Increased Expression of the Enzyme β1–4-Galactosyltransferase is Associated with Human Parotid Neoplasms (43038)

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Abstract. **Human biopsy samples of parotid gland neoplasms were examined for the level of enzyme activity of the glycosyltransferase, β1–4-galactosyltransferase. An analysis of an adenoid cystic carcinoma, Warthin's tumor, mucoepidermoid carcinoma, and five pleomorphic adenomas all revealed elevated levels of enzyme activity. Evidence for plasma membrane β1–4-galactosyltransferase activity was provided by membrane fractionation as well as intact cell enzyme assays. On the other hand, the major protein of human saliva, salivary α-amylase, was substantially reduced in the same tissue compared with adjacent normal parotid gland tissue. The trichloroacetic acid-soluble proteins isolated from gland homogenates were also reduced in two of the carcinoma samples but increased in the pleormophic adenomas. Additionally, the proliferation of these cells, *in vitro*, could be retarded by culturing in media containing the galactosyltransferase specific modifier protein, α-lactalbumin, or the nucleotide sugar, UDP-galactose.** [P.S.E.B.M. 1990, Vol 193]

The functional role of glycosylation and glycosyltransferases in mediating such cellular properties as development, differentiation, adhesion, and proliferation has received substantial attention. Changes in the carbohydrate content of glycoconjugates have been noted but the significance remains essentially unknown (1, 2). The structures of cell surface carbohydrates of oncogenically transformed cells display distinctly different profiles than those of their nontransformed counterparts. In a similar manner, the appearance of the enzymes involved in protein glycosylation, at the cell surface, has yet to be functionally defined.

Membrane-associated glycosyltransferases have classically been localized to the Golgi and endoplasmic reticulum (3, 4) where they have traditionally served in the biosynthesis of the oligosaccharide moieties of glycoconjugates. The cell surface appearance of one of these enzymes, β1–4-galactosyltransferase (4β-galactosyltransferase EC 2.4.1.8), has been proposed to play an important role in the controlled growth of cells (5, 6). Cell-social recognition, including fertilization, appears to be mediated in part by plasma membrane 4β-galactosyltransferase (6–9) . Both cell surface and soluble forms of the enzyme have since been implicated as an abnormality that leads to uncontrolled growth in certain transformed cells (5, 10–12).

Studies of cancer-associated galactosyltransferases have revealed the presence of two 4β-galactosyltransferase isoenzymes (13). One enzyme (galactosyltransferase I) was found to be localized in the Golgi apparatus, while the second form of galactosyltransferase (galactosyltransferase II) was found to be primarily associated with serum from patients with malignant cells (12, 13). The tumor-associated form of galactosyltransferase was shown to be highly conserved in kinetic, chemical, and physical properties with that observed for the Golgi isoenzyme (11).

In our studies of the growth exhibited by rat parotid glands in response to isoproterenol, our attention has been focused on a role for overexpressed β-galactosyltransferase (14–16). Chronic administration of β-adrenergic agonist results in physiologic changes within the parotid gland that include cell hypertrophy and hyperplasia (17, 18). DNA, RNA, and protein synthesis are all increased following isoproterenol administration

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Received June 22, 1989. [P.S.E.B.M. 1990, Vol 193]
Accepted December 11, 1989.

0037-9727/ 90/1934-0293$2.00/0

(19). One of the proteins whose synthesis is stimulated by isoproterenol is 4β-galactosyltransferase, which increases up to 10-fold in activity even though several other Golgi enzymes remain unaffected (16).

Following up on the observations from rodent salivary glands, we here have determined that an increase in cell surface galactosyltransferase accompanies human parotid gland hyperplasia observed with several types of neoplasms. The enzyme activity is enriched in the plasma membrane isolated from these cells. *In vitro* cell culturing of several neoplasms showed that substrates of galactosyltransferase could retard their proliferation. Furthermore, as described by Morley and Hodes (20), we provide evidence for the lack of the major salivary protein, salivary α-amylase (EC 3.2.1.1) as well as the trichloroacetic acid (TCA)-soluble proteins in parotid neoplasms.

Materials and Methods

Material. Ovalbumin, UDP-galactose, fetuin, and CMP-sialic acid were purchased from Sigma Chemical Co. UDP-[1-^{14}C]galactose (300 mCi/mmol) and CMP-[6-^{3}H]sialic acid (60 Ci/mmol) were from Dupont-New England Nuclear. All other reagents were obtained from commercial sources. Human parotid adenomas were obtained 1–3 hr after removal for biopsy from the UAB Cancer Center Tissue Procurement service or Pathology services at Shands Hospital, University of Florida. Upon pathologic examination, samples were divided into normal and disease material whenever possible.

Membrane Preparation. Parotid gland samples to be used in enzyme assays were homogenized at 4°C in 10 m*M* Tris-HCl buffer (pH 8.0) with a Dounce apparatus. Total plasma and Golgi membranes were isolated as described previously (14). In brief, a low-speed centrifugation was performed (200*g*) to remove connective tissue and unlysed cells. Total membranes were obtained by centrifugation of the resulting slurry at 100,000*g* for 1 hr. Plasma and Golgi membranes were isolated by buoyant density differentials in sucrose gradients as described by Arvan and Castle (21). Enrichment for specific membrane fractions were determined by marker enzyme assays as described by Marchase *et al.* (16). Protein assays were performed by a modification of the Lowry method with bovine serum albumin as the standard (22).

Glycosyltransferase Assays. The activity of 4β-galactosyltransferase and sialyltransferase were measured in membrane fractions as described previously by Humphreys-Beher *et al.* (23) and Marchase *et al.* (16). Asialofetuin was prepared by mild acid hydrolysis of the reducing terminus sialic acid (23). Parotid membrane fractions were resuspended in 10 m*M* Tris-HCl (pH 8.0) at a final concentration of 200 μg/ml. The assay mixture (total volume 50 μl) contained 0.1 *M* MES (pH 6.3), 25 m*M* $MnCl_2$, 0.5% Triton X-100, 1 m*M* UDP-[1-^{14}C]galactose (2 mCi/mmol) ovalbumin (as exogenous acceptor for assays of 4β-galactosyltransferase), and 0–0.5 mg of the membrane preparation. In the case of utilization of *N*-acetylglucosamine (GlcNAc) as acceptor, labeled disaccharide product was recovered by column chromatography as described by Humphreys-Beher *et al.* (23) using Biogel AGX-8. For assays of sialyltransferase activity 1 m*M* CMP-[6-^{3}H]sialic acid and asialofetuin were incorporated in the above reaction mix in place of UDP-Gal and ovalbumin, respectively. After incubation at 37°C for 1 hr, the reaction was terminated by the addition of 1 ml of ice-cold trichloroacetic acid and followed by the recovery of ^{14}C- or ^{3}H-incorporated material on glass fiber filters (23). Enzyme activity was expressed as nanomoles of radioligand incorporated per minute/milligram of membrane protein.

Assay of Amylase Activity. α-Amylase activity was assayed by the method of Bernfeld (24). The substrate solution comprised 1 g of soluble starch and 40 mg of NaCl in 100 ml of 0.02 *M* K_2HPO_4 buffer (pH 7.0). The stop solution consisted of 3,5-dinitrosalicylic acid and 30 g of sodium potassium tartrate in 100 ml of 0.4 *M* NaOH. The reactions were carried out at 37°C in a volume of 0.5 ml using 100 μl of the enzyme-containing cell lysate. The reaction was terminated after a 30-min incubation by adding 0.5-μl stop buffer; the solution was boiled to allow color development and absorbance was then measured at a wave length of 540 nm after addition of 5 ml of water. Amylase activity was expressed as units/minute/gram protein. One unit of enzyme activity will liberate 1.0 μmol of maltose from starch per min.

Intact Cell Enzyme Assays. Fresh biopsy tissue was treated with collagenase and hyaluronidase as described previously (14) to isolate intact cells. Intact cells from control and neoplastic tissues were washed into Ca^{2+}-Mg^{2+}-free Hanks' solution containing 10 m*M* $MnCl_2$. They were resuspended in the same buffer and approximately 10^6 cells were added to incubation tubes that also contained 200 μm UDP-[^{14}C]Gal (0.2 Ci/mmol in a final volume of 50 μl). The tubes also contained ovalbumin (1.0 mg/ml final concentration) or GlcNac (10 m*M* final concentration) or neither to assess activity toward exogenous or endogenous acceptors, respectively. Cell protein was determined as described above. Enzyme activities were performed at 37°C for 1 hr with intermittent mixing of the cells. Lactate dehydrogenase assays on postincubation supernatants and sonicated cells were performed to provide a measure of enzyme leakage. The reactions were terminated by the addition of 1 ml of cold 10% trichloroacetic acid and the incorporation of [^{14}C]galactose determined by precipitation on glass fiber filters followed by scintillation counting.

Polyacrylamide Gel Electrophoresis. Protein

samples isolated by solubility in 10% TCA (25) were subjected to electrophoresis in a 1.5-mm-thick 10% polyacrylamide gel using a modified Tris-glycine system of Laemmli, as described by Pugsley and Schnaitman (26). All gels were fixed and stained according to a modification of the method of Fairbanks *et al.* (27) as described by Humphreys-Beher and Wells (28). Samples for gels were made up at 1 mg/ml of sample buffer. Thirty-five micrograms of protein per well were used in gel electrophoresis.

***In Vitro* Cell Culturing.** Fresh biopsy tissues from human parotid gland pleomorphic adenomas were cultured to determine the significance of cell surface galactosyltransferase in regulating cell proliferation. Single cells were recovered after 30-min incubation in collagenase (100 units/ml) and hyaluronidase (25 mg/ml) at 37°C by passing through screens of decreasing pore size. The cells were concentrated by centrifugation, washed once in Dulbecco's modified Eagle's medium and plated for 24 hr in RPMI 1640 containing L-glutathione, 25 m*M* Hepes buffer, 10% fetal bovine serum, and 10 units/ml penicillin/streptomycin in a CO_2 humidified incubator. The cells were subsequently harvested, washed, and resuspended in the same medium to a concentration of 2×10^3 cells/ml. The cell suspensions (300 μl) were transferred to microtiter wells and incubated for 36 hr in the presence of α-lactalbumin, bovine serum albumin, UDP-galactose (UDP-Gal), UDP-glucose (UDP-Glc), or no additives to the medium. Five microcuries of [^{3}H]thymidine were added to the culture medium and the cells were incubated for another 4 hr and subsequently harvested, washed twice in phosphate-buffered saline, and resuspended in 250 μl of phosphate-buffered saline. Samples were removed for scintillation counting of incorporated ^{3}H label as well as protein assays. In a separate experiment, the cells were washed of inhibitors and cultured for an additional 24 hr in fresh medium, labeled for 4 hr with [^{3}H]thymidine, and processed as described above.

Results

4β-Galactosyltransferase Enzyme Activity. An analysis of human parotid gland cellular proliferation, determined to be the result of benign pleomorphic adenoma, mucoepidermoid carcinoma, adenoid cystic carcinoma, or Warthin's tumor, was undertaken in an effort to correlate changes in 4β-galactosyltransferase gene expression with those observed in the rat model of acinar cell proliferation. A survey of 4β-galactosyltransferase enzyme activity was performed on biopsy samples of tissue recovered, over a 2-year period, from parotid neoplasms and the surrounding nontransformed tissue. Our initial results using a total membrane preparation indicated decreased enzyme activity recovered from the pleomorphic adenoma material (Table I). The overall activity of a second glycosyltransferase, sialyltransferase, was also decreased. This was not true, however, for the three carcinomas examined, in which total galactosyltransferase activity was actually elevated when compared with surrounding non-neoplastic tissue. The mucoepidermoid and adenoid cystic carcinomas demonstrated a nearly 4-fold increase in galactosyltransferase activity compared with control tissue (Table I).

Further analysis of the distribution of enzyme activity following membrane fractionation by buoyant density centrifugation indicated a contrary observation. The plasma membrane samples of the pleomorphic adenoma tissues showed increased levels of 4β-galactosyltransferase enzyme activity when compared with control tissue from the same patient (Table I). The inability to detect the sialyltransferase activity in this fraction suggested that the galactosyltransferase activity was not due to contamination by trans-Golgi membrane during purification (16). The plasma membrane samples also showed an enrichment for the marker enzymes γ-glutaminyltranspeptidase (see Table I). The discrepancy in total membrane 4β-galactosyltransferase activity and plasma membrane activity may be a reflection of the tissue necrosis observed in the pleomorphic adenomas when compared with the surrounding normal parotid tissue. The level of galactosyltransferase activity in the plasma membrane fraction from the carcinomas and adenomas were similar to each other and did not depend on the type of neoplasia present.

Intact Cell Enzyme Assays. In order to investigate further the enhancement in galactosyltransferase activity seen in human parotid neoplasms, intact parotid gland acinar cells were isolated from pathogenic and control tissue and assayed for cell surface enzyme activity. As shown in Table II, the incorporation of [^{14}C]Gal into endogenous acceptors by intact cells increase nearly 4-fold for the pleomorphic adenomas isolated for this study. The rise in intact cell enzyme activity again contrasts sharply with the decreased activity present in total membrane lysates. The mucoepidermoid and adenoid cystic carcinomas both had a 6-fold increase in galactosyltransferase activity relative to control tissue. The condition of the Warthin's tumor did not allow for intact cell assays. When the exogenous acceptor ovalbumin was included in the reaction, galactosyltransferase activity increased 6- to 8-fold for the parotid gland adenomas while the carcinomas increased by approximately 11- to 14-fold. The incorporation of radiolabel could be blocked by the specific addition of unlabeled UDP-Gal; however, addition of Gal, CMP-sialic acid, or Gal-1-P had no effect on [^{14}C]Gal incorporation. The addition of monosaccharide, GlcNAc, had the greatest effect on exogeneous galactosyltransferase activity. This was most likely due to the lack of stearic constraints imposed on enzyme-substrate inter-

Table I. Glycosylatransferase Levels in Human Parotid Gland Biopsy Tissue

Sample[a]	Sialyltransferase[b]			4β-Galactosylytransferase[d]		
	Total	Golgi	Plasma[c]	Total	Golgi	Plasma
Mucoepidermoid carcinoma	0.024	0.251	0.002	0.098	0.361	0.517
Control	0.031	0.222	0.003	0.024	0.372	0.023
Adenoma-1	0.012	0.276	0.002	0.015	0.382	0.312
Control	0.037	0.314	0.001	0.034	0.436	0.012
Adenoma-2	0.021	0.297	0.001	0.017	0.394	0.402
Control	0.045	0.286	0.001	0.028	0.450	0.017
Adenoma-3	0.019	0.221	0.001	0.027	0.394	0.357
Control	0.036	0.234	0.002	0.031	0.399	0.025
Adenoma-4	0.017	0.309	0.001	0.027	0.362	0.292
Control	0.041	0.297	0.001	0.039	0.388	0.013
Adenoma-5	0.020	0.214	0.002	0.022	0.314	0.281
Control	0.039	0.224	0.001	0.037	0.301	0.016
Adenoid cystic carcinoma	0.026	0.201	0.002	0.094	0.350	0.462
Control	0.038	0.236	0.002	0.028	0.360	0.033
Warthin's tumor	0.197	0.225	0.031	0.087	0.467	0.279

[a] All assays were performed in triplicate for one experimental determination.
[b] Sialyltransferase was measured using asialofetuin as acceptor species. Values expressed as nanomoles of sialic acid incorporated/hour/milligram protein.
[c] Enrichment for plasma membrane was determined by γ-glutaminyltranspeptidase assay (21).
[d] Galactosyltransferase was measured using ovalbumin as acceptor species. Values expressed as in sialytransferase assays.

actions when GlcNAc is present on glycoprotein acceptor macromolecules. The low level of label incorporated into substrate by leaked medium suggests that the results presented here cannot be attributed to cell breakage, with the subsequent release of galactosyltransferase from cells.

Amylase Activity in Parotid Neoplasms. Salivary α-amylase is the major protein component of parotid gland secretions. Although amylase activity is often enhanced in a variety of human tumors (29–31), the parotid gland, which is one site of normal synthesis, demonstrates a loss of biosynthetic capacity after neoplastic transformation (20). As indicated in Table III, the level of α-amylase enzyme activity in the parotid gland neoplasms investigated in this study was reduced by greater than 95% of control values. A Western blot analysis of cell lysates again failed to detect immunoreactive protein (not shown).

Analysis of TCA-Soluble Proteins from Gland Lysates. The parotid gland is also the source of a number of salivary proteins which collectively have the unique property of TCA solubility. These proteins were isolated from gland homogenates of parotid gland neoplasms and analyzed by sodium dodecyl sulfate- polyacrylamide gels. As shown in Figure 1, the comparison of the two carcinoma samples to normal tissue revealed a nearly complete absence of these proteins from gland extracts. In contrast, the pleomorphic adenomas ap-

Table II. Cell Surface Galactosyltransferase from Human Parotid Neoplasms

	Control[a]	[^{14}C]Galactose transferred to macromolecular acceptors (cpm)						
		P.Ad-1[b]	P.Ad-2	P.Ad-3	P.Ad-4	P.Ad-5	MC	ACC
Endogenous acceptor only	2,000	8,300	7,900	8,100	8,700	9,000	12,000	12,500
1.0 mg/ml ovalbumin[c]	2,700	21,000	24,600	23,400	21,100	18,000	32,600	35,000
Ovalbumin + unlabeled UDP-Gal	100	1,300	1,500	1,400	1,400	1,200	1,000	1,800
GlcNAc[c]	4,500	47,000	52,000	51,000	60,000	54,000	84,000	86,000
Leaked activity in medium[c]	200	900	1,200	700	1,300	1,000	1,110	1,000
Sonicated cell activity	41,000	99,000	117,000	122,000	120,000	125,000	115,000	120,000

[a] Control values are the average for the normal tissue from each sample shown in Table I. Results expressed as average of two experimental determinations performed in duplicate.
[b] P.Ad, Pleomorphic adenoma; MC, mucoepidermoid carcinoma; ACC, adenoid cystic carcinoma.
[c] Cells removed by centrifugation following a mock assay. Culture medium was then assayed for galactosyltransferase activity using ovalbumin as an acceptor.

Table III. α-Amylase Enzyme Activity in Parotid Gland Pathologies

Sample	Activity[a] (units/min/g protein)
Normal parotid[b]	38.6 ± 2.3
Normal tissue pleomorphic adenoma[a]	29.7 ± 3.0
Pleomorphic adenoma[b]	1.3 ± 0.1
Adenoid cystic carcinomas	0.7 ± 0.1
Mucoepidermoid carcinoma	0.5 ± 0.1
Warthin's tumor	1.4 ± 0.73

[a] Values expressed are means of two experimental determinations ± SD.
[b] Average of three tissue samples.

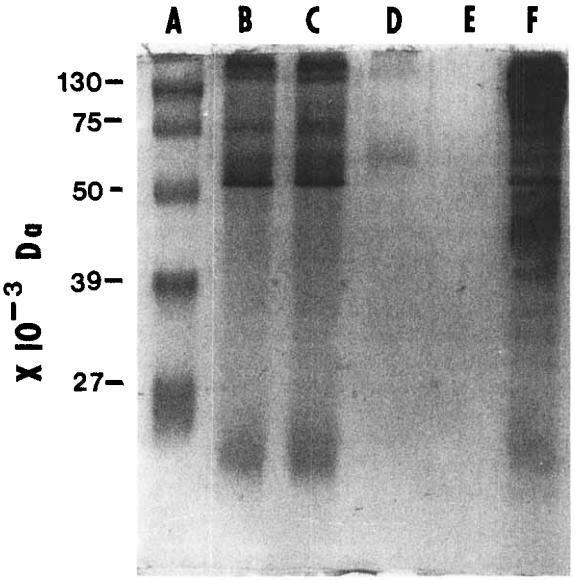

Figure 1. TCA-soluble proteins isolate from human parotid gland pathologies. Proteins (35 μg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. Lane A, molecular weight standards as in Figure 2; Lane B, normal parotid tissue; Lane C, normal parotid tissue from pleomorphic adenoma; Lane D, mucoepidermoid carcinoma; Lane E, adenoid cystic carcinoma; Lane F, pleomorphic adenoma.

peared to overexpress these proteins (Fig. 1, Lane F). The slower mobility of these proteins relative to TCA-soluble proteins isolated from saliva may relate to further proteolytic processing following secretion from the parotid gland.

Inhibition of Neoplastic Cell Growth *In Vitro*. Because of the increase in cell surface-associated galactosyltransferase enzyme activity and the apparent changes in gene expression, experiments were designed to examine the effects of the ectopic expression on cell growth. Stimulation of rat parotid gland acinar cells to proliferate showed inhibition of their growth by galactosyltransferase substrates both *in vivo* and *in vitro*. In the rat studies, the agents with the greatest efficacy of inhibition were UDP-Gal and the modifier protein α-lactalbumin (14, 16). The inclusion of these agents in the medium used to culture adenoma cells resulted in a dose-dependent inhibition of three separate neoplastic isolates (Fig. 2). Cell growth in these experiments was quantitated by incorporation of [^{3}H]thymidine into DNA. Treatment with α-lactalbumin inhibited cell proliferation to a greater extent than UDP-Gal. Typically, α-lactalbumin showed inhibition by approximately 30–40% while UDP-Gal inhibited growth by 15–25%. The most likely explanation for the lower level of growth inhibition would be due to the hydrolysis of the nucleotide sugar by extracellular phosphatases. As a control, the sugar nucleotide UDP-Glc and bovine serum albumin were included in the culture medium. These agents do not affect cell growth over the 48-hr exposure time. As a further control against possible toxic effects of the inhibitors, the cells were washed free of these agents and growth continued for an additional 24 hr. Under these conditions, adenoma cells recovered full proliferation potential.

Discussion

In this study we have demonstrated an abnormal increase in the level of cell surface-associated galactosyltransferase in human parotid gland neoplasms. Similar increases in expression as well as the identification of tumor-associated isoenzymes have been determined by numerous groups (5–10); however, the significance has remained obscure. On the other hand, the major salivary proteins, α-amylase, and TCA-soluble proteins showed a dramatic decline in biosynthesis. More complete studies on amylase expression in parotid glands by Morley and Hodes (20) have shown enzyme synthesis to be blocked at the level of transcription, although there were no detectable changes associated with chromosomal organization. Both amylase and TCA-soluble proteins are hallmarks of salivary gland differentiation (32, 33). While parotid gland tumors appear to lose the ability to express amylase (32, 34), other neoplasms from tissues such as the ovary, lung, and stomach (29–31) ectopically express amylase. The inability to synthesize amylase and TCA-soluble proteins may be related to the loss of differentiated function with parotid neoplastic growth (20). This result suggests that the parotid gland acinar cells have lost the capacity to synthesize necessary factors required for tissue-specific expression of these genes. Thus, the decline in both amylase and TCA-soluble proteins from carcinomas relative to only the loss of amylase activity in parotid gland adenomas may represent a progression to a further dedifferentiated state in salivary gland neoplasia.

Recent studies from our laboratory have provided evidence for the direct participation of cell surface galactosyltransferase in mediating rodent parotid gland acinar cell proliferation. The transition from stasis to active cell growth is accompanied by the appearance of cell surface galactosyltransferase in animals treated with

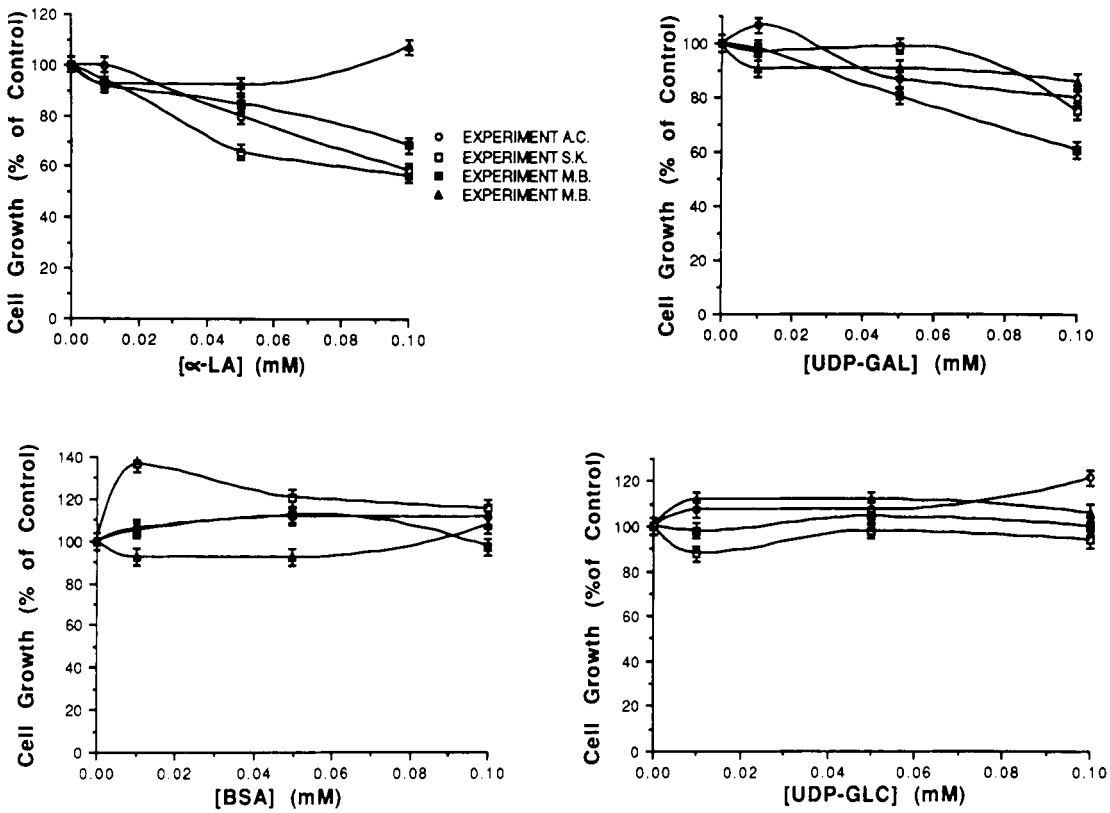

Figure 2. The effects of specific modifiers of galactosyltransferase on cell growth. Cells were treated with increasing concentrations of either α-lactalbumin, bovine serum albumin (BSA), UDP-Gal, or UDP-Glc, then pulsed with [^{3}H]thymidine to assess the effects of these agents on cellular growth. Each point represents the results of two separate experiments run in duplicate. The average deviation is shown by the brackets for each point. The following symbols were used for each of three pleomorphic adenomas: AC, ○; SK, □; MB, ■, ▲. Experiment with MB (▲) was the same cells washed free of inhibitors and cell growth allowed to recover.

β-adrengic receptor agonist (14, 16) and dietary changes which result in hypertrophy and hyperplasia as well (15, 25). In both cases of gland hyperplasia, complete inhibition of cell proliferation occurred by treating animals with specific substrates of 4β-galactosyltransferase (14, 35, 36). The ability of galactosyltransferase substrates such as α-lactalbumin and UDP-Gal to retard the proliferation of acinar cells in pleomorphic adenomas *in vitro* suggest galactosyltransferase may play a direct role in growth control in the parotid gland.

In this report we have again provided evidence for the ectopic expression of galactosyltransferase for human parotid gland neoplasms. These results taken together suggest a possible role for cell surface galactosyltransferase in cellular proliferation. A dramatic increase in the level of this protein on the cell surface leads to the continued growth of hyperplastic and neoplastic cells. The appearance of cell surface enzyme in these dedifferentiated acinar cells (with respect to amylase synthesis) may reflect a common observation for transformed cells, namely, the reexpression of fetal antigens. The implication of galactosyltransferase as a modulator of cell growth suggests the possible designation of this glycosyltransferase as a new type of proto-oncogene product. The involvement of the cell surface component of the enzyme in cell growth, its controlled expression in development (37, 38), and ectopic expression in neoplastic cells provide evidence for such a designation. Indeed, recent observations of lymphoid and bone-derived tumors provide evidence for the presence of galactosyltransferase in approximately 25% of these cell types (39) . As with the parotid gland neoplasms, *in vitro* (and more importantly *in vivo*) growth could be controlled by the interactions of these cells with α-lactalbumin and UDP-Gal (39). The specific function of the cell surface form of galactosyltransferase in the signaling process for cell proliferation remains unclear. However, it has been proposed that it may function as a receptor in certain cells (40). The ability of this protein to interact with specific carbohydrate moieties on the cell surface may be critical to its apparent role in cell proliferation. Numerous cell surface glycoproteins, such as growth factor receptors, could possess the proper terminal *N*-acetylglucosamine residues on the cell surface so as to lead to their activation by interaction with cell surface galactosyltransferase. The implications for changes in carbohydrate structures associated with abnormal cell growth make this an intriguing

mechanism. Therefore, alterations in expression, galactosyltransferase, and glycosyltransferase in general, either increased or decreased, could disrupt normal cell growth.

This research was supported by grants from the NIDR (DE 08778 and DE 00291) and the Interdisciplinary Center for Biotechnology Research, Biotechnology Scholars award to M. G. H-B.

The authors would like to thank Ms. Gloria Robinson and Mr. James Fisher for technical assistance with this work. Additional recognition is provided to Ms. Kathy Sexton of the UAB Tissue Procurement Center and Ms. Marilyn Lietz for assistance in preparation of this manuscript.

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