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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 (AFB₁), 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.

Keywords

CYP1A1 polymorphisms; food carcinogens; budding yeast; DNA damage

Introduction

CYP1A1 is a highly induced extrahepatic P450 enzyme whose expression varies greatly among individuals [1,2,3]. It metabolizes a broad range of xenobiotic and endogenous

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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 N²-guanine DNA adducts [5]. CYP1A1 metabolically activates 7,12-dimethyl benzantracene (DMBA, [4]), and particular food carcinogens, including aflatoxin B1 (AFB₁, [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 [13,14,15,16], 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. Enzymatic 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, 30, 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-fluoro-orotic acid) were described in Burke *et al.* [35].

2.2 Chemical Preparation

Stock solutions of 10 mM aflatoxin B₁ (AFB₁), 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 (AFB₁) 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-*Sa*II fragment from pSB229 into the *Sma*I and *Sa*II sites of pRS424 [37]. pMF::hOR was constructed by inserting the *Bam*HI 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 TGCGTGAGACCGTTGCCCGCTGG, and the reverse primer CCAGCGGGCAACGGTCTCACCGA. We constructed T461N using forward primer TATCGGTGAGAAACATTGCCCGCT and reverse primer AGCGGGCAATGTTCTCACCGATA. 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 AFB₁-associated recombination or mutagenesis was isogenic to S288c; strains used for detecting Rad51 foci were derived from W303. For measuring AFB₁-associated translocation frequencies, diploid strains contained *trp1::hOR* [34] and the recombination substrates [38], *his3-3'* and *his3-5'*, as described [39, 40]. Plasmids containing CYP1A1 and alleles were introduced into yeast strains by selecting for Trp⁺ transformants.

Strains containing *trp1::hOR* were confirmed by PCR using the forward oligo AGGAGACAGACGTGGATCTCTCTG and the reverse oligo AAGCCAAACACACCCAGGAGACTA. Both *MATa* 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 AFB₁ 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 *trp1::hOR*.

2.5 Measuring carcinogen-associated recombination

To measure BaP-DHD and AFB₁-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 10⁸ cells were exposed for four hours to indicated doses of BaP-DHD or AFB₁, previously dissolved in DMSO. Cells were maintained in nutrient media (SC-URA or SC-TRP) during the carcinogen exposure and then washed twice in H₂O. 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 H₂O and resuspended in 10 ml of H₂O and placed in a 81 mm diameter Petri dish. Cells were irradiated at 6 krad using a Nordion 1.8kCi ¹³⁷Cs 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 (TE pH 7.4) buffer, incubated at room temperature for five minutes, and suspended in 0.6 M Sorbitol 50 mM Tris (pH 7.4). The cells were lysed by agitation in a bead beater at 4°C, and centrifuged for 10 minutes at

10000 × g. The supernatant from each sample was made 0.15 M in NaCl, and 20% in polyethylene glycol (MW 3350), and incubated on ice for at least one hour. Each tube was centrifuged at 10000 × g for 20 minutes. The supernatant was removed, and the resulting pellet was suspended in 50mM Tris (pH 7.4) and made 10% in glycerol; supernatants were stored in a -80°C freezer. Protein concentrations from yeast microsomes were determined by the Bradford assay, according to the manufacturer's instructions. NADPH-dependent EROD activity was measured by resorufin fluorescence, as outlined by Mohammadi-Bardbori [44]. Final calculations were determined as picomoles resorufin/minute/mg protein.

2.7 Live cell epifluorescence and microscopy analysis

Cells for microscopic analysis were grown to early to mid-log phase overnight in synthetic medium. Strains harboring pRS424-CYP1A1 were cultured in SC-TRP. Cells were harvested by centrifugation and concentrated five-fold in the growth medium. Immobilization of cells was performed by mixing equal volumes of cell suspension and 1.4% low melt agarose plus growth medium solution. Cover slips were sealed with a wax mixture as described by Lisby et al. [45]. Slides were visualized using a Zeiss LSM 510 META confocal microscope.

2.8 Growth assays in 96 well plate to measure AFB₁ and BaP-DHD sensitivity

In brief, individual saturated cultures were prepared for each yeast strain. Cell density was adjusted to $\sim 0.8 \times 10^7$ cells/ml for all cultures. We maintained the cells in selective medium (synthetic dextrose lacking tryptophan). In each microtiter well, 95 μ l of media and 5 μ l of cells (8×10^4 cells) were aliquoted in triplicate for blank, control and experimental samples. For experimental samples, we added AFB₁, dissolved in DMSO, for a final concentration of 100 nM and 10 μ M. The microtiter dish was placed in a plate reader that is capable of both agitating and incubating the plate at 30° C, as previously described [33, 34]. We measured the A₆₀₀ at 10 minute intervals, for a total period for 24 hrs, 145 readings. Data at 1hr intervals was then plotted. To avoid evaporation during the incubation, the microtiter dishes were sealed with clear optical tape.

2.9 Western blotting

Human CYP1A1 protein was detected in yeast microsomal extracts by Western blots, after semi-dry transfer from a SDS polyacrylamide gel onto nitrocellulose, similar to the protocol used to detect human CYP1A2 by Western blots. To detect CYP1A1, we used a mouse antibody raised against a Rat CYP1A2 protein (Abcam); this antisera contains significant cross-reactivity against human CYP1A1. We used a β -actin antibody (Abcam) that cross reacts with budding yeast actin (Act1), as a control.

3. Results

Previous studies indicated that human CYP1A1, when expressed on high-copy number expression vectors, can metabolically activate both AFB₁ and BaP-DHD in budding yeast [29, 30]. We used genotoxic endpoints in budding yeast to detect differences in metabolic activation conferred by polymorphisms in the coding sequence of CYP1A1. By site-specific mutagenesis, CYP1A1 T461N and CYP1A1 I462V were individually introduced into the

yeast vector pRS424-CYP1A1, and the entire CYP1A1 gene was sequenced to verify the individual base pair substitutions. To verify that equivalent levels of each CYP1A1 protein was expressed in each strain, we isolated yeast microsomes and performed Western blots, using a mouse antibody that recognized human CYP1A1; Act1 was used as an internal control and the ratio of signal (CYP1A1/ Act1) was measured and averaged for two Western blots. For strains expressing CYP1A1, CYP1A1 I462V, CYP1A1 T461N, we observed ratios of 1.3, 1.1, and 1.3, respectively. We conclude that equivalent levels of CYP1A1 protein are expressed in these strains expressing CYP1A1 alleles.

3.1 *rad4 rad51* strains expressing CYP1A1 T461N and CYP1A1 I462V are less sensitive to AFB₁ and BaP-DHD, compared to the strain expressing CYP1A1

Radiation sensitive cells defective in both recombinational repair and nucleotide excision repair are extremely sensitive to DNA damaging agents [33]. Sensitivity to chemical agents can be measured by growth curves, comparing growth in the presence and absence of the agent. We previously showed that *rad4 rad51* cells expressing CYP1A2 are sensitive to 100 nM AFB₁[33]. We exposed *rad4 rad51* strains expressing CYP1A1, CYP1A1 T461N, and I462V, to AFB₁, BaP-DHD, IQ and PhIP.

Results showing differences between strains expressing different CYPs were most notable for cells after chronic exposure to BaP-DHD and AFB₁ (Figure 1). Cells expressing CYP1A1 I462V (YB432, see Table 1) were only sensitive to AFB₁ and BaP-DHD at higher exposure levels, while cells expressing CYP1A1 (YB431) were sensitive at all exposure levels. After exposure to 10 μ M AFB₁, growth was equivalently reduced in cells expressing CYP1A1 I462V or CYP1A1 T461N, while growth was reduced the most (~65%) in cells expressing CYP1A1. After exposure to 37.5 μ M BaP-DHD, growth was reduced the least in cells expressing CYP1A1 I462V, at an intermediate level for cells expressing CYP1A1 T461N (YB433), and at a maximum level for cells expressing CYP1A1. We conclude from these data that all three CYPs activate both BaP-DHD and AFB₁, but growth of cells expressing CYP1A1 I462V were least affected by chronic BaP-DHD exposure.

Expression of CYP1A1, CYP1A1 T461N and CYP1A1 I462V also reduced growth of the *rad4 rad51* strain after chronic exposure to IQ and PhIP (Figure 2). Differences between strains were most notable after exposure to 10 mM IQ. Although a decrease in viability of the *rad4 rad51* mutant expressing all three CYP1A1 alleles after PhIP exposure was detected, differences between these strains were not observed.

3.2 CYP1A1 I462V confers lower levels of carcinogen-associated recombination compared to CYP1A1 and CYP1A1 T461N

Expression of CYP1A1 and the human oxidoreductase (hOR) is sufficient to metabolically activate either AFB₁ or BaP-DHD into potent yeast recombinagens [30]. We previously expressed CYP1A1 in a nucleotide excision repair (NER) mutant and observed enhanced recombination [29], resulting from a deficiency in repair of DNA bulky adducts. Here, we expressed in CYP1A1, CYP1A1 T461N and CYP1A1 I462V in a *rad4* mutant and measured recombination between two *his3* fragments, located on chromosomes II and IV (Figure 3). The frequencies of spontaneous recombination varied between 3.5×10^{-8} and 2×10^{-7}

among strains, consistent with previously observed variations [38]. After exposure to 50 μ M AFB₁, we observed the highest level of recombination in cells expressing CYP1A1 T461N (YB430, fourteen-fold difference). However, cells expressing CYP1A1 (YB428) exhibited a higher-fold increase in recombination (seventeen-fold), due to lower levels of spontaneous recombination. Cells expressing CYP1A1 I462V exhibited the lowest frequency of AFB₁-associated recombination. The survival in all the strains after acute AFB₁ exposure was approximately 60–70%. Since there are essentially equivalent levels of CYP1A1 protein, compared to yeast actin, in all strains, we conclude that CYP1A1 I462V is the least efficient in activating AFB₁ into a recombinagen.

Carcinogen-associated recombination was also observed in *rad4* cells after exposure to BaP-DHD. We detected a ten-fold higher level of carcinogen-associated recombination in cells expressing CYP1A1, while cells expressing CYP1A1 I462V (YB429) only exhibited a two-fold higher level of recombination. Cells expressing CYP1A1 T461N also exhibited BaP-DHD associated recombination; however, there is no significant difference in recombination frequencies compared to cells expressing the other CYP1A1 alleles. Thus, the carcinogen-associated recombination is lower in cells expressing CYP1A1 I462V, compared to cells expressing CYP1A1. These data are consistent with the lower levels of BaP-DHD - associated toxicity observed in the *rad4 rad51* strain (YB429) expressing CYP1A1 I462V.

3.3 EROD activity in strains expressing CYP1A1, CYP1A1 T461N and CYP1A1 I462V

To verify that CYP1A1 variants were enzymatically active in yeast, we performed EROD assays ([44], Figure 4). We compared the EROD activity of cells expressing CYP1A1 I462V (YB429) with those of cells expressing CYP1A1 (YB428) and CYP1A1 T461N (YB430). NADPH-dependent activity was detected in microsome extracts of cells expressing all CYP1A1 variants (see supplemental Figure 1 for an example), and ranged from 0.5 pmol resorufin/min/mg to 2.5 pmol resorufin/min/mg (Figure 4). No EROD activity was detected in cells containing an empty vector (pRS424). We conclude that while EROD activity can be detected in cells expressing each CYP1A1 allele, cells expressing CYP1A1 I462V have comparable if not higher EROD activity compared to that detected in cells expressing other CYP1A1 alleles.

3.4 Rad51 foci appear after AFB₁ exposure in cells expressing CYP1A1, CYP1A1 T461N and CYP1A1 I462V

We previously observed DNA damage-associated Rad51 foci in CYP1A2-expressing cells after exposure to AFB₁ or γ rays (34). While radiation-associated Rad51 foci appeared in cells arrested in G2, AFB₁-associated Rad51 foci appeared in both small-budded cells entering S phase and large dumb-bell shaped cells, indicative of G2- arrested cells. To observe Rad51 foci in cells expressing CYP1A1 alleles, we constructed a *yfp-Rad51* strain in which hOR was integrated in the genome at the *TRP1* locus. Less than one percent of cells (0/153) expressing either CYP1A1 (YB434), CYP1A1 T461N (YB436) or CYP1A1 I462V (YB435) were observed to exhibit Rad51 foci in the absence of DNA damage exposure, consistent with previous studies ([34], Figure 5). After exposure to ionization radiation, 30–90% of the irradiated cells from all strains exhibited Rad51 foci, and these cells were G2 arrested. In the strain that did not express P450, < 1% of the cells exhibited

Rad51 foci after AFB₁-exposure. The percentage of AFB₁-exposed cells expressing either CYP1A1 or CYP1A1 T461N that exhibited Rad51 foci was essentially the same (12–13%), but significantly different from the number of Rad51 foci from cells that were not exposed ($P < 0.05$, Chi square). However, in the strain that expressed CYP1A1 I462V, <5% of the AFB₁-exposed cells exhibited Rad51 foci. These data indicate that CYP1A1-mediated activation that confers the highest level of AFB₁-associated recombination also correlates with the presence of Rad51 foci.

Discussion

Allelic variants of CYP1A1, such as CYP1A1 I462V and CYP1A1 T461N, are alleged risk factors for specific cancers, including endometrial, lung, and breast cancer. The two alleles result from amino acid substitutions that map near the active site of the enzyme [46], and CYP1A1 I462V has been reported to be overly represented in cancer patients [3, 47]. Considering that environmental agents are risk factors for lung and breast cancers, one hypothesis is that these CYP1A1 alleles confer a higher risk for cancer because they encode enzymes that have higher metabolic activities. We expressed CYP1A1 alleles in budding yeast, exposed cells to P450-activated carcinogens and measured different genotoxic endpoints that indicate DNA damage. This is the first time genotoxic endpoints have been directly measured in yeast to phenotype CYP1A1 alleles.

Two main conclusions can be derived from our results: 1) CYP1A1 I462V confers less carcinogen-associated genotoxicity compared to that conferred by the expression of the other alleles in yeast, and 2) phenotypic differences in CYP1A1-mediated activation are carcinogen dependent. These conclusions may appear to contradict the notion that CYP1A1 I462V is a risk factor for cancers associated with xenobiotic exposures. However, we offer two different explanations: 1) higher levels of genotoxicity may be detected in cells expressing CYP1A1 I462V after exposure to chemicals not tested in this study, and 2) in particular human tissues, other CYPs, not coupled to phase II metabolism, may activate genotoxic compounds when CYP1A1 activity is low. Thus, we speculate that altered CYP1A1 metabolism may be a risk factor for environmentally-related cancer for multiple reasons.

CYP1A1 I462V, compared to CYP1A1 and CYP1A1 T461N, conferred lower levels of carcinogen-associated genotoxicity, as indicated by multiple endpoints. The most salient data were growth curves in the presence of BaP-DHD, AFB₁-associated recombination, and carcinogen-associated Rad51 foci. Although growth curves of cells expressing CYP1A1 I462V and CYP1A1 T461N in the presence of AFB₁ did not appear different, CYP1A1 also metabolizes AFB₁ to generate a hydroxylated form (AFM1, [48]) and it is unclear whether the AFB₁-associated metabolites that initiate recombination and those that reduce growth rates are the same. *In vitro* experiments are necessary to elucidate the enzymatic properties of CYP1A1 variants when AFB₁ is a substrate.

Differences in CYP1A1-mediated activation depend on the carcinogen or substrate. While there was no observable difference in AFB₁-associated genotoxic effects in cells expressing CYP1A1 T461N and CYP1A1 I462V, there was a significant difference between BaP-DHD-

associated genotoxic effects in cells expressing CYP1A1 T461N and CYP1A1 I462V. In addition, we observed equivalent levels of EROD activity from extracts derived from CYP1A1 I462V cells, compared to those expressing other CYP1A1 alleles. Higher levels of CYP1A1 I462V-associated EROD activity but lower levels of BaP-associated and BaP-DHD metabolism have been observed in protein extracts derived from ectopic expression of these CYP1A1 variants in bacteria [24, 49]. Considering that amino acid substitutions in exon 7 of CYP1A1 map near the active site, we speculate that the enzyme encoded by CYP1A1 I462V may differentially accommodate different substrates.

Previous studies have suggested that CYP1A1 may have a protective effect against some carcinogens [50]. Nebert *et al.* [50, 51] reported that CYP1A1^{-/-} mice exhibit higher toxicity to BaP; and suggested that CYP1B1 is responsible for metabolic activation of BaP into a potent carcinogen [51]. One explanation is that CYP1A1 and phase II enzymatic activities, such as GSTM, are closely coupled; thus, BaP is more rapidly hydroxylated and genotoxic intermediates detoxified and excreted, reducing the cancer risk for particular xenobiotic exposures [53, 54].

We suggest two reasons for why CYP1A1 I462V may be a risk factor for some cancers. First, due to its lower activity, xenobiotics are metabolized by other CYPs whose activities are not tightly coupled with phase II enzymes. Second, compared to wild type, CYP1A1 I462V more efficiently activates other compounds associated with cancer risk besides BaP. Supporting the first idea are observations that CYP1A1 I462V obtained from ectopic expression in bacteria is less active using BaP and BaP-DHD as substrates *in vitro*, compared to CYP1A1 [50, 51]. Our data supports these observations by showing that, compared to yeast expressing CYP1A1, CYP1A1 I462V confers less BaP-DHD-associated genotoxicity. While the *in vitro* characterization of the CYP1A2 I462V enzyme may confound the interpretation of observations that leukocytes from individuals bearing CYP1A2 I462V have higher levels of DNA adducts [49], it is possible that exposure to other xenobiotics, besides BaP, may be responsible for the increased risk in carrier patients. Thus, additional carcinogens should be tested to identify which compounds are most genotoxic in yeast cells expressing CYP1A1 I462V.

Our studies are an extension of previous work illustrating that AFB₁ is activated by CYP1A2 polymorphisms [34]. Here we have shown that both PAHs and heterocyclic aromatic amines (HAAs) can be activated by CYP1A1 alleles expressed in yeast, extending the phenotypic characterization of these alleles. The availability of multiple genotoxic endpoints in yeast allows for further investigation of CYP1A1-activated substrates and metabolites, with the goal of elucidating xenobiotics that trigger the highest levels of genotoxicity. These studies can be extended to include other CYP1A1 polymorphisms and phase II enzymes..

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights for Review

- Phenotypic characterization of carcinogen activation conferred by CYP1A1 alleles
- CYP1A1 I462V expression confers lower levels of carcinogen-associated genotoxicity compared to CYP1A1 T461N and CYP1A1.
- Expression of CYP1A1 1462V can be measured by EROD assays and is robust.

Figure 1.

Growth curves of the DNA repair *rad4 rad51* mutant expressing CYP1A1 polymorphisms after exposure to BaP-DHD and AFB₁. Approximately 10^5 cells were inoculated in each well in a 96-well plate platform; yeast growth in each well was monitored in a Tecan Plate reader and over a period of twenty-four hours. Absorbance (A_{600}) is plotted against time (hrs). The columns include the *rad4 rad51* strain (YB431) expressing CYP1A1 (Left), the *rad4 rad51* strain (YB432) expressing CYP1A1 I462V, and the *rad4 rad51* strain (YB433) expressing CYP1A1 T461N. The first row (panels A, B, C) is growth curves of cells from each strain after exposure DMSO or indicated doses of AFB₁. The second row (panels D, E, F) is growth curves of cells after exposure to DMSO or BaP-DHD. Error bars represent standard deviation (SD), where $N = 2$.

Figure 2.

Growth curves of the DNA repair *rad4 rad51* mutant expressing CYP1A1 polymorphisms after exposure to IQ and PhIP. Using the protocol described in Panel A, yeast growth in each well was monitored in a Tecan Plate reader over a period of twenty hours. Absorbance (A600) is plotted against time (hrs). The columns include the *rad4 rad51* strain (YB431) expressing CYP1A1 (Left), the *rad4 rad51* strain (YB432) expressing CYP1A1 I462V, and the *rad4 rad51* strain (YB433) expressing CYP1A1 T461N. The first row (panels A, B, C) curves of cells from each strain after exposure to DMSO or indicated doses of PhIP. The second row (panels D, E, F) is growth curves of cells after exposure to DMSO or IQ. Error bars represent standard deviation (SD), N = 2.

Figure 3. Frequencies of AFB₁-associated recombination in diploid cells expressing CYP1A1 (YB428), CYP1A1 I462V (YB429), CYP1A1 T461N (YB430). Figure in panel A is a model of the recombination assay. The oval represents a centromere and the line represents the chromosome; the left arm of the chromosome is not shown for simplicity. The *his3* fragment is shown with arrow and feathers. The shaded areas represent shared homology. An "X" denotes where a cross-over event would occur. The product of the recombination event is shown below where *CEN2* is linked to the long arm of chromosome IV and *CEN4* is linked to the long arm of chromosome II. Figure in panel B shows the recombination frequencies after cells were exposed to DMSO (solid column), 50 μM of AFB₁ (horizontally striped column) and 37.5 μM of BaP-DHD (diagonally-striped column). Figure in panel C shows the net recombination frequencies, which are obtained by subtracting the spontaneous recombination frequency from the carcinogen-associated frequency. The CYP1A1 allele is indicated on the X axis. Error bars represent standard deviation (SD), N = 3.

Figure 4. Ethoxyresorufin-O-deethylase (EROD) activity measured in strains expressing CYP1A1 (YB428), CYP1A1 I462V (YB429), and CYP1A1 T461N (YB430). The measurements (pmol resorufin/min/mg protein) were made using yeast microsomes. Error bars represent standard deviation (SD), N =3.

Figure 5.

Qualitative analysis of yfp-Rad51 foci in cells expressing CYP1A1 (YB434), CYP1A1 I462V (YB435), and CYP1A1 T461N (YB436). Rad51 foci were identified by the appearance of yellow fluorescent foci in the confocal microscope. Left panel shows A) non-irradiated cells containing pRS424-CYP1A1, B) Rad51 foci in AFB₁-treated cells containing CYP1A1, C) Rad51 foci irradiated cells containing CYP1A1, D) Rad51 foci in AFB₁-treated cells containing CYP1A1 T461N. The graph shows the percentage of CYP1A1-expressing cells containing Rad51 foci after either no exposure, exposure to AFB₁ or 10 Gy irradiation. The percentage of cells containing foci is indicated on the y axis and the CYP1A1-expressing strain is identified on the X axis.

Table 1

Yeast Strains

Strain (Synonym)	Genotype	Autonomous plasmid	Reference (Source)
YA101 (MCY7756)	<i>MATa</i> ura3-52 his3- 200 ade2-101 lys2-801		M. Carlson
BY4743	<i>MATa</i> /MATa ura3 - / leu2 - / his3 -1/- LYS2/lys2 0 met15 0 MET15		wiki.yeastgenome.org/index.php/ Commonly_used_strains
YB225	<i>MATa-inc</i> ura3 rad4::KanMX		This laboratory [42]
YB226	<i>MATa-inc</i> trp1 ura3 his3 ade2 rad4::KanMX rad51::URA3		This laboratory [42]
YB318	<i>MATa</i> ura3-52 his3- 200 ade2-n trp1- 1 gal3 leu2-3, 112 GAL1::his3- 5' trp1::his3-3::HOcs		This Laboratory [34]
YB400	<i>MATa-inc</i> trp1 ura3 his3 ade2 rad4::KanMX rad51		Derived from YB226 [42]
YB406	<i>MATa</i> ura3-52 trp1::hOR his3- 200 ade2-101 lys2-801		This Laboratory [34]
LSY1957	<i>MATa</i> YFP-rad51-1345T-URA3-RAD51 ADE2 leu2 trp1 ura3 his3		L.Symington
YB407	<i>MATa</i> ura3-52, trp1::hOR his3- 200,ade2-101 lys2-801		This Laboratory
YB419	<i>MATa</i> YFP-rad51-1345T-URA3-RAD51 ADE2 leu2 trp1::hOR ura3 his3		Meiotic segregant of diploid cross of LSY1957 and YB407
YB420	<i>MATa</i> YFP-rad51-1345T-URA3-RAD51 ADE2 leu2 trp1::hOR ura3 his3	pRS424	Trp ⁺ transformant of YB419
YB425	<i>MATa</i> ura3-52 ura3-52 his3- 200 ade2-n trp1- 1 gal3 leu2-3, 112 GAL1::his3- 5' trp1::his3- 3::HOcs rad4::kanMX		Derived from cross of YB318 and YB225
YB426	<i>MATa</i> ura3-52, trp1::hOR his3- 200,ade2-101 lys2-801 rad4::KanMX		Derived from cross of YB407 and YB225
YB427	<i>MATa</i> /MATa ura3-52/- his3- 200/- ade2-101/ade2-n trp1- 1 trp1::hOR leu2-3,112/ leu2-801 GAL1::his3- 5' trp1::his3- 3::HOcs rad4::kanMX/rad4 kanMX		Diploid cross of YB425 and YB426
YB428	<i>MATa</i> /MATa ura3-52/- his3- 200/- ade2-101/ade2-n trp1- 1 trp1::hOR leu2-3,112/ leu2-801 GAL1::his3- 5' trp1::his3- 3::HOcs rad4::kanMX/rad4 kanMX	pRS424 CYP1A1	Trp ⁺ transformant of YB427
YB429	<i>MATa</i> /MATa ura3-52/- his3- 200/- ade2-101/ade2-n trp1- 1 trp1::hOR leu2-3,112/ leu2-801 GAL1::his3- 5' trp1::his3- 3::HOcs rad4::kanMX/rad4 kanMX	pRS424 CYP1A1 I462V	Trp ⁺ transformant of YB427
YB430	<i>MATa</i> /MATa ura3-52/- his3- 200/- ade2-101/ade2-n trp1- 1 trp1::hOR leu2-3,112/ leu2-801 GAL1::his3- 5' trp1::his3- 3::HOcs rad4::kanMX/rad4 kanMX	pRS424 CYP1A1 T461N	Trp ⁺ transformant of YB427
YB431	<i>MATa-inc</i> ura3 rad4::KanMX rad51	PRS424 CYP1A1	Trp ⁺ transformant of YB400
YB432	<i>MATa-inc</i> ura3 rad4::KanMX rad51	pRS424 CYP1A1 I462V	Trp ⁺ transformant of YB400
YB433	<i>MATa-inc</i> ura3 rad4::KanMX rad51	pRS424 CYP1A1 T461	Trp ⁺ transformant of YB400
YB434	<i>MATa</i> YFP-rad51-1345T-URA3-RAD51 ADE2 leu2 trp1::hOR ura3 his3	pRS424 CYP1A1	Trp ⁺ transformant of YB400
YB435	<i>MATa</i> YFP-rad51-1345T-URA3-RAD51 ADE2 leu2 trp1::hOR ura3 his3	pRS424 CYP1A1 I462V	Trp ⁺ transformant of YB419
YB436	<i>MATa</i> YFP-rad51-1345T-URA3-RAD51 ADE2 leu2 trp1::hOR ura3 his3	pRS424 CYP1A1 T461N	Trp ⁺ transformant of YB419