The Glutathione S-Transferase M1 Genotype in Ovarian Cancer

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Abstract

Glutathione S-transferase mu-1 (GSTM1) is a polymorphic member of the mu class gene family of the glutathione S-transferases. Individuals who are GSTM1 null have increased susceptibility to lung and colon cancer. We hypothesized that: (a) GSTM1 null individuals might also be at increased risk for development of ovarian cancer; and (b) the GSTM1 genotype would influence response to chemotherapy. One hundred and forty-six individuals with invasive epithelial ovarian cancer were genotyped using a three-primer PCR reaction specific for the GSTM1 gene and an internal control glutathione S-transferase mu-4 (GSTM4). The products were analyzed on agarose gels. Healthy individuals without a family history of ovarian, breast, or colon cancer served as unmatched controls (n = 80). The results show that age at diagnosis, histological type, and stage of ovarian cancer were all independent of GSTM1 genotype. The frequency of the GSTM1 null genotype in the ovarian cancer cohort was similar to that in the control population, 51% versus 58%, P > 0.05. Likewise, median survival for individuals with advanced stage ovarian cancer was independent of GSTM1 genotype. We concluded that the GSTM1 null genotype does not increase ovarian cancer risk. These findings suggest that GSTM1 does not play a significant role in detoxifying environmental factors that influence ovarian carcinogenesis and does not play an important role in the resistance of ovarian cancer to chemotherapy.

Introduction

GSTM1 is a member of the mu class gene family of the GSTs. GSTs form a supergene family that can be subdivided into four distinct classes: alpha, mu, pi, and theta (1). GSTs are soluble homodimeric enzymes that aid in detoxifying numerous compounds, including electrophilic carcinogens (2). The mechanism involves the sulfur atom of glutathione acting as a nucleophilic substrate (3). The resulting glutathione conjugate is then either directly excreted or further metabolized to a mercapturic acid (3, 4). Mercapturic acids are excreted in the urine and are the normal products of glutathione conjugates (3–6). This pathway of detoxifying xenobiotics can be extended to the role that glutathione-dependent enzymes play in chemotherapeutic drug resistance (3). Depletion of cellular glutathione levels sensitizes cells to the toxic effects of a wide range of cytotoxic drugs, and augmentation of cellular glutathione enhances resistance against the toxic effects of these compounds. Black and Wolf (3) offer the above evidence to support the role of glutathione dependent enzymes in drug resistance.

Previous studies have shown GSTM1 is polymorphic in humans but expressed by only 12–69% of individuals (7–11). GSTM1 has been primarily studied in lung, bladder, breast, and colon cancers (8–10, 12). The GSTM1 null genotype is defined as the absence of GSTM1 enzyme activity because of the deletion of both copies of the GSTM1 gene. Individuals who are GSTM1 null have been found to carry an increased susceptibility to lung and colon cancer (10, 12). Although definite associations with exposure to carcinogens have not been established, some epidemiological studies suggest ovarian cancer is associated with the use of talcum powder (13–17). However, current biological and epidemiological data do not provide strong evidence for a causal association, because the dose-response trends are lacking (18). Other factors, including body mass index, have been shown to confound the above relationship (19). We hypothesized that GSTM1 null individuals might have an increased risk of developing ovarian cancer. Furthermore, the absence of the GSTM1 detoxification pathway was hypothesized as a mechanism that could make some ovarian cancers more sensitive to chemotherapy. We tested these hypotheses by comparing the frequency of GSTM1 null phenotypes among cohorts of ovarian cancer probands and unaffected individuals in a paid control population. In addition, we evaluated ovarian cancer survival as a function of GSTM1 genotype.

Materials and Methods

The University of Iowa Division of Gynecological Oncology maintains a germ-line DNA bank for a variety of genetic studies related to ovarian cancer. Samples were obtained after informed consent approved by the Hospital Committee for the Protection of Human Subjects. One hundred and forty-six patients with invasive epithelial ovarian cancer were genotyped, along with 80 unmatched controls who had no family history of ovarian, breast, or colon cancer. A representative sample of 33 tumors from GSTM1-positive individuals was also genotyped to define whether a GSTM1 null tumor had developed in an individual who might have lost one or both copies of the GSTM1 gene. All patients were treated by The Division of Gynecological Oncology at The University of Iowa Hospitals and Clinics and were diagnosed with invasive epithelial ovarian cancer before January 1, 1996. Primary cytoreductive surgery included exploration of upper abdomen and pelvis; total abdominal hysterectomy and bilateral salpingo-oophorectomy; cytology from peritoneal washings or ascites; omentectomy; multiple peri toneal biopsies; and selective pelvic and para-aortic lymphadenectomy. The postoperative chemother-

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2 The abbreviations used are: GSTM1, glutathione S-transferase mu 1; GSTP, GST pi.
apy regimen included a platinum compound in combination with cyclophosphamide or paclitaxel.

DNA was extracted from whole blood cells and tumor tissue using conventional techniques of phenol/chloroform/isoamyl alcohol extraction and isopropanol precipitation. All DNA precipitates were resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0; Refs. 20 and 21). PCR amplification of the \textit{GSTM1} and \textit{GSTM4} loci was carried out using the method of Zhong et al. (12). The PCR reaction used three primers: P1, 5'-CGCCATCTTGTCCTACATTGCCCG-3'; P2, 5'-ATCTTCTCCCTTCTGTCTC-3'; and P3, 5'-TTCTGGATTGTAGCAGATCA-3'.

P1 and P3 amplify a 230-bp product that is specific for the \textit{GSTM1} gene. P3 anneals specifically to sequences in the \textit{GSTM1} gene. P1 and P2 can anneal to either the \textit{GSTM1} or \textit{GSTM4} gene and yield a 157-bp product. PCR was carried out in a total volume of 10.0 \mu l containing 20 ng of genomic DNA, 1.0 \mu l of 10X PCR buffer (15 mM MgCl$_2$ at pH 9.0), 2.0 mM of each deoxynucleotide triphosphate, 1.0 \mu l of DMSO, 600 ng of P1, 300 ng each of P2 and P3, 3.12 \mu l of double-distilled H$_2$O, and 0.2 \mu l of Taq polymerase (5 units/\mu l). The reaction was overlaid with 40 \mu l of white light mineral oil. The reaction was then heated to 94°C for 45 s for denaturation, cooled at 52°C for 45 s to allow annealing, followed by extension at 72°C for 45 s. This cycle was repeated 34 times. A terminal 5-min extension at 72°C completed the amplification. The products were then analyzed by electrophoresis on 2% agarose gel and detected by staining with ethidium bromide. If the product only displayed the \textit{GSTM4} (control) gene, it was classified as \textit{GSTM1} null, whereas if the \textit{GSTM1} gene and control were both present on the gel, it was classified as \textit{GSTM1} positive (Fig. 1). \textit{GSTM4} is another member of the GST mu class gene family but does not demonstrate a polymorphism.

\textbf{Family History, Pedigree, Pathological Confirmation of Cancers, and Follow-Up.} A complete pedigree was obtained on each individual studied with pathological follow-up of reported breast, ovary, and colon cancer among family members when possible. Family history of breast, ovary, and colon cancer was determined by reviewing the proband’s pedigree to determine the number of relatives affected by these cancers. For this analysis, we counted only first-, second-, and third-degree relatives. A positive family history was noted if any ovarian, breast, or colon cancers were documented by pathological review or by death certificate.

\textbf{Statistical Analysis.} The sample size to be evaluated was determined by a power analysis. Seidergard et al. (9) reported the prevalence rate of the \textit{GSTM1} null genotype to be 42% in controls. There was a 63% prevalence rate in the lung cancer population they studied (9). Anticipating that we might find a similar difference, we calculated that there was an 80% chance of finding this difference with an $\alpha = 0.05$ if we studied at least 186 individuals. Therefore, we studied 146 ovarian cancer cases and 80 controls.

Statistical calculations were completed using the Kruskal-Wallis test and the two-tailed, $\chi^2$ method. Survival curves were generated with the BMDP statistical software package (BMDP, Los Angeles, CA; 1990) using a Cox proportional hazards model. $P < 0.05$ was considered statistically significant.

\textbf{Results} One hundred and thirty-eight of the 146 germ-line mononuclear DNA samples from individuals with invasive epithelial ovarian cancer were informative (Table 1). Seventy (51%) of informative samples were \textit{GSTM1} null. Eighty healthy females without a family history of ovarian, breast, or colon cancer were analyzed as controls. Seventy-seven were informative, and forty-five of these (58%) were \textit{GSTM1} null. This difference was not significant ($P > 0.05$).

Loss of a viable \textit{GSTM1} coding sequence in ovarian tumors could result in \textit{GSTM1} null tumors developing in individuals who were \textit{GSTM1} positive in the germ line. To evaluate this possibility, tumor DNA was genotyped from 33 \textit{GSTM1}-positive individuals. The same methods described above were used to analyze the tumor DNA. Thirty of the 33 tumors were also \textit{GSTM1} positive, whereas three tumors were \textit{GSTM1} null.

If detoxification mechanisms were dependent on \textit{GSTM1} to prevent carcinogenesis, a younger age of onset of disease would be predicted for \textit{GSTM1} null probands diagnosed with ovarian cancer. The median age of diagnosis was 56.3 years for the \textit{GSTM1} null individuals and 56.8 years for the \textit{GSTM1}-positive individuals. This difference was not statistically significant. Histological subtypes of ovarian carcinoma and stage at diagnosis were also studied to ascertain whether \textit{GSTM1} null individuals develop a more aggressive cancer. These results are summarized in Table 2. There was no statistically significant correlation between ovarian cancer histology ($P > 0.05$) or stage at diagnosis ($P > 0.05$) and the proband’s \textit{GSTM1} genotype.

If \textit{GSTM1} plays a role in detoxifying chemotherapeutics as the pi class gene family of GSTs (\textit{GSTP}) appear to do (22–28), one would predict that \textit{GSTM1} null individuals would show a better initial response to chemotherapy than \textit{GSTM1}-positive individuals. To test this hypothesis, survival rates in the ovarian cancer cohort with stage III or IV disease were generated based on \textit{GSTM1} genotype. These results are shown as Fig. 2. This figure shows that survival is independent of \textit{GSTM1} genotype.
Discussion

Individuals are exposed to a whole host of environmental carcinogens throughout their lives. It is clear that some individuals with genetically compromised detoxification pathways are at increased risk for a variety of cancers. Epidemiological studies have implicated a role for environmental carcinogens in lung, bladder, breast, colon, and ovarian cancer (8, 15–17, 29–34). Smokers, for example, are at an increased risk for the development of lung, bladder, breast, and colon cancer (29–33). Often, individuals with the same occupational and environmental exposures demonstrate different susceptibilities to cancer and may further demonstrate varying responses to the same treatments. These observations are consistent with an important role for genetics in modifying both cancer risk and treatment response.

The glutathione transferases are a family of enzymes that play a key role in detoxifying carcinogens (2). Glutathione transferases were first studied by Seidegard et al. (8), who proposed that expression of phase II detoxification enzymes including the glutathione transferases might help prevent lung cancer. In a series of studies, these investigators reported that smokers who failed to express the GSTM1 genotype in colorectal carcinoma patients relative to a healthy control population. These investigators were unable to confirm Seidegard’s observations in a different lung cancer cohort and emphasized the importance of similar ethnic makeup of various study populations. London et al. (37) have reported that the GSTM1 null genotype is significantly higher in Caucasians than in African-Americans with lung cancer. Likewise, GSTM1 null rates have been reported to range between 31% and 88% when African-Americans and Samoans, respectively, are genotyped (11). The population of ovarian cancer patients treated at The University of Iowa Hospitals and Clinics is almost exclusively of European extraction. This could account for the higher percentage of GSTM1 null individuals in the control population and emphasizes the importance of similar ethnic makeup of study and control populations for studies such as these. A similar phenomenon was observed with regard to the expression of the codon 72 polymorphism of the p53 gene (38).

Others have also postulated that alpha and mu expression might correlate with the response of ovarian cancer to treatment (39). We observed no difference in survival of individuals with advanced stage ovarian cancer genotyped as GSTM1 null compared with those who were GSTM1 positive. This is also consistent with the observations made by Howells et al. (36), who found no association between the GSTM1 null genotype and survival. Howells et al. (36) did report poorer outcomes in women who were both GSTM1 null and GSTT1 null. However, only 12 of the 134 individuals studied were null at both loci (36). Thus, the combination of these genetic markers is not overly helpful in predicting treatment outcomes among the vast majority of ovarian cancer patients.

The present results are contrary to those predicted by published studies of GSTP expression. GSTP expression has been uniformly related to chemotherapy resistance (22–28). However, in both platinum-sensitive and -resistant ovarian cancer cell lines, there is complete absence of GSTM1 mRNA, consistent with

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**Table 2** Comparison of ovarian cancer histology and stage at diagnosis to GSTM1 genotype

<table>
<thead>
<tr>
<th>Histogram</th>
<th>GSTM1 null (n = 70)</th>
<th>GSTM1 positive (n = 68)</th>
</tr>
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<tbody>
<tr>
<td>Serous</td>
<td>28 (40%)</td>
<td>29 (43%)</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>18 (26%)</td>
<td>12 (18%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>12 (17%)</td>
<td>9 (13%)</td>
</tr>
<tr>
<td>Endometroid</td>
<td>9 (13%)</td>
<td>16 (24%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>2 (3%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Transitional</td>
<td>1 (1%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* The staging information on one GSTM1-positive patient was not available.

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**Fig. 2.** Kaplan-Meier estimate of survival in individuals diagnosed with advanced stage ovarian cancer stratified on the basis of GSTM1 genotype.
clinical independence of platinum resistance from \( \text{GSTM1} \). A possible explanation for this discrepancy might be subtle differences in \( \text{GSTM1} \) and \( \text{GSTP} \) function, whereby in the complete absence of \( \text{GSTM1} \), relatively more glutathione is available for \( \text{GSTP} \), which may be more effective than \( \text{GSTM1} \) in shunting chemotherapeutics away from their cytotoxic effects.

Given the failure to demonstrate a relationship between the \( \text{GSTM1} \) genotype and the onset, extent, or aggressiveness of ovarian cancer, we can conclude that \( \text{GSTM1} \) does not play a significant role in detoxifying environmental factors that influence ovarian carcinogenesis. In addition, the absence of an impact upon survival means that the \( \text{GSTM1} \) genotype, contrary to the other GST genotypes, does not modify the overall relative resistance of many epithelial ovarian cancers to chemotherapy.

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References

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