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Human Ovarian Tumors Express γ-Glutamyl Transpeptidase

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ABSTRACT

Gamma-glutamyl transpeptidase (GGT) is a cell surface enzyme that initiates the cleavage of extracellular glutathione, thereby providing the cell with the amino acids necessary for increased synthesis of glutathione. GGT is induced in ovarian tumor cell lines selected in vitro for resistance to cisplatin. No study has examined GGT expression in primary human ovarian tumors. We analyzed frozen sections of 80 normal human ovaries and 56 ovarian tumors for expression of GGT. Histochemical staining showed that GGT was not expressed in the cells of the follicle or surface germinal epithelium of the normal ovary. GGT was expressed in some epithelial inclusion glands and occasionally in a small subset of stromal cells. Granulosa-stromal cell tumors were largely GGT-negative. In contrast, GGT-positive neoplastic cells were observed in 33 of 45 common epithelial ovarian tumors. None of the patients had been treated with chemotherapy. Some of the tumors had only rare GGT-positive cells, while others consisted almost entirely of GGT-positive cells. Among the low malignant potential and invasive tumors, at least one-half of the cells were GGT-positive in 6 of 9 serous borderline tumors (2 with mucinous foci), 0 of 1 borderline mucinous tumor, 3 of 12 serous papillary carcinomas, 2 of 3 mucinous carcinomas, 1 of 2 endometrioid carcinomas, 2 of 2 clear cell carcinomas, 0 of 2 transitional cell carcinomas, and 4 of 5 undifferentiated carcinomas. There was no correlation between the stage of the tumor and GGT expression, indicating that a GGT-negative tumor does not become GGT-positive as it progresses to a more widely disseminated lesion. In addition, there was no correlation between serum levels of CA 125 and GGT expression. These data show that GGT is expressed in many common ovarian epithelial neoplasms. We are currently following the response of these patients to chemotherapy to determine if expression of GGT serves as a marker for identifying neoplasms with enhanced resistance to platinum-based therapy.

INTRODUCTION

GGT (EC 2.3.2.2) is a cell surface enzyme that initiates the cleavage of extracellular glutathione (1). Induction of GGT has been reported in ovarian tumor cells selected for resistance to chemotherapeutic drugs. Godwin et al. (2) selected the human ovarian cell line A2780 for resistance to cisplatin. They found that there was a direct correlation between the increase in GGT mRNA levels and the level of resistance to cisplatin. Lau et al. (3) noted an increase in GGT activity in a subline of the human ovarian carcinoma cell line, ES-2, that was selected for resistance to cytosporone doxorubicin. Lewis et al. (4) reported that GGT activity was increased 6.5-fold in ovarian adenocarcinoma cell lines derived from a patient both before and after the onset of drug resistance to cis-platinum, chlorambucil, and 5-fluorouracil. In each of these studies, increased GGT activity correlated with increased intracellular glutathione concentration.

Hanigan and Piot (1) have proposed that GGT is part of a glutathione detoxification pathway. GGT cleaves the γ-glutamyl bond of glutathione. The cysteinyl-glycine dipeptide that is released by this reaction is susceptible to further cleavage by cell surface dipeptidases. Hanigan and Ricketts (5) have shown that expression of GGT provides the cell with a secondary source of cysteine that can be used for the synthesis of intracellular glutathione. γ-Glutamylcysteine synthetase is the rate-limiting enzyme in the synthesis of glutathione (6). γ-Glutamylcysteine synthetase is also increased in drug-resistant, GGT-positive cell lines (2). With an increased supply of cysteine and an elevated level of γ-glutamylcysteine synthetase, GGT-positive tumor cells have an increased capacity to synthesize glutathione. Elevating levels of intracellular glutathione is one mechanism of resistance to chemotherapeutic drugs such as alkylating agents and platinum derivatives (7).

Detection of the induction of GGT may provide a clinical marker for identifying tumors that are drug resistant due to an enhanced capacity for glutathione synthesis. Intracellular concentrations of glutathione are difficult to measure in clinical samples due to rapid oxidation of the glutathione (8). GGT activity, however, can be detected in frozen sections of tissue specimens (9). A specific histochemical stain produces a bright red precipitate at the site of GGT activity.

We initiated a study to analyze GGT expression in primary human ovarian tumor tissue before and after the onset of resistance to chemotherapy. Previous studies indicated that GGT was induced in ovarian tumor cells in response to treatment with chemotherapeutic agents (2–4). Our analysis of tumor tissue taken at the time of diagnosis showed that some of the tumors expressed high levels of GGT prior to treatment. These findings were unexpected and suggest that some ovarian tumors may be inherently resistant to chemotherapeutic drugs that are detoxified by glutathione.

To investigate the expression of GGT in ovarian tumors, we analyzed 56 ovarian neoplasms as well as 80 normal ovaries. We examined the relationship between GGT expression and the age of the patient, the stage of the tumor, and the serum level of CA 125.

MATERIALS AND METHODS

Specimens. We examined normal and neoplastic ovaries from patients undergoing surgery at the University of Virginia Health Sciences Center from April 1988 to April 1993. Patients ranged in age from 16 to 88 years. When the tissue specimen was received in the Division of Surgical Pathology, a portion was frozen and stored at −80°C. The remainder of the tissue was formalin-fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Ovarian neoplasms were classified according to the modified WHO classification of ovarian tumors (10).

Histochemical Detection of GGT. Three cryostat sections (4 to 6 µm) were cut from each frozen block. The sections were air dried, fixed in acetone at −20°C, and air dried again as described by Groves et al. (11). Sections were stained for GGT activity according to the procedure of Rutenberg et al. (9). To control for nonspecific staining, 5 mM serine and 10 mM borate, pH 7.5, inhibitors of GGT (12), were added to the staining solution. One section from each set was incubated with the inhibitors. Sections from rat kidney were used as positive controls. The sections were counterstained in a 0.05 M aqueous solution of light green yellowish (C.I. no. 42095), rinsed under distilled water, and mounted in glycerol. The third section from each set was stained in Gill’s hematoxylin NO-3 (Polysciences, Inc., Warrington, PA), rinsed in distilled water, and mounted in glycerol. All slides were evaluated and photographed within 3 days of staining.
Assessment of GGT Activity. The histochemical stain leaves an insoluble red dye at the site of GGT activity. Sections were analyzed by 2 observers (M. H. and H. F.). Each ovarian neoplasm was assigned a score from 0 to 4+ based on the percentage of the tumor cells that stained for GGT activity. The scoring system used was: 0, no GGT activity; +, rare to occasional GGT-positive tumor cells; ++, considerably less than one-half of tumor cells were GGT-positive; ++++, approximately one-half of the tumor cells were GGT-positive; ++++, the vast majority of the tumor cells were GGT-positive.

Staging and CA 125 Values. Staging information and CA 125 values were obtained from patient records. Tumors were staged by the surgeon according to the International Federation of Gynecology and Obstetrics staging for carcinomas of the ovary (13). CA 125 was determined by the Special Chemistry Laboratory at The University of Virginia Hospital with the CENTOCOR CA 125 radioimmunoassay (CENTOCOR, Malvern, PA).

Statistical Methods. Correlations were analyzed between GGT expression, histological classification, stage of the tumor, age of the patient, and serum CA 125 values. Correlations were compared by the $x^2$ test with Yates correction for 2 x 2 contingency tables and 0.05 significance level.

RESULTS

We analyzed 80 normal ovaries from 52 patients. The surface germinal epithelium, germ cells, follicles, and blood vessels were all negative for GGT staining. Sections from 7 patients contained a corpus luteum. Six of the corpora lutea stained negative for GGT, while one stained lightly positive. We observed both GGT-positive and GGT-negative epithelial inclusion glands. It was not uncommon to observe both GGT-positive and GGT-negative inclusion glands within the same section. One section from a 77-year-old woman contained 11 inclusion glands: 3 were positive for GGT and 8 were negative. In general, all of the epithelial cells within a single gland stained either GGT-positive or GGT-negative. While most ovarian stromal cells lacked GGT staining, approximately half the sections contained a few elongated GGT-positive cells clustered together within the stroma just below the surface epithelium. Large numbers of these elongated spindle-shaped cells were present within the stroma in sections from several patients with ovarian cysts. We have been unable to identify these cells, although they likely represent ovarian stromal cells.

Table 1 describes histological classification of each tumor, the age of the patient, the stage of the tumor, preoperative serum CA 125 level, and expression of GGT. There was a significant correlation ($P < 0.025$) between the age of the patient and the histological classification of the tumor as benign, LMP, or malignant. Patients with benign lesions were more likely to be less than 50 years of age (mean, 45 years). Patients with LMP tumors and malignant tumors were generally over 50 years of age (mean, 61 years).

Within the epithelial tumors there was a significant correlation ($P < 0.01$) between the histological classification of the tumors and expression of GGT (Table 2). GGT-positive tumors comprised 3 of 9 (33%) of the benign lesions, 7 of 10 (70%) of the LMP tumors, and 23 of 26 (88%) of the malignant neoplasms. Within each histological classification, patients with GGT-negative tumors were younger than those with GGT-positive tumors. The average age of patients with GGT-negative LMP or malignant tumors was 49 years. The average age of patients with GGT-positive LMP or malignant tumors was 64 and 63 years, respectively. These data suggest that GGT-positive tumors are more likely to occur in postmenopausal patients.

Five of 7 benign serous neoplasms lacked GGT-positive staining. Case 7 had a single large serous cyst in which all of the cells lining the cyst were strongly positive for GGT. Among the serous LMP tumors, there was a wide range in the percentage of tumor cells that were GGT-positive. Two of the cases had no GGT-staining, while cases 8 and 10 showed dramatic positive staining in all of the tumor cells. Of the serous papillary carcinomas, only 3 of 12 had one-half or more of their cells that were GGT-positive. Six tumors had no or only rare positive cells.

Among the mucinous tumors, there was an all-or-none distribution with regard to GGT expression. A mucinous cystadenoma (case 27) had a large cyst that was lined by GGT-positive cells, whereas a mucinous LMP tumor was negative. Two of 3 malignant mucinous tumors, cases 30 and 31, had intense GGT-positive cells.

Both of the malignant endometrioid tumors had GGT-positive cells, although the percentage of positive cells differed between the 2 tumors. The strongest GGT-positive staining was seen in the 2 clear cell carcinomas (Fig. 1). In contrast, there was little to no GGT staining in the transitional cell tumors. Each of 4 undifferentiated tumors had GGT-positive cells.
Table 2 Relationship between histological classification and GGT expression common epithelial tumors

<table>
<thead>
<tr>
<th></th>
<th>GGT-negative</th>
<th>GGT-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>LMP</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Malignant</td>
<td>3</td>
<td>23</td>
</tr>
</tbody>
</table>

χ² = 10.4  P < 0.01

*Tumors scored as + or higher were classified as GGT-positive.

The cellular distribution of GGT-positivity varied according to tumor type and degree of differentiation. In most of the GGT-positive cysts and LMP tumors, the enzyme was localized to the luminal surface of the cell (Fig. 1c). This is similar to GGT localization in normal cells such as the brush border of renal proximal convoluted tubule cells and the apical portion of pancreatic acinar cells (9). In the fully malignant ovarian neoplasms, there was often no polarization of enzyme within the cell membrane (Fig. 1d).

The LMP tumors and malignant neoplasms are biologically distinct types of tumors. There was no correlation between the stage of the LMP or malignant neoplasms and GGT expression. This suggests that a GGT-negative tumor does not become GGT-positive as it progresses to a more widely disseminated stage.

GGT expression was also compared to the level of serum CA 125, a high molecular weight glycoprotein that is present in many epithelial ovarian tumors (14). CA 125, detected in the serum, is used to monitor the response of ovarian neoplasms to chemotherapy (15). Values of more than 35 units/ml are considered above normal. As shown in Table 1, patients with benign epithelial tumors had CA 125 values within the normal range, whereas 25 of 29 (86%) of patients with LMP or fully malignant tumors had elevated levels. Expression of GGT in the tumor and serum levels of CA 125 were unrelated.

There was very little expression of GGT in sex-cord and stromal cell tumors (Table 3). Among 4 granulosa cell tumors, only one had rare GGT-positive cells. None of 3 thecoma-fibroma tumors was

Fig. 1. Expression of GGT in frozen sections of human ovarian tumors. The red stain indicates the expression of GGT in case 10, a serous LMP tumor (a); case 31, a mucinous adenocarcinoma (b); case 43, an undifferentiated carcinoma with focal glandular differentiation (c); case 42, an undifferentiated carcinoma (d); case 34, a clear cell carcinoma (e); and case 53, a poorly differentiated Sertoli/Leydig cell tumor (f). Note the polarization of the GGT to the luminal surface of cells in tumors a–c, e, and f. Bars, 100 μm (a) and 50 μm (b–f).
F-GLUTAMYL TRANSPEPTIDASE AND OVARIAN TUMORS

Table 3  Expression of GGT in sex cord-stromal cell and germ cell tumors

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>Patient no.</th>
<th>Patient age</th>
<th>Tumor stage</th>
<th>Preoperative serum CA 125 (units/ml)</th>
<th>GGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex cord-stromal cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulosa-stromal cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulosa cell</td>
<td>46 55</td>
<td>I</td>
<td>ND*</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>47 42</td>
<td>I</td>
<td>116</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 57</td>
<td>IA</td>
<td>44</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>49 74</td>
<td>IC</td>
<td>ND</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thecoma-fibroma</td>
<td>50 83</td>
<td>12</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51 59</td>
<td></td>
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<td>52 54</td>
<td></td>
<td>ND</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sertoli-stromal cell</td>
<td>53 20</td>
<td>IA</td>
<td>51</td>
<td>+++++</td>
<td></td>
</tr>
<tr>
<td>54 64</td>
<td></td>
<td>ND</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germ cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysgerminoma</td>
<td>55 16</td>
<td>III</td>
<td>ND</td>
<td>+++++</td>
<td></td>
</tr>
<tr>
<td>Benign cystic teratoma</td>
<td>56 18</td>
<td>I</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* ND, not done; 0, no GGT activity; +++, approximately half of the tumor cells were GGT positive; +++++, the vast majority of the tumor cells were GGT positive.

GGT-positive. One Sertoli/Leydig cell tumor (case 53) stained strongly for GGT (Fig. 1f), whereas a Leydig cell tumor (case 54) had no GGT staining. Of 2 germ cell tumors analyzed, a dysgerminoma was positive, while a benign cystic teratoma was negative.

DISCUSSION

Our analysis of GGT expression in the normal human ovary has shown that GGT is not expressed in the follicles, germinal epithelium, or most stroma, but is present in some inclusion glands and many ovarian tumors. In an early study on the histochemical localization of GGT expression in the ovary, Glenner et al. (16) noted that in the normal human ovary only the remnants of Wolffian duct epithelium were GGT-positive.

Several investigators have suggested that epithelial inclusion glands are precursors for common epithelial ovarian tumors (17, 18). It is of interest to note that each inclusion gland tended to stain completely GGT-positive or GGT-negative. This same pattern was seen with the mucinous tumors. The serous tumors, however, showed considerable heterogeneity with regard to the percentage of cells that were GGT-positive.

Scully has reported that endometriosis of the ovary is a site of origin of surface epithelial cancers, particularly endometrioid and clear cell carcinomas (18). In humans, the epithelium of normal uterine endometrial glands is intensely GGT-positive (16). It is of interest that 3 of the ovaries with GGT-positive inclusion cysts were from patients with endometriosis or endometrial cancer. The benign serous cyst that was strongly GGT-positive was from a patient with a history of endometrial cancer. In addition, each of the endometrioid and clear cell carcinomas expressed GGT.

GGT was expressed in at least one-half of the cells in 50% of the serous, mucinous, and undifferentiated carcinomas. None of these patients had received chemotherapy. Induction of GGT may be the consequence of activation of a cellular protooncogene. Vincenzeni et al. (19) have reported that transfection of NIH/3T3 fibroblasts with erb B, raf, ras, or src alters glutathione metabolism and results in transformed cells with increased levels of intracellular glutathione. These authors did not analyze their cells for expression of GGT. However, several groups have reported that transfusion of cells with ras results in the induction of GGT (20–22). Activation of ras is a common alteration in human ovarian tumors. Mok et al. (23) found mutations of the K-ras protooncogene in 27 of 58 serous and mucinous human ovarian tumors.

Little is known about the molecular events involved in the initiation and progression of ovarian cancers. If the natural history of ovarian cancer includes activation of cellular oncogenes that affect glutathione levels, this may explain the high level of resistance of some ovarian tumors to chemotherapy. Elevated glutathione increases the resistance of the cell to the toxicity of many drugs including cisplatin, carboplatin, melphalan, and doxorubicin (24). Elevated levels of glutathione have been documented in biopsies from patients whose tumors have become resistant to chemotherapy (14). Clinical studies are currently underway at several cancer centers to test the effectiveness of depleting glutathione as a means of overcoming drug resistance in patients with ovarian tumors (25).

Our study found that the vast majority of cells in transitional cell carcinomas did not express GGT. This is of interest, as these tumors have been found to be very sensitive to chemotherapy. In a study of 53 high-grade carcinomas of the ovary, Robey et al. (26) found that transitional cell carcinomas responded well to chemotherapy, as 83% of the patients were alive without disease 4 to 10 years after diagnosis. In contrast, 77% of the patients with other types of epithelial ovarian tumors died from 6 months to 7 years after diagnosis.

Of all of the glutathione-associated enzymes, GGT is the easiest to assess in a clinical setting. Frozen sections can be stained histochemically for GGT activity (9). The bright red reaction product can be used to identify GGT-positive tumors, as well as subpopulations of GGT-positive cells within a tumor. We are monitoring the response of patients in this study to chemotherapy to determine whether expression of GGT correlates with rapid onset of resistance to alkylating agents and platinum-based therapy. In the future, information on the GGT status of a tumor may prove important in predicting the treatment modality with the greatest probability of success.

ACKNOWLEDGMENTS

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REFERENCES


