splenic injection model for liver colonization (47).<br>BALB/c mice (15-g females; Simonsen Laboratories, Gilroy, CA) were<br>anesthetized BALB/c mice (15-g females; Simonsen Laboratories, Gilroy, CA) were<br>anesthetized with methoxyfluorane by inhalation and prepared sterilely<br>and the spleen was exteriorized through a flank incision. One million<br>and the splee anesthetized with methoxyfluorane by inhalation and prepared sterilely<br>and the spleen was exteriorized through a flank incision. One million<br>and the spleen was exteriorized through a flank incision. One million<br>cells in 10 and the spleen was exteriorized through a flank incision. One million<br>cells in 100  $\mu$ l were slowly injected into the splenic pulp through a 27-<br>gauge needle over 1 min, followed by splenectomy. Animals were<br>sacrificed 2 gauge needle over 1 min, followed by splenectomy. Animals were

Metastatic tumor cells after, the livers were removed and weighed, and<br>tumor nodules were counted.<br>The fate of untreated and desialylated tumor cells after splenic<br>injection was determined by injection of IdUR-labeled tum The fate of untreated and desialylated tumor cells after splenic injection was determined by injection of IdUR-labeled tumor cells.<br>
Metastatic tumor cell line 51B LiM 5 was grown as above and 0.5  $\mu$ Ci/ quality 123IJIdU ml [<sup>125</sup>I]IdUR was added to the culture medium 24 h prior to harvesting.<br>This concentration of isotope was previously shown to label greater<br>than 95% of tumor cells without affecting viability (37). Neuraminidase-<br>treate injection was determined by injection of IdUR-labeled tumor cells. recognometration or cell line 51B LiM 5 was grown as above and 0.5  $\mu$ Ci/<br>ml [<sup>123</sup>I]IdUR was added to the culture medium 24 h prior to harvesting.<br>This We tastatic tumor cell line 51B Lily 5 was grown as above and 0.5  $\mu$ C1/<br>ml [<sup>125</sup>1]IdUR was added to the culture medium 24 h prior to harvesting.<br>This concentration of isotope was previously shown to label greater<br>than min ["-1] HuCk was added to the culture medium 24 h prior to harvesting.<br>
This concentration of isotope was previously shown to label greater<br>
than 95% of tumor cells without affecting viability (37). Neuraminidase-<br>
trea Fins concentration of isotope was previously shown to laber greater<br>than 95% of tumor cells without affecting viability (37). Neuraminidase-<br>treated and control cells were prepared as described above, and  $10^6$  molonizab **(nmarry)** the cells without are extreme that the sphericated and control cells were prepared as described above, and  $10^6$  phor viable cells in  $100 \mu$  were injected into the splenic pulp over 1 min,<br>followed by splenec treated and control cents were prepared as described above, and 10<br>viable cells in 100  $\mu$ l were injected into the splenic pulp over 1 min,<br>followed by splenectomy 1 min later. Animals were sacrificed at various<br>interval Viable cells in 100  $\mu$ I were injected into the spienic pulp over 1 min,<br>followed by splenectomy 1 min later. Animals were sacrificed at various<br>intervals after injection by cervical dislocation, and livers were removed<br> counter.

### **RESULTS**

Electrophoretic Profiles of Radiolabeled Cell Surface Proteins. RESULTS<br>
Electrophoretic Profiles of Radiolabeled Cell Surface Proteins.<br>
Coomassie blue staining of electrophoretically separated cell<br>
surface proteins from parental cell line 51B and its metastatic as<br>
RESULTS<br>
Electrophoretic Profiles of Radiolabeled Cell Surface Proteins.<br>
Coomassie blue staining of electrophoretically separated cell<br>
surface proteins from parental cell line 51B and its metastatic<br>
derivatives 51B RESULTS<br>
Electrophoretic Profiles of Radiolabeled Cell Surface Proteins.<br>
Coomassie blue staining of electrophoretically separated cell<br>
surface proteins from parental cell line 51B and its metastatic<br>
derivatives 51B LiM Electrophoretic Profiles of Radiolabeled Cell Surface Proteins<br>Coomassie blue staining of electrophoretically separated ce<br>surface proteins from parental cell line 51B and its metastati<br>derivatives 51B LiM 5 and 51B LiM 6 Electrophotence Promes of Kaulolabeleu Cell Surface Proteins.<br>Coomassie blue staining of electrophoretically separated cell<br>surface proteins from parental cell line 51B and its metastatic<br>derivatives 51B LiM 5 and 51B LiM Substitutives S1B LiM 5 and S1B LiM 6 did not show substantial<br>derivatives S1B LiM 5 and S1B LiM 6 did not show substantial<br>differences in protein profiles between these cell lines. Lacto-<br>peroxidase-catalyzed iodination o derivatives 51B LiM 5 and 51B LiM 6 did not show substantial<br>differences in protein profiles between these cell lines. Lacto-<br>peroxidase-catalyzed iodination of cell surface proteins fol-<br>lowed by SDS-polyacrylamide electr differences in protein profiles between these cell lines. Lacto-<br>peroxidase-catalyzed iodination of cell surface proteins fol-<br>lowed by SDS-polyacrylamide electrophoresis, however, dem-<br>onstrated quantitative and qualitati peroxidase-catalyzed iodination of cell surface proteins fol-<br>lowed by SDS-polyacrylamide electrophoresis, however, demonstrated quantitative and qualitative differences in the cell<br>surface protein profiles of 51B (low met Formulation of the metastatic potential and amount of<br>lowed by SDS-polyacrylamide electrophoresis, however, dem-<br>onstrated quantitative and qualitative differences in the cell<br>surface protein profiles of 51B (low metastat onstrated quantitative and qualitative differences in the cell<br>surface protein profiles of 51B (low metastatic potential) and<br>its metastatic variants (Fig. 1A). The highly metastatic cell lines<br>had similar protein profile surface protein profiles of 51B (low metastatic potential) and its metastatic variants (Fig. 1A). The highly metastatic cell lines had similar protein profiles, which differed from that of the poorly metastatic parental l its metastatic variants (Fig. 1A). The highly metastatic cell lines<br>had similar protein profiles, which differed from that of the<br>poorly metastatic parental line. Quantitative differences were<br>present in proteins of appro the meaning of the meaning of the meaning of the meaning shift in model in the poorly metastatic parental line. Quantitative differences were present in proteins of approximate molecular weights  $170,000$ ,  $120,000$ ,  $95,$ moorly metastatic paren<br>present in proteins of ap<br>120,000, 95,000, 66,000<br>3, 5, 6, 10, 11, and 14)<br>of the 95,000 protein.<br>Labeling of sialic acid Solve the parameteric metallic acid-containing elements by mild performance molecular weights 170,000,  $\frac{6,0000}{6,0000}, \frac{6,0000}{6,0000}, \frac{55,000}{6,000}$ , and 40,000 (designated bands is 5, 6, 10, 11, and 14) with a sli present in proteins of approximate molecular weights 170,000,<br>120,000, 95,000, 66,000, 55,000, and 40,000 (designated bands into<br>3, 5, 6, 10, 11, and 14) with a slight shift in molecular weight<br>of the 95,000 protein.<br>Labe

55,000 protein.<br>
Labeling of sialic acid-containing glycoproteins by mild per-<br>
Labeling of sialic acid-containing glycoproteins by mild per-<br>
iddate oxidation and sodium borotritide reduction at 4°C sug-<br>
55,000 proteins Labeling of sialic acid-containing glycoproteins by mild periodate oxidation and sodium borotritide reduction at  $4^{\circ}$ C suggested that increases in the  $M$ , 170,000, 120,000, 95,000, and 055,000 proteins (bands 3, 5, 6, iodate oxidation and sodium borotritide reduction at 4°C sug-<br>iodate oxidation and sodium borotritide reduction at 4°C sug-<br>sested that increases in the  $M_r$  170,000, 120,000, 95,000, and dem<br>55,000 proteins (bands 3, 5,

Equivale was mixed with ACS II scintiliation intition counter. Values<br>
obtained without added acceptor were taken as radioactivity transferred<br>
to endogenous acceptors and were subtracted to yield net radioactivity<br>
trans galactose oxidase-sodium borotritide labeling of galactose- and ON CANCER METASTASIS<br>galactose Oxidase-sodium borotritide labeling of galactose- and<br>N-acetylgalactosamine-containing glycoproteins in the presence<br>or absence of prior neuraminidase treatment (Fig. 2). Labeling ON CANCER METASTASIS<br>galactose oxidase-sodium borotritide labeling of galactose- and<br>N-acetylgalactosamine-containing glycoproteins in the presence<br>or absence of prior neuraminidase treatment (Fig. 2). Labeling<br>of bands 3, galactose oxidase-sodium borotritide labeling of galactose- and<br>N-acetylgalactosamine-containing glycoproteins in the presence<br>or absence of prior neuraminidase treatment (Fig. 2). Labeling<br>of bands 3, 5, 6, and 11 was enh galactose oxidase-sodium borotritide labeling of galactose- and<br>*N*-acetylgalactosamine-containing glycoproteins in the presence<br>or absence of prior neuraminidase treatment (Fig. 2). Labeling<br>of bands 3, 5, 6, and 11 was e N-acetylgalactosamine-containing glycoproteins in the presence<br>or absence of prior neuraminidase treatment (Fig. 2). Labeling<br>of bands 3, 5, 6, and 11 was enhanced in all cell lines by prior<br>neuraminidase treatment, indica or absence of prior neuraminidase treatment (Fig. 2). Labeling<br>of bands 3, 5, 6, and 11 was enhanced in all cell lines by prior<br>neuraminidase treatment, indicating that these glycoproteins<br>are sialylated. A proportionately of bands 3, 5, 6, and 11 was enhanced in all c<br>neuraminidase treatment, indicating that the<br>are sialylated. A proportionately greater en<br>neuraminidase treatment was suggested for so<br>cipally 3 and 5) in the metastatic cell uraminidase treatment, indicating that these glycoproteins<br>e sialylated. A proportionately greater enhancement with<br>uraminidase treatment was suggested for some bands (prin-<br>ally 3 and 5) in the metastatic cell lines.<br>Lect Solutionary interesting that these gryceprocentially and the sialylated. A proportionately greater enhancement with<br>neuraminidase treatment was suggested for some bands (principally 3 and 5) in the metastatic cell lines.<br>L

neuraminidase treatment was suggested for some bands (principally 3 and 5) in the metastatic cell lines.<br>Lectin Affinity Chromatography. WGA is a lectin which binds specifically to oligosaccharides containing exposed *N*-a I alternationally 3 and 5) in the metastatic cell lines.<br>
Lectin Affinity Chromatography. WGA is a lectin which binds<br>
specifically to oligosaccharides containing exposed *N*-acetylglu-<br>
cosamine or sialic acid. Affinity c Lectin Affinity Chromatography. WGA is a lectin which binds<br>specifically to oligosaccharides containing exposed N-acetylglu-<br>cosamine or sialic acid. Affinity chromatography of solubilized<br> $^{125}I$ -labeled cell surface pr Eccin Hammy Om omalogation, WON IS a technically to one specifically to oligosaccharides containing exposed N-acetylglucosamine or sialic acid. Affinity chromatography of solubilize  $^{125}$ I-labeled cell surface proteins r cosamine or sialic acid. Affinity chromatography of solubilized<br><sup>125</sup>I-labeled cell surface proteins revealed a 2- to 3-fold increase<br>in WGA-binding proteins, which were sequentially eluted from<br>the column with 0.2 and 0.5 <sup>125</sup>I-labeled cell surface proteins revealed a 2- to 3-fold increase<br>in WGA-binding proteins, which were sequentially eluted from<br>the column with 0.2 and 0.5  $M$  hapten sugar (*N*-acetylglucosain WGA-binding proteins, which were sequentially eluted from<br>the column with 0.2 and 0.5 M hapten sugar (*N*-acetylglucosa-<br>mine), from the highly metastatic cells compared to those from<br>parental 51B (Fig. 3A). Electropho the column with 0.2 and 0.5 M hapten sugar (N-acetylglucosa-<br>mine), from the highly metastatic cells compared to those from<br>parental 51B (Fig. 3A). Electrophoresis of the peak fractions<br>of WGA-binding material eluted from mine), from the highly metastatic cells compared to those from<br>parental 51B (Fig. 3A). Electrophoresis of the peak fractions<br>of WGA-binding material eluted from the column again sug-<br>gested that glycoproteins corresponding parental 51B (Fig.  $3A$ ). Electrophoresis of the peak fractions of WGA-binding material eluted from the column again suggested that glycoproteins corresponding to sialoproteins are altered on the metastatic cells (Fig.  $3B$ ). When solubilized cell surface proteins were applied to a succinylated WGA column (which binds  $N$ -acetylglucosamine but not sialic acid), the quantitative differences previously seen between the cell lines on the

The fate of untreated and desialylated tumor cells after splenic Elderberry bark lectin (SNA) is a lectin which preferentially<br>injection was determined by injection of IdUR-labeled tumor cells. recognizes oligosaccharides WGA column were abolished, suggesting that those differences<br>were due to differences in the sialic acid content of the cell<br>membrane fractions.<br>Elderberry bark lectin (SNA) is a lectin which preferentially<br>recognizes olig membrane fractions.<br>
Elderberry bark lectin (SNA) is a lectin which preferentially<br>
recognizes oligosaccharides which contain the terminal squence<br>
Neu-5-Ac( $\alpha$ 2-6)Gal/GalNAc (48, 49). Affinity chromatography of solubili Elderberry bark lectin (SNA) is a lectin which preferentially<br>recognizes oligosaccharides which contain the terminal se-<br>quence Neu-5-Ac( $\alpha$ 2-6)Gal/GalNAc (48, 49). Affinity chro-<br>matography of solubilized cell surface p recognizes oligosaccharides which contain the terminal sequence Neu-5-Ac( $\alpha$ 2-6)Gal/GalNAc (48, 49). Affinity chromatography of solubilized cell surface proteins on agarose-<br>bound SNA revealed increased SNA binding by th quence Neu-5-Ac( $\alpha$ 2-6)Gal/GalNAc (48, 49). Affinity chromatography of solubilized cell surface proteins on agarose-<br>bound SNA revealed increased SNA binding by the highly metastatic cells compared to parental 51B (Fig. matography of solubilized cell surface proteins on agarose-<br>bound SNA revealed increased SNA binding by the highly<br>metastatic cells compared to parental 51B (Fig. 4A). Electro-<br>phoresis of SNA-binding proteins eluted from bound SNA revealed increased SNA binding by the highly metastatic cells compared to parental 51B (Fig. 4A). Electro-<br>phoresis of SNA-binding proteins eluted from the column demonstrated quantitative increases in labeling o metastatic cells compared to parental<br>phoresis of SNA-binding proteins elu<br>onstrated quantitative increases in la<br>sialoglycoproteins (3, 5, 6) previously<br>the metastatic cell lines (Fig. 4B).<br>Sialic Acid Content of Cell Mem Since Sialic Acid Content of Cell Membranes. Neuraminidated members of SNA-binding proteins eluted from the column destrated quantitative increases in labeling of three of the flogly<br>coproteins (3, 5, 6) previously shown t photosis of SAT shang process cated from the column dem<br>onstrated quantitative increases in labeling of three of the four<br>sialoglycoproteins (3, 5, 6) previously shown to be increased in<br>the metastatic cell lines (Fig. 4B)

sialoglycoproteins (3, 5, 6) previously shown to be increased in<br>the metastatic cell lines (Fig. 4B).<br>Sialic Acid Content of Cell Membranes. Neuraminidase-re-<br>leasable membrane-associated sialic acid was determined for<br>par the metastatic cell lines (Fig. 4B).<br>Sialic Acid Content of Cell Membranes. Neuraminidase<br>leasable membrane-associated sialic acid was determined<br>parental murine colon cancer cell line 51B and its metas<br>derivatives 51B LiM Sialic Acid Content of Cell Membranes. Neuraminidase-re-<br>leasable membrane-associated sialic acid was determined for<br>parental murine colon cancer cell line 51B and its metastatic<br>derivatives 51B LiM 5 and 51B LiM 6. Neuram leasable membrane-associated sialic acid was determined for parental murine colon cancer cell line 51B and its metastatic derivatives 51B LiM 5 and 51B LiM 6. Neuraminidase-releasable sialic acid was approximately 2-fold h rental murine colon cancer cell line 51B and its metastatic<br>rivatives 51B LiM 5 and 51B LiM 6. Neuraminidase-releas-<br>le sialic acid was approximately 2-fold higher on the met-<br>tatic cell lines compared to the parental cell derivatives 51B LiM 5 and 51B LiM 6. Neuraminidase-releas-<br>able sialic acid was approximately 2-fold higher on the met-<br>astatic cell lines compared to the parental cell line (Table 1).<br>Sialyltransferase Activity. Sialyltra

able sialic acid was approximately 2-fold higher on the met-<br>astatic cell lines compared to the parental cell line (Table 1).<br>Sialyltransferase Activity. Sialyltransferase activities associ-<br>ated with parental cell line 5 able stand actu was approximately 2-10tu inglier on the inet-<br>astatic cell lines compared to the parental cell line (Table 1).<br>Sialyltransferase Activity. Sialyltransferase activities associ-<br>ated with parental cell line Sialyltransferase Activity. Sialyltransferase activities associated with parental cell line 51B and metastatic lines 51B LiM<br>5 and 51B LiM 6 were determined using asialotransferrin<br>([Gal $\beta$ 1-4GlcNAc<sub>2</sub>)Man<sub>3</sub>] and asialo ated with parental cell line 51B and metastatic lines 51B LiM<br>5 and 51B LiM 6 were determined using asialotransferrin<br>((Gal $\beta$ 1-4GlcNAc<sub>2</sub>)Man<sub>3</sub>] and asialo-OSM (GalNAc $\alpha$ 1-Ser/<br>Thr) as acceptors. Sialyltransferase spe 5 and 51B LiM 6 were determined using asialotransferrin [(Gal $\beta$ 1-4GlcNAc<sub>2</sub>)Man<sub>3</sub>] and asialo-OSM (GalNAc $\alpha$ 1-Ser/Thr) as acceptors. Sialyltransferase specific activities using either acceptor were higher for the meta cell membrane-associated sialidation of the cell membrane-cell (Gal $\beta$ 1-4GlcNAc<sub>2</sub>)Man<sub>3</sub>] and asialo-OSM (GalNAc $\alpha$ 1-Ser/<br>Thr) as acceptors. Sialyltransferase specific activities using<br>either acceptor were higher for t Thr) as acceptors. Sialyltransferase specific activities using either acceptor were higher for the metastatic cell lines and rrelated with both the metastatic potential and amount of<br>Il membrane-associated sialic acid of the cell lines (Table 2).<br>contrast, there was no difference in the  $\beta$ 1-4-galactosyltrans-<br>rase activities of the cell lines cell membrane-associated sialic acid of the cell lines (Table 2).<br>In contrast, there was no difference in the  $\beta$ 1-4-galactosyltrans-<br>ferase activities of the cell lines.<br>WGA Binding to Experimental Primary and Metastati

3, 5, 6, 10, 11, and 14) with a slight shift in molecular weight<br>of the 95,000 protein.<br>Iabeling of sialic acid-containing glycoproteins by mild per-<br>Labeling of sialic acid-containing glycoproteins by mild per-<br>(Fig. 5A) In contrast, there was no difference in the  $\beta$ 1-4-galactosyltrans-<br>ferase activities of the cell lines.<br>WGA Binding to Experimental Primary and Metastatic Tu-<br>mors. Parental cell line 51B is heterogeneous *in vitro* (37 In contract, the cell intervalsed in the particular of the cell lines.<br>
WGA Binding to Experimental Primary and Metastatic T<br>
mors. Parental cell line 51B is heterogeneous in vitro (37) are<br>
forms moderately to poorly dif WGA Binding to Experimental Primary and Metastatic Tumors. Parental cell line 51B is heterogeneous in vitro (37) and forms moderately to poorly differentiated tumors when injected into the ceca of syngeneic animals. Examin mors. Parental cell line 51B is heterogeneous in vitro (37) and forms moderately to poorly differentiated tumors when injected into the ceca of syngeneic animals. Examination of hematoxy-<br>lin- and eosin-stained sections of the primary tumor formed<br>after cecal injection of 51B revealed two major cell populations<br>(Fig. 5A). Immunohistochemical analy lin- and eosin-stained sections of the primary tumor formed<br>after cecal injection of 51B revealed two major cell populations<br>(Fig. 5A). Immunohistochemical analysis using biotinylated<br>wheat germ agglutinin and streptavidin after cecal injection of 51B revealed two major cell populations<br>(Fig. 5A). Immunohistochemical analysis using biotinylated<br>wheat germ agglutinin and streptavidin peroxidase staining<br>demonstrated preferential binding of WG (Fig. 5.4). Immunohistochemical analysis using biotinylated wheat germ agglutinin and streptavidin peroxidase staining demonstrated preferential binding of WGA to the cell surface of one population (Fig. 5*B*). Examination wheat germ agglutinin and streptavidin peroxidase staining<br>demonstrated preferential binding of WGA to the cell surface<br>of one population (Fig. 5B). Examination of liver metastases<br>from 51B and its more metastatic derivati







DI:<br>
Fig. 2. Galactose- and *N*-acetylgalactosamine-containing glycoproteins from<br>
parental (51B) and metastatic (51B LiM 5, 51B LiM 6) murie colon cancer cell<br>
lines labeled by the galactose oxidase-sodium borotritide met Fig. 2. Galactose- and *N*-acetylgalactosamine-containing glycoprote parental (51B) and metastatic (51B LiM 5, 51B LiM 6) murine colon c:<br>lines labeled by the galactose oxidase-sodium borotritide method in the<br> $(+N)$  or ab parental (51B) and metastatic (51B LiM 5, 51B LiM 6) murine colon cancer cell<br>lines labeled by the galactose oxidase-sodium borotritide method in the presence<br> $(+N)$  or absence  $(-N)$  of prior neuraminidase treatment to rem lines labeled by the galactose oxidase-sodium borotritide method in the presence  $(+N)$  or absence  $(-N)$  of prior neuraminidase treatment to remove cell surface sialic acid. Equal amounts of protein were applied to paralle

amine).

 $51B$  LiM5 LiM6 B<br>Cell Surface Sialic Acid Effect on Liver Colonization. In order<br>to determine whether removal of sialic acid from the cell surface<br>would affect the ability of colon cancer cells to form secondary Cell Surface Sialic Acid Effect on Liver Colonization. In order<br>to determine whether removal of sialic acid from the cell surface<br>would affect the ability of colon cancer cells to form secondary Cell Surface Sialic Acid Effect on Liver Colonization. In order<br>to determine whether removal of sialic acid from the cell surface<br>would affect the ability of colon cancer cells to form secondary<br>foci in the liver, cells of Cell Surface Sialic Acid Effect on Liver Colonization. In order<br>to determine whether removal of sialic acid from the cell surface<br>would affect the ability of colon cancer cells to form secondary<br>foci in the liver, cells of Cell Surface Sialic Acid Effect on Liver Colonization. In order<br>to determine whether removal of sialic acid from the cell surface<br>would affect the ability of colon cancer cells to form secondary<br>foci in the liver, cells of Le Surface Stanc Acid Errect on Liver Colonization. In order<br>to determine whether removal of sialic acid from the cell surface<br>would affect the ability of colon cancer cells to form secondary<br>foci in the liver, cells of hi to determine whether removal of stail acid from the cell surface<br>would affect the ability of colon cancer cells to form secondary<br>foci in the liver, cells of high and low metastatic ability were<br>treated with neuraminidase would affect the ability of colon cancer cells to form secondary<br>foci in the liver, cells of high and low metastatic ability were<br>treated with neuraminidase *in situ*, and liver colonization was<br>tested after splenic inject liver in the liver, cells of high and low metastatic ability were<br>treated with neuraminidase *in situ*, and liver colonization was<br>tested after splenic injection. Control cells were handled iden-<br>tically but were not treat treated with neuraminidase *in situ*, and liver colonization was<br>tested after splenic injection. Control cells were handled iden-<br>tically but were not treated with neuraminidase prior to injec-<br>tion. Animals were sacrifice rested arter spienic injection. Control cells were nandied identically but were not treated with neuraminidase prior to injection. Animals were sacrificed 2 weeks after injection and the livers were examined for tumor. Few tically but were not treated with neuraminidase prior to injection. Animals were sacrificed 2 weeks after injection and the livers were examined for tumor. Few tumor nodules were noted in the livers of animals injected wit tion. Animals were sacrificed 2 weeks after injection and the<br>livers were examined for tumor. Few tumor nodules were noted<br>in the livers of animals injected with parental cell line 51B<br>(range, 3 to 12), and almost none we livers were examined for tumor. Few tumor nodules were noted<br>in the livers of animals injected with parental cell line 51B<br>(range, 3 to 12), and almost none were present after prior<br>neuraminidase treatment (range, 0 to 1). in the livers of animals injected with parental cell line 51B (range, 3 to 12), and almost none were present after prior neuraminidase treatment (range, 0 to 1). Animals injected with cell line 51B LiM 5 had extensive tum neuraminidase treatment (range, 0 to 1). Animals injected with<br>cell line 51B LiM 5 had extensive tumor throughout the liver<br>(range, 220 to >500 nodules), which was dramatically decreased<br>by prior removal of cell surface si cell line 51B LiM 5 had extensive tumor throughout the liver<br>(range, 220 to >500 nodules), which was dramatically decreased<br>by prior removal of cell surface sialic acid (range, 6 to 68<br>nodules) (Table 3, Fig. 6). Injectio (range, 220 to >500 nodules), which was dramatically decreased<br>by prior removal of cell surface sialic acid (range, 6 to 68<br>nodules) (Table 3, Fig. 6). Injection of  $[^{125}]$ IJdUR-labeled tumor<br>cells demonstrated that prio by prior removal of cell surface sialic acid (range, 6 to 68 nodules) (Table 3, Fig. 6). Injection of  $[^{125}1] \text{IdUR-labeled tumor}$  cells demonstrated that prior neuraminidase treatment did not alter the number of viable tumor cells cells demonstrated that prior neuraminidase treatment did not<br>alter the number of viable tumor cells leaving the spleen and<br>initially reaching the liver (Table 4). Rather, it appeared that,<br>by 12 h after injection, fewer d

## **DISCUSSION**

The state injection, fewer desialylated cells remained in the<br>Metastasis is a selective process by which certain tumor cells<br>SCUSSION<br>Metastasis is a selective process by which certain tumor cells<br>seminate to form secondar liver compared to the untreated cells.<br>
DISCUSSION<br>
Metastasis is a selective process by which certain tumor cells<br>
disseminate to form secondary foci at distant sites. Cell mem-<br>
brane glycoproteins and glycolipids play a DISCUSSION<br>Metastasis is a selective process by which certain tumor cells<br>disseminate to form secondary foci at distant sites. Cell mem-<br>brane glycoproteins and glycolipids play an important role in<br>many biological functio DISCUSSION<br>Metastasis is a selective process by which certain tumor cells<br>disseminate to form secondary foci at distant sites. Cell mem-<br>brane glycoproteins and glycolipids play an important role in<br>many biological functio Metastasis is a selective process by which certain tumor cells<br>disseminate to form secondary foci at distant sites. Cell mem-<br>brane glycoproteins and glycolipids play an important role in<br>many biological functions such as Metastasis is a selective process by which certain tumor cells<br>disseminate to form secondary foci at distant sites. Cell mem-<br>brane glycoproteins and glycolipids play an important role in<br>many biological functions such as disseminate to form secondary foci at distant sites. Cell membrane glycoproteins and glycolipids play an important role in many biological functions such as cell-cell interactions, growth regulation, differentiation, and m brane glycoproteins and glycolipids play an important role in<br>many biological functions such as cell-cell interactions, growth<br>regulation, differentiation, and malignant transformation, and<br>tumor cell metastasis may requir many biological functions such as cell-cell interactions, growth<br>regulation, differentiation, and malignant transformation, and<br>tumor cell metastasis may require alterations in membrane<br>properties determined by cell surfac regulation, differentiation, and malignant transformation, and<br>tumor cell metastasis may require alterations in membrane<br>properties determined by cell surface glycoconjugates (1–4).<br>While an universal alteration in cell me



Fig. 3. Affinity chromatography of solubilized <sup>125</sup>1-labeled cell surface proteins<br>on an agarose-bound WGA column. *A*, WGA-binding material was sequentially<br>eluted from the column with 0.2 and 0.5 M hapten sugar (*N*-ac 51B, parental murine colon cancer cell line; 51B LiM 5, metastatic colon cancer cell line. Similar differences were found when comparing 51B to metastatic line 51B LiM 6 (data not shown). B, SDS-PAGE of cell membrane prot rig. 3. Attintly chromatography of solutilized <sup>72</sup>-l-labeled cell surface proteins<br>on an agarose-bound WGA column. A, WGA-binding material was sequentially<br>eluted from the column with 0.2 and 0.5 M hapten sugar (*N*-acet For the Similar differences were found when comparing 51B<br>cell line. Similar differences were found when comparing 51B<br>51B LiM 6 (data not shown). B, SDS-PAGE of cell membrar<br>tially eluted from a wheat germ agglutinin aff SIB LIM 6 (data not shown). B, SDS-PAGE of cell membrane proteins sequentially elucid from a wheat germ agglutinin affinity column. A and A', proteins from metastatic line SIB LiM 5. A and B represent proteins eluted with

The final venture in the wiest germ aggiuding animaly column. A and A, potential line 51B; B and B', proteins from metastatic line 51B LiM 5. A and B represent proteins eluted with 0.25 M N-acetylglucosamine; B and B' rep represent proteins eluted with 0.5 M  $N$ -acetylglucosamine.<br>
sialylated oligosaccharides are commonly associated with me-<br>
tastasis (5–20, 23, 34, 50). The current study compares the cell<br>
surface proteins and glycoprotei epresent proteins eluted with 0.5 M N-acetylglucosamine.<br>
sialylated oligosaccharides are commonly associated with me-<br>
tastasis (5–20, 23, 34, 50). The current study compares the cell<br>
surface proteins and glycoproteins o sialylated oligosaccharides are commonly associated with me-<br>tastasis (5–20, 23, 34, 50). The current study compares the cell<br>surface proteins and glycoproteins of murine colon cancer cell<br>lines which differ in their spont sialylated oligosaccharides are commonly associated with me-<br>tastasis (5–20, 23, 34, 50). The current study compares the cell<br>surface proteins and glycoproteins of murine colon cancer cell<br>lines which differ in their spont tastasis (5–20, 23, 34, 50). The current study compares the cell<br>surface proteins and glycoproteins of murine colon cancer cell<br>lines which differ in their spontaneous liver-metastasizing abil-<br>ity during cecal growth. Met surface proteins and glycoproteins of murine colon cancer cell<br>lines which differ in their spontaneous liver-metastasizing abil-<br>ity during cecal growth. Metastatic lines were derived by serial<br>selection from a heterogeneo lines which differ in their spontaneous liver-metastasizing abil-<br>ity during cecal growth. Metastatic lines were derived by serial<br>selection from a heterogeneous parental cell line in an animal<br>model for colon cancer meta selection from a heterogeneous parental cell line in an animal<br>model for colon cancer metastasis (37). Thus, all cell lines<br>compared are genetically related but were selected for altera-<br>tions in their metastatic phenotyp

model for colon cancer metastasis (37). Thus, all cell lines<br>compared are genetically related but were selected for altera-<br>tions in their metastatic phenotype.<br>SDS-polyacrylamide gel electrophoresis of <sup>125</sup>I-labeled cel compared are genetically related but were selected for alterations in their metastatic phenotype.<br>
SDS-polyacrylamide gel electrophoresis of <sup>125</sup>I-labeled cell<br>
surface proteins revealed alterations in several proteins f tions in their metastatic phenotype.<br>
SDS-polyacrylamide gel electrophoresis of <sup>125</sup>I-labeled cell<br>
surface proteins revealed alterations in several proteins from<br>
the metastatic lines compared to the less metastatic par SDS-polyacrylamide gel electrophoresis of <sup>125</sup>I-labeled cell<br>surface proteins revealed alterations in several proteins from<br>the metastatic lines compared to the less metastatic parent. In<br>the case of at least four of the surface proteins revealed alterations in several proteins from<br>the metastatic lines compared to the less metastatic parent. In<br>the case of at least four of these proteins (approximate  $M_1$ <br>170,000, 120,000, 95,000, and 5 170,000, 120,000, 95,000, and 55,000), this represented an<br>increase in the radioactive labeling of sialoglycoproteins from<br>the metastatic lines (mild periodate oxidation-sodium borotri-<br>ide reduction). This was confirmed the case of at least four of these proteins (approximate  $M_r$ , 170,000, 120,000, 95,000, and 55,000), this represented an increase in the radioactive labeling of sialoglycoproteins from the metastatic lines (mild periodat 170,000, 120,000, 95,000, and 55,000), this represented an increase in the radioactive labeling of sialoglycoproteins from the metastatic lines (mild periodate oxidation-sodium borotritide reduction). This was confirmed b increase in the radioactive labeling of sialoglycoproteins from<br>the metastatic lines (mild periodate oxidation-sodium borotri-<br>tide reduction). This was confirmed by galactose oxidase label-<br>ing of galactose- and *N*-acety the metastatic lines (mild periodate oxidation-sodium borotri-<br>tide reduction). This was confirmed by galactose oxidase label-<br>ing of galactose- and *N*-acetylgalactosamine-containing glyco-<br>proteins. All cell lines were p fide reduction). This was confirmed by galactose oxidase label-<br>ing of galactose- and *N*-acetylgalactosamine-containing glyco-<br>proteins. All cell lines were poorly labeled by this method prior<br>to neuraminidase treatment, ing of galactose- and *N*-acetylgalactosamine-containing glyco-<br>proteins. All cell lines were poorly labeled by this method prior<br>to neuraminidase treatment, suggesting that the nonreducing<br>termini of the oligosaccharide



Fig. 4. Affinity chromatography of solubilized <sup>125</sup>I-labeled cell surface proteins<br>on an agarose-bound SNA column. A, material bound to the column eluted with<br>0.5 M lactose. B, SDS-PAGE of cell membrane proteins eluted fr

Fig. 4. Affinity chromatography of solubilized <sup>125</sup>I-labeled cell surface proteins on an agarose-bound SNA column.  $A$ , material bound to the column eluted with 0.5 M lactose.  $B$ , SDS-PAGE of cell membrane proteins elut those labeled by mild periodate oxidation, and a proportionally greater enhancement was seen for some proteins in the metastatic colon cancer cell lines.

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ANIMAL MODEL FOR COLON CANCER METASTASIS<br>An increase in cell surface sialoglycoproteins on the met- sialyltransferase activities associated with the metastatic cell ANIMAL MODEL FOR CONTROLLATED STANDAL MODEL FOR CONTROLLATED SUPERFORM CONTROLLATED ANIMAL MODEL FOR C<br>An increase in cell surface sialoglycoproteins on the met-<br>astatic variants was further supported by lectin affinity chro-<br>matography of solubilized cell membrane proteins. WGA binds ANIMAL MODEL FOR COLON C<br>ANIMAL MODEL FOR COLON C<br>astatic variants was further supported by lectin affinity chro-<br>ine<br>matography of solubilized cell membrane proteins. WGA binds<br>to the oligosaccharides of glycoconjugates c ANIMAL MODEL FOR COLON CALL<br>
ANIMAL MODEL FOR COLON CALL<br>
astatic variants was further supported by lectin affinity chro-<br>
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matography of solubilized cell membrane proteins. WGA binds<br>
to the oligosaccharides of glyc An increase in cell surface sialoglycoproteins on the met-<br>sialyl<br>astatic variants was further supported by lectin affinity chro-<br>ines.<br>matography of solubilized cell membrane proteins. WGA binds<br>A f<br>to the oligosaccharide An increase in cell surface sialoglycoproteins on the met-<br>siaty astatic variants was further supported by lectin affinity chro-<br>lines.<br>matography of solubilized cell membrane proteins. WGA binds<br>A to the oligosaccharides astatic variants was further supported by lectin affinity chro-<br>matography of solubilized cell membrane proteins. WGA binds<br>to the oligosaccharides of glycoconjugates containing exposed live<br>N-acetylglucosamine or sialic a matography or solubilized cell membrane proteins. WGA binds<br>to the oligosaccharides of glycoconjugates containing exposed liver<br>N-acetylglucosamine or sialic acid. A 2- to 3-fold increase in used<br>wheat germ binding was obs to the oligosaccharides of glycoconjugates containing exposed in<br>
N-acetylglucosamine or sialic acid. A 2- to 3-fold increase in<br>
wheat germ binding was observed in the metastatic lines when<br>
compared to the poorly metasta N-acetyigiucosamine or sialic acid. A 2- to 3-101d increase in used<br>wheat germ binding was observed in the metastatic lines when<br>compared to the poorly metastatic parental line. This difference (sple<br>was not seen after app wheat germ binding was observed in the metastatic lines when<br>compared to the poorly metastatic parental line. This difference (spl<br>was not seen after application of membrane proteins to a crea<br>succinylated WGA column (bind compared to the poorly metastatic parental line. This difference (splet was not seen after application of membrane proteins to a creass succinylated WGA column (binds *N*-acetylglucosamine but not parentiallic acid), sugge was not seen after application of membrane proteins to a crea<br>succinylated WGA column (binds *N*-acetylglucosamine but not<br>pare<br>sialic acid), suggesting that the differences seen in WGA binding<br>liver<br>were due mainly to di succinylated WGA column (binds *N*-acetylglucosamine but not<br>sialic acid), suggesting that the differences seen in WGA binding<br>were due mainly to different degrees of sialylation of membrane<br>glycoproteins. Application of sialic acid), suggesting that the differences seen in WGA binding<br>were tumor foci after removal of sialic acid from the surface of<br>were due mainly to different degrees of sialylation of membrane<br>these cells by neuraminida were due mainly to different degrees of sialylation of membrane<br>glycoproteins. Application of solubilized membrane proteins to<br>glycoproteins. Application of solubilized membrane proteins to<br>tially binds  $\alpha$ 2-6-linked sial glycoproteins. Application of solubilized membrane proteins to<br>agarose-bound *Sambucus nigra* lectin, a lectin which preferen-<br>tially binds  $\alpha$ 2-6-linked sialic acid, again confirmed increased liver<br>sialylation of the me agarose-bound Sambucus nigra lectin, a lectin which preferen-<br>tially binds  $\alpha$ 2-6-linked sialic acid, again confirmed increased liver<br>sialylation of the metastatic cell lines. SDS-PAGE of material cells<br>eluted from the W tially binds  $\alpha$ 2-6-linked sialic acid, again confirmed increased<br>sialylation of the metastatic cell lines. SDS-PAGE of material cells<br>eluted from the WGA and SNA columns demonstrated en-<br>port<br>hancement of proteins from sialylation of the metastatic cell lines. SDS-PAGE of material cells<br>eluted from the WGA and SNA columns demonstrated en-<br>porta<br>hancement of proteins from the metastatic cells corresponding Im<br>in molecular weight to the pr imately 2-fold higher neuraminidase-releasable sialic acid and

Solid CANCER METASTASIS<br>sialyltransferase activities associated with the metastatic cell<br>dines. lines.

CANCER METASTASIS<br>Ilyltransferase activities associated with the metastatic cell<br>es.<br>A functional relationship between cell surface sialic acid and<br>er-colonizing ability of the murine colon cancer cell lines N CANCER METASTASIS<br>
sialyltransferase activities associated with the metastatic cell<br>
lines.<br>
A functional relationship between cell surface sialic acid and<br>
liver-colonizing ability of the murine colon cancer cell lines<br> sialyltransferase activities associated with the metastatic cell<br>lines.<br>A functional relationship between cell surface sialic acid and<br>liver-colonizing ability of the murine colon cancer cell lines<br>used in this study was s stalyltransterase activities associated with the metastatic cell<br>lines.<br>A functional relationship between cell surface sialic acid and<br>liver-colonizing ability of the murine colon cancer cell lines<br>used in this study was s Innes.<br>
A functional relationship between cell surface sialic acid and<br>
liver-colonizing ability of the murine colon cancer cell lines<br>
used in this study was suggested by comparing their ability to<br>
form liver nodules aft A functional relationship between cell surface sialic acid and<br>liver-colonizing ability of the murine colon cancer cell lines<br>used in this study was suggested by comparing their ability to<br>form liver nodules after their in used in this study was suggested by comparing their ability to form liver nodules after their introduction into the portal system (splenic injection). This experiment confirmed the greatly increased ability of 51B LiM 5 to form liver nodules after their introduction into the portal system form liver nodules after their introduction into the portal system (splenic injection). This experiment confirmed the greatly in<br>creased ability of 51B LiM 5 to colonize the liver, compared to<br>parental line 51B, and demons (splenic injection). This experiment confirmed the greatly in-<br>creased ability of 51B LiM 5 to colonize the liver, compared to<br>parental line 51B, and demonstrated a dramatic reduction in<br>liver tumor foci after removal of s creased ability of 51B LiM 5 to colonize the liver, compared to<br>parental line 51B, and demonstrated a dramatic reduction in<br>liver tumor foci after removal of sialic acid from the surface of<br>these cells by neuraminidase tre parental line 51B, and demonstrated a dramatic reduction in<br>liver tumor foci after removal of sialic acid from the surface of<br>these cells by neuraminidase treatment. Tracking of iododeox-<br>yuridine-labeled tumor cells demon liver tumor foci after removal of sialic acid from the surface of<br>these cells by neuraminidase treatment. Tracking of iododeox-<br>yuridine-labeled tumor cells demonstrated that the decrease in<br>tumor burden by neuraminidase t yuridine-labeled tumor cells demonstrated that the decrease in<br>tumor burden by neuraminidase treatment was due to decreased<br>liver colonization and not due to a difference in the number of<br>cells leaving the spleen and reach ridine-labeled tumor cells demonstrated that the decrease in<br>mor burden by neuraminidase treatment was due to decreased<br>er colonization and not due to a difference in the number of<br>lls leaving the spleen and reaching the l formed after colonization and not due to a difference in the number of<br>cells leaving the spleen and reaching the liver after splenic<br>portal injection.<br>Immunohistochemical staining with WGA of primary tumors<br>formed after ce

mmunonistochemical staining with WGA of primary tumors<br>med after cecal injection of parental cell line 51B revealed<br>Table 3 Effect of neuraminidase treatment on hepatic tumor burden after<br>intrasplenic injection of primary

*iter cecal injection of parental cell line 51B reve*<br>*Effect of neuraminidase treatment on hepatic tumor burden aft*<br>*intrasplenic injection of primary and metastatic cell lines*<br>Liver weight Number of tumo

|  |  | Table 1 Neuraminidase-releasable membrane-associated sialic acid in primary |  |
|--|--|---|--|
|  |  |   |  |
|  | and metastatic colon cancer cell lines |   |  |



Cell line Condition 51B +Neuraminidase<sup>c</sup> 5 Cell line Condition (g)<br>  $51B$  - 0.96 ± 0.1<br>  $51B$  - Neuraminidase<sup>c</sup> 1.03 ± 0.1<br>  $51B$  LiM 5 - 2.22 ± 0.7<br>  $51B$  LiM 5 + Neuraminidase 1.12 ± 0.1<br>
Mean ± SD. *n* = 6 animals/group. Liver weight  $\frac{(g)}{0.96\pm0.15^a}$  $1.03 \pm 0.16$ ±0.75 1.12 ±0.17'Number Table 3 *Effect of neuraminidase treatment on hepatic tumor burden after*<br>intrasplenic injection of primary and metastatic cell lines<br>Liver weight Number of tumor<br>Cell line Condition (g) nodules (range)<br>51B  $-0.96 \pm 0.15^$ nodules (range)  $0 - 1$ 6-68' **SIB.**  $\rightarrow$  Mean  $\rightarrow$  Mean  $\rightarrow$  Mean  $\rightarrow$  SD. 16 **SIB** LiM 5  $\rightarrow$  2.22 ± 0.75 **SIB** LiM 5  $\rightarrow$  Mean  $\rightarrow$  SD.  $n = 6$  animals/group.<br>
<sup>2</sup> Mean  $\pm$  SD.  $n = 6$  animals/group.<br>
<sup>2</sup> Macroscopic nodules visible to the naked eye **51B LiM 5**  $\rightarrow$  **Heuraminidase**<br> **d** Mean  $\pm$  SD.  $n = 6$  animals/group.<br> **b** Macroscopic nodules visible to the<br> **c** Neuraminidase type X from *C*. perf<br> **d**  $P < 0.05$  versus untreated cells.<br> **c**  $P < 0.01$  versus untre





 $\sum_{n=6}^{6} Mean \pm SD$ .

# Table 4 *Distribution of [<sup>125</sup>IJIdUR-labeled tumor cells after splenic injection*<br>IB LiM 5: 10<sup>6</sup> viable tumor cells/animal = 81.000 to 84.000 com) were injected in

<sup>d</sup>  $P < 0.01$  compared to 51B.<br> **Table 4** Distribution of  $[1^{125}]]IdUR$ -labeled tumor cells after splenic injection<br>  $[1^{221}]IdUR$ -labeled tumor cells (cell line 51B LiM 5; 10<sup>6</sup> viable tumor cells/animal = 81,000 to 84,000 Table 4 Distribution of  $[135]$  IIdUR-labeled tumor cells after splenic injection<br>  $[135]$ IIdUR-labeled tumor cells (cell line 51B LiM 5; 10<sup>6</sup> viable tumor cells/animal = 81,000 to 84,000 cpm) were injection<br>
followed by *Abbeled tumor cells after splenion*<br>
inimal = 81,000 to 84,000 cpm<br>
s and livers were removed. The<br>
<u>amma-counting</u>.<br>
% of input radioactivity<br>
Liver



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Fig. 5. WGA binding to experimental primary and metastatic tumors. Bars, 50  $\mu$ m. A, hematoxylin- and eosin-stained tissue section from experimental cecal primary tumor formed after injection of murine colon cancer Fig. 5. WGA binding to experimental primary and metastatic tumors. *Bars*, 50  $\mu$ m. *A*, hematoxylin- and exercision of murine colon cancer may tumor formed after injection of murine colon cancer cell line 51B. Note two metastatic tumors. *Bars*, 50  $\mu$ m. *A*, hematoxylin- and<br>eosin-stained tissue section from experimental cecal pri-<br>mary tumor formed after injection of murine colon cancer<br>cell line 51B. Note two cell populations separat

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Fig. 6. Livers from BALB/c mice 2 weeks after in<br>colon cancer cell lines.  $A$ , livers injected with 5<br>neuraminidase prior to injection to remove cell sur<br>5; D, 51B LiM 5 pretreated with neuraminidase. Fig. 6. Livers from BALB/c mice 2 weeks after intrasplenic injection of murine<br>colon cancer cell lines. A, livers injected with 51B; B, 51B pretreated with<br>neuraminidase prior to injection to remove cell surface sialic ac

Primary cecal tumor. Spontaneous liver métastases in the same method of metastase prior to injection to remove cell surface sialic acid;  $C$ , 51B LiM furth function is necessarily and the metastases in the same in dbindin meuraminidase prior to injection to remove cell surface sialic acid; C, 51B LiM furth<br>
5; D, 51B LiM 5 pretreated with neuraminidase.<br>
in de<br>
binding of this lectin to only a subpopulation of cells in the<br>
primary cecal tu 5; *D*, 51B LiM 5 pretreated with neuraminidase. <br>
in discriming of this lectin to only a subpopulation of cells in the<br>
primary cecal tumor. Spontaneous liver metastases in the same<br>
animals, however, demonstrated strong binding of this lectin to only a subpopulation of cells in the<br>primary cecal tumor. Spontaneous liver metastases in the same<br>animals, however, demonstrated strong homogeneous binding<br>of WGA, again suggesting selective met binding of this lectin to only a subpopulation of cells in the<br>primary cecal tumor. Spontaneous liver metastases in the same<br>animals, however, demonstrated strong homogeneous binding<br>of WGA, again suggesting selective met primary cecal tumor. Spontaneous liver metastases in the same<br>animals, however, demonstrated strong homogeneous binding<br>of WGA, again suggesting selective metastasis by WGA-binding<br>tumor cells. Although it is possible that animals, however, demonstrated strong homogeneous binding<br>of WGA, again suggesting selective metastasis by WGA-binding<br>tumor cells. Although it is possible that the difference in WGA<br>binding between the cells of the primar of WGA, again suggesting selective metastasis by WGA-binding<br>tumor cells. Although it is possible that the difference in WGA<br>binding between the cells of the primary cecal tumor and the<br>liver metastases was a result of env tumor cells. Although it is possib<br>binding between the cells of the<br>liver metastases was a result of en<br>is unlikely since cells selected for<br>strongly bound WGA when grov<br>data not shown) and *in vitro*.<br>We found an approxim nding between the cells of the primary cecal tumor and then the reflects was a result of environmental factors (34), this unlikely since cells selected for liver-metastasizing potential congly bound WGA when grown in other liver metastases was a result of environmental factors  $(34)$ , this<br>is unlikely since cells selected for liver-metastasizing potential<br>strongly bound WGA when grown in other sites (s.c., cecum;<br>data not shown) and *in vit* 

is unlikely since cells selected for liver-metastasizing potential<br>strongly bound WGA when grown in other sites (s.c., cecum;<br>data not shown) and *in vitro*.<br>We found an approximately 2-fold increase in neuramini-<br>dase-rel strongly bound WGA when grown in other sites (s.c., cecum;<br>data not shown) and *in vitro*.<br>We found an approximately 2-fold increase in neuramini-<br>dase-releasable membrane-associated sialic acid and sialyltrans-<br>ferase ac data not shown) and *in vitro*.<br>We found an approximately 2-fold increase in neuramini-<br>dase-releasable membrane-associated sialic acid and sialyltrans-<br>ferase activity associated with the metastatic colon cancer cell<br>lin We found an approximately 2-fold increase in neuramini-<br>dase-releasable membrane-associated sialic acid and sialyltrans-<br>ferase activity associated with the metastatic colon cancer cell<br>lines, compared to the poorly metas dase-releasable membrane-associated sialic acid and sialyltrans-<br>ferase activity associated with the metastatic colon cancer cell<br>lines, compared to the poorly metastatic parent. While no<br>significant difference in  $\beta$ 1-4 ferase activity associated with the metastatic colon cancer cell<br>
lines, compared to the poorly metastatic parent. While no<br>
significant difference in  $\beta$ 1-4-galactosyltransferase (lactose syn-<br>
thetase) was found betwee lines, compared to the poorly metastatic parent. While no<br>
significant difference in  $\beta$ 1-4-galactosyltransferase (lactose syn-<br>
thetase) was found between the cell lines, we cannot exclude<br>
differences in other galactos significant difference in  $\beta$ 1–4-galactosyltransferase (lactose syn-<br>thetase) was found between the cell lines, we cannot exclude<br>differences in other galactosyltransferases. Sialic acid is known<br>to be present in various thetase) was found between the cell lines, we cannot exclude<br>differences in other galactosyltransferases. Sialic acid is known<br>to be present in various linkages on a variety of *N*-linked and 8. Y<br>*O*-linked oligosacchari differences in other galactosyltransferases. Sialic acid is know<br>to be present in various linkages on a variety of *N*-linked al<br>*O*-linked oligosaccharides of glycoproteins. Glycoproteins wi<br>*N*-linked oligosaccharides c to be present in various linkages on a variety of *N*-linked and<br> *O*-linked oligosaccharides of glycoproteins. Glycoproteins with<br> *N*-linked oligosaccharides containing the nonreducing terminal<br>
sequence Gal $\beta$ 1-4GlcNA *O*-linked oligosaccharides of glycoproteins. Glycoproteins with<br> *N*-linked oligosaccharides containing the nonreducing terminal<br>
sequence Gal $\beta$ 1-4GlcNAc may serve as acceptors for the ad-<br>
dition of sialic acid cataly *N*-linked oligosaccharides containing the nonreducing terminal 9. Sequence Gal $\beta$ 1-4GlcNAc may serve as acceptors for the addition of sialic acid catalyzed by  $\beta$ -galactoside  $\alpha$ 2-6-sialyltrans-<br>ferase, and nearly sto sequence Gal $\beta$ 1-4GlcNAc may serve as acceptors for the addition of sialic acid catalyzed by  $\beta$ -galactoside  $\alpha$ 2-6-sialyltrans-<br>ferase, and nearly stoichiometric amounts of sialic acid can be<br>incorporated into asialot dition of sialic acid catalyzed by  $\beta$ -galactoside  $\alpha$ 2-6-sialyltrans-<br>ferase, and nearly stoichiometric amounts of sialic acid can be<br>incorporated into asialotransferrin by this enzyme (51). While<br>it is likely then tha ferase, and nearly stoichiometric amounts of sialic acid can be<br>incorporated into asialotransferrin by this enzyme (51). While<br>it is likely then that such a sialyltransferase is elevated in the<br>cells studied (as suggested incorporated into asialotransferrin by this enzyme (51). While<br>it is likely then that such a sialyltransferase is elevated in the<br>cells studied (as suggested by SNA affinity chromatography),<br>other sialyltransferases may a it is likely then that such a sialyltransferase is elevated in the<br>cells studied (as suggested by SNA affinity chromatography),<br>other sialyltransferases may also be responsible for increased<br>incorporation into asialotrans cells studied (as suggested by SNA affinity chromatography),<br>
other sialyltransferases may also be responsible for increased<br>
incorporation into asialotransferrin. The sequence NeuAc $\alpha$ 2-<br>
3Gal $\beta$ 1-4GlcNAc may be found incorporation into asialotransferrin. The sequence NeuAc $\alpha$ 2-<br>3Gal $\beta$ 1-4GlcNAc may be found in the *N*-linked chains of<br>certain glycoproteins (52). Although asialotransferrin has not<br>been shown to be an efficient accept 3Gal $\beta$ 1-4GlcNAc may be found in the *N*-linked chains of<br>certain glycoproteins (52). Although asialotransferrin has not<br>been shown to be an efficient acceptor for such an enzyme (51,<br>53), our data do not exclude an elev certain glycoproteins (52). Although asialotransferrin has not<br>been shown to be an efficient acceptor for such an enzyme (51,<br>53), our data do not exclude an elevation in a  $\beta$ -galactoside  $\alpha$ 2-<br>3-sialotransferase. Sinc been shown to be an efficient acceptor for such an enzyme (51,<br>53), our data do not exclude an elevation in a  $\beta$ -galactoside  $\alpha$ 2-<br>3-sialotransferase. Since sialic acid was also transferred to<br>asialo-OSM by the metasta 53), our data do not exclude an elevation in a  $\beta$ -galactoside  $\alpha$ 2-<br>3-sialotransferase. Since sialic acid was also transferred to<br>asialo-OSM by the metastatic colon cancer cells, *O*-linked<br>glycoproteins in these cells 3-sialotransferase. Since sialic acid was also transferred to<br>asialo-OSM by the metastatic colon cancer cells,  $O$ -linked<br>glycoproteins in these cells may also contain greater amounts<br>of terminal sialic acid, compared to

ANIMAL MODEL FOR COLON CANCER METASTASIS<br>
some cases are responsible for tumor-associated antigenicity STRIG CANCER METASTASIS<br>Some cases are responsible for tumor-associated antigenicity<br>(57). The gastrointestinal cancer-associated antigen recognized (57). The gastrointestinal cancer-associated antigenicity<br>(57). The gastrointestinal cancer-associated antigen recognized<br>by monoclonal antibody 19-9, for example, is a sialyl Le<sup>4</sup> blood EXAMPLE METASTASIS<br>some cases are responsible for tumor-associated antigenicity<br>(57). The gastrointestinal cancer-associated antigen recognized<br>by monoclonal antibody 19-9, for example, is a sialyl Le\* blood<br>group antigen GANCER METASTASIS<br>some cases are responsible for tumor-associated antigenicity<br>(57). The gastrointestinal cancer-associated antigen recognized<br>by monoclonal antibody 19-9, for example, is a sialyl Le<sup>4</sup> blood<br>group antigen some cases are responsible for tumor-associated antigenicity<br>(57). The gastrointestinal cancer-associated antigen recognized<br>by monoclonal antibody 19-9, for example, is a sialyl Le<sup>4</sup> blood<br>group antigen expressed as a ga (57). The gastrointestinal cancer-associated antigen recognized<br>by monoclonal antibody 19-9, for example, is a sialyl Le<sup>4</sup> blood<br>group antigen expressed as a ganglioside in tissues but found<br>in mucin-type glycoproteins i (57). The gastrointestinal cancer-associated antigen recognized<br>by monoclonal antibody 19-9, for example, is a sialyl Le<sup>4</sup> blood<br>group antigen expressed as a ganglioside in tissues but found<br>in mucin-type glycoproteins i by monoclonal antibody 19-9, for example, is a sialyl Le<sup>4</sup> blood<br>group antigen expressed as a ganglioside in tissues but found<br>in mucin-type glycoproteins in serum (58). Sialylated mucin-<br>type glycoproteins have been asso group antigen expressed as a ganglioside in tissues but found<br>in mucin-type glycoproteins in serum (58). Sialylated mucin-<br>type glycoproteins have been associated with metastatic poten-<br>tial in at least one murine colon ca in mucin-type glycoprotein<br>type glycoproteins have bee<br>tial in at least one murin<br>alkaline-labile carbohydrat<br>sialoglycoproteins have be<br>colon cancer cells (34).<br>Our findings are consis be glycoproteins have been associated with metastatic poten-<br>
I in at least one murine colon cancer cell line (17), and<br>
staline-labile carbohydrate chains on high molecular weight<br>
loglycoproteins have been described on m tial in at least one murine colon cancer cell line (17), and<br>alkaline-labile carbohydrate chains on high molecular weight<br>sialoglycoproteins have been described on metastatic human<br>colon cancer cells (34).<br>Our findings are

Fig. 6. Livers from BALB/c mice 2 weeks after intrasplenic injection of murine<br>Fig. 6. Livers from BALB/c mice 2 weeks after intrasplenic injection of murine<br>olon cancer cell lines. A, livers injection is the extent of me sialoglycoproteins have been described on metastatic human<br>colon cancer cells (34).<br>Our findings are consistent with observations from other<br>laboratories, which have demonstrated increases in total or<br>neuraminidase-releasa sialoglycoproteins have been described on metastatic human<br>colon cancer cells (34).<br>Our findings are consistent with observations from other<br>laboratories, which have demonstrated increases in total or<br>neuraminidase-releasa colon cancer cells (34).<br>
Our findings are consistent with observations from other<br>
laboratories, which have demonstrated increases in total or<br>
neuraminidase-releasable sialic acid and increases in the sialy-<br>
lation of p Our findings are consistent with observations from other<br>laboratories, which have demonstrated increases in total or<br>neuraminidase-releasable sialic acid and increases in the sialy-<br>lation of penultimate galactose residue laboratories, which have demonstrated increases in total of neuraminidase-releasable sialic acid and increases in the sialy lation of penultimate galactose residues on metastatic murine tumor cells (5, 8, 15, 17). Wheat ge neuraminidase-releasable sialic acid and increases in the sialy-<br>lation of penultimate galactose residues on metastatic murine<br>tumor cells (5, 8, 15, 17). Wheat germ agglutinin binding by<br>metastatic tumor cell variants is lation of penultimate galactose residues on metastatic murine<br>tumor cells (5, 8, 15, 17). Wheat germ agglutinin binding by<br>metastatic tumor cell variants is also, in at least some cases,<br>due to the presence of sialic acid tumor cells (5, 8, 15, 17). Wheat germ agglutinin binding by<br>metastatic tumor cell variants is also, in at least some cases,<br>due to the presence of sialic acid on terminal *N*-acetyllactosa-<br>mine structures, the loss of wh metastatic tumor cell variants is also, in at least some cases,<br>due to the presence of sialic acid on terminal *N*-acetyllactosa-<br>mine structures, the loss of which leads to decreased metastatic<br>potential (20, 50). In the due to the presence of sialic acid on terminal *N*-acetyllactosa-<br>mine structures, the loss of which leads to decreased metastatic<br>potential (20, 50). In the case of some tumor cells, overall levels<br>of membrane-associated mine structures, the loss of which leads to decreased metastatic<br>potential (20, 50). In the case of some tumor cells, overall levels<br>of membrane-associated sialic acid are not elevated, but the<br>position of sialic acid on t which sialic acid appears may be most important in determining<br>the extent of metastasis  $(9, 13, 16, 19)$ . The present study<br>further emphasizes the importance of cell membrane sialylation<br>in determining the metastatic pot position of sialic acid on terminal carbohydrate structures on 1. Nicolson, G. L. Cancer metastasis. Organ colonization and cell-surface<br>
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