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CYP1A1 I462V polymorphism is associated with reduced genotoxicity in yeast despite positive association with increased cancer risk

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ABSTRACT

CYP1A1 functions in detoxifying xenobiotics but occasionally converts compounds into potent genotoxins. CYP1A1 activates polyaromatic hydrocarbons, such as benzo[a]pyrene 7,8 dihydrodiol (BaP-DHD), rendering them genotoxic. Particular alleles of CYP1A1, such as CYP1A1 I462V have been correlated with a higher incidence of breast and lung cancer, but it is unknown whether these variants express enzymes *in vivo* that are more potent in generating genotoxins. We individually expressed CYP1A1 (CYP1A1.1), CYP1A1 T461N (CYP1A1.4) and I462V (CYP1A1.2) alleles in wild-type and DNA repair deficient mutant strains of *Saccharomyces cerevisiae* (budding yeast) and asked which yeast strains exhibited the highest levels of carcinogen-associated genotoxicity after exposure to BaP-DHD, aflatoxin B1 (AFB_1), and heterocyclic aromatic amines (HAAs). We measured carcinogen-associated recombination, Rad51 foci, and carcinogen-associated toxicity in a DNA repair mutant deficient in both nucleotide excision repair and recombinational repair. CYP1A1 activity was confirmed by measuring ethoxyresorufin-O-deethylation (EROD) activities. Our data indicate that CYP1A1 I462V allele confers the least carcinogen-associated genotoxicity, compared to CYP1A1; however, results vary depending on the chemical carcinogen and the genotoxic endpoint. We speculate that the cancer-associated risk of CYP1A1 I462V may be caused by exposure to other xenobiotics.

1. Introduction

CYP1A1 is a highly induced extrahepatic P450 enzyme whose expression varies greatly among individuals [1–3]. It metabolizes a broad range of xenobiotic and endogenous compounds including carcinogens, pharmaceuticals, and hormones, with a preference for aromatic substrates in a planar configuration [4]. Its importance in cancer etiology is underscored as the activator of the well-known polyaromatic hydrocarbon (PAH) benzo[a]pyrene, which is ultimately converted into BaP-dihydrodiol epoxide, the active metabolite that forms mutagenic N^2-guanine DNA adducts [5]. CYP1A1 metabolically activates 7,12-dimethyl benzanthracene (DMBA, [4]), and particular food carcinogens, including aflatoxin B1 (AFB_1, [6]) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP, [4]). Alternatively, CYP1A1 converts anticancer drugs into potent therapeutics, such as aminoflavone [7] and dacarbazine [8]. Its metabolism of polyunsaturated fatty acids aids in controlling blood pressure [9]. Thus, CYP1A1's metabolic functions have significance in cancer etiology, pharmacology, and cardiovascular physiology.

The importance of CYP1A1 in carcinogen metabolism has motivated investigators to identify CYP1A1 variants that are associated with disease. The CYP1A1 variant, CYP1A1*2C (CYP1A1 I462V [10]), has been correlated with the increased cancer incidence of adult leukemia [11], lung cancer [12], breast cancer [13–16], and renal cancer [17]. The CYP1A1 variant, CYP1A1*4 (CYP1A1 T461N, [18]), has been correlated with an increased risk for endometrial cancer [19]; see [20] for a list of CYP1A1 variants. CYP1A1 I462V is found in approximately 20% of the Japanese population and 9% of the Caucasian population while CYP1A1 T461N is found in 2–8% of the Caucasian population [21]. Considering that exposure to polyaromatic hydrocarbons (PAHs) is a risk factor for lung cancer (for review, see [22]), one hypothesis is that cancer-associated CYP1A1 alleles have higher catalytic activity in converting xenobiotics, such as PAHs, to potent genotoxins [23].

To test this hypothesis, investigators characterized enzymatic properties of CYP1A1 alleles expressed *in vitro*, and measured DNA adducts and quantified chromosomal abnormalities in human leukocytes from individuals bearing different CYP1A1 alleles. Enzy-

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matic properties, such as K_{cat} and K_m, support the notion that CYP1A1 enzymatic variants may have higher EROD activities but still lower activity towards BaP, compared to wild-type CYP1A1 ([24,25]). However, other studies indicated that the m2 variant (CYP1A1*2C, 2454A > G, Ile462Val) is clearly associated with 6- to 12-fold higher enzymatic activity towards 17β-estradiol and estrone [26]. Studies using human lymphocytes indicate that particular CYP1A1 alleles, such as CYP1A1*2A, may confer higher levels of DNA PAH-adducts and chromosomal abnormalities, compared to individuals bearing the wild type allele [27]; however, there has been inconsistent reports on levels of PAH adducts in leukocytes from individuals bearing either CYP1A1*2C or *2B [28]. Differential expression of phase II enzymes that detoxify compounds, such as GSTM1, has been indicated to be an additional variable in evaluating CYP1A1 alleles [28] Thus, particular CYP1A1 alleles may confer higher levels of genotoxic activation depending on the exposure to the particular carcinogenic compound or substrate.

We used budding yeast to determine whether CYP1A1 alleles correlated to higher cancer incidence conferred higher levels of genotoxicity for particular carcinogens, compared to wild type. Yeast does not express Phase II enzymes that can rapidly detoxify the activated compounds nor does it express multiple P450s, as in the lung or liver, which can metabolically activate compounds. However, the expression of individual human P450s greatly enhances the genotoxicity of PAHs in yeast cells [29–31]. The full-length cDNA of the CYP1A1 was expressed and active enzyme was measured from yeast microsomal extracts [32]. We chose genotoxic endpoints that were particularly sensitive in detecting DNA damage, including growth in the *rad4 rad51* mutant after carcinogen exposure, carcinogen-associated recombination, and carcinogen-associated Rad51 foci [33,34]. We previously used this methodology to phenotype CYP1A2 polymorphisms [34]. Our studies indicate that phenotypic differences between CYP1A1 alleles depend on the xenobiotic.

2. Materials and methods

2.1. Media

Standard media were used for the culture of yeast cells. YPD (yeast extract, peptone, dextrose), SC-TRP (synthetic complete lacking tryptophan), SC-URA (synthetic complete lacking uracil) and FOA (5-fluro-orotic acid) were described in Burke et al. [35].

2.2. Chemical preparation

Stock solutions of 10 mM aflatoxin B_1 (AFB_1), 37.5 mM benzo[a]pyrene-7,8-dihydrodiol (BaP-DHD), 50 mM 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and 100 mM 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) were dissolved into dimethyl sulfoxide (DMSO). Chemicals were purchased from Sigma (AFB_1) and Santa Cruz Biotechnology (IQ, MeIQx, PhIP). BaP-DHD was obtained from the National Cancer Center Repository for Chemical Carcinogens (Kansas City, MO), as a powder. It was protected from light at −20 °C, and stock solutions were made as required.

2.3. Plasmid constructions and site-specific mutagenesis

Standard molecular biology techniques [36] for DNA isolation and bacterial transformation were used to construct vector pRS424-CYP1A1 and pMF-human oxidoreductase (hOR). The human CYP1A1 was inserted into pRS424-CYP1A1 by subcloning the 2.3 kb CYP1A1 *Sma*I-SalI fragment from pSB229 into the *Sma*I and *Sal*I sites of pRS424 [37]. pMF:hOR was constructed by inserting the *Bam*H1 fragment containing the hOR gene in pSB229 into pMF [34] so that it is flanked by DNA sequences both centromere proximal and distal to *TRP1*. pRS424-CYP1A1 was introduced into yeast strains expressing hOR by selecting for Trp^+ transformants.

Site-specific mutagenesis was performed using QuickChange kit (Stratagene) according to the manufacturer's instructions. We constructed I462V using the forward primer TGCGTGAGACC**G**TTGCCCGCTGG, and the reverse primer CCAGCGGGCAA**C**GGTCTCACCGA. We constructed T461N using forward primer TATCGGTGAGA**A**CATTGCCCGCT and reverse primer AGCGGGCAATG**T**TCTCACCGATA. The specific mutations were then verified, and the entire gene was sequenced to verify that only the base substitutions specific for T461N and I462V were introduced.

2.4. Yeast strains

The genotypes of yeast strains used in this study are listed in Table 1. Strains used to measure AFB_1-associated recombination or mutagenesis was isogenic to S288c; strains used for detecting Rad51 foci were derived from W303. For measuring AFB_1-associated translocation frequencies, diploid strains contained *trp1:hOR* [34] and the recombination substrates [38], *his3-Δ3′* and *his3-Δ5*,' as described [39,40]. Plasmids containing CYP1A1 and alleles were introduced into yeast strains by selecting for Trp^+ transformants.

Strains containing *trpl:hOR* were confirmed by PCR using the forward oligo AGGAGACAGACGTGGATCTCTCTG and the reverse oligo AAGCCAAACACACCCAGGAGACTA. Both *MAT***a** and *MATα* strains were made by genetic crosses to the S288c-related strains. Since it is easier to detect DNA damage-associated recombination in diploid strains [41], the indicated strains were made by diploid crosses, as indicated in Table 1.

The *rad4 rad51* strains for measuring BaP-DHD and AFB_1 sensitivity are derived from YB226, which contains *his3* recombination substrates in tandem at *TRP1* [38]. A Ura^- derivative (YB400) of the *rad4 rad51* strain (YB226, [42]) was selected on 5-fluoroorotic acid (FOA) medium.

Strains used to detect Rad51 foci were derived from LSY1957, a gift of L. Symington [43]. This strain was crossed with YB407 and the meiotic segregant YB419 was obtained that contains both yfp-RAD51 and *trpl:hOR*.

2.5. Measuring carcinogen-associated recombination

To measure BaP-DHD and AFB_1-associated recombination events, log phase yeast cells ($A_{600} = 0.5 - 1$) were centrifuged and concentrated five-fold in synthetic media (SC-URA or SC-TRP). Approximately 10^8 cells were exposed for four hours to indicated doses of BaP-DHD or AFB_1, previously dissolved in DMSO. Cells were maintained in nutrient media (SC-URA or SC-TRP) during the carcinogen exposure and then washed twice in H_2O. For measuring recombination, cells were directly plated on SC-HIS and an appropriate dilution was inoculated onto YPD to measure viability. Statistical significance was determined using the Student's *t-test.*

To determine whether ionizing radiation stimulates the formation of Rad51 foci, cells were washed once in H_2O and resuspended in 10 ml of H_2O and placed in a 81 mm diameter Petri dish. Cells were irradiated at 6 krad using a Nordion 1.8kCi ^{137}Cs irradiator (6 krad/hr). After irradiation cells were concentrated in YPD medium and immobilized on glass slides.

2.6. Preparation of yeast microsomes and quantifying ethoxyresorufin O-deethylation (EROD) activities

Strains were grown to saturation in 75 ml SC-TRP media. The cells were pelleted, resuspended in 5 ml 50 mM Tris 1 mM EDTA