ABSTRACT

Previous immuno- and lectin-histochemical studies using mAbs and Ulex europaeus lectin I, which recognize various fucose-containing blood group antigens, have shown an increased expression of Lewis and H blood group antigens in endometrial carcinoma. We investigated the biochemical basis of aberrant fucose-containing antigen expression by comparing the activity of fucosyltransferases (FTase) and a-L-fucosidase in tissue biopsies from normal (n = 18) and malignant (n = 20) endometrium. Alteration of FTase activity in tumor tissue homogenates was evaluated by using a panel of FTase substrates including N-acetyllactosamine (type 2), lacto-N-biose I (type 1), and phenyl-ß-D-galactoside. Based on histological subtyping, the endometrioid group (n = 14) showed a significant (P < 0.05) increase in tumor FTase activity with all three substrates, while no significant increase was detected for the papillary serous group (n = 4). Matched pair analysis of normal and tumor tissue from a subgroup (n = 5) of the patients with increased tumor enzyme activity also showed higher FTase activity (P < 0.05) in the tumor tissue when the type 1 substrate was used. Regression analysis showed a correlation between the FTase activities acting on type 2 or type 1 substrates (r = 0.821 and r = 0.722, respectively) and the endogenous fucose levels in tumor homogenates. Spectrophotometric analysis of a-1-fucosidase activity using p-nitrophenyl-ß-D-l-fucoside revealed a higher activity in tumor homogenates than in normal homogenates (P < 0.05) and, therefore, could not account for the enhanced expression of fucose-containing antigens. The current study suggests that aberrant expression of fucose-containing antigens, such as the H and the Lewis blood-group antigens, have shown an increased expression of Lewis and H blood group antigens, in endometrial carcinoma is consequential to the change in FTase rather than in a-L-fucosidase activity. In addition, the investigation suggests that different glycosylation mechanisms are operative in different subtypes of endometrial cancer.

INTRODUCTION

Alteration in cellular glycosylation patterns is a feature routinely shared by diverse types of neoplastic diseases (1–6). Such changes may occur in the form of: (a) incomplete synthesis of carbohydrate structures; (b) reexpression of oncofetal antigens that normally exist during the embryonic development but either disappear or become minimally expressed in the corresponding adult tissues; or (c) organizational changes, as shown by immuno- and lectin-histochemical staining, due to demasking or increased expression of normally expressed carbohydrate structures (7).

Previous immuno- and lectin-histochemical comparison of normal and malignant uterine endometrium have demonstrated that fucose-containing blood group antigens (H and Lex(h)) consistently shown higher and differential expression (cytoplasmic versus apical or luminal surface) in malignant tumor cells (8–12). No correlations have been found, however, between the aberrant expression of fucose-containing antigens and the age, ABO status, or the menstruation cycle of the patients (8–10). Recently, a positive correlation between the invasion has been reported in endometrial carcinoma (12). Collectively, these observations of malignant endometrial tissue provide indirect evidence for an abnormality in the activity of either the fucosyltransferases responsible for synthesis of the fucose-containing antigens or a fucosidase that catalyzes the hydrolytic cleavage of the terminal fucose from the carbohydrate chain.

The fucose-containing blood group antigens (H and Lex(h)) are synthesized from two carbohydrate precursors: type 1 (Galβ1→3GlcNAc-R) and type 2 (Galβ1→4GlcNAc-R) precursors (13–16). Distinctive FTases are involved in the process for fucose in either α1→2 linkage to terminal Gal and/or in α1→3/4 linkage to subterminal GlcNAc and are, therefore, designated as α-2, α-3, and α-4-L-FTase, respectively (16–19). Cancer-associated increase of these FTases have been observed frequently in serum or tumor tissues from patients with various types of carcinomas (20–31). In addition, it has been shown that, in patients with breast, colon, or gastric cancers, the removal of tumors was followed by a significant reduction in serum activity of FTases, while recurrence of cancer or metastasis tended to occur in patients who displayed increased activity of the FTases (20, 28, 31). Similar to the observations with the FTases, abnormal levels of α-l-fucosidase have also been found in tissue extracts of ovarian, cervical, endometrial, colon, and liver carcinomas (32–35).

The present study has been undertaken to determine the activity of FTases in normal and endometrial tumor tissues. Three different fucose-acceptors with the precursor structures of type 1, type 2, and pGal were used to detect FTases with different acceptor substrate preferences (18, 36, 37). In addition, a-1-fucosidase activity was measured using p-nitrophenyl-α-D-fucoside. Total fucose concentration in tissue homogenate was assessed by HPAEC. Our data show an increase in activity of FTases as well as α-L-fucosidase in the endometrioid subtype of carcinomas. Furthermore, the enhanced FTase activities are associated with an increase in tissue fucose content.

MATERIALS AND METHODS

Reagents. pGal was obtained from Fluka Chemical Corp. (Ronkonkoma, NY). Two shipments of GDP-[3H]fucose (6700 and 8100 Ci/mol) were purchased from New England Nuclear (Wilmington, DE). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless stated otherwise.

Patient Material. Eighteen normal and 20 tumor biopsies of endometrium were obtained from the Albany Medical Center Tumor Bank based upon a random sampling of endometrial tissue collected from surgical cases at the Albany Medical Center. Demographic and clinical information regarding the age of the patients, stage of the disease, as well as tumor grade and the histological type were obtained after biochemical analysis was completed (Table 1). The endometrioid subtype predominated in the sample population due to the relatively higher frequency of occurrence for this histological subtype of tumors in endometrial carcinoma.

Tissue Processing. Hematoxylin and eosin-stained sections from OCT-embedded (Baxter Diagnostics Inc., McGaw Park, IL) tissue samples were stained with the Ulex europaeus lectin I staining and myometrial and vascular...
prepared and reviewed microscopically to confirm the normal or neoplastic characteristics of the tissue used for biochemical analysis. OCT compound was removed by placing the tissue in ice-cold, double-distilled water before the homogenization procedure. All subsequent steps were performed on ice.

Each specimen was finely minced with surgical scissors, and the tissue was suspended in 50 mM HEPES buffer (pH 7.0) at a concentration of 100 mg of tissue/3 ml of buffer. The tissue was homogenized with a two-speed Biospec homogenizer (Biospec Products, Inc., Bartlesville, OK) for two and four 10-s bursts at low and high speed, respectively. Triton X-100 was added to the homogenate at a final concentration of 0.1% (v/v). The homogenate was centrifuged for 30 s in a Sero-fuge bench-top centrifuge (Clay-Adams, New York, NY), and the supernatant was aliquoted and stored at −70°C in the freezer until analyzed. The bicinechonic acid assay (Pierce Chemical, Rockford, IL) was used to determine protein concentrations.

**Fucosyltransferase Assays.** A modification of a previously described assay was used for the detection of FTases in the homogenates (38). The FTase reaction mixture (80 μl) consisted of 50 mM HEPES (pH 7.0), 10 mM ATP, 12.5 mM MnCl₂, 12.5 mM NaN₃, 6.25 mM acceptor substrate (type 1, Galβ1→3GlcNAc; type 2, Galβ1→4GlcNAc; pGal), 1.8 μM GDP-[3H]fucose, and 20 μl of enzyme extract. Prior to the experiment, GDP-[3H]fucose was diluted 6-fold with unlabeled GDP-β-L-fucose of equimolar concentration. In addition, prior to analysis, the homogenates were diluted 2- or 4-fold for normal and tumor tissues, respectively, with the homogenization buffer [50 mM HEPES (pH 7.0)-0.1% Triton X-100]. After equilibration at 37°C, the reaction was initiated by the addition of GDP-[3H]fucose. For validation of zero-order kinetics throughout the 37°C incubation period, the reaction product was monitored at 0, 30, and 60 min. At the end of the appropriate incubation time, the reaction was stopped by placing the tubes in an ice bath. Control assays for each sample were run in the absence of acceptor substrate.

Separation of the radiolabeled products from unused GDP-[3H]fucose was achieved by using anion exchange column chromatography. Bond Elute columns (1.5 ml capacity; Analytichem International, Harbor City, CA) contained a 20 μm pore frit and were packed with a 0.5-ml bed volume of anion exchange resin (Dowex 1 × 2–400, formate form). The total reaction mixture volume was applied to the column, and the flow-through fraction was collected directly into a scintillation vial. The column was washed four times with 500 μl of distilled water. Fifteen milliliters of Scintisol (Krackeler Scientific, Inc., Albany, NY) were then added to each scintillation vial to obtain a clear solution. The radioactivity was counted for 10 min using a Beckman LS 5000 TD counter (Beckman Instrument, Inc., Fullerton, CA). The specific activity of fucosyltransferase was expressed as the micromoiies/mg protein. A micromol of enzyme activity is the amount of enzyme that will catalyze the conversion of one pmol of substrate to product/min.

Table 1 Clinical data of patients with/without endometrial cancer

<table>
<thead>
<tr>
<th>Normal</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient no.</td>
<td>Age</td>
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<tr>
<td>N1⁻</td>
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</tr>
<tr>
<td>N2⁻</td>
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<tr>
<td>33</td>
<td>84</td>
</tr>
</tbody>
</table>

Mean age, 59 ± 13

Mean age, 68 ± 13

¹ Normal and tumor matched pairs from five patients were available for the current investigation. The specimens were designated as N1 to N5 for normal tissue and T1 to T5 for corresponding tumor tissue.

² MMMT, malignant mixed mesodermal tumor; pap-serous, papillary serous tumor.

NA, not available.

RESULTS

**Fucosyltransferase Activities in Normal and Endometrial Tissue.** Biochemical assessment of FTase activity in normal (n = 18) and tumor (n = 20) tissue homogenates revealed a significant increase in tumor tissue activity with all three acceptor substrates (Fig. 1). Statistical analysis of tissue activity was also performed based on the histological subtype of the tumors. The results showed a significant increase in FTase activity with all three substrates for the endometrioid type (n = 14) of carcinoma (Fig. 1). Furthermore, with type 1 acceptor substrate, the mean FTase activity in endometrioid carcinoma homogenates was 11-fold higher than in the normal tissue homogenates. In contrast, no significant difference in enzyme activity was observed between the normal homogenates and papillary serous carcinoma homogenates independent of the substrate used (Fig. 1).

To determine whether the increase in FTase activities in endometrial tissues is a general phenomenon in both normal and tumor tissues from patients with endometrial cancer, normal and tumor matched pairs of tissue were obtained from different sites of surgically resected
endometrium from five patients. Again, mean FTase activity was higher in tumor homogenates with all three substrates, but the difference reached statistical significance only with the type 1 acceptor (Fig. 1).

In addition to the increase in FTase activity, tumor tissue also displayed differential patterns of activity between the three FTase substrate assays. As shown in Fig. 2, for example, tumor tissues T2 and 21 displayed higher FTase activity with type 2 acceptor as compared to the type 1 precursor. In sharp contrast, samples T4, 22, and 27 showed a prominent increase in FTase activity with type 1 precursor. In a number of tumor preparations, including T3 and 32, FTase activities increased proportionately in the type 1 and 2 acceptor assays. Therefore, the activity patterns varied significantly among patients with the endometrioid carcinoma. In contrast, patients who had papillary serous carcinoma (patients 23 to 26) showed no significant change in FTase activity nor any differential change in enzyme activity patterns for the three substrates (Fig. 2).

Total Fucose Measurement and Comparison to FTase Activities. The direct measurement of fucose in tissue from patients with and without endometrial carcinoma showed an increased fucose concentration in homogenates from patients with endometrial cancer (Fig. 3). A significant increase in fucose levels in tumor homogenates was also observed in matched pair comparisons of normal and endometrial cancer sites within the same patient. In contrast, the mean fucose level in the papillary serous carcinoma homogenates was comparable to the normal controls. Linear regression analyses of data for all tumor homogenates showed a strong correlation between total fucose levels and FTase activity with either type 2 or type 1 acceptor substrate ($r = 0.82$ and $r = 0.72$, respectively). Furthermore, total FTase activity as determined by summation of activity for the three substrate assays showed the highest correlation with fucose level in tumor tissue ($r = 0.88$).

α-L-Fucosidase Activities in Normal and Malignant Uterine Endometrium. A comparison of fucosidase activity in normal and tumor tissue showed an activity increase in the tumor group (Fig. 4). Similar to FTase findings, histological subtyping showed a significant activity increase in the endometrioid but not the papillary serous type of endometrial carcinoma. The increase in fucosidase activity in endometrioid-type tumor homogenates, however, averaged only 2-fold higher than control tissue. A weak positive correlation ($r = 0.57$) was observed between the tissue fucose levels and enzyme activity for the patients with endometrial carcinoma.
A number of studies have found an enhanced expression of Le\(^{a/b/y}\) and H-antigens, which are fucose-containing blood group antigens, in endometrial cancer cells (8–11). It was further suggested that increased Lewis antigen expression results from increased fucose transfer onto type 1 precursor substrates (10). The present biochemical findings provide direct evidence in support of the hypothesis that the increases in fucose-containing antigen profiles in endometrial cancer are, at least in part, related to alteration in FTase activities. The increased expression of tumor-associated fucose-containing antigens in endometrial cancer cells cannot be explained by a decrease in \(\alpha\)-fucosidase activity since this degradative enzyme also showed enhanced activity in tumor homogenates. This observation is in agreement with the result of Vesce and Biondi (35), who also found an increased \(\alpha\)-fucosidase activity in endometrial cancer tissues. Since certain glycosidases may act only on natural oligosaccharides but not on synthetic glycosides such as \(p\)-nitrophenyl-glycosides, natural oligosaccharides with different \(\alpha\)-fucosyl linkages may be required to determine the possible substrate specificity of the tumor-associated \(\alpha\)-fucosidase (42).

Although the use of three acceptors in our FTase assay allowed the assessment of changes in \(\alpha\)-2-, \(\alpha\)-3-, and \(\alpha\)-[3/4]-FTase (Lewis-type FTase), only the assay using pGal as acceptor was specific since this precursor allows only a single fucose substitution by \(\alpha\)-2-1-FTase, the FTase responsible for synthesizing H antigen (37). Nevertheless, the results with pGal precursor, which showed very low \(\alpha\)-2-1-FTase activity in most of the tumor homogenates, indicate that the major increase in FTase activity is in FTase with \(\alpha\)-3- or \(\alpha\)-[3/4]-FTase activity. The predominate increases in \(\alpha\)-3- or \(\alpha\)-[3/4]-FTase activities are consistent with enhanced expression of Lewis antigens by endometrial cancer cells as previously observed in histochemical studies (8–11).

The present study also provides evidence indicating that aberrant FTase activity in endometrial cancer cells is a specific, rather than a general, process in that differential patterns of enzyme activities toward type 1 and type 2 acceptors were observed in a number of tumor homogenates (Fig. 2). These differential patterns of enzyme activities may be explained by either a potential loss in substrate specificity in transformed tumor cells or by the possible co-expression of multiple FTases within the tumor mass (43, 44). Specific fucosylation mechanisms in endometrial cancer cells are further supported by the observations that increases in FTase activity was measured only in specific histological subtypes of endometrial carcinoma (Fig. 2). Higher FTase activities were found in the endometrioid subtype, which is associated with hyperestrinism and arises on a background of hyperplasia. In contrast, levels of FTase activity in papillary serous carcinoma, which are not associated with hyperestrinism or endometrial hyperplasia, were comparable to levels in normal homogenates.

In conclusion, the current investigation provides direct evidence that increased FTase activities are associated with endometrioid carcinoma and thus provide the biochemical basis for the increased expression of fucose-containing antigens observed previously by other investigators (8–11). Future molecular and biochemical characterization of the tumor-associated FTases (18, 38) in malignant uterine endometrium will be necessary to elucidate the potential significance of enhanced FTase activities and their differential pattern of activity in endometrial cancer genesis and subsequent metastasis.

### REFERENCES


Fucosyltransferase and α-L-Fucosidase Activities and Fucose Levels in Normal and Malignant Endometrial Tissue


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