Cytochrome P450 and Glutathione Transferase Expression in Human Breast Cancer

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ABSTRACT

Purpose: The cytochrome P-450 (CYP) and glutathione S-transferase (GST) enzyme systems may influence the biological effects of carcinogens, including estrogens. As such, these enzymes may predict the developmental risk of breast cancer, as well as be potential targets for chemoprevention. The purpose of this study was to compare the expression of GST-Pi and CYPs 1A1, 2B6, 2E1, and 3A4 in paired samples of normal and malignant breast tissue from patients with breast cancer and women undergoing reduction mammoplasty.

Experimental design: Expression of CYPs 1A1, 2B6, 2E1, 3A4, and GST-Pi was quantified in breast tissue from 33 patients with breast cancer and in 17 women without history of cancer who underwent reduction mammoplasty. The expression of CYP 1A1, 2B6, 2E1, 3A4, and GST-Pi was quantified by immunoblotting.

Results: CYP 1A1, 2E1, and 3A4 expression was significantly lower (P < 0.05) in malignant tissue as compared with morphologically normal adjacent tissue. Conversely, GST-Pi expression was marginally lower in the normal tissue (P = 0.08). No significant difference in enzyme expression was seen between the tissue from reduction mammoplasty and normal tissue from breast cancer patients. There was a trend for higher expression of CYP 2B6 and GST-Pi in the estrogen receptor expressing tumors than those tumors without expression (P > 0.28).

Conclusion: The expression of these enzymes was similar in morphologically normal breast tissue from patients with or without breast cancer. The expression of CYPs was down-regulated in the tumor tissue. The clinical significance of CYP alterations in breast cancer will need further characterization.

INTRODUCTION

Approximately 190,000 new cases of breast cancer will be diagnosed every year in the United States (1). Established risk factors for breast cancer include a family history of the disease, the age at menarche, the age at menopause, and the age at the first pregnancy (2). However, these risk factors are present in ~25% of the patients with breast cancer (3). Understanding mechanisms of susceptibility and thereby identifying new risk factors for breast cancer can have an impact on the prevention and screening for this disease.

The carcinogen-metabolizing enzymes are involved in the activation and deactivation of diverse chemical carcinogens, including endogenous sex hormones (3–5). Interindividual variations in the metabolism of carcinogens may occur from various activities of metabolizing enzymes in the liver and target tissues. These variations may result in different susceptibilities to breast cancer development. CYP9 is a superfamily of heme-containing mono-oxygenases that is involved in the synthesis and metabolism of a wide variety of endogenous and exogenous compounds (6). Enzymes of the CYP family may have different but overlapping substrate specificities (Ref. 7; Table 1). The regulation of CYP expression is in part tissue specific (7). Marked enzymatic activities of CYPs 1A1 (8–10), 2B6 (11), 2E1 (12), and 3A4 (8) have been demonstrated in the human mammary epithelium. GSTs are involved in the Phase II metabolism that generally detoxifies carcinogens (9, 10). GST-π and -μ have been shown to be expressed in the human breast tissue (11, 12).

Carcinogen-metabolizing enzymes may also be targets for chemoprevention. Up-regulation of detoxifying enzymes and/or the down-regulation of activating enzymes may reduce the activity of carcinogenic compounds at the target sites (3, 13). Recently, the down-regulation of CYP2D6 and CYP2C9 by tamoxifen has been reported as a possible mechanism of chemoprevention (14).

This study compared the expression of CYP 1A1, 2B6, 2E1, and 3A4 enzymes and GST-π in breast cancer cells and morphologically normal adjacent breast tissue. We also compared morphologically normal breast tissue from cancer patients with the breast tissue from reduction mammoplasty specimens. The study also compared the expression of these carcinogen-metabolizing enzymes in breast cancer cells in relation to the sex receptor expression. We hypothesized that the expression of the CYPs would be higher in normal tissue from breast cancer patients than from patients without history of breast cancer and the opposite for GST-π expression. The second hypothesis was that the expression of CYPs would be lower in the breast cancer tissue as compared with the normal adjacent tissue.

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3 The abbreviations used are: CYP, cytochrome P450; GST, glutathione S-transferase; RT-PCR, reverse transcriptase-PCR; PR, progesterone receptor; ER, estrogen receptor; IHC, immunohistochemistry.
MATERIALS AND METHODS

Study Population. Thirty-three consecutive patients with breast cancer and 17 women undergoing reduction mammoplasty were enrolled in this study. Patients had no previous therapy for breast cancer. The reduction mammoplasty group included women with no personal history of breast cancer.

Tissue Collection. The pathologic material from patients with breast cancer was obtained from either lumpectomy or modified radical mastectomy specimens. Mammary tissue removed at the time of surgery was submitted fresh. Tissue from patients with breast cancer was histologically examined by the pathologist. Tumor samples were obtained from unequivocal sites within the tumor nodule. Normal tissue samples were obtained from morphologically normal breast tissue that was 1 cm away from the cancer site. All samples were covered with aluminum foil, coded, and stored at −70°C until analysis. Assays were done in batches of 10–20 samples and without knowledge of the sample source.

Western Blot Analysis (Immunoblotting). The minimum tissue weights sufficient for all intended analyses were 200 mg. Tissue samples were suspended in 2 ml of homogenizing buffer (pH 7.4) containing 50 mM Tris, 0.25 M sucrose, and 1 mM EDTA. The samples were then homogenized twice followed by centrifugation at 12,000 rpm for 30 min at 4°C. The supernatant was centrifuged at 45,000 rpm for 1 h at 4°C. The microsome pellet was dissolved by sonication in 50 mM Tris, 0.25 M sucrose, and 1 mM EDTA. The concentration of proteins was determined by bicinchoninic acid assay using spectrophotometer and measured at an absorbance of 562 nm.

Approximately 20 μg of protein from each sample was loaded onto a 10% SDS-polyacrylamide gel along with three different concentrations of each of the known enzyme standards. Gels were run at 120 V for 90 min and subsequently transferred onto a nitrocellulose membrane. The membrane was blocked for 1 h at room temperature by PBS/0.05 Tween 20 containing 5% powdered milk. It was then treated with either of the primary antibodies 3A4, 1A1, and 2E1 (Gentest Corp., Woburn, MA) GST-π (Oxford Biomedicalals, Rochester Hills, MI), or 2B6 (gift from NIH) to the enzymes using optimized dilutions. Membranes were washed and treated with a secondary antibody (horseradish peroxidase conjugate). Protein bands were detected by enhanced chemiluminescent substrate. Cytokeratin 19 expression was used to standardize for the epithelial cell component of the tissue sample while loading the proteins.

The intensity of the protein band was quantified by an image analysis system (Storm System; Molecular Dynamics, Piscataway, NJ). The intensity of the bands from the patient samples was determined from the standard linear curve encompassing the actual experimental measurements and corrected for the expression of cytokeratin 19 in each sample. Enzyme levels were expressed in relative arbitrary units.

Statistical Methods. Nonparametric methods of statistical analyses were used because of the non-normality of the enzyme distributions. Comparison of enzyme levels between tumor and adjacent normal tissue from patients with breast cancer was performed via the Wilcoxon signed-rank test for paired data (15). Comparison of enzyme levels between the two sources of normal breast tissue (mammoplasty patients versus breast cancer patients) was performed via the two-sample Wilcoxon rank-sum test (15). As the sample sizes were relatively small for comparing enzyme levels by ER or PR status, an exact inference version of the Wilcoxon rank-sum test was used (16). Linear associations among the enzymes were assessed via Spearman rank correlation coefficients (15). Each set of inferences for the five enzymes was adjusted for multiple comparisons using the method of Holm (17), but that did not change any of the conclusions of the study. Statistical significance was assumed for a P ≤ 0.05.

RESULTS

Fig. 1 demonstrates a representative immunoblot of the five enzymes that were studied. No significant relationship between any of the CYP enzymes and GST-π was identified in this study, indicating that the regulation of the CYP system is independent of the GST-π activity. Wide interindividual variations in the expression of enzymes were noted. Expression of CYP enzymes 1A1, 2B6, 2E1, 3A4, and GST-π was compared in breast cancer and adjacent normal tissue (Fig. 2; Table 2). The mean levels of the enzyme expression for 1A1, 2B6, 2E1, and 3A4 were higher in the normal tissue as compared with the breast cancer tissue. Conversely, the mean GST-π level was 7% lower in the normal tissue as compared with the tumor tissue. These differences were statistically significant only for CYPs 1A1, 2E1, and 3A4 (P < 0.05, even after adjustment for multiple comparisons).

The levels of expression of all five enzymes in the normal tissue from patients with breast cancer were compared with the levels of the respective enzymes in the breast tissue from women undergoing reduction mammoplasty (Fig. 3; Table 2). The mean CYP 1A1, 2E1, and 3A4 enzyme levels were higher in normal tissue samples from breast cancer patients as compared with women undergoing reduction mammoplasty; the differences in expression were 17, 56, and 47%, respectively. However, the mean levels of CYP2B6 and GST-π in normal tissue samples from breast cancer women were lower than in the control group. None of these differences in enzyme levels between patients with and without breast cancer were statistically significant.

The expression levels of CYPs and GST-π in the sex hormone receptor-positive and -negative tumors were compared (Fig. 4). ER-positive tumors had higher mean levels of CYP2B6 and GST-π. None of these differences were statistically signif-
icant \( (P > 0.43 \text{ for each}) \) because of the small sample sizes. A similar analysis was performed to compare the PR-positive and -negative tumors. Again, a statistically nonsignificant difference was found for all five enzymes \( (P > 0.41 \text{ for each}) \). Only the CYP 2B6 and GST-\( \pi \) means were higher in the progesterone-positive tumors (data not shown).

**DISCUSSION**

Lifetime estrogen exposure is a known risk factor for human breast cancer (2). Estrogen promotes mitotic activity in the breast epithelium, increasing the risk of cancer (18). Furthermore, recent evidence indicates that estrogen metabolites may form DNA adducts, resulting in DNA mutations (19). Concentrations of active estrogenic metabolites in the mammary tissue are critical for their biological activity. The influences of the CYPs and GST enzyme systems on the biological effects of carcinogens, including estrogen, make these enzymes predictors of breast cancer development, as well as potential targets for chemoprevention. Tissue metabolism by locally expressed enzymes in the breast tissue may be more important than metabolism in the more distant organs, such as the liver. Estrogen is metabolized via oxygenation at one of multiple positions by different CYPs (Ref. 20; Table 1). Although oxygenation at the C-2 position results in an inactive hormone, oxygenation on C-4 or C-16 results in a potent hormone with mutagenic potential (20). Estrogens are also metabolized through conjugation, which renders them more water soluble, facilitating their excretion. GSTs detoxify carcinogens, including estrogens (9). GST expression varies in different tissues (21, 22). Most of the recent published studies assessed the risk of breast cancer in relation to CYP or GST genotypes. However, interindividual variations in the expression and activity of the CYPs and GST are dependent on genotypic as well as post-transcriptional factors, which are tissue specific (23, 24). In addition, polymorphisms that do not influence the enzyme activity or the expression level may have no clinical significance. Studies to determine the expression of CYP or GST in breast cancer have generated contradictory conclusions (7). Possible interpretations of these discrepancies include the small sample sizes and/or various methods of measuring the CYP and GST-\( \pi \) expression levels. Immunoblotting, IHC, or RT-PCR have been used to determine CYP or GST expression. Interpretation of the results from different studies may be influenced by the nonspecificity of the antibodies (8) and semiquantitative nature of IHC and RT-PCR. In addition, RT-PCR and immunoblots carry the disadvantage of measuring the expression of these enzymes in the tumor tissue, as well as the nonmalignant epithelial and stromal cells admixed with tumor cells. Molina et al. (9) compared GST-\( \pi \) expression by IHC, immunoblotting, and RT-PCR in breast cancer tissue. Although GST-\( \pi \) was expressed in the stromal elements, there still was a strong correlation between the measurements obtained by the different assays. Murray et al. (25) used IHC staining to evaluate the expression of the CYP in breast tumor and adjacent stromal elements. No expression of CYP was reported in the adjacent stromal elements. Both these studies indicate that the stromal contribution to the expression of GST-P and CYP might be negligible in breast cancer tissue.

We hypothesized that the expression of CYPs will be higher in mammary tissue from patients with breast cancer. In
our study, no significant difference was seen in any of the five enzymes in the normal tissue from breast cancer patients and healthy women. The lack of difference in the enzyme expression suggests that measuring expression levels of these enzyme levels may not help assess the risk of breast cancer in a given individual. Alternatively, morphologically normal tissue from patients with breast cancer may contain cells that are genetically altered with respect to CYP/GST expression. Future studies may address the influence of the enzymatic activities of other enzymes involved in estrogen metabolism. In this study, the expression of CYPs 1A1, 2B6, 2E1, and 3A4 was lower in the tumor tissue than in the adjacent normal tissue. Several studies on the expression of the CYP enzymes in breast cancer using IHC and other studies showed no significant differences in the expression of CYPs in breast tumors and adjacent normal tissue (8, 26). Results of our study are consistent with other experimental (7, 20) and clinical evidence (3, 24) of down-regulation of CYPs in tumor tissue relative to morphologically normal tissue. GST-π level was increased in the tumor tissue as compared with the adjacent normal breast tissue, although this difference was not statistically significant. Helzlsouer et al. (12) reported an increased risk of breast cancer in association with GST-π-null genotype. Maugard et al. (27) conducted a study comparing the prevalence of the different GST gene polymorphisms in 361 patients with breast cancer versus 437 female controls. A significantly higher predominance of the null genotype was observed in the group of women 55 years with breast cancer. On the other hand, a larger study conducted in the United States found no association between GST-π-null genotype and the risk of breast cancer (28). Alternatively, GST-π expression by IHC was present in 47% of breast cancer tissue compared with 100% of normal breast epithelium (29).

Estrogens can inhibit CYP 1A1 transcription (30) and regulate the expression of mRNA in breast cancer cells (30, 31). Therefore, lower expression of the CYPs and GST-π may be expected in the ER-positive tumors. Previously published studies

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### Table 2   
A summary of enzyme expression levels in breast tumor, normal breast tissue adjacent to tumor, and breast tissue from patients who underwent reduction mammoplasty

<table>
<thead>
<tr>
<th>Source</th>
<th>CYP1A1 n</th>
<th>CYP2B6 n</th>
<th>CYP2E1 n</th>
<th>CYP3A4 n</th>
<th>GST-π n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast tumor</td>
<td>29</td>
<td>25</td>
<td>25</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>Median</td>
<td>71.6</td>
<td>73.0</td>
<td>19.1</td>
<td>98.4</td>
<td>10.0</td>
</tr>
<tr>
<td>Mean</td>
<td>84.8</td>
<td>105.8</td>
<td>24.9</td>
<td>157.3</td>
<td>11.4</td>
</tr>
<tr>
<td>SE</td>
<td>13.5</td>
<td>21.2</td>
<td>4.4</td>
<td>29.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Minimum</td>
<td>2.1</td>
<td>3.3</td>
<td>4.4</td>
<td>15.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Maximum</td>
<td>336.3</td>
<td>412.0</td>
<td>83.0</td>
<td>595.0</td>
<td>25.9</td>
</tr>
<tr>
<td>Adjacent normal tissue</td>
<td>29</td>
<td>25</td>
<td>25</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>Median</td>
<td>110.3</td>
<td>101.0</td>
<td>35.1</td>
<td>144.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Mean</td>
<td>114.9</td>
<td>134.3</td>
<td>48.6</td>
<td>258.3</td>
<td>10.6</td>
</tr>
<tr>
<td>SE</td>
<td>14.9</td>
<td>19.9</td>
<td>11.0</td>
<td>65.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Minimum</td>
<td>4.5</td>
<td>11.5</td>
<td>3.3</td>
<td>28.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Maximum</td>
<td>345.0</td>
<td>415.0</td>
<td>243.3</td>
<td>1784.0</td>
<td>43.2</td>
</tr>
<tr>
<td>Mammoplasty</td>
<td>17</td>
<td>13</td>
<td>6</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Median</td>
<td>91.8</td>
<td>202.0</td>
<td>32.3</td>
<td>150.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Mean</td>
<td>98.4</td>
<td>179.3</td>
<td>31.2</td>
<td>175.8</td>
<td>10.9</td>
</tr>
<tr>
<td>SE</td>
<td>7.3</td>
<td>25.9</td>
<td>11.7</td>
<td>31.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Minimum</td>
<td>42.9</td>
<td>27.5</td>
<td>0.2</td>
<td>43.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Maximum</td>
<td>155.0</td>
<td>379.0</td>
<td>379.0</td>
<td>510.0</td>
<td>58.4</td>
</tr>
</tbody>
</table>

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**Note:** CYPs 1A1, 2B6, 2E1, 3A4, and GST-π levels were determined by immunoblotting. Results are represented by arbitrary units from densitometric measurements of protein bands relative to CK 19 expression in each sample.
demonstrated that GST-\(\tau\) expression by IHC, Western blot, or PCR in ER-positive tumors was lower than in ER-negative tumors (9, 29, 32). In the present study, there was no significant relationship between estrogen or PR status of the breast cancer tissue and the expression of any of the CYPs or GST-\(\tau\). Similarly, no relation between CYP3A and either ER or PR was found (24). No previous studies have examined the expression of CYP 2B6 or 2E6 in relation to the ER or PR expression in tumor cells. The relatively few subjects in each subgroup in our study might have limited the ability to detect significant differences of expression of CYP and GST-\(\tau\) based on the receptor status of the tumor.

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