RNA Expression of Cytochrome P450 in Breast Cancer Patients

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Abstract. *Background: The expression pattern of cytochrome P450 genes (CYPs) affected by tumorigenesis may have an important role in the progression of cancer and in the metabolism of anticancer drugs. The aim of the study was to determine the expression patterns of four cytochrome P450 genes (CYP1B1, 2C9, 2E1 and 3A4) in breast cancer patients. Patients and Methods: mRNA expression was quantified by real-time PCR. Analyses of 40 sets of human breast tumors, adjacent non-tumor tissues and of 18 peripheral blood lymphocyte samples were performed. Expression levels were tested for correlation with clinical and pathological data of patients. Results: Expression levels of CYP2C9 and CYP3A4 were negligible. CYP1B1 expression was on average 50-fold higher than that of CYP2E1 with overexpression detected in one third of the tumors. Correlation of CYP1B1 expression in lymphocytes with that in non-tumor tissues was found. Significantly higher CYP2E1 expression was associated with an invasive lobular type of tumor, locally advanced disease as well as with non-tumor tissue of progesterone receptor-negative patients. Conclusion: CYP2E1 expression has a potential role as a breast cancer prognosis marker. The observed high CYP1B1 expression in tumor cells may evoke changes in their response to drugs which are substrates of P450 1B1 and influence metabolism or activation of environmental carcinogens.*

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Breast cancer is the most common malignant tumor in the female population in the Western world responsible for 15% of cancer-related deaths (1). The expression of tumor-specific proteins in mammary glands may play a critical role in the development of breast cancer 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 breast cancer.

Cytochrome P450 (protein name P450, EC 1.14.14.1; gene name *CYP*) enzymes catalyze a large number of reactions modifying biological activities of environmentally related xenobiotics, including drugs and endobiotics, *e.g.* fatty acids, steroids, prostaglandins and bile acids (2). Another aspect of importance of P450s is their ability to metabolize a variety of anticancer drugs. The expression of major P450s is polymorphic and results in the generation of multiple population-specific phenotypes with different drug metabolizing capabilities. Thus, it is important to determine the expression patterns in different populations.

Human cytochrome P450 1B1 is an extrahepatic enzyme involved in the production of reactive estrogen metabolites, *e.g.* 4-hydroxyestradiol (3), and in the activation of environmental procarcinogens (4). P450 1B1 is highly expressed in estrogen-sensitive tissues such as the mammary gland, uterus and ovary (4, 5, 6). Immunohistochemistry revealed a localization of P450 1B1 in breast cancer microsomes (7). P450 1B1 activates prodrugs, such as resveratrol, and inactivates others, such as tamoxifen and docetaxel (8-11).

P450 2C9 is highly abundant in the human liver and was also found in the kidney, adrenals, pancreatic islets, pituitary gland, prostate ducts, and gastrointestinal tract (12). P450 2C9 contributes to biotransformation of pharmacologically important drugs and metabolizes endogenous fatty acids, prostanoids, and steroid hormones (13). P450 2C9 expression in human mammary gland may

lead to activation of anticancer prodrug ifosfamide (14).

P450 2E1 is predominantly expressed in human liver, but it has also been found in esophagus, kidney, lung (15), and brain (16). *CYP2E1* is expressed at an early stage of human fetal development (17), indicating its importance for cellular proliferation and differentiation. P450 2E1 is responsible for the metabolic activation of low molecular weight procarcinogens, *e.g.* chlorinated hydrocarbons and nitrosamines (18). Etoposide and dacarbazine are inactivated by P450 2E1 (8).

P450 3A4 metabolizes a majority of prescription drugs (19). P450 3A4 converts endogenous estrone and estradiol to 16-hydroxy metabolites (20). *CYP3A4* is expressed in the liver, gut, colon, prostate and mammary gland (6, 21). P450 3A4 in tumors may inactivate drugs, *e.g.* etoposide, gefitinib, paclitaxel, tamoxifen, topotecan, vincristine, and vinblastine, or activate them, *e.g.* cyclophosphamide, doxorubicin, ifosphamide, imatinib and thiotepa (2, 8, 9, 11, 22).

In this study, we determined the mRNA expression of *CYP1B1, CYP2C9, CYP2E1* and *CYP3A4* in samples of human carcinomas of the mammary gland and paired surrounding tissue without morphological signs of presence of tumor cells. Peripheral blood lymphocytes were tested as potential surrogates. A highly sensitive method with absolute quantification and internal normalization was used for the first time. The potential of *CYP* expression patterns as markers of prognosis was also examined.

Table I. *Clinical and histological characteristics of patients involved in the study.*

Characteristic	N(%)
Average age at diagnosis (years), mean±SD	
62.3±11.5	40 (100]
≤40	1 (2.5)
40-49	3 (7.5)
50-59	15 (37.5)
>60	21 (52.5)
Menopausal status	
Pre/Post	6 (15)/34 (85)
Histological type	
Invasive ductal carcinoma	30 (75)
Invasive lobular carcinoma	7(17.5)
Other type	3 (7.5)
Average tumor size, mean±SD	
23.9±11.6 mm	
≤20 mm	20 (50)
>20 mm	20 (50)
Histological grade†	
1	5 (12.5)
2	20 (50)
3	9 (22.5)
Not assessed	6 (15)
Clinical stage††	
I	12 (30)
II	24 (60)
III	3 (7.5)
IV	1 (2.5)
Estrogen receptor	
Positive/Negative	26 (65)/14 (35)
Progesterone receptor	
Positive/Negative	18 (45)/22 (55)

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

Patients and Methods

Materials. Histopaque (Ficoll) and chemicals for preparation of buffers were purchased from Sigma-Aldrich (Prague, Czech Republic). Deoxynucleotides (dATP, dCTP, dGTP and dTTP) for polymerase chain reaction (PCR) and molecular weight standards for electrophoresis (ΦX174DNA/HaeIII digest, λEcoRI/HindIII) were products of New England Biolabs, Inc. (Ipswich, MA, USA). Ultrapure agarose was supplied by Life Technologies (Paisley, UK). Quantitative real-time PCR (QRTPCR) of *CYP1B1, CYP2C9, CYP3A4* and *cyclophilin A* (*CYCA*) was performed using commercially available TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA) including sets of specific probe and primers for *CYP1B1* (kit no. Hs00164383_m1), *CYP2C9* (kit no. Hs00426397_m1), *CYP3A4* (kit no. Hs00430021_m1) and *CYCA* (kit no. 4310857). TaqMan Universal PCR Master Mix (kit no. 4304437) was used for these assays as well. QRTPCR for *CYP2E1* was performed using Thermostart Polymerase kit (AB Gene, Surrey, UK) and fluorescent probe with primers was purchased from Integrated DNA Technologies (Coralville, IA, USA). Sequences of primers and probe for QRTPCR of *CYP2E1* have been described elsewhere (23).

Samples. Samples of human mammary carcinomas and paired adjacent normal tissue without morphological signs of carcinoma were obtained from 40 breast cancer patients diagnosed at the Motol Faculty Hospital in Prague during the period 2003-2005. Peripheral lymphocytes were isolated by centrifugation with Histopaque from 18 of these patients. Tissue samples were collected during surgery and snap-frozen in liquid nitrogen. In each sample containing carcinoma more than 50% of vital tumor tissue was present. The histological classification of the carcinomas, as well as the evaluation of non-tumor breast lesions, was made according to standard diagnostic procedures. Non-tumor samples were without morphologically detected tumor cells. Characteristics of patients are shown in Table I. 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 Prague.

Isolation of total RNA and cDNA synthesis. Total RNA was isolated from tissue slices and from peripheral blood lymphocytes using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the procedure supplied by the manufacturer. The quality of RNA was verified by horizontal agarose gel electrophoresis. RNA quantity was assessed by UV spectrophotometry on a Cary 300 spectrophotometer (Varian, Palo Alto, CA, USA). cDNA was synthesized using 0.5 μg of total RNA using random hexamer primers with the help of RevertAid™ First Strand cDNA Synthesis

Table II. *Programs for the QRTPCR of human CYPs and CYCA performed on RotorGene 2000.*

	PCR program					
Gene	Initial hold	Initial denaturation	Number of cycles	Denaturation	Annealing	Extension
CYP1B1	2 min 50˚C	15 min 95˚C	55	10 s 95˚C	60 s 60˚C	15 s 72˚C
CYP2C9	2 min 50˚C	10 min 95˚C	50	15 s 95˚C	60 s 60˚C	none
CYP2E1	none	15 min 95˚C	45	15 s 95˚C	50 s 60˚C	none
CYP3A4	2 min 50˚C	10 min 95˚C	50	15 s 95˚C	60 s 60˚C	15 s 72˚C
CYCA	none	15 min 95˚C	55	15 s 95˚C	45 s 62˚C	none

Kit from MBI Fermentas (Vilnius, Lithuania). The quality of cDNA in terms of DNA contamination was then confirmed by PCR amplification of *ubiquitin C* fragment discriminating between product from cDNA (190 bp) and from genomic DNA (1009 bp) as described elsewhere (24).

Quantitative real-time PCR assays. The resulting cDNA was used for QRTPCR performed in a RotorGene 2000 (Corbett Research, Sydney, Australia) under conditions shown in Table II. *CYCA* was used as a reference gene. The PCR reaction mixtures contained cDNA diluted 10-fold for determination of expression levels of *CYP1B1* and *CYCA* and 5-fold for determination of *CYP2E1, CYP2C9* and *CYP3A4*. The non-template control consisted of a reaction tube in which water was used in place of cDNA. Negative cDNA controls were also employed to reveal possible carry-over contamination.

Preparation of standards for quantitative real-time PCR. Bacterial plasmids containing full-length coding sequences of *CYP1B1, CYP2C9, CYP2E1* and *CYP3A4* were used for construction of calibration curves. The use of these standards has been described elsewhere (24). Plasmid containing subcloned fragments of exons 1 and 2 of *CYCA* in *pCR2.TOPO* plasmid was kindly provided by D. Lison (Center of Occupational Medicine, Catholic University, Louvain, Brusel, Belgium). Plasmids were propagated in *Escherichia coli* DH5α Maximum Efficiency Cells (Invitrogen) and isolated by Plasmid Midi Kit (Qiagen, Hilden, Germany). The concentration of plasmids was determined spectrophotometrically.

Data analysis. All data were analyzed using RotorGene v6 software (Corbett Research) and evaluated by absolute quantification with external standard curves. Standard curves were generated from five decimal dilutions of the corresponding plasmid clone; the number of copies ranged from 10^2 to 10^6 copies/reaction. The amount of target gene expression was calculated from the standard curve and quantitative normalization of the amount of cDNA in each sample was performed using the expression of *CYCA* as internal control ($copies_{target}/copies_{control}$). Each sample was assayed in duplicate and the mean value was used for the calculation.

Analyses of associations among clinical and histological variables and mRNA expression in tumor and non-tumor adjacent pairs were also performed. Determined variables were as follows: age at diagnosis; menopausal status, pre- *vs.* post-; tumor size in mm; tumor size ≤20 mm *vs.* >20 mm; histological type, invasive ductal *vs.* lobular; histological grade, G1 *vs.* G2 *vs.* G3; clinical stage, I *vs.* II, III and IV combined (*i.e.* regional *vs.* advanced disease); expression of estrogen and progesterone receptors, positive *vs.* negative. One-way ANOVA tests and two-tailed Pearson's bivariate correlation analysis were used for evaluation of the significance of these associations. For statistical analyses, SPSS v12.0 program (SPSS Inc., Chicago, IL, USA) was used.

Results

Characteristics of patients and tumors. Available clinical and histological data on all patients are summarized in Table I. Tissue samples were collected from Caucasian females who ranged from 39 to 83 years of age at diagnosis with more than half of the patients being older than 60 years; the average age at diagnosis of breast cancer patients was 62 years (median 62.5). Almost all patients (85%) had post-menopausal status at diagnosis. The average size of their tumors was 23.9 mm (median 20.5) and 75% of cases were invasive ductal carcinomas, with 65% of tumors being estrogen receptor-positive. Progesterone receptor expression was positive in 45% of tumors.

Expression of CYP1B1, CYP2C9, CYP2E1, and CYP3A4 in human mammary gland. CYP2C9 and *CYP3A4* were present at very low levels in all kinds of samples (see Table III for *CYCA* normalized levels). *CYP2C9* expression was below the limit of quantification (50 copies per μg of RNA) in 1 sample of tumor and 9 samples of non-tumor tissue. *CYP3A4* was present at slightly higher levels than *CYP2C9* (Table III). *CYP3A4* expression was below the limit of quantification (50 copies per μg of RNA) in 14 samples of 40 tumor samples and 5 samples of 40 non-tumor tissue.

CYP2E1 was unequivocally expressed in all samples of mammary tumor and paired non-tumor tissue from the same patients (Table III). *CYP1B1* expression was the highest in all samples, with mean values exceeding those of *CYP2E1* expression by almost 50-fold (Table III). One non-tumor sample showed no *CYP1B1* expression (Figure 1b).

Table III. *CYP expression levels in human mammary tissue and peripheral blood lymphocytes.*

	Expression of *CYP*/*CYCA* (per μg of total RNA)				
Gene	Sample	Minimum value	Maximum value	Mean±S.E.	Individual variability[b]
CYP1B1	Lymphocytes	5.0	483.2	89.2±32.0	97
	Tumor	0.2	300.5	47.6±11.5	1252
	Non-tumor	4.1	292.7	55.1±11.2	72
CYP2C9	Lymphocytes	BLQ	$1.2x10^{-3}$	$0.2x10^{-3}±0.1x10^{-3}$	-[a]
	Tumor	BLQ	$279.3x10^{-3}$	$19.3x10^{-3}±7.1x10^{-3}$	-[a]
	Non-tumor	BLQ	$404.7x10^{-3}$	$50.9x10-3±13.9x10^{-3}$	-[a]
CYP2E1	Lymphocytes	0.1	77.1	4.6±4.3	857
	Tumor	0.1	3.5	1.0±0.1	25
	Non-tumor	0.1	3.2	0.9±0.1	53
CYP3A4	Lymphocytes	BLQ	$0.5x10^{-3}$	$0.4 x10^{-3}±0.3x10^{-4}$	-[a]
	Tumor	BLQ	$217.6x10^{-3}$	$24.1x10^{-3}±8.1x10^{-3}$	-[a]
	Non-tumor	BLQ	$910.8x10^{-3}$	$136.3x10^{-3}±36.1x10^{-3}$	-[a]

[a]No variability is shown because the lowest value was below limit of quantification (BLQ). [b]Fold difference.

The expression levels of examined *CYPs* in paired mammary tumor and non-tumor tissue from the same individuals were also compared. *CYP2C9* and *CYP3A4* expressions were usually higher in the morphologically confirmed non-tumor tissue than in the tumor in 22/30 and 32/40 of cases, respectively. We found only one patient with a 3-fold and one with negligible (1.08-fold) *CYP3A4* overexpression in the tumor compared to non-tumor tissue. In the rest of the patients, *i.e.* 32 of 34, the level of *CYP3A4* was lower in their tumor tissue.

The *CYP2E1* expression was higher in non-tumor than in tumor tissue of 23 out of 40 patients and lower in 17 samples (Figure 1a). In four of forty patients, *CYP2E1* was overexpressed more than 3-fold in their tumors (3.3-, 3.4-, 4.0-, and 7.6-fold) compared to non-tumor tissue. *CYP1B1* expression levels were higher in non-tumor than in tumor tissue of 27 out of 40 patients (Figure 1b). More than a 3-fold overexpression of *CYP1B1* was detected in tumors of three patients (3.3-, 7.2-, and 22.9-fold).

We compared the *CYP2E1* and *CYP1B1* expression in peripheral blood lymphocytes with that of the mammary gland. We observed higher *CYP2E1* expression in lymphocytes than in mammary gland tissue (almost 5-fold on average, Table III). *CYP2E1* expression in lymphocytes did not correlate with that in either tumor or non-tumor mammary tissue (results not shown). Similarly to mammary gland tissue, the expression of *CYP1B1* mRNA in peripheral blood lymphocytes of all patients was significantly higher than the expression of *CYP2E1* (almost 20-fold higher on average, Table III). *CYP1B1* expression in lymphocytes was up to 2-fold higher on average than that in mammary gland tissue (Table III). Interestingly, *CYP1B1* expression positively correlated with that in non-tumor samples (R^2=0.67). The correlation of *CYP1B1* expression in lymphocytes with that in tumors was weaker (R^2=0.42).

Association of expression of CYPs with clinical and histological characteristics of patients. Expression levels of *CYP1B1* and *CYP2E1*, as the highest among the studied *CYPs*, were compared to clinical and histological characteristics of patients. *CYP1B1* expression did not correlate with any of characteristics inspected (data not shown). *CYP2E1* expression was significantly higher in invasive lobular types of tumors in comparison with invasive ductal ones (p=0.028; Table IV). *CYP2E1* expression was higher in non-tumor tissue of progesterone receptor-negative patients than in receptor-positive patients (p=0.026; Table IV). *CYP2E1* expression in tumors of patients with clinical stage I was higher than the expression in those with more advanced stages (II, III, and IV; p=0.070; Table IV). Age and menopausal status did not modify any of inspected associations.

Discussion

Our data suggest that of the four *CYPs* studied, *CYP2C9* and *CYP3A4* were present at very low levels. The low, but detectable expression of *CYP2C9* in tumors and non-tumor samples of mammary gland found by our study is in accordance with previous studies (13, 19). Similarly, *CYP3A4* mRNA was detected in 8 out of 11 examined normal mammary tissue samples and in 2 out of 13 tumors by PCR determined with Southern blotting (25). By far largest study reported recently found positive immunohistochemical P450 3A4/5 staining in 25% (97 out of 393) of inspected mammary tumors (6). However, it remains unclear whether there is any correlation between mRNA and protein levels in mammary tumors because mRNA expression levels were not analyzed. From another point of view, it is also worth to mentioning the issue of the specificity of antibodies used for immunohistochemistry. The human P450 family includes

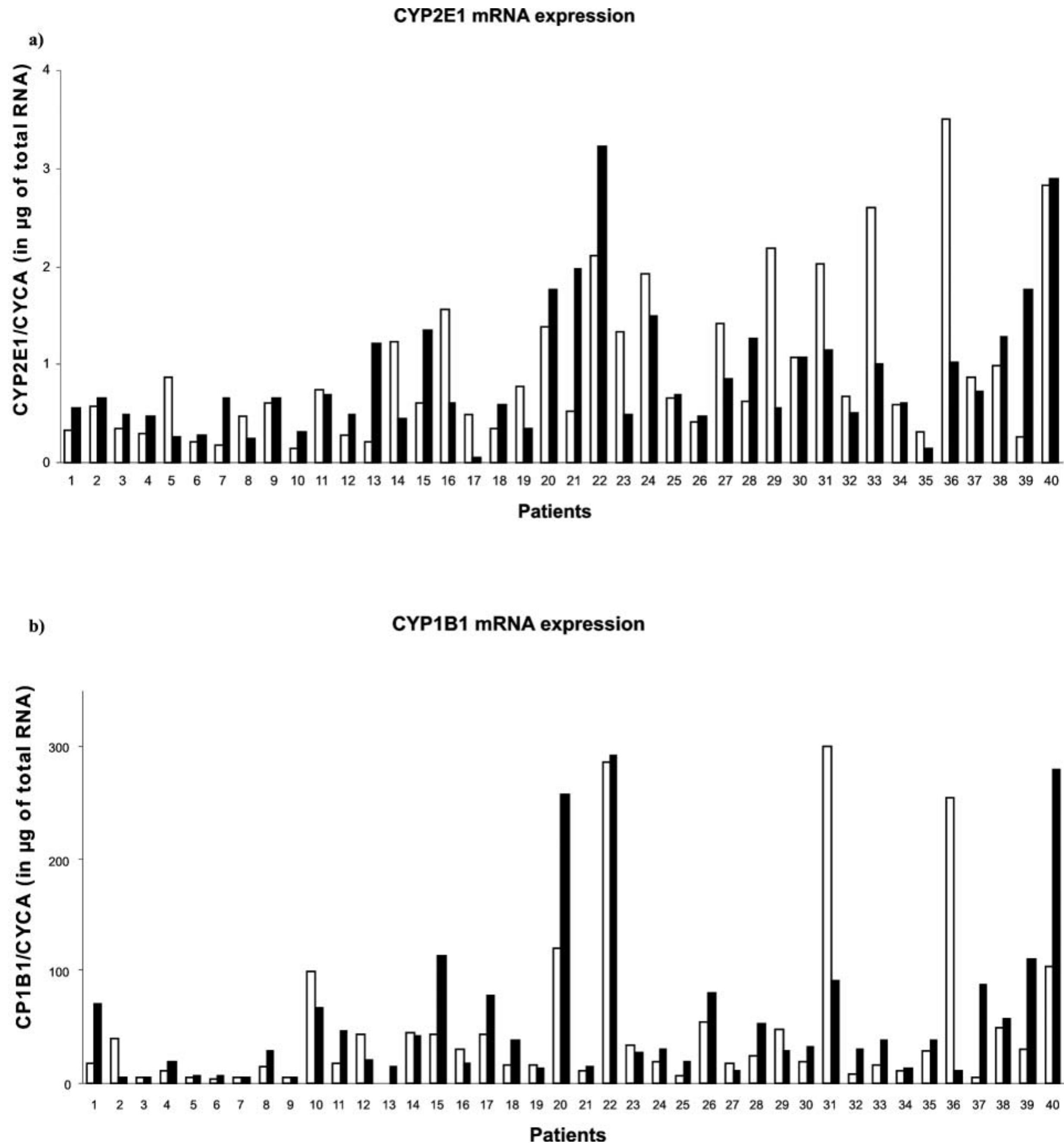

Figure 1. *Levels of mRNA expression of (a) CYP2E1 and (b) CYP1B1 in tumor and adjacent non-tumor mammary tissue. CYP1B1 and CYP2E1 levels were normalized to the level of the control gene CYCA and expressed per µg of total RNA. Values represent means with standard deviation of the mean of expression levels in 40 samples of non-tumors (■) and tumors (□) determined in duplicates.*

about 60 CYP genes (http://drnelson.utmem.edu/hum.html) with some members sharing more than 90% homology in their primary protein sequences. In fact, the study of Haas *et al.* (6) used polyclonal antibodies, raised in rabbits, which did not distinguish between P450 3A4 and the highly homologous 3A5.

There were quite contradictory findings on *CYP2E1* expression in mammary gland tissue in the literature. *CYP2E1* was not detected in tumor or non-tumor tissue (20) while two previous studies showed its expression in both tumor (26) and in normal tissue from reduction mammaplasties (27). The presence of P450 2E1 protein in mammary gland tissue was

Table IV. *Associations of CYP2E1 expression with clinical and histological characteristics of patients.*

Tissue	Characteristics	N	*CYP2E1* expression	95% Confidence interval	
			Mean±S.E[a]	Lower Bound	Upper Bound
Tumor	Ductal invasive	30	0.85±0.12	0.60	1.10
F=5.29; *p*=0.028	Lobular invasive	7	1.61±0.45	0.51	2.71
Non-tumor	Progesterone receptor positive	18	0.62±0.08	0.45	0.79
F=5.33, *p*=0.026	Progesterone receptor negative	22	1.10±0.17	0.73	1.46
Tumor	Stage I	12	1.32±0.32	0.60	2.03
F=3.47, *p*=0.070	Stages II-IV	28	0.81±0.11	0.58	1.04

N=number of patients, [a]*CYP2E1/CYCA* (per μg of total RNA), mean and standard error of the mean is presented.

also found by immunohistochemistry (28) and in 45%, *i.e.* 175 out of 393 tumors (6). In our study, *CYP2E1* was unequivocally expressed in all samples of mammary tumor and paired non-tumor tissue from the same patients.

CYP1B1 expression was the highest *CYP* on average with mean values exceeding those of *CYP2E1* expression almost 50-fold. Principally, data on high *CYP1B1* expression in mammary gland tissue well support the fact that P450 1B1 is extrahepatic enzyme. Our data are also consistent with previous studies in which *CYP1B1* expression in both tumor and non-tumor adjacent breast tissue was observed (20, 25, 26). In an earlier study, Murray *et al.* (7) detected immunostaining for P450 1B1 in (12/12) breast invasive ductal carcinomas in contrast to normal tissue (0/12). It is quite interesting that a recent immunohistochemical study found positive P450 1B1 staining in only 16.5% (65 of 393) of inspected mammary tumors (6) while our study detected transcripts in all inspected tumors. In our opinion, this result suggests that detection of *CYP1B1* transcripts may be more sensitive than detection of protein by immunohistochemistry and thus better reflect tissue characteristics.

The expression levels of the examined *CYPs* in paired mammary tumor and non-tumor tissue from the same individuals were compared in order to reveal signatures of individual tumors which may then be used for targeted therapy design.

CYP2C9 and *CYP3A4* levels were usually higher in the morphologically confirmed non-tumor tissue than in the tumor. Differences in *CYP2C9* expression between mammary tumor and non-tumor tissue were not previously found (20). Our data on *CYP3A4* support results of others who found higher levels in morphologically normal adjacent tissue than in tumors (20, 29). Similarly, Miyoshi *et al.* (30) found significantly higher *CYP3A4* levels in normal mammary tissue (n=44) than those in tumors (n=70). However, the fact that normal tissue was obtained from different patients than were the tumors might have biased the results. In contrast, Kapucuoglu *et al.* (31) concluded that P450 3A4 protein levels assessed by immunohistochemistry were higher in tumors than in normal mammary tissue (n=25).

Our data, together with findings of others, suggest that *CYP2C9* and *CYP3A4* expression is mostly down-regulated during carcinogenesis. The level of P450 3A4 was reported to be associated with a low response rate to docetaxel treatment (32). In our group of patients, the extremely low expression of *CYP3A4* in all tumors suggests that its predictive value for any estimation of response to anticancer drugs metabolically inactivated by P450 3A4 is negligible unless significant mRNA stabilization occurs. The same applies to *CYP2C9*.

In four out of forty patients, *CYP2E1* was overexpressed more than 3-fold in their tumors. These patients could potentially benefit from P450 2E1-activated prodrugs. On the other hand, it is also worth pointing out that metabolism of a number of P450 2E1 substrates as well as a P450 2E1 futile cycle in the absence of substrate is known to lead to an increased production of reactive oxygen species and deregulation of apoptotic pathways (33).

CYP1B1 expression levels were higher in non-tumor than in tumor tissue of the majority of patients. Our data comply with a recent study that found significantly higher *CYP1B1* mRNA levels in adjacent non-tumor tissue than in tumors (20). In our study, more than 3-fold overexpression of *CYP1B1* (3.3-, 7.2-, and 22.9-fold) was detected in tumors of three patients. These patients could potentially benefit from P450 1B1-based therapeutic interventions. In fact, P450 1B1-directed immunotherapeutics, P450 1B1-activated prodrugs and P450 1B1 inhibitors are currently being developed to exploit the presence and activity of this enzyme in tumor cells (34). In a Phase I clinical trial, all patients where anti-P450 1B1 increased had significantly improved response to their next therapy possibly associated with clinical benefit (35).

This study is the first to compare the expression of *CYPs* in mammary glands with that in lymphocytes from the same patients as potential surrogate marker. Expressions of *CYP2C9* and *CYP3A4* in lymphocytes from a subset of 18 examined patients were negligible. Previously detected *CYP1B1* and *CYP2E1* expression in human lymphocytes was confirmed (5, 23). We observed on average almost a 5-fold higher *CYP2E1* expression in lymphocytes than in mammary gland tissue. Due to lack of correlation, *CYP2E1* expression in lymphocytes cannot be used as surrogate marker. On the contrary, correlation of *CYP1B1* expression in non-tumor samples with that in lymphocytes found by our study suggests that lymphocytes may serve as marker of the *CYP1B1* level in the human mammary gland. The correlation of *CYP1B1* expression in lymphocytes with that in tumors was weaker. However, our study was performed on quite a small number of lymphocyte samples and thus a confirmation study on a larger number of paired samples is necessary.

Expression levels of *CYP1B1* and *CYP2E1* were compared with clinical and histological characteristics of patients in order to identify possible associations between expression levels and prognostic markers of breast cancer. *CYP1B1* expression did not correlate with any of characteristics inspected. *CYP2E1* expression seemed to co-segregate with locally advanced disease, because in tumors of patients with clinical stage I it was higher than that in patients with stages II, III and IV. The fact that *CYP2E1* expression was down-regulated in hepatocellular carcinomas and its decreased expression was associated with poor prognosis suggests that the relevance of this finding may be more general (36). Interestingly, *CYP2E1* expression was higher in the non-tumor tissue of our progesterone receptor-negative patients. Higher P450 1B1 levels were observed in estrogen receptor-negative tumors than in positive ones (p=0.006; 6). Significantly higher *CYP2E1* expression observed in our study in the invasive lobular types of tumors in comparison with invasive ductal ones suggests that *CYP2E1* expression may be an indicator of different types of breast carcinogenesis. Higher *CYP2E1* expression may be caused by unknown events leading to deregulation of receptors or other *trans*-acting factors interacting with *CYP2E1* expression in lobular carcinomas. Association of *CYP2E1* expression with breast cancer type has not been reported to date. Recently, no significant differences between 274 ductal and 73 lobular cases in P450 1A1/2, 1B1, 2E1 and 3A4/5 protein levels were found by immunohistochemistry (6). There is a lack of information on any correlation between CYP mRNA and P450 protein expression levels in the literature. Thus, in our opinion, mRNA and protein levels should be considered as independent markers of prognosis until mechanistic studies clarify these discrepancies. Indeed it seems that gene expression profiling will soon become a highly valuable tool in the molecular pathology of breast cancer (37).

In conclusion, *CYP2C9* and *CYP3A4* expression was negligible in most of the examined samples. *CYP2E1* was expressed at a significant level in mammary tumors and non-tumor adjacent tissues and associated with some of the clinical and pathological data on patients. The observed high *CYP1B1* expression in tumor cells may evoke changes in their response to drugs which are substrates of P450 1B1 as well as perhaps influencing estrogen metabolism or activation of environmental carcinogens. The potential implications of *CYP1B1* expression in prodrug activation and vaccination strategies to combat tumor cells are envisaged. Detailed association studies of *CYP1B1* and *CYP2E1* expression with response to various chemotherapy regimens in the long-term perspective are currently being carried out in our laboratory.

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