Interindividual variation in CYP1A1 expression in breast tissue and the role of genetic polymorphism

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The cytochrome P4501A1 (CYP1A1) enzyme is regulated at the transcriptional level and its expression is influenced by genetic factors, polymorphisms in the structural and regulatory genes, and by environmental factors such as exposure to polycyclic aromatic hydrocarbons (PAHs). To investigate the role of CYP1A1 in breast cancer, we studied CYP1A1 expression in breast tissue, thereby taking all possible modifying factors into account. We measured CYP1A1 expression in 58 non-tumor breast tissue specimens from both breast cancer patients (n = 26) and cancer-free individuals (n = 32) using a newly developed reverse transcription–polymerase chain reaction assay. CYP1A1 expression varied between specimens ~400-fold and was independent of age. CYP1A1 expression was somewhat higher in tissue from breast cancer patients than in that from cancer-free individuals, but this difference was not statistically significant. Analysis for CYP1A1 genetic polymorphisms revealed eight variants, seven in the cancer-free group and one in the patient group. The variant genotype was not a good predictor of expression level. We conclude that high CYP1A1 expression could be a risk factor for breast cancer and that the known CYP1A1 polymorphisms are not good predictors of CYP1A1 expression.

Polycyclic aromatic hydrocarbons (PAHs), a class of chemicals that includes potent carcinogens, could have a role in breast cancer because they accumulate in breast adipose tissue (1) and because normal human mammary cells in culture activate PAHs efficiently (2). PAH–DNA adduct levels have been found to be significantly higher in normal breast tissue of breast cancer patients than in that of non-cancer controls (3). The mutational spectrum in the p53 gene in breast tumors resembles that of lung cancers where there is a well-established role for environmental agents, such as tobacco smoke (4). The major metabolic pathway for ingested or inhaled PAHs to water-soluble derivatives is oxidative activation by CYP1A1 followed by detoxification by phase II enzymes. There is evidence supporting a role of CYP1A1 in breast cancer from recent animal experiments: using a rat model to identify loci that control breast cancer susceptibility, one of the four loci mapped to CYP1A1 or a nearby locus (5).

Interindividual variation in carcinogen metabolism has been recognized as a determinant of susceptibility to various cancers (6). Genetic polymorphism is one potential source of variation. For CYP1A1, four genetic polymorphisms consisting of single base changes have been described (7); two of them have been studied extensively as genetic biomarkers of susceptibility to various cancers, including breast cancer (8). The first described variant, CYP1A1*2, is located in the 3′ non-coding region of the CYP1A1 gene and introduces an MspI restriction endonuclease site (9,10). The second variant, CYP1A1*3, is strictly linked to CYP1A1*2 (7) and consists of an A→G transition in exon 7 that results in an amino acid substitution of Val462 to Ile462 (11). Several studies have suggested that this genotype increases susceptibility to various cancers, but the biochemical basis is unclear. It has been assumed that the CYP1A1*2 and CYP1A1*3 alleles lead to higher inducibility. Expression of CYP1A1 is regulated by the aryl hydrocarbon receptor, together with several other regulatory proteins. Increased transcription of the CYP1A1 gene reflects induction of the enzyme (12). CYP1A1 expression can be induced by exposure to PAHs and organochlorines (13). Besides environmental factors, genetic factors can modify CYP1A1 expression; these include the genotype of the structural gene and the genotype of regulatory genes, including the aryl hydrocarbon receptor. Therefore determining the amount of transcript or the actual level of the enzyme captures the influence of all potentially modifying factors and is a more sensitive tool than the analysis of the genotype of a single gene.

We have examined CYP1A1 expression as a possible breast cancer risk factor by comparing CYP1A1 expression in non-tumor breast tissue from 27 breast cancer cases and 32 cancer-free individuals. Although we did not measure CYP1A1 protein levels or CYP1A1 enzyme activity, mRNA levels and enzyme activities are known to be closely related (14,15). The case specimens were derived from 22 mastectomies (peripheral non-tumor tissue) and five contralateral to carcinomatous breast. The control specimens were obtained from 32 reduction mammoplasties. Tissue specimens were dissected and isolated from adipose and connective tissue, so that only epithelial material was stored frozen as organoids (16). The pathological diagnosis of the excised tumors was intraductal carcinomas for two cases and infiltrating ductal carcinoma for the other 20 cases. In two of the 22 cases, metastasis to axillary lymph nodes was observed, indicating more advanced disease. Samples were collected without respect to age and race. Only the age and disease status of the specimen donors are known. No information is available on donors’ race, lifestyle, smoking habits or other potential confounding factors. Individuals undergoing reduction mammoplasty ranged in age from 15 to 68 years, and mastectomy patients ranged in age from 30 to 87 years.

To determine CYP1A1 transcript levels, we developed a reverse transcription–polymerase chain reaction (RT–PCR) assay that determines CYP1A1 expression relative to the

Abbreviations: CYP1A1, cytochrome P4501A1; PAHs, polycyclic aromatic hydrocarbons.

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constantly expressed β-actin gene, thus controlling for varying sample sizes and RNA yield. Previously published primers designed to span an intron (thus excluding amplification of any contaminating genomic DNA) were used and generated products of 320 bp for CYP1A1 and 273 bp for β-actin (17, 18). PCR conditions and cycle numbers were optimized separately for each target sequence to ensure that the reaction was in the linear phase of product accumulation. A five-fold serial dilution of cDNA was amplified in separate reactions for CYP1A1 and β-actin. After amplification, the products were mixed together before electrophoresis on a 10% native polyacrylamide gel. The gel was stained with SYBR Gold nucleic acid stain and scanned on a Molecular Dynamics STORM 860 optical scanner. The fluorescent signal for each band was quantified using ImageQuant software (Figure 1). We found that this assay for CYP1A1 expression is sensitive, reproducible and has a broad dynamic range. CYP1A1 expression was measured in 59 non-tumor breast tissues from individuals with breast cancer (n = 27) and from cancer-free individuals (n = 32). Only one of the 59 samples did not have amplifiable RNA. CYP1A1 quantification was repeated in a blinded assay for 20% of samples. The correlation between the original measurements and the respective repeats was 0.9878, indicating that the assay is highly reproducible. In experiments with human mammary epithelial cells in culture, we found that the amount of β-actin transcript was independent of benzo[a]pyrene exposure, whereas CYP1A1 transcript levels increase in proportion to the dose (data not shown). In the present study, β-actin transcript levels in the 58 specimens could be evaluated from one of the first two dilutions of the cDNA. In contrast, the whole range of dilutions was needed to determine the CYP1A1 transcript levels in all specimens, indicating the large variations between individuals in CYP1A1 expression. The CYP1A1:β-actin transcript ratio varied between the lowest value of 0.17 to the highest value of 70, a >400-fold range. As seen in Figure 2, individuals in the control group were younger than in the small sample studied. Comparing the distribution of CYP1A1:β-actin ratio as function of age of specimen donors; [] represent values of reduction mammoplasty controls and • represent values of breast cancer cases.

Fig. 2. CYP1A1/β-actin ratio as function of age of specimen donors; [] represent values of reduction mammoplasty controls and • represent values of breast cancer cases.

Fig. 3. Distribution of CYP1A1 expression levels among breast cancer cases and reduction mammoplasty controls. The upper two histograms show the untransformed data. The bottom two histograms show the log-transformed data.
CYP1A1 expression in breast tissue

Fig. 4. CYP1A1:β-actin ratio ranked for all individuals. Open bars represent the CYP1A1*1 (wild-type) genotype. Solid bars represent CYP1A1 polymorphic variants of the following categories: (a) CYP1A1*2 heterozygotes; (b) CYP1A1*2/CYP1A1*1 heterozygotes; (c) CYP1A1*2 homozygotes. CYP1A1:β-actin ratios are given in parentheses for the polymorphic variants. The origin of each tissue specimen is given below the bar, R, reduction mammoplasty; P, peripheral to carcinoma; C, contralateral.

CYP1A1 expression in breast tissue

The CYP1A1*2 and CYP1A1*3 alleles have been associated with a phenotype of high gene induction in response to PAHs (11). To investigate to what extent the CYP1A1 genotype modifies CYP1A1 expression, the CYP1A1 genotype of all specimens was determined using PCR/restriction fragment length polymorphism analysis according to published procedures (7). A total of eight CYP1A1*2 and CYP1A1*3 alleles in 58 samples were detected: three CYP1A1*2 heterozygotes, three CYP1A1*2/CYP1A1*1 heterozygotes and two CYP1A1*2 homozygotes. The case group had only one CYP1A1*2/CYP1A1*1 heterozygote while the control group had seven variants. When all CYP1A1 values are ranked (Figure 4), the CYP1A1 variants are distributed between the lowest and highest expression values. All heterozygous variants and the one homozygous CYP1A1*2 variant have CYP1A1 values below the mean CYP1A1 values. Only one homozygous CYP1A1*2 variant was among the five specimens with the highest CYP1A1 expression values, indicating that the polymorphism has at most a minor role in determining CYP1A1 expression.

The CYP1A1*2 variant is located in the non-coding region of the gene, suggesting that the CYP1A1*2 polymorphism alters the inducibility of CYP1A1. The CYP1A1*3 variant is located in exon 7, which codes for the heme-binding region. A change in amino acids in this region could possibly result in a change in enzyme activity. An earlier study reported a 50% higher enzyme activity (11). However, using purified human recombinant CYP1A1*1 and CYP1A1*2, a more recent study did not find different benzo[a]pyrene activation (23). Another study reported no difference in the kinetics of the CYP1A1 polymorphic variants (24). Therefore, any change in CYP1A1 expression in females was more than twice that in males (22).

CYP1A1 levels in CYP1A1*3 seem to be the result of strict linkage to CYP1A1*2 polymorphism (7), which presumably alters the inducibility of the enzyme. Our data suggest that CYP1A1*2 polymorphism has a minor, if any, role in modifying CYP1A1 expression. If individuals with the CYP1A1 variant genotype were exposed to much lower levels of PAHs than individuals with the wild-type genotype, the impact of genotype on expression might be masked. In an earlier study, human mammary epithelial cells derived from 18 individuals were treated with benzo[a]pyrene and DNA adducts quantified (2). Among the strains examined were six derived from donors tested here for CYP1A1 expression and CYP1A1 genotype, including the two homozygous CYP1A1*2 and one of the heterozygous CYP1A1*2 variants identified here. Contrary to expectations, the two homozygous CYP1A1*2 alleles had the lowest amount of adducts, indicating that the CYP1A1*2 genotype did not increase DNA adduct formation. Besides activating xenobiotics, CYP1A1 also metabolizes 17 β-estradiol to the less active 2-hydroxy estradiol (25). A recent study suggests that CYP1A1*2 may be a marker of altered estradiol metabolism and of increased susceptibility to estrogen-related breast cancer in African-Americans (26).

In conclusion, this study shows that breast tissue expresses a considerable range of CYP1A1 levels independent of age and genotype, reinforcing the importance of evaluating both genotype and phenotype. Although the results are not statistically significant in the small unselected specimen groups avail-
able, they suggest that increased PAH activation by CYP1A1 might play a role in initiation of breast cancer. Larger sample sizes will be required to corroborate these suggestive findings.

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