IN VITRO SYNTHESIS AND SECRETION OF GLYCOPROTEIN
BY HUMAN MAMMARY TISSUE

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SUMMARY

Organ cultures of human surgical specimens can be used to investigate glycoprotein production in vitro under conditions in which three-dimensional tissue structures and cell-cell interactions resemble those present in vivo. In this report, an organ-culture system is used to investigate the synthesis, transport and release of glycoprotein by normal and benign hyperplastic human mammary epithelium. Autoradiography of explants pulse-labeled with individual glycoprotein precursors (\[^{3}H\]glucosamine, \[^{3}H\]fucose, \[^{3}H\]acetylmannosamine) and maintained in organ culture for intervals up to 72 hr revealed that glycoprotein is synthesized and then secreted by mammary epithelium. Incorporation of each isotope took place in the Golgi apparatus. Most of the newly synthesized glycoprotein, labeled with each of the three precursors, then was transported to apical cell surfaces and secreted into gland lumina. Observations were indistinguishable in normal and benign hyperplastic glands. Thus nonlactating human mammary epithelium exhibits a glycoprotein secretory activity. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of \[^{3}H\]glucosamine-labeled macromolecules released into the medium showed a group of glycoproteins with a molecular weight of 48,000±6,000 daltons plus high-molecular-weight glycosylated components at the top of gels. The nature of gp48 is not known, but similar molecular-weight glycoproteins also are released by surgical specimens of human mammary cancer maintained in organ culture.

Key words: mammary gland; glycoproteins; synthesis; secretion; autoradiography.

INTRODUCTION

Organ cultures of human surgical specimens can be used to investigate glycoprotein production in vitro under conditions in which three-dimensional tissue structures and cell-cell interactions resemble those present in vivo. In this report, an organ-culture system is used to investigate the synthesis, transport and release of glycoprotein by normal and benign hyperplastic human mammary epithelium. Autoradiography of explants pulse-labeled with individual glycoprotein precursors (\[^{3}H\]glucosamine, \[^{3}H\]fucose, \[^{3}H\]acetylmannosamine) and maintained in organ culture for intervals up to 72 hr revealed that glycoprotein is synthesized and then secreted by mammary epithelium. Incorporation of each isotope took place in the Golgi apparatus. Most of the newly synthesized glycoprotein, labeled with each of the three precursors, then was transported to apical cell surfaces and secreted into gland lumina. Observations were indistinguishable in normal and benign hyperplastic glands. Thus nonlactating human mammary epithelium exhibits a glycoprotein secretory activity. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of \[^{3}H\]glucosamine-labeled macromolecules released into the medium showed a group of glycoproteins with a molecular weight of 48,000±6,000 daltons plus high-molecular-weight glycosylated components at the top of gels. The nature of gp48 is not known, but similar molecular-weight glycoproteins also are released by surgical specimens of human mammary cancer maintained in organ culture.

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INTRODUCTION

Organ cultures of human mammary tissue survive intact for 4 months (1). By means of these in vitro preparations (1–4), the effects of hormones on gland maintenance and DNA synthesis have been studied. Little attention has been given, however, to other biosynthetic activities of human mammary epithelium within intact glands such as the synthesis and turnover of membrane and secretory components. Appropriate studies would define active biosynthetic processes which could be compared to biosynthetic activities of malignant human mammary epithelial cells (5). Of particular interest to us is the question of whether nonmalignant human mammary epithelium produces the same glycoproteins that have been found to be synthesized and released into the medium by organ cultures of human mammary cancer (6, 7). Since studies performed on mammary glands from pregnant (8, 9) and virgin (10) mice have shown that hormones effect differentiation of glandular epithelium in vitro with evidence of secretory activity, conditions necessary to produce the events seen in the differentiated human gland also might be investigated.

Since glycoproteins are important functional components of plasma membranes and make up part of the secretions of lactating mammary gland (11), we have investigated the in vitro glycoprotein synthetic and secretory activity of normal and benign hyperplastic nonlactating human mammary tissue by autoradiographic and biochemical techniques. Partial biochemical characterization of released, radioactive macromolecules carried out by electrophoresis in sodium dodecyl sulfate-polyacrylamide disc gels reveals a group of glycoproteins that may be related to similar molecular-weight glycoproteins released by human mammary cancer organ cultures (6, 7).
GLYCOPROTEINS OF HUMAN MAMMARY TISSUE

MATERIALS AND METHODS

Tissue was obtained under sterile conditions minutes after surgery from two benign fibroadenomas, one reduction mammoplasty specimen which contained essentially normal epithelium, and from uninvolved areas of six mastectomy specimens containing breast cancer. Three uninvolved areas contained essentially normal glands, whereas the other three exhibited epithelial hyperplasia of ducts and alveoli. Four of the patients were premenopausal, five were postmenopausal and none were pregnant or lactating.

Organ culture. Tissue samples were cut into 0.5-mm cubes which were divided into three parts and placed on stainless-steel screens within three 35- by 10-mm petri dishes (Falcon Plastics, Oxnard, Calif.) containing 1.5 ml of medium. The cultures were carried out at 37° C in a water-saturated atmosphere of 95% air: 5% CO2. Medium 199 (Flow Laboratories, Rockville, Md.) supplemented with 2 mM glutamine, 100 U per ml penicillin and 100 μg per ml streptomycin was the basic medium. The cultures were supplemented additionally with patient serum obtained a day before surgery to a final concentration of 10%.

Glycoprotein synthesis and secretion. At the start of culture, labeled glycoprotein precursors obtained from New England Nuclear Corp. (Boston, Mass.) were added individually to the three cultures. Fucose[L-(1-3H) specific activity 1 to 5 Ci per mmol], 20 to 60 μCi; glucosamine hydrochloride [D-[6-3H(N)] specific activity 10 to 30 Ci per mmol], 20 to 60 μCi; and acetyld-Mannosamine [N-[3H(G)] specific activity 2 to 10 Ci per mmol], 20 to 60 μCi; were used. Some explants were harvested after 4, 8, 20 and 24 hr incubation with label and fixed; others were transferred to cold basic medium containing patient serum to a final concentration of 10% before surgery to a final concentration of 10%.

 Autoradiography. For light-microscopic autoradiography using Ilford L4 emulsion (Ilford, Ltd., Ilford, Essex, England) by the loop method of Maunsbach (13). Distribution of radioactivity in glands was described qualitatively in light-microscopic autoradiographs as being located primarily either in the paranuclear Golgi region or apical cytoplasm of the epithelium or in the gland lumina and brush border. The intensity of autoradiographic reactions over connective tissue cells and matrix as well as over the periphery of glands, which corresponds to the location of basal plasma membranes and basement membranes, also was evaluated. At each time interval studied, 50 epithelial cells from each experiment were scored by a single observer without knowledge of diagnosis or labeling conditions.

Characterization of glycoprotein secretion products. Additional tissue samples from each of the two fibroadenomas were cultured as in the autoradiographic experiments in 35- by 10-mm petri dishes containing 10μCi [3H]glucosamine. After 72 hr the culture medium, containing labeled material released by the explants, was collected and centrifuged to remove cellular debris. Solid urea was added to the supernate, 10 M final concentration, and after 30 min at 37° C, the urea-treated medium was extensively dialyzed at 4° C for 3 days against 0.01% ammonium bicarbonate and lyophilized. Portions of the lyophilized samples were subjected to urea-sodium dodecyl sulfate electrophoresis (SDS-PAGE) (14) by means of 7.5% precast polyacrylamide gels (Bio-Rad, Richmond, Calif.) under reducing conditions. Molecular-weight markers were heavy and light polypeptide chains from human immunoglobulin. After electrophoresis, the gels were cut into 2-mm slices, and radioactivity within each slice was determined using scintillation counting after treatment with Beckman Tissue Solubilizer.

RESULTS

Most of the explants used for experimentation from the nine breasts were found to contain ducts and alveoli embedded in a connective tissue stroma. The explants from benign fibroadenomas contained branching, tubular ducts with substantial epithelial proliferation. Epithelial proliferation without significant cellular atypia also was marked in hyperplastic glands within explants from three of the six mastectomy specimens. Explants from the reduction mammoplasty and the