Cell Surface Glycosyltransferase Activity in Normal and Neoplastic Intestinal Epithelium of the Rat

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SUMMARY

Cell surface glycosyltransferase enzymes and lectin agglutination with concanavalin A and wheat germ agglutinin were studied in normal and neoplastic rat intestinal cells. Adenocarcinomas of the large and small bowel of inbred rats were induced by weekly injections of dimethylhydrazine (10 mg/kg). Small intestinal cell surface glycosyltransferases showed higher levels in crypt and tumor cells, compared to differentiated villus cells. However, in the colon, tumor cells showed a marked decrease in cell surface glycosyltransferase activity compared to normal colon epithelial cells. Lectin agglutination studies revealed that small intestinal crypt and tumor cells, but not villus cells, were preferentially agglutinated by Concanavalin A; colon tumor cells were agglutinated by wheat germ agglutinin. These results demonstrate that neoplastic transformation of rat intestinal cells is accompanied by alterations of lectin agglutination and cell surface glycosyltransferase activity and that these alterations differ in the small and large intestine.

INTRODUCTION

Glycoproteins of the plasma membrane play an important role in cell-to-cell contact, growth regulation, and binding sites for hormones and lectins. Alterations of cell surface glycoproteins in neoplasia, such as loss of transplantation antigens and appearance of tumor-specific antigens, have been extensively studied. Certain tumor-associated glycoproteins, such as the carcinoembryonic antigen of Gold (3), are found normally in the fetal state, suggesting that their presence on tumor cells represents reversion or dedifferentiation to an embryonic type of plasma membrane structure.

In previous studies from this laboratory (14, 15) it was shown that small intestinal crypt cells, which are undifferentiated and mitotically active, could be separated from villus tip cells, which are differentiated and mitotically inactive. The intact, metabolically active crypt cells were found to have higher levels of plasma membrane glycosyltransferases capable of incorporating monosaccharides into cell membrane endogenous glycoprotein acceptors when compared to the corresponding low activities found in the differentiated villus cells.

Crypt cells were also distinguished from villus cells by their preferential agglutination with ConA (7). Human fetal intestinal cells also have plasma membrane galactosyltransferase and are agglutinated by ConA (13).

In this study we have extended our observations to the neoplastic intestinal epithelial cell in both small intestine and colon of rats. The carcinogen DMH was used to induce colon and small intestinal adenocarcinomas, which are histologically similar to human intestinal neoplasms (12). Using this model of neoplasia we were able to compare surface membrane glycosyltransferases and lectin agglutinability in normal undifferentiated (crypt) and differentiated (villus) cells with dedifferentiated (neoplastic) intestinal cells.

MATERIALS AND METHODS

Materials. Inbred ARS Fischer 344 rats were obtained from the ARS Sprague Dawley Co., Madison, Wis. DMH (symmetrical) was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. Radioactive nucleotide sugars were obtained from New England Nuclear, Boston, Mass. ConA and wheat germ acid phosphatase were purchased from Miles-Yeda, Kankakee, III. Purified WGA was prepared from wheat germ acid phosphatase by the method of Burger and Goldberg (2). DTT was purchased from Calbiochem, San Diego, Calif.

Intestinal Carcinogenesis with DMH. Male ARS Fischer 344 rats were given weekly s.c. or i.m. injections (10 mg/kg) starting at 8 weeks of age and continuing for 6 to 12 weeks. The carcinogen was dissolved in deionized water and the pH was adjusted to 6 with sodium hydroxide. No acute toxicity was observed at this dosage level and the entire intestinal tract appeared grossly normal during the injection period. The 1st tumors appeared at age 6 months and reached a diameter of approximately 1.0 cm at 8 to 12 months of age. The animals were sacrificed by rapid etherization and cardiac puncture, and the small and large intestine were removed rapidly and placed in ice-cold 0.15 M NaCl prior to cell harvesting.

Preparation of Intestinal Cell Suspensions. Suspensions of small intestinal crypt and villus cells were prepared as previously described (14). Suspensions of normal colon cells were prepared by first flushing the colon with ice-cold 0.15 M NaCl containing 1.5 mM DTT until clear. The colon was then filled with citrate buffer, pH 7.4, containing 1.0 mM EDTA.

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The epithelial cells were washed in PBS, filtered once through coarse gauze to remove larger clumps, and once through fine silk to remove aggregates of cells. To assay glycosyltransferases, the colon cells were washed once in 0.15 M NaCl: 0.1 M sodium cacodylate buffer, pH 7.4, and resuspended in the same buffer at a concentration of 1 to 5 x 10^6 cells/ml.

To prepare suspensions of neoplastic intestinal cells, tumors of small or large intestine were rinsed thoroughly in iced 0.1 M NaCl: 1.5 mM EDTA to remove adherent stool and mucus and then incubated for 10 min in Solution A at 37° and in Solution B at 37° for 10 min. The cells were removed by gentle teasing with a scalpel blade into warm Solution B. The tumor cells were washed, filtered, and suspended as for normal cells.

Glycosyltransferase Assays. Cell surface membrane glycosyltransferase enzymes were measured in intact normal and neoplastic cells by methods previously described (14). Cell suspensions (100 µl), 0.1 M manganese chloride (10 µl), and nucleotide sugars radioactively labeled in the carbohydrate portion with ^14C (10 µl) were incubated at 37° for 30 min. The concentrations and specific activities of the nucleotide sugars were as follows: UDP-galactose-^14C, 6.8 µM, 1.93 x 10^6 cpm/µmole; UDP-N-acetylglucosamine-^14C, 15.3 µM, 3.3 x 10^7 cpm/µmole; CMP-sialic acid-^14C, 7.5 µM, 2.24 x 10^8 cpm/µmole. There was no further addition of unlabelled nucleotide sugar. The reactions were terminated by addition of 3 ml cold 5% TCA and the precipitated proteins were washed with 10 ml of cold TCA and 10 ml cold absolute ethanol.

Radioactivity was counted in a toluene-based scintillation fluid (14) with a counting efficiency for ^14C of 91 to 96%. Identically prepared control tubes incubated for 0 time were subtracted from each experimental value. Protein concentrations of whole cell suspensions were determined by the method of Lowry et al. (5) using bovine serum albumin as a standard.

Agglutination Studies. Suspensions of isolated intestinal cells in PBS were adjusted to a concentration of 1 to 2 x 10^6 cells/ml. The lectins were dissolved in PBS at a concentration of 1 mg/ml and agglutination at 37° was scored as previously described (13).

RESULTS

Intestinal Tumors. DMH treatment of male inbred rats caused intestinal neoplasms in 90% of the animals by age 12 months. There were no generalized morphological changes in the intestine during the injection period or during the early latency period prior to the appearance of tumors. Approximately 10% of animals developed bowel obstruction; these animals were not used for biochemical studies. Weight loss, diarrhea, and blood in the stool were common in tumor-bearing animals. Metastases to regional lymph nodes and peritoneum occurred in 5% of animals, and squamous cell carcinomas of the external ear canal were found in 43%.

Cell Surface Glycosyltransferases in the Small Intestine. Glycosyltransferases were measured in intact cell suspensions of normal and neoplastic cells, 70 to 80% of which excluded Trypan blue. Enzyme activity was not released into the medium during the incubation. In addition, nucleotide sugars are believed not to be transported into cells (8). This evidence, together with evidence previously presented for intestinal cells (14), suggests that most of the glycosyltransferase activity measured in whole-cell suspensions represents cell surface enzyme. The normal gradient of cell surface galactosyltransferase in the small intestine was not altered in DMH-treated rats prior to appearance of tumors. Chart 1 shows a typical gradient for cells obtained from the villus and crypt zones in a DMH-treated and untreated control rat. As previously shown (15) cells from the crypt zone have a markedly increased galactosyltransferase:endogenous acceptor activity compared to cells from the villus zone. This difference is maintained in the animal treated with DMH for 8 weeks. Thus, treatment with the carcinogen did not appear to cause any generalized alteration in intestinal cell surface galactosyltransferase activity prior to appearance of tumors or in normal-appearing tissues adjacent to tumors (see below).

Several glycosyltransferases were studied in a group of small intestinal adenocarcinomas and compared to the normal crypt and villus cell populations obtained either from DMH-treated rats or from untreated controls (Chart 2). The galactosyltransferase and N-acetylglucosaminyltransferase:endogenous acceptor activities for the tumors were comparable or higher than the levels obtained for the normal crypt cell population. The levels for surface membrane sialyltransferase in the tumor cells were less consistent. In 2 tumors sialyltransferase:endogenous acceptor activity could not be detected, while in 3 tumors the levels were comparable to those normally found on the villus cell surface (data not shown).

Cell Surface Glycosyltransferase in the Large Intestine. Normal colon cells had generally higher specific activities for...
the glycosyltransferases when compared to small intestine (Chart 3). Since the colon lacks a villus structure and is composed of short crypts and a single layer of superficial epithelium it was not possible to achieve a separation of differentiated from undifferentiated epithelial cells as in the small intestine. Therefore the activities for cell surface glycosyltransferases on the tumor cell external membranes were compared to cells harvested from normal tissue adjacent to the tumors or from untreated age matched controls. As shown in Chart 3, there is a striking reduction of cell surface glycosyltransferases in 3 tumors of the rectum when compared to normal tissue. The levels for the tumors, however, were similar to those measured in the crypt and neoplastic cells of the small intestine.

**Lectin Agglutination.** Cell suspensions of the different types of small intestinal and colonic cells were tested for agglutination by ConA or WGA (Table 1). A remarkable difference was noted between small intestinal and colonic cells. WGA appeared not to distinguish cells of villus, crypt, or tumor cell origin in the small intestine and agglutinated all 3 cell types. However, ConA markedly agglutinated small intestinal crypt and tumor cells but had little effect on the differentiated villus cell. Colonic cells had a different pattern of agglutination than did small intestinal cells. The normal colonic cell was not agglutinated by either WGA or ConA, while the colonic tumor cell was agglutinated by WGA and not by ConA. Thus, small intestinal tumor cells appear to be preferentially agglutinated by ConA, while colonic tumor cells are preferentially agglutinated by WGA.

**DISCUSSION**

Evidence has been previously presented from this laboratory (14, 15) that rat intestinal cells have cell surface glycosyltransferases that transfer monosaccharides from nucleotide sugars to incomplete glycoprotein acceptors located on the cell surface. As intestinal cells become differentiated during their migration from crypt to villus, the enzyme activities for galactosyl- and N-acetylglucosaminyltransferase are strikingly reduced, while sialyltransferase activity is increased. In a recent study (8) we showed a correlation between ConA agglutination of various cells and the level of surface membrane galactosyltransferase. Agglutinable cells, such as the rabbit erythrocyte, rat intestinal crypt cell, and TA3-ST ascites cells had significantly higher surface galactosyltransferase activity than did nonagglutinable cells, such as human erythrocytes, intestinal villus cells, or TA2H ascites cells. Furthermore, purified rabbit erythrocyte membrane galactosyltransferase could impart ConA agglutinability to nonagglutinable human erythrocytes. Thus in several cell types there appears to be a correlation of cell surface galactosyltransferase with the degree of differentiation and with ConA agglutinability.

**Table 1**

Agglutination of normal and neoplastic intestinal cells by WGA and ConA

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Whole cell suspensions (in volume of 100 μl PBS) were mixed with the lectins as described in "Materials and Methods" and agglutination was scored under the microscope. Data are the pooled results of 3 to 5 separate observations for each group. The concentration of ConA and WGA was 1 mg/ml.
Our data suggest that the plasma membrane of small intestinal tumor cells resembles the normal crypt cell in that both cell types have high galactosyl- and \( N \)-acyethylglucosaminyltransferase activities. On the other hand, sialyltransferase activity, which is higher in the differentiated villus cell plasma membrane, was quite variable in small intestinal tumors. Further similarity between small intestinal crypt and tumor cells was demonstrated by ConA agglutination of crypt and tumor cells, but not the villus cells. Thus, the plasma membranes of the undifferentiated small intestinal crypt cells and adenocarcinomas produced by DMH are similar as regards glycosyltransferase activity, availability of "incomplete" endogenous acceptors, and agglutinability by ConA. In the rat colon, however, we found reduced activity of galactosyl-, \( N \)-acyethylglucosaminyl-, and sialyltransferase in the malignant cells as compared to normal cells. Interpretation of this finding is made difficult by lack of an effective method to separate the mitotically active colonic crypt cells from the mature surface epithelium, although we did develop such a separation for small intestinal cells. Hence the normal control population of colon cells is composed of a mixture of mitotically active undifferentiated cells and nondividing, mature cells. It is possible that the decrease of surface transferase activity in the colon tumor cells might reflect a reduction of mitotic activity in the tumors. This explanation seems unlikely in view of autoradiographic studies by others which show an increased cellular proliferation in DMH-induced colon cancers (12). Another explanation is that the colon cancer cell regresses to a fetal state wherein the glycosyltransferase levels are similar along the entire length of the gut. This concept is supported by our finding that small and large intestinal tumors have similar levels of galactosyl- and \( N \)-acyethylglucosaminyltransferase: endogenous acceptor activity.

Furthermore, alterations of the membrane glycoprotein in these cell populations were implied by the differences in lectin agglutinability in normal and neoplastic cells of small and large intestine. The agglutination of small intestinal crypt and tumor cells by ConA is consistent with the observation by others that normal tissue culture cells in mitosis share with transformed cells the capacity to be agglutinated by ConA (11). In the colon, however, only WGA is able to agglutinate tumor cells. Moscona (6) showed that embryonic liver and neural retinal cells were strongly agglutinated by ConA but not WGA. When the same cell lines were treated with trypsin the cells were still strongly agglutinated by WGA, while ConA agglutination was diminished. This was interpreted as showing that the WGA receptor site is buried in the membrane in both fetal and adult cell lines and is unmasked in both the fetal and neoplastic state. This situation may also obtain in the intestinal tract, since human fetal intestinal cells are agglutinated by ConA but not WGA (13).

The function of intestinal cell surface glycosyltransferases is unknown. Roth, McGuire, and Roseman (9) have postulated that surface glycosyltransferases have a role in cellular recognition and adhesion of neural retina cells. Roth and White (10) presented evidence that a surface galactosyltransferase on mouse embryo cells in tissue culture may be involved in contact inhibition of growth. Bosmann et al. (1) have recently shown that surface glycosyltransferases were higher in melanoma cells with increased rates of metastases as compared to a nonmetastasizing melanoma cell line. We have recently shown (4) that the blast transformation of neonatal rat lymphocytes by ConA is accompanied by a marked increase in cell surface galactosyltransferase. Further studies are in progress to determine the significance of surface glycosyltransferases in various cell populations and their relationship to growth and differentiation.

REFERENCES

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