Radioimmunoassay for Tissue Distribution of a Human Mammary
Tumor-specific Glycoprotein

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ABSTRACT

The candidate tumor-specific soluble mammary tumor glycoprotein with a molecular weight of approximately 20,000 (MTGP20) has been isolated from human breast carcinomas and characterized biochemically. Although with the use of xenoantisera this glycoprotein has previously been demonstrated only in breast carcinomas, analyses of body fluids and conclusions regarding putative tumor specificity have been limited by the sensitivity of assays. In the present study, a specific radioimmunoassay has been developed. With appropriate selection of buffer, the assay has a sensitivity threshold of <0.1 unit of MTGP20 per ml, equivalent to <250 pg type I MTGP20 per ml or <530 pg type II MTGP20 per ml, which is more than 200-fold more sensitive than previously described assays. MTGP20 type I, which contains tyrosine, was labeled with 125I and used as the immunochemical ligand in a double antibody competitive inhibition assay. A partial weak cross-reaction was observed with a mixture of placental glycoprotein (perchloric acid extract), but this reaction was abolished by absorption of antisera with placental glycoprotein. Normal tissue and tumors of other than breast origin were devoid of MTGP20-related antigens. MTGP20-related antigens were not detectable in sera or concentrated urine specimens from normal individuals or patients with metastatic breast carcinomas. The present studies further support the probable tumor specificity of this glycoprotein but indicate that it does not represent a circulating secretory product of the cancer cell and does not provide a serum marker for breast carcinoma.

INTRODUCTION

In the search for tumor-specific molecules, and investigation of their molecular structure and genomic derivation, a candidate molecule has been identified and partially characterized. Soluble MTGP20 is a cellular glycoprotein (MW about 20,000) which has been purified to homogeneity from metastatic human ductal carcinomas of the breast (9, 10). MTGP20 has been observed to occur in tumor cells not only in the soluble or cytosol form but also as an ultracentrifugally sedimentable form of plasmalemma derivation (8). Two types of soluble MTGP20 have been identified. These differ in isoelectric point, buoyant density, and carbohydrate composition but differ little in amino acid composition and share the major antigenic determinants of the molecule (10). Using antisera specific for MTGP20 and reactive with both types, the soluble glycoprotein has been identified in biopsies of 76% of breast carcinomas using qualitative electroimmunodiffusion (7). In contrast, it has not been possible to identify MTGP20-related antigens in benign breast tissue or breast milk or in tumors or tissues of origin other than the breast (7, 10). Because of the need for a highly sensitive assay for analysis of the molecular biology and neoplastic significance of this tumor-associated glycoprotein, we have developed a specific radioimmunoassay.

MATERIALS AND METHODS

Mammary Tumor Glycoprotein. MTGP20 types I and II were isolated in a method similar to that previously described (10). Briefly, 20 g of breast carcinoma tissue were homogenized in 40 ml of 0.0005% PMSF, passed through a 50 mesh sieve, and centrifuged for 1 hr at 100,000 x g. To the supernatant at 4° was added 0.5 volume of cold 1.8 M PCA. Following centrifugation, the supernatant was neutralized with 0.1 M sodium carbonate and exhaustively dialyzed against water. MTGP20 was isolated by successive: (a) preparative isoelectric focusing; (b) affinity chromatography on insolubilized IgG fraction of antisera to human plasma, human plasma glycoproteins, α1-antitrypsin, CEA, NCA, and Blood Group A and B antigens; and (c) using the unbound fraction, molecular exclusion chromatography on ACA-44 (10).

Antiserum G-300 was obtained by immunization of a goat with 10 μg of partially purified MTGP20 emulsified in complete Freund’s adjuvant. The goat was boosted 5 times at monthly intervals with 10 μg MTGP20 in incomplete adjuvant. An aliquot of antiserum was absorbed with a panel of lyophilized homogenates of normal tissues then further absorbed with glycoprotein fractions of human lung and colon which had been immobilized on Bio-Gel P-6 or Sepharose 4B (3, 18). The antigen gave a single precipitin upon reaction with the concentrated PCA-soluble fraction of all of a panel of 6 breast carcinomas, and the precipitin exhibited identity with purified MTGP20.

Electroimmunodiffusion Assay. Electroimmunodiffusion assay was performed on 7.5- x 10-cm glass slides using absorbed G-300 antiserum as previously described (10). Precision (coefficient of variation) varied from 6.6% at 500 units MTGP20 per ml sample to 21.5% at 125 units per ml.

Radioiodination. MTGP20 was radioiodinated according to a modified chloramine-T procedure (2). To 5 μg of MTGP20 type I (5 μl) in 5 μl of 0.5 M phosphate buffer, pH 7.4, was added 0.5 mCi of Na125I (5 μl) at 4° and then 3 μl of chloramine-T (5 μl). After 5 min, the reaction was terminated by addition of 3.1 μg of sodium metabisulfite (5 μl). BSA was added to a 1% final protein concentration. Following dialysis, the solution was passed through Dowex AG 1-X2, and the MTGP20 was recovered by chromatography on ACA-54. The 125I-MTGP20 peak was pooled, adjusted to 3.5% BSA and 0.3 M glycine in 0.015

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2 The abbreviations used are: MTGP20, mammary tumor glycoprotein of about 20,000 molecular weight; PMSF, phenylmethylsulfonyl fluoride; PCA, perchloric acid; CEA, carcinoembryonic antigen; NCA, nonspecific cross-reacting antigen; BSA, bovine serum albumin; TCA, trichloroacetic acid; NGS, normal goat serum.

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Radioimmunoassay assay of the double antibody type was used (14). Twelve different solvent systems were evaluated with final adoption of 0.01 \( \text{m} \) sodium borate, pH 8.0 (conductance, 1 mmho)-0.01% sodium azide, containing 0.0005% PMSF. The reagents were added to 12- x 70-mm glass tubes in sequence: (a) the inhibitor compartment containing 0.5 ml of competing antigen in 1\% BSA-borate buffer; (b) the \( 125^\text{I} \)-labeled compartment containing 125 \( \mu\text{l} \) of \( 125^\text{I} \)-labeled glycoprotein (2 ng) in 1:60 NGS in borate buffer, and (c) the antiserum compartment containing 125 \( \mu\text{l} \) of the appropriate dilution of anti-MTGP in 1:60 NGS borate buffer. Seventy-two hr later was added the second antibody compartment containing 250 \( \mu\text{l} \) of rabbit anti-goat IgG antiserum or 40\% TCA (for estimation of maximum precipitable counts), or 1:4 dilution of NGS (for estimation of minimum precipitable counts). Four hr later, tubes were centrifuged for 30 min at 1000 \( \times \text{g} \) at \( 4^\circ \), and 500 \( \mu\text{l} \) of the supernatant were quantitatively recovered, and \( 125^\text{I} \) was quantitated in a gamma counter. In analysis of antigen-binding capacity, the inhibitor compartment contained only BSA-borate buffer and the antibody compartment contained anti-MTGP at serial 1:2 dilutions. In competitive inhibition analyses, the antibody compartment contained anti-MTGP at 1:100 dilution. The same standard breast tumor extract used in standardization of quantitative electromuno-diffusion assays (10) was used. This PCA-soluble fraction of T-124 breast carcinoma at a protein concentration of 218 \( \mu\text{g} /\text{ml} \) was designated at 1000 units/ml. Precision (coefficient of variation) varied from 2.8\% at 0.1 5 units MTGP per ml to 8.4\% at 2.5 units per ml.

Soluble Tissue Glycoproteins. These were prepared by homogenization of 2 g of neoplastic, benign, or normal tissue in 12 ml of water containing 0.0005% PMSF at \( 4^\circ \). To 12 ml of ultracentrifuged supernatant (100,000 \( \times \text{g} \) for 30 min) were added 6 ml of 1.8 \( \text{m} \) PCA at \( 4^\circ \). The soluble glycoprotein fraction was recovered by centrifugation at 10,000 \( \times \text{g} \) for 30 min (10). This was neutralized; the PCA-soluble glycoproteins were extensively dialyzed, lyophilized, and redissolved; and the glycoprotein was adjusted to 1 mg protein per ml by Lowry analysis (11).

Sedimentable Tissue Fractions. The sedimentable fractions of the tissue homogenates were recovered after ultracentrifugation of the homogenates (above) at 100,000 \( \times \text{g} \) for 30 min. The pellets were washed 3 times with 12 ml 0.0005% PMSF at \( 4^\circ \) and resuspended in an equal volume of distilled water. The protein content of the suspensions was estimated by a modified Lowry assay (12) and adjusted to 1 mg/ml.

Cytosols. Cytosols of breast carcinomas were prepared by homogenization of 1 g of frozen sample in 5 ml of 10 mm Tris-HCl-1.5 mm EDTA-1 mm dithiothreitol, pH 7.4, at \( 4^\circ \) according to the method of Johnson et al. (6). The homogenates were ultracentrifuged at 100,000 \( \times \text{g} \) at \( 4^\circ \) for 30 min to obtain clear lipid-free supernatants which were adjusted to 1 mg protein per ml by standard Lowry assays (11).

Urine and Serum Samples from Patients with Breast Carcinomas. Sera were collected and stored at \( -20^\circ \). Urine samples, usually 24 hr collections, were filtered (E. & S. No. 15 filter paper) followed by 0.2-\( \mu\text{m} \) Amicon No. DPOZ filters and then concentrated 400-fold with an Amicon PM-10 membrane under positive pressure.

Serum Samples. Serum samples from patients with breast carcinomas were stored at \( -20^\circ \).

Cell Culture. Human breast carcinoma line AY-726 and BF-964, a control fibroblast line from the breast of donor Hs-578T (5, 10), were originally provided by Dr. Walter Nelson-Rees, Cell Culture Laboratory, University of California, School of Public Health, Naval Bioscience Laboratory, Oakland, Calif., and were grown in minimum essential medium supplemented with 10\% fetal calf serum, penicillin (50,000 units/liter), streptomycin (50,000 \( \mu\text{g} /\text{l} \)), 0.2 \( \text{mM} \) l-glutamine, 20 \( \text{mM} \) 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Grand Island Biological Co., Grand Island, N. Y.), gentamicin (2.5 mg/liter, Schering Corp., Kenilworth, N. J.), and 0.5\% Fungizone (Flow Laboratories, Rockville, Md.). Cultures were propagated in Falcon 75-sq cm flasks (Becton-Dickinson, Oxnard, Calif.) seeding at about 10\( ^6 \) cells/flask at 37\% and 5\% \( \text{CO}_2 \) in air.

Polyacrylamide Gel Electrophoresis. Analytical polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed according to the method of Weber and Osborn (17), as described previously (10). Following electrophoresis, gels were sliced at 2-mm intervals and counted in a gamma counter.

Other. Murine mammary tumor virus was obtained from Dr. J. Schlom (National Cancer Institute). NCA was obtained from Dr. P. Burtin of the Institute for the Scientific Research of Cancer, France. CEA and CEA species were purified in this laboratory as described earlier (14).

RESULTS

Radioiodination of MTGP for use as the labeled ligand is critical. Under a variety of conditions and with commonly used methods of radioiodination such as the lactoperoxidase (13) and Bolton-Hunter (1) methods, less than 1\% of the \( 125^\text{I} \) was incorporated into TCA-precipitable protein eluting with the MTGP peak from an ACA-54 column. The cause of the failures of effective oxidative labeling by lactoperoxidase or direct labeling by the Bolton-Hunter reagent have not been identified but might result from local steric hindrance by the abundant surface carbohydrate. Type I MTGP was labeled with the chloramine-T method under selected conditions, whereas type II did not incorporate \( 125^\text{I} \), presumably reflecting the lack of a tyrosine in the latter (10). Significant degradation of MTGP type II following labeling was indicated by the progressive appearance with storage at \( 4^\circ \) of \( 125^\text{I} \) which was not concordant with the MTGP band when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis or subjected to molecular exclusion chromatography on ACA-54. With carefully limited quantities of chloramine-T, degradation was minimized, the \( 125^\text{I} \) recovered from the ACA-54 column was concordant to MTGP, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis gave a single radiolabeled band with a relative migration identical to that of unlabeled MTGP. \( 125^\text{I} \)-MTGP had limited stability in 0.9\% NaCl solution, but when stored in 3.5\% BSA-0.3 \( \text{m} \) glycine, pH 5.5, the TCA precipitability and immunoprecipitability of \( 125^\text{I} \)-MTGP remained greater than 90\% for 3 weeks at \( -20^\circ \).

Immunoprecipitation of MTGP was observed at high concentration of antigen and antiserum in gel diffusion (8); however, antisera to MTGP appeared to have relatively low affinity as indicated by limited maximum binding of \( 125^\text{I} \)-MTGP at low.
concentration (0.1 pmol/assay) and shallow binding curves upon dilution of antisera in a variety of buffers. The possibility of conformational subsets of MTGP\textsubscript{20} that differ in surface exposure of antigenic epitopes can also be considered to explain the partial maximum binding. Twelve solvent-buffer systems were explored including borate, acetate, and phosphate ions over a variety of ion concentrations, incubation periods, and at temperatures of 4, 22, or 37°. The pH range 5.5 to 8.0 was examined as was the use of polyethylene glycol 6000 and dextran T-500 as augmenting agents. Hyperimmune goat anti-MTGP\textsubscript{20} antiserum (G-300) bound \textsuperscript{125}I-MTGP\textsubscript{20} to a greater degree than did 4 different rabbit antisera. By comparison of all solvent systems: (a) binding was 0.4- to 2.0-fold greater at 3 days than at 1 day incubation; (b) binding was 1.8- to 5.0-fold greater at 4° than at 37°; and (c) nonspecific binding was <1%. Maximal binding was observed with low-ionic-strength solvents and optimal for 10 mM sodium borate buffer (conductance, 1 mmho). Neither dextran nor polyethylene glycols were capable of further augmentation of binding of \textsuperscript{125}I-MTGP in the 10 mM borate buffer, although they did augment specific and background binding in a number of higher-ionic-strength buffers. Binding of \textsuperscript{125}I-MTGP\textsubscript{20} is illustrated for 72 hr assay at 4° and shows a relatively shallow binding curve on dilution (Chart 1).

Antiserum G-300 at 1:100 dilution bound 50% of the \textsuperscript{125}I-MTGP\textsubscript{20} and was used at this concentration in equilibrium competitive inhibition assays. Standard PCA-soluble glycoprotein from breast tumor T-124 produced typical competitive inhibition over a progressive range of concentrations, and complete inhibition of the binding of \textsuperscript{125}I-MTGP\textsubscript{20} by anti-MTGP\textsubscript{20} was approached at high concentration (Chart 2). The limit of sensitivity of the assay was 0.05 to 0.1 unit/ml, and the maximum assayable concentration was about 5 units/ml. Both forms of purified MTGP\textsubscript{20} inhibited binding with a mean of 2.5 ng/unit for type I MTGP\textsubscript{20} and 5.3 ng/unit for type II (average, 3.9 ng/unit). MTGP\textsubscript{20} type II has been shown to differ slightly from MTGP\textsubscript{20} type I by physicochemical analysis (10). The highest concentration of MTGP\textsubscript{20} type II that was the same as MTGP\textsubscript{20} type I did not achieve complete inhibition in this analysis, which could reflect this difference. The competitive inhibition slopes of the purified glycoproteins were not significantly different from the arbitrary standard.

Reactivity in the radioimmunoassay was observed with the PCA-soluble glycoprotein from each of 3 human placenatas with an average of 980 pg MTGP\textsubscript{20} equivalent antigen per mg placental glycoprotein (range, 570 to 1220 pg/mg). Titration of these placental glycoproteins gave a very shallow inhibition profile, which was not parallel with the standard curve and only partially inhibited the assay (approximately 15%), indicative of an immunonochemical cross-reaction rather than the presence of MTGP\textsubscript{20} molecules. The weak cross-reaction was abolished by absorption of 1 ml of G-300 anti-MTGP\textsubscript{20} antiserum with 100 μg of placental PCA-soluble glycoprotein. This absorbed anti-MTGP\textsubscript{20} antiserum, bound \textsuperscript{125}I-MTGP\textsubscript{20} and binding was completely inhibited by MTGP\textsubscript{20} or breast carcinoma extracts containing MTGP\textsubscript{20} at equivalent concentration, but not by placental glycoprotein. All subsequent analyses used anti-MTGP\textsubscript{20} absorbed with placental glycoprotein.

Panels of normal tissue homogenates and their PCA-soluble glycoprotein fractions were assayed at 10 mg protein per ml homogenate or 1 mg glycoprotein per ml, respectively. None of 37 preparations of 24 different tissues [colon, 3; uterus, 1; testes, 1; placenta, 3; prostate, 1; spleen, 3; ovary, 1; thyroid, 1; urinary bladder, 1; liver, 2; kidney, 1; muscle, 1; seminal vesicle, 1; diabetic kidney, 1; brain, 1; heart, 1; gall bladder, 1; salivary gland, 3; plasma, 2; adrenal, 1; pancreas, 3; breast, 4 (Table 1)], including human breast tissue or milk, contained detectable MTGP\textsubscript{20} as indicated by a maximum inhibition of less than 3% (Chart 2) or less than 125 to 500 pg/mg glycoprotein.

Soluble MTGP has been undetectable in 12 tumors of other than breast origin, including epidermoid and adenocarcinoma: pancreas, 1; liver, 1; kidney, 1; bowel, 1; lung, 1; tongue, 1; testes, 1; stomach, 1; colon, 2; as well as 2 sarcomas. In contrast, it was readily demonstrated in the cytosol preparations of 9 MTGP\textsubscript{20}-positive and not in 10 MTGP\textsubscript{20}-negative biopsies of breast carcinomas when reassayed by radioimmunoassay. The MTGP\textsubscript{20}-positive and -negative biopsies were determined by electroimmunodiffusion assay in a previous study (7), where 66 of 83 breast carcinoma biopsies were positive (Table 1). Comparison of an electroimmunodiffusion assay and a radioimmunoassay is also shown in Chart 3. There is a statistically significant correlation (r = 0.87; p < 0.01) between assay of cytosol preparations of 10 MTGP\textsubscript{20}-positive breast carcinoma biopsies, although concordance is by no means precise. The disagreement between quantitation is...
Table 1

MTGP<sub>20</sub> content of tumors and normal tissues by radioimmunoassay

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. tested</th>
<th>No. positive</th>
<th>Concentration&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsies (cytosol), MTGP&lt;sub&gt;20&lt;/sub&gt; positive&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
<td>9</td>
<td>400–1400 units/mg protein</td>
</tr>
<tr>
<td>Biopsies (cytosol), MTGP&lt;sub&gt;20&lt;/sub&gt; negative&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10</td>
<td>0</td>
<td>&lt;0.1 unit/mg protein</td>
</tr>
<tr>
<td>Cell line HS-578T (AY-726)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble fraction</td>
<td>1</td>
<td>1</td>
<td>1.7 units/10&lt;sup&gt;6&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>Sedimentable fraction</td>
<td>1</td>
<td>1</td>
<td>3.9 units/10&lt;sup&gt;6&lt;/sup&gt; cells</td>
</tr>
<tr>
<td>Sera</td>
<td>10</td>
<td>0</td>
<td>&lt;0.1 unit/ml</td>
</tr>
<tr>
<td>Urine</td>
<td>16</td>
<td>0</td>
<td>&lt;0.0025 unit/ml</td>
</tr>
<tr>
<td>Non-breast tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble fraction</td>
<td>12</td>
<td>0</td>
<td>&lt;0.1 unit/mg glycoprotein</td>
</tr>
<tr>
<td>Sedimentable fraction</td>
<td>5</td>
<td>0</td>
<td>&lt;0.1 unit/mg glycoprotein</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sera</td>
<td>10</td>
<td>0</td>
<td>&lt;0.1 unit/ml</td>
</tr>
<tr>
<td>Non-breast tissue, sedimentable fraction</td>
<td>10</td>
<td>0</td>
<td>&lt;0.1 unit/mg protein</td>
</tr>
<tr>
<td>Non-breast tissue, soluble fraction</td>
<td>35</td>
<td>0</td>
<td>&lt;0.1 unit/mg glycoprotein</td>
</tr>
<tr>
<td>Breast tissue&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6</td>
<td>0</td>
<td>&lt;0.1 unit/mg glycoprotein</td>
</tr>
</tbody>
</table>

<sup>a</sup> 250 pg MTGP<sub>20</sub> type I per unit; 526 pg MTGP<sub>20</sub> type II per unit.
<sup>b</sup> Ref. 7.
<sup>c</sup> Includes fibrocystic disease and gynecomastia.

somewhat greater than would be expected from the 2.8 to 8.4% precision of the radioimmunoassay and the 6.6 to 21.5% precision of the electropherophoretic diffuse assay, suggesting somewhat different quantitative reactivity of MTGP<sub>20</sub> from different tumor cytosols.

The possibility that membrane-associated MTGP<sub>20</sub> (8) alone might be present in some tissues was examined using the ultracentrifuged pellets of normal tissues and non-breast tumors. In none of these could MTGP<sub>20</sub>-related antigens be demonstrated by radioimmunoassay, although present in breast carcinomas and breast carcinoma cell lines, i.e., 6.0 units/mg protein of tumor C-261 sedimentable fraction and 3.9 units (1.56 ng)/10<sup>6</sup> HS-578T (AY-726) cells. In contrast, fibroblast line BF-924 and breast myoepithelial cell line BF-724, both from donor breast tissue HS-578T, were devoid of MTGP<sub>20</sub> (<0.1 unit/10<sup>6</sup> cells).

Carcinoembryonic antigen, both CEA and CEA-species, as well as NCA at 1000 ng/ml did not react in the competitive inhibition assay, further demonstrating the immunochemical dissimilarity between MTGP<sub>20</sub> and these tumor-associated molecules. In addition, a preparation of murine mammary tumor virus was assayed. At 500 µg/ml, there was no observed reactivity for MTGP<sub>20</sub>.

MTGP<sub>20</sub> was not detected (0.1 unit/ml) in neat normal sera or in the PCA-soluble serum glycoprotein fractions at 1 mg/ml. In addition, MTGP<sub>20</sub> was undetectable in the serum from 10 patients or in the concentrated urine (0.0002 unit or 0.08 pg/ml) of 16 patients with metastatic breast carcinoma. The results are summarized in Table 1.

**DISCUSSION**

Detailed analysis of conditions of radiiodination of MTGP<sub>20</sub> and appropriate selection of a buffer solvent system has led to a competitive inhibition radioimmunoassay capable of measuring as little as 0.05 to 0.1 unit equivalent to 0.12 to 0.25 ng type I MTGP<sub>20</sub> or 0.26 to 0.53 ng type II MTGP<sub>20</sub> per ml. The assay is not perturbed by normal tissue glycoprotein at concentrations as high as 1 mg/ml. MTGP<sub>20</sub> could not be detected in the homogenate, soluble glycoprotein fraction, or membrane-rich fractions of normal tissues or tumors of other than mammary gland epithelial derivation.

The results of this study continue to support the hypothesis that MTGP<sub>20</sub> may be a specific marker for breast carcinomas. The radioimmunoassay provided a greater than a 200-fold increase in sensitivity and a greater precision than did previously used electropherophoretic assays; however, MTGP<sub>20</sub> could not be detected in normal tissues, non-breast tumors, serum from patients with breast neoplasms, or normal sera or serum glycoprotein fractions. It was, however, found in both soluble and insoluble extracts of breast tumors. Immunochemical independence from CEA, NCA, murine mammary tumor virus, and other tumors demonstrates that MTGP<sub>20</sub> is a discrete marker for breast neoplasms.
It is conceivable that some breast carcinomas such as the 10 MTGP\textsubscript{20}-negative tumors (Table 1) do not synthesize soluble MTGP\textsubscript{20}. However, it is also possible that MTGP\textsubscript{20} occurs in concentrations lower than that of the detection threshold. In the present analysis, we have demonstrated modest concentrations lower than that of the detection threshold. In some tumors still might account for the negative results; however, the degree of sensitivity and precision of the radioimmunoassay permits more critical analysis of tumors.

It appears from the present study that MTGP\textsubscript{20} is not secreted or shed from the neoplastic cell to circulate in serum in significant concentration. Its absence from the urine and sera of breast carcinoma patients suggests either internalization of the molecule from the cell surface during membrane metabolism or shedding with very rapid clearance. Although the catabolism of many cell surface molecules includes shedding or secretion (16), there is also evidence indicating that some cell surface glycoproteins may be internalized subsequent to exposure on the surface. Doyle et al. (4) have observed in hepatoma tissue culture cells the presence of cell surface glycoproteins in substantial quantities in an internal membrane compartment. Ronzio et al. (15) demonstrated that a plasma membrane protein of the pancreatic exocrine cell was found at higher quantities in the zymogen granules than in smooth microsomal fractions or plasma membrane. The relationship between the cytosol and cell surface forms of MTGP\textsubscript{20} as well as their role as target antigen in tumor immunobiology are under investigation.

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