RESEARCH COMMUNICATION

RNA Expression of Cytochrome P450 in Mexican Women with Breast Cancer

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Abstract

Involvement of cytochrome P450 genes (CYPs) in breast cancer (BCa) may differ between populations, with expression patterns affected by tumorigenesis. This may have an important role in the metabolism of anticancer drugs and in the progression of cancer. The aim of this study was to determine the mRNA expression patterns of four cytochrome P450 genes (CYP2W1, 3A5, 4F11 and 8A1) in Mexican women with breast cancer. Real-time PCR analyses were conducted on 32 sets of human breast tumors and adjacent non-tumor tissues, as well as 20 normal breast tissues. Expression levels were tested for association with clinical and pathological data of patients. We found higher gene expression of CYP2W1, CYP3A5, CYP4F11 in BCa than in adjacent tissues and only low in normal mammary glands in our Mexican population while CYP8A1 was only expressed in BCa and adjacent tissues. We found that Ki67 protein expression was associated with clinicopathological features as well as with CYP2W1, CYP4F11 and CYP8A1 but not with CYP3A5. The results indicated that breast cancer tissues may be better able to metabolize carcinogens and other xenobiotics to active species than normal or adjacent non-tumor tissues.

Keywords: CYP2W1 - CYP3A5 - CYP4F11 - CYP8A1 - mRNA - breast cancer

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Introduction

Breast cancer (BCa) is the most common malignant tumor in the 25 years and over Mexican female population with an increase of 30% of cancer-related deaths in the last 20 years (WHO, 2008; SINAIS, 2010). The expression of tumors specific proteins in mammary glands may play a critical role in the development of BCa as well as in the success of chemotherapy treatment. To date, very few critical markers have been validated for the prediction of drug efficiency in BCa (Vaclavikova et al., 2007).

Cytochrome P450 (CYP) system is a multigene superfamily of enzymes that play a central role in activating and detoxifying a wide variety of xenobiotics as well as endobiotics (Nebert, 1991). Cytochrome P450s have been proposed to be of importance both in carcinogenesis, by activating precarcinogens, and as determinants of cancer chemotherapy, where they participate in activation or inactivation of anti-cancer drugs (Oyama et al., 2004; Rooseboom et al., 2004). The relevance of P450 modulation to cancer risk, tumorigenesis, cancer progression and metabolizing of different anticancer drugs has not been well understood in humans. The expression of major P450s is polymorphic and results in the generation of multiple population-specific phenotypes with different drug metabolizing capabilities (Vaclavikova et al., 2007). Thus, it is important to determine the expression patterns in different populations.

Cytochrome P450 2W1 (CYP2W1) has been shown to participate in the oxidation of arachidonic acid and catalysts the reductive activation of AQ4N to AQ4 (an anticancer drug) and in the bioactivation of several pro-carcinogens including polycyclic aromatic hydrocarbons and aflatoxin B1 (Wu et al., 2006; Nishida et al., 2010). mRNA expression was detected in tumor tissues from colon, adrenal, and lung, while no expression was seen in a normal tissues like brain, heart, colon, kidney, and placenta (Karlgren et al., 2005). Recent data obtained by RT-PCR of whole mice RNA indicate that CYP2W1 mRNA is expressed in mouse embryos but not in adult animals (Huang et al., 1998), suggesting a role for CYP2W1 in fetal life. CYP2W1 mRNA was here found to be expressed in rat fetal tissues, especially in colon but also in lung where the expression increased by fetal age and transiently in brain at day 16. After birth, the CYP2W1 gene gradually becomes silent in rat and mouse, and in support of such silencing also occurring in human is the fact that we found no or only very small amounts of CYP2W1 mRNA in all adult non-transformed tissues examined. It appears that CYP2W1 provides the most specific form of cytochrome P450 for tumor expression hitherto found (Karlgren et al., 2005).

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Cytochrome P450 3A5 (CYP3A5) has been shown to play a role in the 16α-hydroxylation of estrogens in humans. Since 16-hydroxy-estrogen 1 (16-OHE1) is a putative breast carcinogen, knowledge of the enzymes involved in its synthesis provides a basis for blocking its synthesis in vivo (Huang et al., 1998). CYP3A4 and CYP3A5 are both expressed in human female breast tissue, but not in all individuals (Huang et al., 1996), these results also provide a basis for selection of potentially susceptible individuals. Recent study found that expression of CYP3A4⁄5 was significantly associated with lymph node metastases in BCa (Murray et al., 2010).

Cytochrome P450 4F11 (CYP4F11) showed catalytic activity against endogenous eicosanoids (leukotriene B4) arachidonic acid, prostaglandins and lipoxins, and hydroxyeicosatetraenoic acids and commonly used drugs (erythromycin, benzphetamine, and chlorpromazine) (Kalsotra et al., 2004; Choudhary et al., 2005). CYP4F11 is thought to be primarily involved in the metabolism of fatty acids (3-hydroxypalmitate) and arachidonic acid metabolites (Dhar et al., 2008; Uno et al., 2011). mRNA is mostly found in liver and kidney; minor expression was noted in skeletal muscle, placenta, and heart (Cui et al., 2000).

Cytochrome P450 8A1 (CYP8A1; Prostacyclin I2 synthase; PGIS) gene encodes for an enzyme that acts as an isomerase and rearranges PGH2 to PGI2. PGIS is considered to be an atypical CYP because it does not possess oxygenase activity (Ullrich et al., 1981; Smith et al., 1995). PGI2 has many important biological functions. It is the most important endogenous inhibitor of platelet aggregation discovered to date, and also causes vascular relaxation (Moncada et al., 1976; Cathcart et al., 2010). PGI2 is also anti-mitogenic and inhibits DNA synthesis in smooth muscle cells (Libby et al., 1988). The presence of PGIS at the nuclear and endoplasmic reticular membrane suggests multiple signaling pathways for this enzyme via PGI2 generation, involving both cell surface and nuclear receptors. However, the cellular signaling initiated by this class of compounds is probably the least understood of all the primary prostanoids (Bos et al., 2004; Cathcart et al., 2010). A number of studies have demonstrated that the prostanoid biosynthesis profile of malignant cells is different compared to normal tissues (Wang and Chen, 1996; Yokoyama et al., 1996). CYP8A1 signaling through arachidonic acid metabolism affects a number of tumor cell survival pathways including cell proliferation and apoptosis as well as, tumor cell invasion, metastasis and angiogenesis. PGI2 is a potent antimitogenic and anti-metastatic cancer agent (Honn et al., 1981). The association between CYP8A1 SNP and cancer (lung, colorectal, thyroid and breast) has been well-documented (Keith et al., 2004; Abraham et al., 2009).

In this study, we determined the mRNA expression, for the first time, of CYP2W1, CYP3A5, CYP4F11 and CYP8A1 in samples of human carcinomas of the mammary gland, surrounding tissue without morphological signs of presence of tumor cells and in normal mammary gland tissues from Mexican women. A highly sensitive method with absolute quantification and internal normalization was used. Moreover, our study is the first to evaluate the possible association between CYP expression patterns with some clinic-pathological risk factors.

Materials and Methods

Biological Samples

All samples of human mammary carcinomas and paired adjacent normal tissue without morphological signs of carcinoma were obtained from 31 BCa patients diagnosed at the Pathology Service, Clínica de Especialidades de la Mujer, Secretaría de la Defensa Nacional (SEDENA) in Mexico City. Normal tissues of the mammary gland were obtained for reduction of mammary gland in the Service of Plastic Surgery, Hospital Central Militar (SEDENA). Tissue samples were collected during surgery and frozen at -80°C. 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 four pathologists. Non tumor samples were without morphologically detected tumor cells. Patients were asked to read and sign an Informed Consent in agreement with requirements of the Ethical Commission of the National Institute of Public Health in México. This project has the approval of Hospital Research Ethical commission (ref. no. SI-378).

Isolation of total RNA from human tumor and its adjacent normal tissues

Approximately 100 mg of human tumor, adjacent normal tissue and mammary normal tissues were separated and homogenized individually in a Trizol reagent (TRI Reagent® Solution, RNA/DNA/Protein Isolation Reagent, Ambion). Total cellular RNA was extracted according to the manufacture's protocol. The concentration of total RNA in each sample was measured by Nanodrop Spectrophotometer (Delaware, USA) using the ratio of 260-nm/280-nm. The quality of the isolated RNA was accessed based on the integrity of the 28S, 18S, and 5S bands after ethidium bromide staining electrophoresis of a 1% formaldehyde denaturating gel and visualized under a UV transilluminator (EDAS 290 KODAK, New Haven, CT). Total RNA (1 μg) was successively supplemented with 0.5 μL RNase inhibitor (Boehringer Mannheim Gmbh Germany). All extracted RNAs were stored at -80°C.

Quantitative real-time PCR assays

Specific oligonucleotides of the CYP2W1, CYP3A5, CYP4F11 and CYP8A1 genes and for the reference genes: subunit ribosomal 18S, glyceraldehyde-3 phosphate dehydrogenase (GAPDH), glucose 6 phosphate dehydrogenase (G6PDH) and β-actin (BACT) were designed and optimized at 59 ºC (Table 2). The sequences were obtained from GenBankTM. Endogenous gene (GAPDH) was validated with BestKeeper software as was reported by Pfaff et al. (Pfaff et al., 2002). Conditions for the RT-PCR were optimized with a thermal cycler (gradient Px2 Thermal Cycler Hybaid, Franklin, MA). The reaction mixture contained 12.5 μL of 2X SYBR® Green Reaction Mix with ROX, 0.5 μL of SuperScript™ III RT/Platinum® Taq Mix (Invitrogen, Carlsbad, CA), 1 μl of $MgSO_4$ and 1 μL of free water, 1 μL of sense and 1 μL of antisense primers and 8 μL of total RNA. Reverse transcription was performed at 52°C for 5 min. PCR was performed at 95°C for 6 min and 40 cycles of: 95°C for 20 s, 59°C for 30 s and 76°C for 15 s, melt temperature 60-90°C and finally 30°C for 2 min. The size of PCR products are shown in Table 1. The results of the amplifications such as temperature, primer concentrations, dNTPs and volumes were transferred to the amplification protocol in real time with the Rotor Gene 6.0 detection system (Corbett Life Science, Sydney City, Australia). The amplification products by real time RT-PCR were displayed by electrophoresis on a 2% agarose gel and studied with the electrophoresis EDAS 290 analysis system.

Quantitative determination of CYP2W1, CYP3A5, CYP4F11 and CYP8A1 mRNA

Data from the CP of endogenous candidates genes (GADPH, G6PDH, BACT and 18S) and the CYP2W1, CYP3A5, CYP4F11 and CYP8A1 gene , were exported from Rotor-Gene 6.0 software (Corbett Life Science, Sydney City, Australia), to calculate efficiencies with the REST© statistical model (Pfaff et al., 2002; Floriano-Sánchez et al., 2009) and data were plotted constructing a linear regression which compares the logarithmic concentration (total RNA) against CP (CP is defined as the number of cycles in which the fluorescence intensity increases above the baseline fluorescence of the sample). To correlate the candidate endogenous genes and determine the more stable gene, the BestKeeper software was used, exporting the CP of Rotor-Gene 6.0 software at Excel tool to show the melting temperature (Tm) characteristic of each amplified product. Determination of HKG was realized using BestKeeper statistical model analized CP values by Pearson correlation (Table 3) (Tinzl et al., 2004).

Statistics

Data from the absolute quantification of all samples were normalized with the HKG and were analyzed by Student's t test. Chi-square test or Fisher exact test was used to estimate the association between individual clinic-pathological factors and risk of BCa. U de Mann Withney was used for compare means between groups. Spearman test were used for correlations between histopathological markers and the cytochrome expression. Statistical analysis was performed using SPSS v17 for Windows XP (SPSS UK, Ltd, Woking, UK). p= 0.05 was regarded as significant.

Table 1. Sequence of Primers for Endogenous Genes and CYP2W1, CYP3A5, CYP4F11 and CYP8A1. From Left to right: Gene Name, Primer Sequence, Fragment Size and Primer Efficiency

Symbol	Nucleotide sequence: Forward primer Reverse primer	Amplicon size (pb)	Primers efficiency
BACT	CTGGCACCCAGCACAATG GGGCCGGACTCGTCATAC	143	2.70
18S	GTAACCCGTTGAACCCCAT CCATCCAATCGGTAGTAGC	151	2.60
GAPDH	GAGCCAAAAGGGTCATCATC CCTTCCACGATACCAAAGTT	175	2.04
G6PDH	CCTGGAGGAGCTGAGAATG CGGGCTCTCTCGGTACTTG	159	2.66
CYP 2W1	CCATCTTCCAGCTCATCCAG CCCAGAGAGGCATTTCAGC	162	2.16
CYP 3A5	GCTGTCTCCAACCTTCACC TCACATCCATGCTGTAGGC	156	1.82
CYP4F11	CCTCAGAGGGCAGCGCCAGAC AAGCTGAAGACACATTTCTGC	86	2.16
CYP8A1	CTCACGAGGATGAAGGAGAAG ATGAGGAAGATGGCATAGGC	153	2.60

*N=number of patients. †According to histological type of tumor and tumor necrosis.

Table 2. Clinical and Histological Characteristics of Patients Involved in the Study

Characteristic		N	(%)
Average age at diagnosis (years), mean±SD	59.52 ± 10.25	31	100%
	50	31	16.10%
	>50	31	83.90%
Menopausal status	Pre	31	29%
	Post	31	71%
Overweight and Obesity	Yes	31	74.20%
	No	31	25.80%
Histological type	Invasive ductal carcinoma	31	74.20%
	Invasive lobular carcinoma	31	16.10%
	Other type	31	9.70%
Histological grade†	1	31	16.2%
	2	31	41.9%
	3	31	41.9%
Oestrogen receptor status	Positive	31	51.60%
	Negative	31	48.40%
Progesterone receptor status	Positive	31	61.20%
	Negative	31	38.80%
HER2 status	Positive	31	41.90%
	Negative	31	58.10%
Nottingham Prognostic Index	Good = 3.4	31	0%
	Med 3.4–5.4	31	19.40%
	Poor > 5.4	31	80.60%

Results

Characteristics of patients and tumors

Available clinical and histological data on all patients are summarized in Table 2. Tissue samples were collected from Mexican females who ranged from 44 to 83 years of age at diagnosis with more than three quarters of the patients being older than 50 years; the average age at diagnosis of BCa patients was 59 years (mean 59.52). Almost all patients (71%) had postmenopausal status at diagnosis. Overweight or obesity were found in 74.2% of our patients. The histological type more common was invasive ductal carcinomas with 74.2 % of cases and 41.9% of the tumors were grade 2 and grade 3 respectively. Progesterone receptor expression was positive in 61.2% of tumors and 51.6% of tumors being estrogen receptor-positive. Triple-negative breast cancer (TNBC), which is characterized by negativity for estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2), is a high risk breast cancer that lacks specific targets for treatment selection were 16% of our

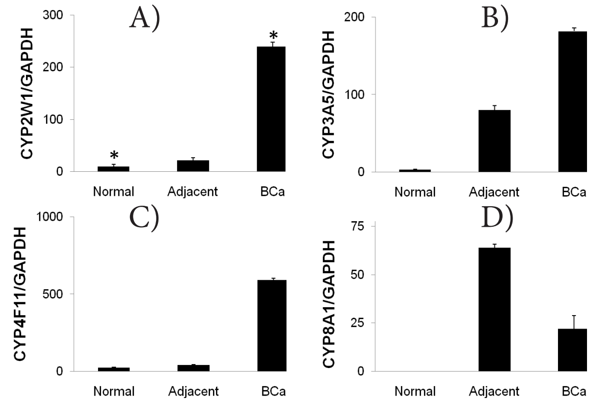

Figure 1. Levels of Normalized mRNA Expression of (A) CYP2W1, (B) CYP3A5, (C) CYP4F11 and (D) CYP8A1 in BCa, Adjacent and Normal Mammary Tissues. CYPs levels were normalized to the level of the control gene GAPDH and expressed per 8 μg of total RNA. Values represent means with standard deviation of the mean of expression levels in 31 samples. We found a difference in CYP2W1 between the BCa and normal mammary (*p =0.01), but not in CYP3A5, 4F11 and 8A1.

patients. Nottingham Prognostic Index was poor in the 80.6% of our patients.

Housekeeping gene determination

The GAPDH gene showed better stability than the BACT, G6PDH and 18 S with a Pearson correlation of 0.953 (p=0.007) (Table 3).

Gene expression of CYP2W1, CYP3A5, CYP4F11, and CYP8A1 in human mammary gland

CYP2W1, CYP3A5 and CYP4F11 genes were expressed in all tissues (BCa, Adjacent and normal mammary gland) but CYP8A1 only was expressed in BCa and adjacent tissues (Figure 1). CYP expression levels in human mammary tissue are showed in Table 4. BCa samples, CYP2W1 gene was overexpressed 230-fold than in normal mammary gland tissues (p=0.01). Despite in BCa CYP3A5, CYP4F11 and CYP8A1 are overexpressed than in normal mammary tissues, we did not find statistical significant differences, neither between adjacent tissues and BCa nor between adjacent tissues and normal mammary tissues (Figure 1).

We determine CYPs associations between means with clinical and histological characteristics, we only show the statistical significance results (Table 5).

CYP2W1 in BCa expression was associated with the age of patients (F=6.054, p=0.02), with cell proliferation marker Ki67 (F=4.102, p=0.01), Triple-positive breast cancer (TPBC), which is characterized by positive for estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2) (F=4.74, p=0.03) and with histological type (KW=8.407, p=0.01). CYP3A5 in BCa was correlated with the age (Rho=0.383, p=0.03) and with the Menarche age (Rho= -0.350, p=0.05). Also, we found associated CYP3A5 with histological type of tumor (KW=6.937, p=0.03) and TPBC patients, (F=4.99, p=0.03).

CYP4F11 in BCa was associated with cell proliferation marker Ki67 (F=4.136, p=0.01), with alcohol consumption (F=4.65, p=0.03), TPBC, (F=4.75, p=0.03); in contrast to other CYPs we did not find association for histological type.

Table 3. Determination of Stable HKG

	BACT vs BK	18S vs BK	GAPDH vs BK	G6PDH vs BK
R	0.940	0.868	0.953	0.070
Slope	1.522	1.083	1.100	0.044
SD	0.565	0.636	0.136	0.643
p	0.008	0.025	0.007	0.895

Table 4. CYP Expression Levels in Human Mammary Tissue

Gene	Sample	Mean ± S.D.
Expression of CYP/GAPDH (per 8 μg of total RNA)		
CYP2W1	BCa	240 ± 4.04
	Adjacent	22 ± 4.58
	Normal	10 ± 8.38
CYP3A5	BCa	182 ± 4.16
	Adjacent	80 ± 6.02
	Normal	3 ± 0.57
CYP4F11	BCa	591 ± 12.7
	Adjacent	20 ± 2.51
	Normal	12 ± 1.52
CYP8A1	BCa	22 ± 6.80
	Adjacent	64 ± 1.73
	Normal	0

Table 5. Associations of CYPs Expression with Clinical and Histological Characteristics of Patients.

Cytochrome P450	Characteristics (BCa)	Statistical analysis	
CYP2W1:	Edad	F= 6.054	p= 0.02
	Ki67	F= 4.10	p= 0.01
	Histological Type	KW= 8.40	p= 0.01
CYP3A5:	Histological Type	F= 6.054	p= 0.02
CYP4F11:	Ki67	F= 4.13	p= 0.01
CYP8A1:	Edad	F= 4.13	p= 0.01
	Ki67	F= 4.08	p= 0.01
	Histological Type	KW= 7.21	p= 0.02
Ki67:	Tobacco exposure	F= 7.54	p= 0.01
	Alcohol exposure	F= 3.63	p= 0.06
	Biomass exposure	F= 5.22	p= 0.03
	Non physical activity	F= 4.09	p= 0.01
	Metastasis	F= 4.75	p= 0.03
	Triple negative patientes	F= 26.41	p= 0.0001
	Oestrogen Receptor	F= 7.23	p= 0.01
	Progesterone Receptor	F= 6.11	p= 0.02
	p53	F= 3.94	p= 0.05
	CD34	F= 3.38	p= 0.07

CYP8A1 in BCa gene expression was associated with the age of patients (F=4.13, p=0.01), with cell proliferation marker Ki67 (F=4.08, p=0.01), histological type (KW=7.21, p=0.02) and with TPBC in BCa (F=4.69, p=0.03) and adjacent tissues (F=4.60, p=0.04). Nottingham prognosis index was associated with adjacent tissues gene expression of 3A5 (KW=5.84, p=0.05), CYP8A1 (KW=6.36, p=0.04) and we found tendency in association with adjacent tissues of CYP2W1 (KW=4.86, p=0.08).

In our study, we found that Ki67 protein expression was associated with clinical-pathological features (Table

5) like tobacco (F=7.54, p=0.01) and alcohol consumption (F=3.63, p=0.06), biomass exposition (F=5.22, p=0.03), Non physical activity (F=4.09, p=0.01), Metastasis (F=4.75, p=0.03), TNBC patients, (F=26,41, p=0.0001), RE (F=7.23, p=0.01), RP(F=6.11, p=0.02), p53(F=3.94, p=0.05) and association tendency with CD34 (F=3.38, p=0.07).

Discussion

This study has defined the gene expression of CYP2W1, CYP3A5, CYP4F11 and CYP8A1 in Mexican women with breast cancer and associated expression of individual cytochrome P450s with some histopathological markers like Ki67, p53, ER and PR status, HER2 and key clinicopathological factors, namely age, tumor grade, histological type and Nottingham Prognostic Index.

In Mexican women, the age range of the risk for BCa is between 40-69 years, the median age of menarche is 12 years and the median age of menopause has been reported to be between 47 and 48.2 years. With respect to the clinicopathological factors the patients included in this study, the average age, age of menarche and menopause, and the most common histological type were consistent with the literature. (Garrido-Latorre et al., 1996; Torres-Mejía et al., 2005). The TNBC has been reported that comprises about 15% of breast cancer. According to this study we found that 16% of our patients were TNBC. It is associated with a poor prognosis compared with tumors that are positive for hormone receptors or HER2 (Rakha and Chan, 2011). This means that despite being sensitive to chemotherapy, many women with metastatic triple-negative breast cancer (TNBC) relapse quickly, and commonly develop visceral metastasis, including lung, liver and brain metastasis.

In our results we observed that CYP8A1 only was expressed in BCa and adjacent tissues. This evidence indicates that CYP8A1 is involved in inflammatory mechanisms in BCa. It has been observed that arachidonic acid pathway is responsible for the generation of a wide variety of bioactive metabolites (Cathcart et al., 2010). These metabolites, otherwise known as eicosanoids, have been shown to be involved in a wide different pathology, including inflammation and cance. The COX enzymes catalyse the first step in the synthesis of prostanoids from arachidonic acid (Cathcart et al., 2010). Prostacyclin synthase (PGIS) or cytochrome P450 8A1 (CYP8A1) act downstream of COX signaling to catalyse the formation of prostanoids prostacyclin (Stearman et al., 2007). COX-derived prostanoids, prostaglandins and thromboxanes, are biologically active lipid mediators involved in a wide range of physiological processes such as modulation of vascular tone, the inflammatory response and gastric cytoprotection. Prostanoids have also been implicated in various disease states such as arthritis, heart disease and pulmonary hypertension (Needleman and Isakson, 1997). PGI2 is a potent antimitogenic and anti-metastatic agent in cancer, and has been implicated as a potential chemopreventive agent in NSCLC (Honn et al., 1981; Keith et al., 2002). It has recently been established that there are two main signaling pathways for prostacyclin following its production through PGIS activity. The first pathway is through the prostacyclin (IP) receptor, and the second is at the nuclear membrane via the peroxisome-proliferator-activated receptors (PPARs) (Gupta et al., 2000). Probably, in BCa, induction of CYP8A1 is for some of these ways. It have been demonstrated that in piglets exposed to short hypoxia the protein expression of CYP8A1 decrease in the endothelium of pulmonary arteries in according with adult humans (Fike et al., 2011). A shift in production of arachidonic acid metabolites (and in inflammatory process) occurs during exposure to hypoxia in tumoral cells of breast cancer.

CYP2W1, CYP3A5 and CYP4F11 were overexpressed in BCa tissues; CYP2W1 is a cytochrome that is involved in arachidonic acid metabolism. CYP2W1 catalyzed arachidonic acid oxidation to a mixture of several products that have not been defined (Wu et al., 2006). In this cancer, CYP2W1 is involved in inflammatory mechanisms like CYP8A1. It have been demonstrated that CYP2W1, which is exclusively expressed in transformed tissue in the adult human, mainly in colon tumors (Karlgren et al., 2006). CYP2W1 mRNA was here found to be expressed in rat fetal tissues, especially in colon but also in lung where the expression increased by fetal age (Choudhary et al., 2005). After birth, the CYP2W1 gene gradually is silent in rat and mouse. It appears that CYP2W1 provides the most specific form of cytochrome P450 for tumor expression hitherto found. Bioactivation of AQ4N, an anticancer prodrug, to AQ4 for CYP2W1 is an evidence that this cytochrome is overexpressed in tumors (Nishida et al., 2010).

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 aforementioned issues of expression, polymorphism, and clinical impact (Guengerich, 1999; Wrighton et al., 2000). CYP3A5 in expressed in breast and have been demonstrated that possession of single nucleotide polymorphisms that cause alternative splicing and protein truncation have the greatest influence on regulation of CYP3A5 (Williams and Phillips, 2000; Kuehl et al., 2001).

CYP4Fs family catalyze the metabolism of both endogenous and exogenous molecules (Kalsotra and Strobel, 2006). They inactivate the leukotriene and prostaglandin prompts for the inflammation cascade playing an anti-inflammatory role, and they also catalyze the metabolism of many drugs (Hashizume et al., 2002; Kalsotra et al., 2004). Among the human CYP4F enzymes, CYP4F11 is most active in metabolizing therapeutic drugs and has been demonstrated that retinoids down-regulate CYP4F11 expression in HaCaT cells for its anti AP-1 activity and supports the positive regulation of this cytochrome through the AP-1 complex by inflammatory cytokines TNF-α and IL-1β in accord with the literature (Wang et al., 2010). CYP4F11 catalyzes N-hydroxylations of leukotriene-B4, arachidonic acid, lipoxin-A4, and 8-hydroxyeicosatetraenoic acid (Wang et al., 2010).

Ki67 is expressed in all active phases of the cell cycle except the G0 phase (Gerdes et al., 1984). In contrast to other cell cycle-associated proteins, Ki67 is neither present in quiescent cells nor during DNA repair (Hall et al., 1993). Several studies (Charpin et al., 1988; Isola et al., 1990) describe an association of Ki67 expression with parameters of tumour aggressiveness, such as aneuploidy, histopathological grade, lymph node status (Barnard et al., 1987; Locker et al., 1992) and steroid receptor negativity (Railo et al., 1993; Wiesener et al., 1998). In contrast to this studies we find no association of Ki67 with histopathological grade but we found associated with metastasis, Triple-negative breast cancer (TNBC), and p53. We also found association between Ki67 and some factor risk like tobacco and alcohol consumption, biomass exposition and non physical activity. The search for parameters which might be used as additional decisive factors is still continuing. In our study we found that CYP2W1, CYP4F11 and CYP8A1 were associated with Ki67. Recently studies has describe that Ki67 was correlated with HIF-2a, this protein is involved in the hypoxia HIF-2a could regulate ABCG2 in breast cancer cells, and could be a novel potential bio-marker to predict chemotherapy effectiveness. The hypoxia/HIF-2a/ABCG2 pathway could be a new mechanism of breast cancer multidrug resistance (Xiang et al., 2012). In this regard, in humans, indirect evidence suggests that hypoxia reduces the rate of biotransformation of drugs cleared by cytochrome P450 (P450) subfamilies CYP1A, 2B, and 2C in liver (Fradette et al., 2007). Further investigation on the molecular mechanism of possible regulation relationships between them should be done.

In conclusion, we found the gene expression of CYP2W1, CYP3A5 and CYP4F11 in BCa, adjacent tissues and normal mammary gland in Mexican population while CYP8A1 only was expressed in BCa and adjacent tissues. Further we found that Ki67 was associated with CYP2W1, CYP4F11 and CYP8A1.

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