Carcinoma-related Alterations of Glycosyltransferases in Human Tissues

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INTRODUCTION

Glycosyltransferases catalyze the addition of sugars to glycolipids and glycoproteins. Although their physiological roles are uncertain, these macromolecules are significant components of membranes, as are their biosynthetic enzymes (12, 20). The ABO(H) blood group phenotypes are designated by terminal nonreducing sugars on such molecules, and their synthesis is catalyzed by specific glycosyltransferases (10, 39). Specific ABO(H) blood group phenotypes can be expressed only in the presence of their designated glycosyltransferases, which are the actual products of the blood group genes. Alterations in ABO antigens and in their transferases, which are the actual products of the blood group genes, have been reported (2, 8, 13, 15, 22, 25, 33, 34, 37). The customary picture has been one of deficiencies or losses, coupled with continued biosynthesis of precursor molecules (27). Exceptions to this pattern have been reported (2, 19, 24, 31).

The potential importance of these markers in cancer diagnosis or prognosis is recognized but may not be fully realized until precise relationships to normal cell structure and function have been established. Unique characteristics of specific normal tissues can then be made available as criteria to evaluate their transformed counterparts.

In the present study, we report results of A, B, and H glycosyltransferase assays carried out on 23 primary carcinomas from various anatomic locations and on their normal tissue counterpart. Deficient enzyme patterns in relation to the normal characterized most, but not all, tumor tissue. The results of enzyme assays on normal mucosa from diverse tissues confirm earlier studies of the variable distribution of blood group substances.

MATERIALS AND METHODS

Procurement of Tissue

Tissues were obtained fresh at New York Veterans Administration Hospital and at New York University and Bellevue Center Hospitals from surgical operative procedures. All cases selected were considered to be primary tumors. In most cases, malignant tissue was surrounded by a zone of normal tissue, thus permitting histological comparisons between tumor cells and normal cells in the same patient. Normal tissues were obtained from autopsied material from accident victims.

Tissues Prepared for Enzyme Studies

Tissues were washed with cold phosphate buffer, pH 7.0, and tumor tissue was carefully dissected away from normal adjacent tissue. The distinctions were established on the basis of biopsies and microscopic observation in order to determine boundaries and to minimize contamination of either normal or carcinomatous tissue by unwanted cell populations. The mucosal epithelial portions of normal and diseased tissue were dissected away from the remaining muscularis and serosal portions. Tissues were immediately frozen in dry ice and stored at -70°C until used. One-g quantities of thawed tissue maintained at 2-4°C were broken with 10 to 12 strokes of a Dounce homogenizer in 10 volumes of 0.1% Triton X-100 (3) with 0.001 M EDTA and 0.014 M mercaptoethanol buffered at pH 7.0. The mixture was stirred overnight at 4°C and then centrifuged at 30,000 × g for 1 hr (3). The supernatant from this spin was decanted, lyophilized, and made up in solution to contain 200 mg of lyophilized material per ml. Protein determinations using the technique of Lowry et al. (32) and enzyme assays were carried out on these samples.

Radioactive Nucleotide Sugars and Low-Molecular-Weight Acceptors

UDP-N-acetyl[14C]galactosamine (43 Ci/mol) and UDP-α-[14C]galactose were purchased from New England Nuclear,
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Frankfurt, Germany, and GDP-[\(\text{\textsuperscript{14}C}\)]fucose (170 Ci/mol) was obtained from the Radiochemical Centre, Amersham, England. The low-molecular-weight blood group acceptor 2'-fucosyllactose used for A and B transferase assays [\(\alpha\text{-L-fucosyl(1} \rightarrow \text{2)}\) \(\beta\text{-D-galactosyl(1} \rightarrow \text{4)}\)-D-glucoside] was isolated from human milk (26). The low-molecular-weight acceptor for H transferase assays, phenyl-\(\beta\)-D-galactopyranoside (6), was obtained from the Sigma Chemical Co. The products were visualized with alkaline silver nitrate (38).

Assays for Glycosyltransferases

A and B Transferases. The \(\alpha\)-3-N-acetylgalactosaminyltransferases (A enzymes) were assayed at both pH 6 and pH 8 to provide the conditions required for the differentiation of the \(A_1\) and the \(A_2\) gene-specified transferases (35); the \(\alpha\)-galactosyltransferase (B enzyme) was assayed at pH 6.5 (5); each assay utilized its respective nucleotide \(\text{\textsuperscript{14}C}\)-sugar in the presence of 2'-fucosyllactose and Mn\(^{2+}\) (39). All mucosal extracts were tested immediately following their reconstitution from the frozen or lyophilized state. At the end of the incubation, the reaction mixtures were streaked onto Whatman No. 40 filter paper and dried, and the total radioactivity was measured in the liquid scintillation counter. Neutral sugars were removed from charged components by resolution on ion-exchange papers as described (35). The neutral sugars were chromatographed on Whatman No. 40 filter paper in Solvent A [ethyl acetate:pyridine:water (2:1:2, by volume) upper phase]. Under these conditions, residual isotope-labeled nucleotide sugar (UDP-N-acetyl[\(\text{\textsuperscript{14}C}\)]galactosamine) was readily separated from acceptor-sugar product. Radioactive peaks were detected with a radiochromatogram scanner and counted in a liquid scintillation counter. The At and A2 enzymes transfer N-acetyl-D-\(\text{\textsuperscript{14}C}\)galactosamine to the acceptor substrate 2'-fucosyllactose to give a tetrasaccharide product with an flecto\(\text{\textsubscript{4}}\) of 0.5 in Solvent A. The radioactive incorporation into the tetrasaccharide product was expressed as nmol radioactive sugar incorporated into product (6).

B Transferase. The \(\alpha\)-galactosyltransferase (B enzyme) was assayed in mucosal extracts as described above except that assays were performed using buffer at pH 6.5. The B enzyme transfers D-galactose from nucleotide to the acceptor substrate 2'-fucosyllactose to give a tetrasaccharide product with \(R_{\text{f} \text{act}\text{ose}}\) of 0.4 in Solvent A (5).

H Transferase. The \(\alpha\)-2-L-fucosyltransferase (H enzyme) was assayed as described by Chester et al. (6). Details of the reaction mixtures and incubation times are given in the charts. The radioactive products were chromatographed on Whatman No. 40 paper in Solvent B [ethyl acetate:pyridine:water (10:4:3, by volume)], a mixture which permitted separation of isotope-labeled components into residual GDP-N-\(\text{\textsuperscript{14}C}\)fucose and acceptor-sugar product. The product of [\(\text{\textsuperscript{14}C}\)]fucosyl transfer to the acceptor substrate phenyl-\(\beta\)-D-galactoside has an \(R_{\text{f} \text{act}\text{ose}}\) of 1.54 in this solvent. The radioactive peaks were detected and measured as described for the A transferase assays, and the results were expressed as the percentage of the total recovered radioactivity incorporated into the low-molecular-weight acceptor (6) or as nmol radioactive sugar incorporated into acceptor substrate.

Glycosidases

\(\alpha\)-L-Fucosidase and \(\beta\)-D-galactosidase activities of mucosal extracts were assayed with \(p\)-nitrophenyl-\(\alpha\)-L-fucopyranoside and \(p\)-nitrophenyl-\(\beta\)-galactopyranoside, respectively. The nitrophenyl glycosides (0.33 M; 50 \(\mu l\)) were incubated for 2 hr at 37\(^\circ\)C with 50 \(\mu l\) of serum or of tissue extract and 500 \(\mu l\) of 0.1 M Tris-HCl buffer, pH 7.2. At the end of the incubation time, the reaction was stopped by the addition of 1.5 ml of sodium bicarbonate, and the absorbance was read in 1-cm light-path cells at 420 nm in a Unicam AP.600 spectrophotometer. To determine whether the substrate for the H transferase assay, phenyl-\(\beta\)-D-galactopyranoside, was being broken down under the conditions used to assay the \(\alpha\)-2-fucosyltransferase, 5.0 nmol of this substrate were incubated for 5 hr with 20 \(\mu l\) of tissue extract plus ATP, Tris-HCl buffer (pH 7.2), and NaN\(\text{\textsubscript{3}}\) at the concentrations used in the reaction mixture for the H transferase assay (see legends to tables and charts). The product of [\(\text{\textsuperscript{14}C}\)]fucosyltransfer, phenyl-2-O-(\(\alpha\)-L-[\(\text{\textsuperscript{14}C}\)]galactopyranosyl-\(\beta\)-D-galactopyranoside was similarly incubated with 20 \(\mu l\) of tissue extract and the other constituents of the reaction mixture except GDP-[\(\text{\textsuperscript{14}C}\)]fucose. At the end of the incubation time, the reaction products were examined by descending chromatography on Whatman No. 40 paper in ethyl acetate:pyridine:water (10:4:3, by volume) for 5 hr. The products obtained with phenyl-\(\beta\)-D-galactoside were visualized with alkaline silver nitrate (38). The radioactive products obtained with phenyl[\(\text{\textsuperscript{14}C}\)]fucosyl-\(\beta\)-galactoside were detected with a radiochromatogram scanner and counted in a liquid scintillation counter. To determine whether the substrate for the A and B transferase assays, 2'-fucosyllactose, was broken down under assay conditions, reaction mixtures were prepared as described, the tetrasaccharide product was incubated with mucosal extract and the other constituents of the reaction mixture used for A or B enzyme assays except that UDP-galactose or UDP-N-acetylgalactosamine was omitted. Chromatography was then carried out as described above using ethyl acetate:pyridine:water (2:1:2, by volume). The products were visualized and quantitated as described.

Incorporation of Radioactive Sugars into Endogenous Acceptors in Mucosal Extracts

To test whether there was competition between incorporation of [\(\text{\textsuperscript{14}C}\)]fucose from GDP-[\(\text{\textsuperscript{14}C}\)]fucose into endogenous acceptor substrates and the low-molecular-weight acceptor used in the H transferase assay, incubation mixtures were set up with and without phenyl-\(\beta\)-D-galactoside. The reaction products were examined by descending chromatography on Whatman No. 40 paper in Solvent C [propan-1-ol:ethyl acetate:pyridine:water (5:1:1:3, by volume)] for 16 hr. In this solvent, the neutral endogenous products remain on the origin, whereas GDP-fucose had an \(R_{\text{f} \text{act}\text{ose}}\) of 0.42 and phenyl-2-O-(\(\alpha\)-L-[\(\text{\textsuperscript{14}C}\)]galactosyl-\(\beta\)-D-galactoside had an \(R_{\text{f} \text{act}\text{ose}}\) of 1.2.

In the case of A and B transferase assays, incubation mixtures were set up with and without 2'-fucosyllactose. Descending chromatography was carried out as described above to separate neutral endogenous products from UDP-N-acetylgalactosamine and its tetrasaccharide products (\(R_{\text{f} \text{act}\text{ose}}\) 0.5 in Solvent A) or from UDP-D-galactose and its tetrasaccharide product (\(R_{\text{f} \text{act}\text{ose}}\) 0.4 in Solvent A).
RESULTS

Distribution of Glycosyltransferases in Extracts Derived from Normal Mucosae of the Gastrointestinal Tract. Normal mucosa was available from a Group O and a Group A individual from the following locations: esophagus; stomach; jejunum; cecum; ascending, transverse, and descending colon; and rectum. Levels of distribution of α-2-fucosyltransferase (H enzyme) ranged from 2.1 to 82.2 nmol per mg protein per hr, being highest in Group A stomach mucosa (82.2 nmol) and lowest in rectal mucosa (2.1 to 2.6 nmol). The discrepancy between H enzyme content in Group O and A mucosae was large in stomach (O = 25.1 nmol and A = 82.2 nmol) but negligible in colorectal mucosae (in rectal mucosa O = 2.1 nmol and A = 2.6 nmol) (Chart 1). N-Acetylgalactosaminyltransferases (A enzymes) from mucosae of individuals with the corresponding erythrocytic blood group showed a distribution pattern similar to that of H enzyme. Thus, A enzyme values at pH 6 and pH 8, respectively, were 60.6 and 28.5 nmol in stomach and 0.3 and 0.5 nmol in rectal mucosa.

Comparisons of Glycosyltransferases Derived from Mucosal Extracts of Tumor and Normal Adjacent Tissue. Tumor and normal adjacent mucosae were available from 23 cases of carcinoma in mouth (2 cases), tongue, larynx (2 cases), esophagus, lung, stomach, cervix, and lower gastrointestinal tract (14 cases) (Charts 2 and 3). The erythrocytic groups of these patients were distributed as follows: Group O, 11; Group A, 9; Group B, 3. The distribution of H enzyme was found to parallel that found in normal individuals (range: tumor extracts, 0.5 to 70.3 nmol; normal adjacent tissue extracts, 0.5 to 51.6 nmol), the highest levels being found in stomach (70.3 nmol in tumor, 51.6 nmol in normal adjacent tissue) and the lowest level being found in rectum (0.5 nmol). Although significant differences were frequently observed between H enzyme in normal versus cancer tissue, exceptions were noted. In stomach extracts (Case 8), H enzyme derived from tumor exceeded that found in normal adjacent tissue. In cases of colorectal carcinoma, exclusive of rectum and sigmoid, significantly lower H enzyme values were observed in tumor in contrast to normal tissue (Cases 10 to 19). Normal H enzyme values were low in tissue derived from sigmoid and rectum and thus afforded a less satisfactory basis for tumor versus normal comparisons, but in most instances normal values were superior to those obtained from tumor tissue extracts. Exceptions were noted in Cases 20 and 21 (normals, 0.5 and 0.5 nmol, respectively; tumor extracts, 0.8 and 0.5 nmol, respectively). Nine carcinomas occurred in Group A individuals (Cases 2, 3, 12, 13, 16, 18, 19, 22, and 23). Tumor tissue extracts proved deficient in A enzymes relative to normal adjacent tissue in carcinomas of mouth, tongue, ascending colon, and transverse colon. A enzymes were not demonstrated in normal or tumor tissue in descending colon, sigmoid, and rectum. Three carcinomas occurred in Group B patients (Cases 4, 6, and 17). Carcinoma tissue extracts from carcinomas of larynx and esophagus contained smaller quantities of B enzymes than did normal adjacent tissue extracts. Normal and tumor extracts derived from a carcinoma of the sigmoid contained no demonstrable B enzyme.
Incorporation of \[^{14}C\]Fucose into Endogenous Acceptors in Tumor Tissue Extracts and in Normal Adjacent Tissue in Carcinoma Patients. Enzyme assay mixtures were incubated with and without the exogenous acceptor, phenyl-\(\beta\)-galactoside, in order to test the possibility that competition existed between incorporation of \[^{14}C\]fucose from GDP-[\[^{14}C\]fucose into endogenous acceptor substrates and the phenyl-\(\beta\)-galactoside used in the H enzyme assay. The products were examined by chromatography in Solvent C which separated endogenous high-molecular-weight substances from other components of the reaction mixture including GDP-[\[^{14}C\]fucose. The level of endogenous incorporation of \[^{14}C\]fucose with tumor and normal tissue extracts was similar in the presence of phenyl-\(\beta\)-galactoside and in the absence of this acceptor (Chart 2). The low-molecular-weight acceptor therefore appeared to be competing very effectively with the endogenous high-molecular-weight acceptors for the \[^{14}C\]fucose transferred from GDP-[\[^{14}C\]fucose. The decreased levels of \(\alpha\)-2-\(\beta\)-fucosyltransferase activity in the tumor tissue extracts from carcinoma patients were therefore not related to the inability of phenylgalactopyranoside to compete with an increased level of endogenous acceptors.

The low-molecular-weight acceptor assay involves ethanol precipitation of the incubation mixture. Assays carried out on washed precipitates derived from incubation mixtures prepared from normal and tumor tissue extracts failed to show any significant differences. The low levels of radioactivity in the precipitates indicated that this precipitable portion of the incubation mixture possessed little or no endogenous acceptor.

Effects upon Normal H Enzyme Activity of Mixtures of Normal or Tumor Tissue Extracts. Assay mixtures were prepared which contained equal amounts of normal or tumor extract and normal Group O serum. The latter serum diluted with an equal quantity of 0.1 M Tris-HCl buffer at pH 7.2 served as a positive control. Tests of mixtures prepared with either normal extract or tumor extract failed to demonstrate suppression of enzyme in comparison with normal serum controls. It was concluded from these findings that these extracts did not possess an inhibitor for normal H enzyme expression.

\(\alpha\)-L-Fucosidase and \(\beta\)-o-Galactosidase Activities in Normal and Carcinomatous Tissue Extracts. Normal and carcinoma tissue extracts were tested for \(\alpha\)-L-fucosidase activity on the phenyl-2-o-[\[^{14}C\]fucosyl-\(\beta\)-o-galactoside acceptor-sugar product formed in the H transferase assays. The radioactive acceptor-sugar complex used in each assay (1100 cpm/3 nmol) was derived by chromatographic separation of labeled products from reaction mixtures and then eluted in water and concentrated. Under conditions used in the standard assay, no liberation of \[^{14}C\]fucose was observed when extracts of tumor and normal adjacent tissues from the following sources were tested: larynx, tongue, esophagus, stomach, cervix, cecum, ascending and transverse colon, sigmoid and rectum. Likewise, free \[^{14}C\]fucose proved to be a minor component of H enzyme incubation mixtures (Chart 3). Confirmatory results were obtained in tests of normal and tumor extracts using p-nitrophenoxy-\(\alpha\)-L-fucoside and p-nitrophenyl-\(\beta\)-o-galactoside as substrates. The results indicated that low levels of \(\alpha\)-2-fucosyltransferase demonstrated in tumor extracts and in extracts from lower bowel mucosa were not caused by breakdown of acceptor-sugar product by fucosidase or by galactosidase.

Tests of extracts on phenyl-\(\beta\)-o-galactoside acceptor itself failed to demonstrate any breakdown of this product since it functioned normally in enzyme assays following preincubation with tumor extracts. In these experiments, separate acceptor-extract mixtures were incubated, and assays were carried out using normal and carcinoma tissue extract, or acceptor alone was incubated. Acceptor which was derived chromatographically from each incubation was utilized in H enzyme assays with a serum which in prior tests had been shown to contain a positive inhibitor for normal H enzyme expression. Tests of mixtures prepared which contained equal amounts of normal or tumor extract and normal Group O serum. The latter serum diluted with an equal quantity of 0.1 M Tris-HCl buffer at pH 7.2 served as a positive control. Tests of mixtures prepared with either normal extract or tumor extract failed to demonstrate suppression of enzyme in comparison with normal serum controls. It was concluded from these findings that these extracts did not possess an inhibitor for normal H enzyme expression.
normal adjacent tissue in patients of blood groups A, B, and O. Exceptions in the 23 cases studied were noted in the one case of carcinoma of the stomach and in 2 of 7 cases of carcinoma of the rectum and sigmoid. In 4 of 9 patients (carcinoma of the mouth, tongue, ascending and transverse colon), N-acetylgalactosaminyltransferases (A enzymes) were demonstrated but were deficient in relation to the normal adjacent counterpart. A enzymes were not demonstrable in normal and tumor extracts from distal colon in 5 cases. Differences between tumor extracts and normal adjacent tissue were noted in α-galactosyltransferase (B enzyme) derived from carcinomas of larynx and esophagus (Cases 4 and 6), but B enzyme was not demonstrated in tumor or normal adjacent tissue derived from the sigmoid colon (Case 17). The cases of Groups A and B, although numerically small, reflected the abnormal change observed for H enzyme. Histological studies for the distribution of tissue blood group phenotypes by the method of specific RBC adherence confirmed these findings. High percentages of tumor cells (70 to 90%) could be identified, but in most cases mitotic figures approximated those found in normal adjacent tissue, suggesting by this parameter that these tumors were not in a phase of rapid growth.

The differences either in tissue enzyme distribution of in normal versus tumor tissue extracts could not be accounted for on the basis of endogenous inhibitors functioning as competitors for substrate (Chart 2). The occurrence of free radioactive sugars in low amounts relative to product and residual nucleotide sugars suggested that, under the conditions used, glycosidases did not play a significant role in the observed differences (Chart 3). The results of mixing experiments showed little or no difference between normal and tumor extracts upon H enzyme expression. Consistent with the finding of diminished colonic blood group enzyme has been our observation that cultured colonic cell extracts were deficient in glycosyltransferases, judged from their inability to synthesize blood group antigens in the presence of specific nucleotide sugars and low-molecular-weight acceptors.

Alterations in the composition and properties of plasma membrane are common sequelae of cell transformation by carcinogens (29, 36). This is reflected in changes of glycolipids and glycoproteins which are major membrane constituents (7, 11, 29, 36). The changes could be due to decreased synthesis or increased degradation and in some cases increased synthesis (7, 11, 30). Decreases in glycosyltransferases have been observed frequently along with decreases in complex forms of glycoconjugates (4, 8, 17, 25, 27, 37). This does not appear to be a uniform finding in cancers (2, 19, 24, 31), particularly those characterized by high growth rates (2, 24).

These studies did not establish the presence of tumor-associated A-like antigen in stomach or colonic tissue. In cases studied by Hakomori et al. (16), some tumor mucosae were found to contain Forsman A-like glycolipid in contrast to normal adjacent mucosa which was Forsman negative. Presumably, such tumor mucosa contains the glycosyltransferase which catalyzes the addition of N-acetylgalactosamine to globoside. It is not yet clear whether this is related to the fetal A antigen recently described in colonic tumors (9).

The present findings have established that the capability for A and H antigen synthesis by normal mucosa from the distal colon was low in comparison with that of the stomach judging from the distribution of H enzyme. This is consistent with the observations of Hartman (18) that ABO antigens in colon were diminished in relation to their levels in the upper gastrointestinal tract. Confirmation of these results would raise the possibility that cells differ with regard to the potential for expressing phenotypes and that such differences may be of importance in antigenic recognition and cell-to-cell adhesion.

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REFERENCES


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