CYP3A4 Expression in Breast Cancer and its Association with Risk Factors in Mexican Women

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Abstract

\textbf{Background:} In Mexico, breast cancer (BCa) is the leading type of cancer in women. Cytochrome P450 (CYP450) is a superfamily of major oxidative enzymes that metabolize carcinogens and many antineoplastic drugs. In addition, these enzymes have influence on tumor development and tumor response to therapy. In this report, we analyzed the protein expression in patients with BCa and in healthy women. Links with some clinic-pathological characteristic were also assessed. \textbf{Materials and Methods:} Immunohistochemical analyses were conducted on 48 sets of human breast tumors and normal breast tissues enrolled in Hospital Militar de Especialidades de la Mujer y Neonatología and Hospital Central Militar, respectively, during the time period from 2010 to 2011. Informed consent was obtained from all participants. Statistical analysis was performed using $\chi^2$ or Fisher exact tests to estimate associations and the Mann Whitney U test for comparison of group means. \textbf{Results:} We found a significant CYP3A4 overexpression in BCa stroma and gland regions in comparison with healthy tissue. A significant association between protein expression with smoking, alcoholism and hormonal contraceptives use was also observed. Additionally, we observed estrogen receptor (ER) and progesterone receptor (PR) positive association in BCa. \textbf{Conclusions:} We suggest that CYP3A4 expression promotes BCa development and can be used in the prediction of tumor response to different treatments. One therapeutic approach may thus be to block CYP3A4 function.

Keywords: Breast cancer - CYP3A4 - protein expression - gene environment interactions - Mexico

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Introduction

In 2011, World Health Organization (WHO) estimated that the leading cause of worldwide death during 2008 was cancer with 7.6 million cases mainly lung, stomach, liver, colon and breast cancer (BCa) (World Health Organization, 2012). In Mexico, BCa is the most common malignancy in women, followed by cervical cancer. Currently, BCa etiology has been attributed to factors such as advanced age, reproductive, personal and family history, estrogen metabolism, geographic area and diet (Lopez-Carrillo, 2003). These observations suggest that environmental factors, diet, physical inactivity and obesity, as well as the increase in the consumption of alcohol and saturated fat and sugar diet are involved in BCa progression (Blacam et al., 2012; Canchola et al., 2012; Nelson et al., 2012; Xing et al., 2014).

Cytochromes P450 (CYP450) is a superfamily of constitutive and inducible enzymes that metabolize a large amount of environmental chemicals and endogenous substances such as environmental toxins, therapeutic drugs and steroid hormones. This group of enzymes is involved in several biological processes, including carcinogenesis, drug metabolism and cell signaling (Mc Fadyen et al., 2004). Therefore, CYP450 may be a valuable therapeutic target, since they may influence the response of tumors to chemotherapeutic agents (Mc Fadyen et al., 2004; Bruno and Njar, 2007; Michael and Doherty, 2007).

CYP3A4 is involved in the metabolism of therapeutic agents, environmental and endogenous compounds. CYP3A4 is expressed in the prostate, breast, stomach, colon and small intestine and liver (Huang et al., 1996; Lown et al., 1997; Guengerich, 1999). CYP3A4 plays an important role in the testosterone (2β, 6β, o 15β-hydroxytestosterone) and estrogen (4-y 16α-hydroxylation) oxidation (Shou et al., 1997; Niwa et al., 1998). In recent years have been described several genetic polymorphisms that affect the expression of this gene (Chu and Fyles, 2007). CYP3A4 protein expression has been observed in cancer tissues (Takahara et al., 2011; Weiss and Haefeli, 2011) due the induction of different kind of substances, resulting in an increase in transcription and expression of CYP3A4.

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The evidence suggests that overexpression of CYP3A4 could be associated with BCa. Huang et al. (1996) studied CYP’s gene expression levels of mRNA in BCa; CYP3A4 mRNA was more expressed in normal breast tissues (70%) than tumoral tissues (18%) (Huang et al., 1996). Zheng et al. (2001) evaluated the association between urinary cortisol levels and the risk of BCa in a subgroup of women in Shanghai, China. They found a strong association between BCa risk and urinary levels of 6β-hydroxycortisol. It also showed that increasing in the risk of dose-response manner. This association appears to be higher in older women (Zheng et al., 2001). Currently, there are no data in Mexico about the CYP3A4 expression and its possible association with the development of BCa. The aim of this study was to evaluate, protein expression of CYP3A4 in Mexican women with BCa and in normal mammary gland.

Materials and Methods

Biological samples

All samples of human mammary carcinomas were obtained from 48 BCa patients with radical mastectomy surgery diagnosed in the Department of Pathology, Hospital Militar de Especialidades de la Mujer y Neonatologia, Secretaria de la Defensa Nacional (SEDENA) in Mexico City. 48 normal tissues were obtained for reduction of mammary gland donated by the Department of Plastic Surgery, Hospital Central Militar (SEDENA).

The histological classification of the carcinomas, as well as the evaluation of non-tumor breast lesions, were made according to standard diagnostic procedures and confirm by three pathologists. The sample collection was conducted from October 2010 to November 2011 and was considered inclusion, exclusion and elimination criteria. Patients were asked to read and sign an Informed Consent in agreement with requirements of the Ethical approval provided by the Bioethics and Research Committees of the Hospital Militar de Especialidades de la Mujer y Neonatologia in Mexico with registration number SI-378. Collection information of demographic status, tumor characteristics, as well as medical history and lifestyle behavior in patients was used. The human experimentation guidelines of these committees were followed.

Immunohistochemistry study

Immunohistochemical analyzes were performed on slide prepared with histological tissues previously fixed with 10% formalin and embedded in paraffin. The cuts included on slides were deparaffinized and heated to unmask antigenic sites; the endogenous peroxidase activity was blocked with 0.03% H2O2 in absolute methanol. Tissue sections were incubated overnight at 4°C with a 1:100 dilution of polyclonal antibody against CYP3A4 (Santa Cruz Biotechnology, CA, U.S.A.) and were washed twice repeated with TRIS solution. Bound antibodies were detected with the Avidin-Biotin complex (ABC-kit Vectastain) and diaminobenzidine as the substrate. After washing repeatedly with TRIS solution, sections were counterstained with hematoxylin. All sections were incubated under the same conditions with the same concentration of antibody in the same run and therefore, immunostaining was comparable. All specimens were examined by Axiosvert 200M inverted confocal microscope (Carl Zeiss, Jena, Germany). For automated morphometric analysis the percentage of positive cells (brown color) was determined with a computerized image analyzer KS-3003.0 (Carl Zeiss, Jena, Germany). This equipment automatically detects positive cells per field by determining their percentage. Five random fields were examined at a magnification of 40x (total area 1,584,000 m2). The results were expressed as a percentage.

Statistical analysis

χ2 test or Fisher exact test was used to estimate the association between individual clinicopathological factors and risk of BCa. Mann Whitney U test was used for compare means between groups. Statistical analysis was performed using SPSS v19 for Windows XP (SPSS UK, Ltd, Woking, UK). p<0.05 was regarded as significant.

Results

Available clinical and histological data on all patients are summarized in Table 1. Tissue samples were collected from patients with BCa.

### Table 1. Characteristics of Patients with BCa

<table>
<thead>
<tr>
<th>Factors</th>
<th>N</th>
<th>Mean</th>
<th>Deviation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48</td>
<td>58.3</td>
<td>10.90</td>
<td>119.500</td>
</tr>
<tr>
<td>Height (meters)</td>
<td>45</td>
<td>1.5</td>
<td>0.06</td>
<td>0.004</td>
</tr>
<tr>
<td>Weight (Kilograms)</td>
<td>46</td>
<td>67.7</td>
<td>13.40</td>
<td>179.500</td>
</tr>
<tr>
<td>BMI (Kilograms/meters²)</td>
<td>45</td>
<td>29.2</td>
<td>5.90</td>
<td>34.900</td>
</tr>
<tr>
<td>Menarche (years)</td>
<td>48</td>
<td>12.5</td>
<td>1.40</td>
<td>2</td>
</tr>
</tbody>
</table>

BMI (Body Mass Index)

### Table 2. Descriptive Analysis of Patients Diagnosed with BCa

<table>
<thead>
<tr>
<th>Region</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center</td>
<td>32</td>
<td>66.7</td>
</tr>
<tr>
<td>North</td>
<td>9</td>
<td>18.8</td>
</tr>
<tr>
<td>South</td>
<td>6</td>
<td>12.5</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 3. Estrogen Receptor Association with Other Receptors, Markers and Sociodemographic Variables in Samples from Patients with BCa

<table>
<thead>
<tr>
<th>Variables</th>
<th>Positive (N=21)</th>
<th>Negative (N=27)</th>
<th>p</th>
<th>OR (IC95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>11 (52.4%)</td>
<td>4 (14.8%)</td>
<td>0.007*</td>
<td>6.3 (1.6-4.7)</td>
</tr>
<tr>
<td>K67</td>
<td>6 (28.6%)</td>
<td>15 (55.6%)</td>
<td>0.05*</td>
<td>0.3 (0.0-1.0)</td>
</tr>
<tr>
<td>ERBB2</td>
<td>8 (40.0%)</td>
<td>13 (50.0%)</td>
<td>0.35*</td>
<td>0.6 (0.2-2.1)</td>
</tr>
<tr>
<td>Smoking</td>
<td>2 (9.5%)</td>
<td>8 (29.6%)</td>
<td>0.08*</td>
<td>0.2 (0.0-1.3)</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>1 (4.8%)</td>
<td>7 (25.9%)</td>
<td>0.05*</td>
<td>0.1 (0.0-1.2)</td>
</tr>
<tr>
<td>Metastases</td>
<td>12 (57.1%)</td>
<td>8 (30.8%)</td>
<td>0.06*</td>
<td>3.0 (0.9-9.9)</td>
</tr>
<tr>
<td>Weight</td>
<td>71.9 (9.3%)</td>
<td>64.7 (15.0%)</td>
<td>0.06**</td>
<td></td>
</tr>
</tbody>
</table>

*Chi Square was applied or, where appropriate the Fisher’s Exact test in all contrasts, Estrogen receptor (ER), Progesterone receptor (PR). **Mann-Whitney U test was applied
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**Table 4. Association of CYP3A4 Expression with Some Clinicopathological Factors**

<table>
<thead>
<tr>
<th>Factor</th>
<th>p value</th>
<th>Test Used</th>
<th>Test Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>0.018</td>
<td>Chi Square</td>
<td></td>
</tr>
<tr>
<td>Alcoholism</td>
<td>0.008</td>
<td>Chi Square</td>
<td></td>
</tr>
<tr>
<td>Hormonal Contraceptives</td>
<td>0.042</td>
<td>Chi Square</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.096</td>
<td>Chi Square</td>
<td></td>
</tr>
</tbody>
</table>

CYP3A4 PROTEIN EXPRESSION IN MAMMARY GLAND

A) in (a) BCa tissue immunohistochemistry; in (b) densitometric analysis in cancer tissue expression; in (c) healthy breast tissue immunohistochemistry; in (d) densitometric analysis of the healthy breast expression. In both groups was determined immunoreactivity percentage per field (40x). B) We showed a significant increase in immunoreactivity of CYP3A4 in BCa group (p<0.0001)

CYP3A4 PROTEIN EXPRESSION IN MAMMARY STROMA

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**Discussion**

This study has defined the protein expression of CYP3A4 in Mexican women with breast cancer and associated expression of this cytochrome with some epidemiological factors namely smoking, alcoholism, hormonal contraceptives and age. Additionally, we evaluated estrogen receptor (ER) association with histopathological markers such as progesterone receptor (PR), Ki67 and HER2 and with sociodemographic factors smoking, alcoholism, metastases, and body weight.

The results obtained in the characteristics of patients are agree with those reported in studies in Mexican women, in which, the age range of risk for developing BCa is between 40 to 69 years, the median age of menarche is 12 years and the median age of menopause has been reported between 47 and 48.2 years (Garrido-Latorre et al., 1996; Torres-Mejia et al., 2005; Mendez-Estrada et al., 2006). The BCa is more common in the north and center of Mexico (Sistema Nacional de Informacion en Salud, 2012).

We observed that the majority of patients had a sedentary life and overweight/obesity. The lifestyle behavior such as smoking, use of oral contraceptives or thyrotropin-releasing hormone, obesity or overweight and hyperglycemia are associated with the risk of BCa development as reported in other studies (Llatrakis et al., 2011; Nelson et al., 2012). In other study, we demonstrated that BCa risk was associated with chronic diseases, family history of cancer, body mass index, nulliparity, chemotherapy, menopause and metastases. Figure 1 and 2 shows CYP3A4 protein expression. This cytochrome was overexpressed significantly in BCa stroma and gland regions (p<0.05).

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tumors appeared to have a better survival but did not offer any differences in disease-free survival. In our results no association existed between ER expression with Ki67, HER-2, smoking, alcoholism, metastases and body weight.

The human CYP3A forms have been extensively studied from several perspectives. They are collectively the most abundant P450s, have the largest number of drug substrates, and illustrate many of the before mentioned issues of expression, polymorphism, and clinical impact (Guengerich, 1999; Hassan and Yusoff, 2011). In relation with BCa, the level of CYP3A4 immunohistochemistry expression was reported to be associated with response rate to docetaxel treatment (Miyoshi et al., 2005).

In this study, we observed CY3A4 overexpression in BCa in comparison with healthy gland. Recently, CYP3A4 expression has been identified in BCa growth using specific RNAi gene silencing in cell lines MCF-7, T47D and MDA-MB-231 (Mitra et al., 2011). These authors showed that CYP3A4 synthesizes epoxycosatetraenic acids that promote BCa growth. In other work, CYP3A4 gene expression was observed, although in low levels, in BCa. The authors suggest that CYP3A4 expression is predictive value for any estimation of response to anticancer drugs metabolically inactivated by this cytochrome (Vaclavikova et al., 2007). In other study found positive immunohistochemical CYP3A4/A5 staining in 25% of inspected mammary tumors (Haas et al., 2006).

Moreover, we showed an association between CYP3A4 expression with alcohol consumption, tobacco consumption and oral contraceptives use. These results suggest that a change in the CYP3A4 gene expression, or polymorphisms, in BCa alters the function of this CYP in the alcohol, tobacco and oral contraceptives metabolism (Ragin et al., 2010; Bandala et al., 2012).

In summary, we suggest that the expression of CYP3A4 promotes BCa development and can use clinically in predicting tumor response to treatment against BCa. Moreover, one potential therapeutic approach may be to block CYP3A4 function.

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References


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