AUTOGRAPHIC LOCALIZATION OF GLYCOPROTEIN IN HUMAN BREAST CANCER CELLS MAINTAINED IN ORGAN CULTURE AFTER INCUBATION WITH $[^{3}H]$FUCOSE OR $[^{3}H]$GLUCOSAMINE

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SUMMARY

Explants of nine infiltrating duct carcinomas of the human female breast, maintained in organ culture, were exposed to the glycoprotein precursors, L-$[^{3}H]$fucose and $[^{3}H]$glucosamine, in order to determine the cellular distribution of newly synthesized glycoprotein as revealed by autoradiography with the light and electron microscopes. Explants were incubated with a single isotope for 24 hr, at which time some of the labeled explants were removed for autoradiographic analysis while the rest were transferred to nonradioactive medium for an additional 24 hr.

After exposure to label for 24 hr, autoradiography with each isotope was similar and showed strong reactions over most tumor cells. The reactions were due to clumps of silver grains over intracytoplasmic lumina within single tumor cells and silver grains over Golgi sacculs, cytoplasmic vesicles, lysosome-like bodies, lateral and basal plasma membranes, and microvilli. Extracellular ductular structures were also heavily labeled. At the later sampling time, Golgi sacculs often showed a reduced reaction while the reactions over other organelles and intracellular and extracellular ductular structures remained strong.

The observations suggest that in our in vitro system the tumor cells are metabolically active and complete the synthesis of the carbohydrate side chains of glycoproteins within the Golgi apparatus. From there, some of the newly synthesized glycoprotein appears to migrate to plasma membranes and lysosome-like bodies. Furthermore, our data support the notion that many duct carcinomas of the breast exhibit secretory activity by showing that some newly synthesized glycoprotein also appears to become products that are secreted into intracellular and extracellular ductular structures.

INTRODUCTION

Within a cell, glycoproteins are found primarily as components of plasma membranes (4), lysosomes (8), and extracellular material (22). Fucose is a terminal residue (17) of some of the oligosaccharide side chains of glycoproteins and is incorporated directly (5, 12) into newly synthesized glycoprotein within sacculs of the Golgi apparatus (2, 3). Glucosamine is also efficiently incorporated into glycoprotein (10, 11), but in this case some glucosamine is converted to other hexosamines or sialic acid before incorporation into the growing oligosaccharide chains (10). Since these hexosamine residues are located nearer the polypeptide chain than terminal fucose or sialic acid residues are, they are incorporated earlier into newly synthesized glycoprotein. This occurs within the rough endoplasmic reticulum as the polypeptide migrates through the endoplasmic reticulum to the Golgi apparatus. Autoradiographic studies (1-3) of many normal cell types have shown that, after completion of the sugar side chains in the Golgi apparatus, glycoprotein migrates to cell surfaces, to lysosomes, and into secretory products. The intensity of the autoradiographic reaction over organelles depends on the cell type (3).

Here we report our experiments designed to find out the cellular distribution of newly synthesized glycoprotein within human breast carcinoma cells maintained for several days in organ culture.

MATERIALS AND METHODS

The specimens used in this study consisted of small pieces of human female breast tumors obtained under sterile conditions minutes after mastectomy. The data presented here are confined to observations from 9 infiltrating duct carcinomas. The tumor pieces were sliced under sterile conditions into 1-mm cubes and explanted on triangular stainless steel grids within organ culture dishes (Falcon Plastics Co., Oxnard, Calif.) containing 1 ml of medium and placed in an incubator at 37°. The culture medium (16) consisted of Eagle's double-strength amino acid medium supplemented with 20% calf serum and 5% beef embryo extract ultrafiltrate. Also incorporated were glucose, 500 mg/100 ml; penicillin, 100 units/ml; and streptomycin, 100 µg/ml. After culture for 24 hr, 100 µCi of L-$[^{6}H]$fucose (New England Nuclear, Boston, Mass.; specific activity, 13.4 Ci/m mole) or 100 µCi of D-$[^{6}H]$glucosamine hydrochloride (New England Nuclear; specific activity, 10.13 Ci/m mole) were added to different dishes each of which...
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contained 4 to 6 explants. Incubation with the labeled compounds proceeded for 24 hr, at which time several explants from each dish were removed for autoradiographic analysis at the light and electron microscopic level. The remaining explants were transferred to nonradioactive medium for an additional 24 hr and then also processed for subsequent autoradiographic analysis.

The initial steps in processing involved fixing the explants for 1 to 2 hr in a 2% solution of glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.3), postfixing for 2 hr in 1% osmium tetroxide in cacodylate buffer, dehydrating in acetone, and embedding in the polyester resin Vestopal W.

For light microscope autoradiography, 1.0-μm-thick unstained sections of the Vestopal-embedded explants were coated with Kodak NTB 2 emulsion (Eastman Kodak Co., Rochester, N. Y.) by dipping the slides on which the sections had been placed into melted emulsion diluted 1:1 with distilled water (6). The slides were stored at 5° in lightproof boxes for 1 week and then developed. After autoradiography, the sections were stained through the emulsion with toluidine blue.

For electron microscope autoradiography, thin (silver to gold) sections from the same explants were autoradiographed with Ilford L4 emulsion (Ilford Ltd., Ilford, Essex, England) by the loop method of Maunsbach (14). Sections placed on Formvar-coated grids were stained with uranyl acetate and lead citrate and then covered with a thick coat of carbon. By means of the loop, a monolayer film of emulsion was then placed over individual grids. Emulsion melted at 42° was used diluted 1:1 with distilled water. The grids were then stored in lightproof boxes at 5° for about 1 month and then developed for 90 sec in undiluted Kodak Microdol-X and fixed for 4 min in 30% sodium thiosulfate.

DISCUSSION

The strong autoradiographic reactions we have observed over the cytoplasm of tumor cells within explants after incubation with [3H]fucose or [3H]glucosamine indicate that, in our organ culture system, the cells are first of all metabolically active. Furthermore, the cells take up and incorporate sugars into glycoproteins since free fucose, its nucleotide sugar derivative, and glycolipids are for the most part extracted from tissue during histological processing, while glycoproteins are retained (3, 20). Although some free glucosamine, as opposed to fucose, may be retained by tissue (20), the similarity of the autoradiographic patterns observed with both isotopes indicates that the reaction following [3H]glucosamine addition is again due primarily to labeled glycoproteins.

The most striking observation was the finding of intense autoradiographic reactions over intracytoplasmic lumina within single tumor cells. These structures, rarely found in normal human breast epithelium (7, 9, 13, 19), have been considered to represent a departure from normal ductular structures and are believed to contain secretions (7). Our data are in accord with a secretory activity and suggest that malignant human breast epithelial cells synthesize glycoproteins, perhaps similar to 1 or more components of milk, which are secreted into intracytoplasmic lumina. The strong reactions over microvilli at surfaces of intracytoplasmic lumina are consistent with a secretory activity.
lumina could be largely due to recently released secretory products. Moreover, the findings also imply that the cytoplasmic vesicles (1–3) around lumina may be carriers of secretion products. Secretion is also suggested by the strong autoradiographic reactions found over extracellular ductular structures, reminiscent of normal cells active in glycoprotein secretion (3, 20). However, other explanations to account for the localization of grains over intracellular and extracellular ductular structures are nevertheless possible.

The strong autoradiographic reaction over Golgi saccules of breast carcinoma cells after 24 hr exposure to isotope suggests that, as in normal cells (1–3), the Golgi apparatus may be the primary site of completion of the oligosaccharide side chains of glycoprotein. The observation that the reaction over Golgi zones decreases after labeled explants are cultured for an additional 24 hr in nonradioactive medium supports this view and indicates that, as in normal cells, newly synthesized glycoprotein migrates from the Golgi apparatus to other cellular sites and into secretory products.

Newly synthesized glycoprotein also appears to be incorporated into plasma membranes of malignant human breast epithelium. Since we do not know the growth rate of the tumor cells in vitro, we cannot say whether the plasma membrane reaction was due primarily to a turnover of glycoprotein components of plasma membranes as observed in nongrowing cells or to an increase in substance of plasma membranes as seen in growing cells (18). Strong autoradiographic reactions over lysosome-like bodies within the tumor cells suggest that, as in normal cells (3), newly synthesized glycoprotein (presumably hydrolyases (8)) migrates to this organelle.

Although different techniques were used, some observations of cell cultures of mouse mammary tumors seem to parallel our observations regarding the secretory activity of human breast carcinomas. The mouse cell cultures exhibited “domes,” which appeared to be periodically expanding and collapsing secretory structures (21). Furthermore, the domes contained milk constituents (15) that seemed to be produced by the cells participating in the dome structure.

ACKNOWLEDGMENTS

The authors wish to acknowledge the excellent technical assistance of Carole Kaufman and Valda Richters.

REFERENCES

The autoradiographs are from explants of infiltrating duct carcinomas of the human breast that were maintained in organ culture and incubated either with L-[3H]fucose or [3H]glucosamine. Sections examined in the light microscope (Figs. 1 and 2) were stained with toluidine blue while the electron autoradiographs (Figs. 3 to 9) were from sections stained with uranyl acetate and lead citrate.

Fig. 1. Explant incubated with [3H]fucose for 24 hr. Besides a general autoradiographic reaction over the cytoplasm of tumor cells, clumps of silver grains are found over intracytoplasmic lumina (arrows). Original magnification, × 1,000.

Fig. 2. Explant incubated with [3H]glucosamine for 24 hr and then transferred to nonradioactive medium for an additional 24 hr. Compared to Fig. 1, the cytoplasmic autoradiographic reaction is decreased while many silver grains are still found over the lumen of a ductular structure. Original magnification, × 1,000.

Fig. 3. Explant incubated with [3H]glucosamine for 24 hr. An intense autoradiographic reaction is found over an intracytoplasmic lumina within a single tumor cell. Cytoplasmic vesicles around the lumina also exhibit a reaction. × 11,000.
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Fig. 4. Explant incubated with [3H]glucosamine for 24 hr and then transferred to nonradioactive medium for an additional 24 hr. Similar to Fig. 3, but vesicles around the intracytoplasmic lumina show few silver grains. × 11,000.

Fig. 5. Explant incubated with [3H]glucosamine for 24 hr and then transferred to nonradioactive medium for an additional 24 hr. The small extracellular lumen bounded by several tumor cells exhibits a strong autoradiographic reaction. × 11,000.

Fig. 6. Explant incubated with [3H]glucosamine for 24 hr. Similar to Fig. 5, but cytoplasmic vesicles near luminal surfaces also exhibit an autoradiographic reaction. × 16,500.

Fig. 7. Explant incubated with [3H]fucose for 24 hr. Many silver grains are found over Golgi saccules and associated smooth-surfaced vesicles. × 16,500.

Fig. 8. Explant incubated with [3H]glucosamine for 24 hr and then transferred to nonradioactive medium for an additional 24 hr. Lysosome-like bodies within a tumor cell exhibit an autoradiographic reaction. × 11,000.

Fig. 9. Explant incubated with [3H]fucose for 24 hr. Silver grains are found over lateral plasma membranes. × 11,000.
Autoradiographic Localization of Glycoprotein in Human Breast Cancer Cells Maintained in Organ Culture after Incubation with [3H]Fucose or [3H]Glucosamine

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