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Elevation of Glycoprotein Galactosyltransferase Activity in Human Lung Cancer Related to Histological Types[1]

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ABSTRACT

Uridine diphosphogalactose:glycoprotein galactosyltransferases were examined in human lung adenocarcinoma and squamous cell carcinoma. The galactosyltransferase activities in tissue homogenates from both carcinomas were higher than in adjacent normal control with asialoagalactofetuin as a substrate. This activity in adenocarcinoma (27 cases) was two times higher than that in squamous cell carcinoma (19 cases) with statistical significance ($p < 0.001$). Using Triton-solubilized enzymes from a particulate fraction, similar differences in the activity were observed with ovalbumin, asialoagalactofetuin, and its β-eliminated derivative as acceptors but not with bovine submaxillary mucin. These observations mean that the higher activity of galactosyltransferase(s) in lung carcinomas (especially in adenocarcinoma) is mainly responsible for galactosylation of carbohydrate chains in *N*-glycoside-type but not *O*-glycoside-type glycoproteins.

INTRODUCTION

Glycosyltransferases have been investigated for elucidation of metabolic deviations in neoplastic changes and for diagnostic purposes in cancer (2, 6, 15, 17). A cancer-associated GT[3] isoenzyme in serum has been demonstrated to be increased in human cancers of various organs (24), and some properties of the isolated isoenzyme were reported (23). Elevated activity of GT was also demonstrated in human ovarian (4, 8) and bladder (21) cancer tissues.

However, there are few reports on GT or other glycosyltransferases studied in relation to the histology of the carcinoma in an organ. It is known that there are several histological types in human lung cancer. Previous investigations in this laboratory have demonstrated alterations in contents of glycosaminoglycans (12, 13) and glycolipids (30) and in the level of the cerebroside sulfotransferase activity (9) which correlated with histological types of human lung cancer.

Of interest would be to compare the glycosyltransferase activities of adenocarcinoma with other carcinoma types, because normal glandular tissues produce secretory glycoproteins and would be assumed to have higher activity levels of glycosyltransferases as required for glycoprotein synthesis. If adenocarcinoma still expresses these differentiated properties, then an adenocarcinoma tissue would also possess higher levels of glycosyltransferase activities when compared to other carcinoma types.

In this paper, GT level was assayed in histologically different human lung cancers, and some properties of GT solubilized from tissues are reported.

MATERIALS AND METHODS

Materials. Glycoproteins differing in their linkage region between protein and carbohydrate moieties were used as acceptors. They were ASGF, having both *N*- and *O*-glycosidic saccharides; ASGF-βE and OVA, having *N*-glycosidic saccharides; and ASBSM, having *O*-glycosidic saccharides. Fetuin (type III), OVA (type VII), and BSM (type I) were purchased from Sigma Chemical Co., St. Louis, Mo. ASGF and ASBSM were prepared according to the previous method (27). To obtain fetuin derivative (ASGF-βE), having only *N*-glycosidically linked carbohydrate chains, ASGF was subjected to β-elimination reaction by the procedure described previously (7). When ASGF-βE was assayed for hexosamine (5) in relation to the structure of fetuin, 1 mg of ASGF-βE, as determined as protein, contained about 100 nmol of *N*-acetylglucosamine as acceptor sites. Ganglioside G_{M2} and its asialo derivative, G_{A2} were prepared from Tay-Sachs brain (14). UDP-D-[*U*-^{14}C]galactose (274 Ci/mol) was obtained from New England Nuclear, Boston, Mass.

Tumor and Normal Tissues. Human lung tissue obtained at surgery was separated into tumor and the macroscopically normal portions. The tumors were characterized histologically as described previously (30). Histology of individual tumors used in this study is described in the legend to Chart 1.

Enzyme Preparation. Tumor and normal lung tissue adjacent to tumor were homogenized into 0.25 M sucrose and centrifuged at 500 × *g* for 10 min as described previously (10). The supernatant (Fraction S_1) was spun at 105,000 × *g* for 1 hr to obtain a membrane particulate fraction (Fraction P) and a supernatant (Fraction S_2).

Solubilization of Enzyme. To the Fraction P pooled from tumors of the same histological type was added Triton X-100 to a final concentration of 2%. The mixture was sonicated with a Kontes sonicator (10 kHz) for 2 min, stirred on ice for 30 min, and spun at 105,000 × *g* for 1 hr. The supernatant was designated solubilized enzyme.

Enzyme Assay. Incubation mixture for the assay of GT

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[3] The abbreviations used are: GT, galactosyltransferase; ASGF, fetuin free of terminal sialic acid and penultimate galactose (asialoagalactofetuin); ASGF-βE, β-eliminated asialoagalactofetuin; OVA, ovalbumin; ASBSM, bovine submaxillary mucin free of terminal sialic acid (asialo bovine submaxillary mucin); BSM, bovine submaxillary mucin; G_{M2}, ganglioside G_{M2} [Tay-Sachs ganglioside; *N*-acetylgalactosaminyl $\xrightarrow{\beta}$ (*N*-acetylneuraminyl) $\xrightarrow{\alpha}$ galactosyl $\xrightarrow{\beta}$ glucosyl $\xrightarrow{\beta}$ ceramide]; G_{A2}, asialoganglioside G_{M2} (Tay-Sachs globoside; *N*-acetylgalactosaminyl $\xrightarrow{\beta}$ galactosyl $\xrightarrow{\beta}$ glucosyl $\xrightarrow{\beta}$ ceramide); G_{M1}, ganglioside G_{M1} [galactosyl $\xrightarrow{\beta}$ *N*-acetylgalactosaminyl $\xrightarrow{\beta}$ (*N*-acetylneuraminyl) $\xrightarrow{\alpha}$ galactosyl $\xrightarrow{\beta}$ glucosyl $\xrightarrow{\beta}$ ceramide]; G_{A1}, asialoganglioside G_{M1} (galactosyl $\xrightarrow{\beta}$ *N*-acetylgalactosaminyl $\xrightarrow{\beta}$ galactosyl $\xrightarrow{\beta}$ glucosyl $\xrightarrow{\beta}$ ceramide).

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activity contained 0.8 to 1.5 mg of acceptor glycoprotein, 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.0), 10 mM $MnCl_2$, 50 nCi of UDP-[*U*-^{14}C]galactose (5 nmol), and enzyme protein with or without Triton X-100 in a final volume of 100 μl. The mixture was incubated at 37° for 30 or 60 min. The reaction was terminated by boiling for 3 min after the addition of 70 μl of bovine serum as carrier proteins. When BSM or ASBSM was used as an acceptor, acetone:50% trichloroacetic acid (4:1, v/v) was added to the assay mixture to terminate the reaction. The precipitates obtained by boiling or acetone:trichloroacetic acid treatment were washed 3 times with 5 ml each of water or acetone, respectively. To the precipitates, 6 ml of a toluene:Triton X-100 (2:1, v/v) cocktail were added, and the radioactivity incorporated into the precipitates was counted in a liquid scintillation spectrometer. The synthesis of G_{M1} and G_{A1} from G_{M2} and G_{A2}, respectively, was assayed by the previous method (16). In all the experiments, enzyme activities are expressed by averages in duplicate determinations. Protein was determined by the method of Lowry *et al.* (20).

RESULTS

Conditions for GT Assay. For these experiments, ASGF as acceptor and Fraction S_1 as enzyme were used. The GT activity was proportional to the amounts of enzyme protein up to about 200 (adenocarcinoma) or 400 μg (squamous cell carcinoma and normal control) and to the time of incubation up to about 60 min. The reaction proceeded proportionally with the concentrations of ASGF and UDP-galactose. Enzyme (180 μg protein) from adenocarcinoma was saturated at 0.8, 0.5, 1.4, and 0.4 mg of ASGF, ASGF-βE, OVA, and BSM (or ASBSM), respectively, giving an apparent K_m 1.3 × 10^{-4} M with ASGF-βE. Mn^{2+} was required for GT reaction, giving 10 mM for the optimum concentration. Triton X-100 at 0.2 to 2% stimulated the GT activity of membrane-bound enzyme (Fraction P) about 1.5 to 2 times the activity in the absence of the detergent.

GT Activities in Tumors. Chart 1 shows GT activities toward ASGF as an acceptor in human lung tumors from individual patients. GT activities in all the primary lung tumors examined were elevated when compared to those in the normal tissues from the same individuals irrespective of histological types and difference of assay conditions, with the exception of Tumor 911 (adenosquamous carcinoma). The results of statistical analysis of the enzyme activities are summarized in Table 1.

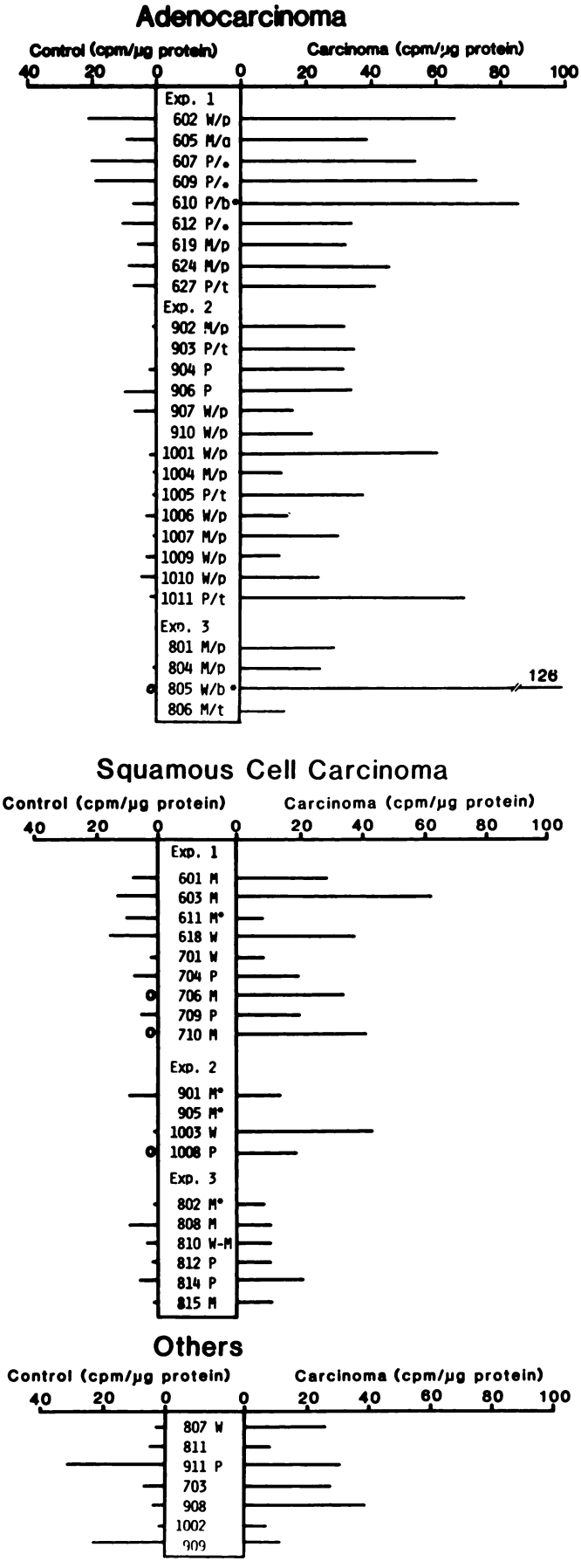

Chart 1. UDP-galactose:ASGF GT in individual human lung tumors. In Experiment 1, GT activity was assayed with 0.8 mg protein of ASGF and about 100 μg protein of Fraction S_1 without detergent for 60 min. Experiment 2 was the same as Experiment 1 except that the lot of ASGF was different. In Experiment 3, Fraction P was assayed for GT in the presence of 2% Triton X-100 for 30 min with 0.8 mg of ASGF. *W*, *M*, and *P*, after each tumor number, indicate well-, moderately, and poorly differentiated carcinomas, respectively. *a*, *b*, *p*, and *t* indicate histological subtypes of acinar, bronchioloalveolar, papillary, tubular adenocarcinomas, respectively. In the tumors indicated by •: *607*, adenocarcinoma with predominance of large-cell carcinoma; *609*, adenocarcinoma with predominant area of giant cells; *610*, adenocarcinoma of mucus-secretory bronchioloalveolar, goblet cell type; *612*, adenocarcinoma with marked desmoplasia; *805*, adenocarcinoma of bronchioloalveolar, goblet cell type; *611*, squamous cell carcinoma with predominant feature of small-cell type; *901*, squamous cell carcinoma with partially small-cell type; *802* and *905*, squamous cell carcinoma of peripheral type. In other carcinomas: *807*, colon adenocarcinoma metastasized to lung; *811*, mucoepidermoid carcinoma of low-grade malignancy; *911*, adenosquamous carcinoma; *703*, large-cell carcinoma; *908*, giant-cell carcinoma (undifferentiated large-cell carcinoma); *1002*, small-cell carcinoma; *909*, malignant thymoma. O, not determined because of lack of material. No *bar* indicates no activity.

The elevation of GT activity was statistically significant ($p < 0.001$) in adenocarcinoma and squamous cell carcinoma compared to the normal control. When lung carcinoma was compared in terms of histological type, the GT level in the adenocarcinoma was significantly ($p < 0.001$) higher than in squamous cell carcinoma. These results were demonstrated with the enzyme either of homogenates (in Experiments 1 and 2) or Fraction P with Triton X-100 (in Experiment 3). Examination of the individual adenocarcinomas revealed that tumors of Tumors 805 and 610 which were a goblet cell type of adenocarcinoma and 609 which had a giant-cell area contained the highest GT activity. Large-cell carcinoma (Tumors 703 and 908) and a mixed type of adenocarcinoma and large-cell carcinoma (Tumors 607) showed high GT activity, while small-cell carcinoma (Tumor 1002) and squamous cell carcinoma with features of the small-cell type (Tumors 611 and 901) showed low GT activity.

To examine whether the subcellular distribution of GT's is similar in carcinoma and normal control, Fraction S_1 was fractionated into a Fraction P and Fraction S_2. The ratio of the distribution of total activity in Fractions P and S_2 was about 1:2 in normal control as well as in 2 types of carcinoma. Therefore, the increased GT activity in carcinoma was not due to the difference in subcellular distribution of the enzyme, confirming the results obtained using the different enzyme preparations (S_1 in Experiments 1 and 2 and P in Experiment 3 in Table 1).

Solubilization and Properties of GT. To examine properties of GT from carcinoma and control tissues, the enzyme was solubilized from Fraction P to avoid contamination of serum which has been shown to have a high GT activity for ASGF (22). Sixty % of the total GT activity was solubilized with Triton X-100 under conditions described in "Materials and Methods." The solubilized enzymes of lung carcinomas were more heat labile as compared to the enzymes of the control lung. The pH optimum of GT from either lung cacinomas and normal control was at 7.0.

GT activities using different acceptor glycoproteins and the solubilized membrane preparation are summarized in Table 2. The solubilized membrane enzyme preparation catalyzed transfer of galactose from UDP-galactose not only to glycoproteins (OVA and ASGF-βE) with *N*-glycosidically linked carbohydrate chains but also to those containing *O*-glycosidic saccharides, such as ASBSM and BSM. GT activities were significantly higher in adenocarcinoma than in squamous cell carcinoma when OVA, ASGF-βE, and ASGF served as acceptors, while the differences in the activities among histological types and carcinoma and the control were not clear when ASBSM and BSM were acceptors. GT activity toward ASGF-βE, which possesses only *N*-glycosidic linkage, was reduced to about 80% of that toward ASGF, which possesses both *N*- and *O*-glycosidic types of linkages (18, 28). Preparation from human lung and its carcinomas also transferred galactose to glycolip-

Table 1

Statistical analysis of GT activities in human lung tumors

Histological type	Specific activity (cpm/μg protein) Experiment 1	Experiment 2	Experiment 3	% of activity[a]
Adenocarcinoma	51.0 ± 6.2[b] (9)[c]	31.3 ± 4.5 (14)	48.8 ± 26.0 (4)	100 ± 10.8[d] (27)
Control	12.3 ± 2.1 (9)	2.5 ± 0.7 (14)	0.1 ± 0.1 (3)	17.7 ± 5.6 (26)
Squamous cell carcinoma	30.1 ± 5.6 (9)	19.8 ± 9.1 (4)	12.0 ± 2.0 (6)	48.8 ± 8.4[e,f] (19)
Control	8.7 ± 1.9 (9)	3.6 ± 3.2 (3)	3.4 ± 1.4 (6)	12.5 ± 2.7 (16)

[a] The individual activities were converted into percentages relative to the mean values of adenocarcinoma (100%) in each experiment (for Experiments 1, 2, and 3, see the legend to Chart 1.), and then the percentage values in 3 groups were subjected to statistical analysis all together.
[b] Mean ± S.E.
[c] Numbers in parentheses, number of tumors.
[d] $p < 0.001$ (*t* test; adenocarcinoma *versus* its control).
[e] $p < 0.001$ (*t* test; squamous cell carcinoma *versus* its control).
[f] $p < 0.001$ (*t* test; squamous cell carcinomas *versus* adenocarcinoma).

Table 2

Substrate specificity of GT solubilized from human lung and its carcinomas

Activities indicated had been subtracted for blank values in which heat-inactivated enzyme was used. Blank values were 0.57, 0.56, 0.75, 2.31, and 2.62 cpm/μg protein/30 min for OVA, ASGF-βE, ASGF, ASBSM, and BSM, respectively. Amounts of substrates used were 1 mg each of glycoproteins except 1.5 mg of OVA and 50 μg each of glycolipids which were enough to saturate the enzymes. Amounts of enzyme protein used were 68 μg in normal control, 90 μg in adenocarcinoma, and 78 μg in squamous cell carcinoma.

Substrate	Probable reaction	Activity (cpm/μg protein/30 min) Normal control	Adenocarcinoma	Squamous cell carcinoma
OVA	Gal[a] → GlcNAc---GlcNAc-*N*-Asn	3.1	24.3	14.9
ASGF-βE	Gal → GlcNAc—GlcNAc-*N*-Asn	4.3	53.2	40.0
ASGF	Gal → GlcNAc—GlcNAc-*N*-Asn Gal → GalNAc-*O*-Ser	5.3	69.5	48.1
ASBSM	Gal → GalNAc-*O*-Ser	72.5	83.2	95.4
BSM	Gal → GalNAc-*O*-Ser AcNeu	65.1	83.2	95.7
G_{M2}	Gal → GalNAc—Cer	0.41	0.24	0.30
G_{A2}	Gal → GalNAc—Cer	0.95	0.60	0.96

[a] Gal, galactose; GlcNAc, *N*-acetylglucosamine; Asn, asparagine; GalNAc, *N*-acetylgalactosamine; Ser, serine; AcNeu, *N*-acetylneuraminic acid; Cer, ceramide.

ids, such as G_{M2} and G_{A2}, although the activity was much lower compared to those toward glycoproteins. However, glycolipid GT levels did not show differences between carcinoma and the control. The above results strongly suggest that the increased GT activity, which is correlated with histological types of human lung carcinoma, is an activity directed toward glycoproteins having *N*-glycosidic saccharides.

DISCUSSION

In this study, it was demonstrated that GT activities with ASGF were considerably increased in 2 histologically different types of human lung cancer as compared to those in normal control ($p < 0.001$). Increased GT levels with ASGF have been demonstrated in human ovarian adenocarcinoma (4, 8) and transitional cell carcinoma of bladder (21) when compared to the respective normal tissues. Elevations of GT level, however, do not appear to be a general finding for all neoplasms. The GT activities in human colonic adenocarcinoma were decreased in comparison to the uninvolved colonic tissue, although those in cultured colonic carcinoma cells were higher than those in colonic fetal cells (29). GT levels in neoplasms may be increased or decreased in a tissue-specific manner.

The results in the present study also demonstrated that the increase of GT activity was significantly larger in adenocarcinoma than in squamous cell carcinoma ($p < 0.001$). This elevation of GT activity, as well as raised activity of cerebroside sulfotransferase in lung adenocarcinoma type (9), could be accounted for by one of the functional characteristics of lung tumors with adenomatous histology. The results observed in the lung adenocarcinoma group indicate that the histological features correlate with the biochemical features. The adenocarcinoma group, Tumors 805 and 610, which exhibited the highest activity, were histologically bronchioloalveolar, goblet cell types. The goblet cell is a mucin-producing cell and would contain enriched amounts of glycosyltransferases implicated in biosynthesis of carbohydrate moieties in mucin-type glycoproteins. With regard to correlation of mucin production and glycosyltransferase level, Baker and Sawyer (1) demonstrated that lung, as well as other respiratory tissues, from patients with hypersecretions of respiratory mucin exhibited significantly increased levels of GT and other glycosyltransferase activities.

GT's consist of multienzymes which are different in substrate specificity and also in physical properties such as particulate-bound GT (26), soluble GT (3), or charge heterogeneity (11, 29). Our GT's solubilized with Triton X-100 from lung and its tumors exhibited a heterogeneous profile on electrophoresis (data not shown), although the characterization of the elevated enzyme in lung cancer still remained to be studied. GT's may be distinguished into 2 groups with respect to substrate specificity of the enzymes. One group catalyzes the synthesis of plasma-type glycoproteins having *N*-glycosidic saccharides to form galactosyl-*N*-acetylglucosaminyl linkage, and the other catalyzes the synthesis of mucin-type glycoproteins having *O*-glycosidic saccharides to form galactosyl-*N*-acetylgalactosaminyl linkage. The majority of glycoproteins secreted by glandular tissue is believed to be of the mucin-type glycoproteins (19). Therefore, it was presumed that the elevated GT activity observed in lung adenocarcinoma over squamous-cell carcinoma synthesized mucin-type glycoproteins, rather than plasma-type ones. Contrary to this assumption, the enhanced GT activity in adenocarcinoma was found only for plasma-type glycoproteins but not for mucin-type glycoproteins, similar to the differences between carcinoma group and normal lung. This suggests that the increase in GT activities is not a reflection of differentiation to mucin-secreting glandular tissue; it may be an indication of a more fundamental change in glycoprotein metabolism by tumor tissue (25).

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