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Perspective

Origins of Individual Variability in P4501A Induction

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Contents

1. Introduction

The P450 family of enzymes plays important roles in the metabolism of drugs, carcinogens, steroid hormones, and environmental/occupational chemicals (*1*−*5*). P4501A1 and P4501A2, two members of the 1A family, catalyze oxygenation of PAHs[1] and HAAs, as well as dealkylation of phenacetin and caffeine. The reactions serve as initial steps in the conversion of chemicals to more polar metabolites for excretion from the body and thus represent an adaptive response to changes in the chemical environment of cells. On the other hand, oxygenation of carcinogenic PAH and HAA (procarcinogens) can generate arene oxide, dioepoxide, and other electrophilic reactive species (ultimate carcinogens), leading to tumor formation or cell toxicity (*6*−*8*). The formation of more toxic or carcinogenic species as a result of metabolism is known as metabolic activation. Humans are exposed to carcinogens from a variety of sources, such as tobacco

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[1]Abbreviations: PAH, polycyclic aromatic hydrocarbon; HAA, heterocyclic aromatic amine/amide; P450, cytochrome P450; AHH, aromatic hydrocarbon hydroxylase; AhR, aryl hydrocarbon receptor; Arnt, Ah receptor nuclear translocator; AIP, AhR interacting protein; AhRR, Ah receptor repressor; ADPF, AhR degradation promoting factor; Hsp90, heat shock protein 90; p23, heat shock protein 23; Mybbp1a, Myb-binding protein 1a; CBP, CREB-binding protein; pRB, retinoblastoma protein; HIF1α, hypoxia inducible factor 1α; bHLH, basic helix loop helix; PAS, Per-Arnt-Sim; DRE, dioxin responsive element; PM, poor metabolizer; EM, extensive metabolizer; B(a)P, benzo[*a*]pyrene; 3-MC, 3-methylcholanthrene; BNF, β-naphthoflavone; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; DMBA, 7,12-dimethylbenz[*a*]anthracene; ITE, 2-(1′H-indole-3′-carbonyl)thiazole-4-carboxylic acid methylester; 7-KC, 7-ketocholesterol; APAP, *N*-acetyl-*p*-aminophenol; RFLP, restriction fragment length polymorphism.

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smoke, automobile exhaust, smoked and cooked food, and industrial processes. High levels of exposure to PAHs and HAAs contribute to increased incidence of cancer in certain populations such as smokers (*6*, *9*). Because of the unique role of P4501A enzymes in the metabolic activation of carcinogens, variations in P4501A activities in human tissues can, in principle, influence the susceptibility of individuals to chemical carcinogenesis.

A main feature of P4501A action in the metabolism of xenobiotics is their inducibility by a variety of chemicals (*10*, *11*). P4501A1 is expressed at low levels in extrahepatic tissues in humans but is highly inducible in liver and extrahepatic tissues. P4501A2 is constitutively expressed and is inducible in liver. However, induction of P4501A in human tissues exhibits large variations. Early studies by Conney and associates revealed that the activity of AHH, which measures 3-hydroxylation of B(a)P, was not detectable in human placentas obtained after birth from nonsmoking mothers but was found in placentas from smokers; the higher AHH activity in smoking mothers is attributed to induction of AHH by PAHs present in tobacco smoking (*12*). Moreover, AHH activities in placentas from mothers who smoke the same amount of cigarettes exhibit variations as large as 84-fold (*12*). In a separate study, it was found that neonatal human foreskin contains AHH activities that are inducible by PAH. The basal AHH activity showed a 3-fold variability among tested individuals, whereas induction of the enzyme by benz[*a*]anthracene in cultured skin cells from the individuals varied from 180 to 530% as compared with uninduced controls (*13*). Because P4501A1 is responsible for AHH activities in extrahepatic tissues, the observed variations reflect individual variability in the induction of P4501A1. These studies clearly demonstrated that a large variability exists in the induction of P4501A in human populations. Although the critical role of P4501A in the metabolic activation of PAH and HAA has been established in vitro, it remains controversial whether induction of P4501A in humans can be positively correlated to human cancer incidences. In some studies, a high inducibility of P4501A was linked to increased incidence of lung cancer in certain populations (*14*−*16*); in others, the extent of P4501A induction and cancer incidence could not be correlated (*17*, *18*). In view of the apparent importance of P4501A induction in human cancer risk debate, it is important to examine the major determinants responsible for human P4501A induction variability. Such analyses can reveal new aspects of the mechanism by which individual susceptibility to carcinogens and toxic and therapeutic chemicals is determined. To facilitate our discussion in this paper, we chose to use the term "P4501A" instead of P4501A1/2. Although the induction mechanisms for P4501A1 and P4501A2 may not be totally identical, induction of the two enzymes is mostly mediated by the Ah receptor. Major determinants responsible for individual variability of induction should apply to both enzymes. In addition to 1A1 and 1A2, P4501B1, a new member of the P4501 family, is active in the metabolism of certain PAHs, such as DMBA. P4501B1 is poorly expressed in liver, lung, and kidney but is constitutively expressed in extrahepatic mesodermal cells, such as steroidogenic and steroid responsive tissues; thus, 1B1 appears to contribute to carcinogenicity by DMBA in bone marrow and lymphoid tissues (*19*, *20*). Because of its limited spectrum of tissue expression, weak induction by AhR agonists, and a dearth of information on expression and induction in humans, individual variability of P4501B1 induction is not discussed in this paper.

2. Major Determinants of Individual Variability of Induction

The recognition of P4501A induction was first provided by Conney and Millers, when they discovered that PAHs induce their own metabolism (*21*). Studies on the induction of P4501A and other drug-metabolizing enzymes over several decades led to the development of a number of concepts that have broadly influenced the fields of drug metabolism, cancer research, receptor biology, and chemical toxicity. A substrate/inducer relationship was revealed as a common theme in the induction of drug-metabolizing enzymes. Substrate-induced synthesis of P450 enzymes is usually advantageous in that it enhances the metabolism of the chemical, leading to increased detoxification and elimination of the chemical from the body. Moreover, induction of a P450 enzyme by substrates is often limited in duration as the inducers are metabolized by the enzyme, thereby allowing cells to increase and maintain high levels of enzyme activity only as needed. However, in the case of PAH metabolism, induction of AHH can be disadvantageous because it increases the formation of ultimate carcinogens via metabolic activation of PAHs. In addition, P450 enzymes often have a broad spectrum of substrate specificity; induction of the enzymes by one substrate may result in enhanced metabolism of other chemicals, altering the pharmacokinetic properties and therapeutic/toxic effects of other chemicals (*22*).

During the past several decades, a combination of genetic, pharmacological, and molecular biochemical approaches have been utilized to elucidate the molecular mechanism of P4501A induction (*23*). These studies have revealed a ligand-activated, receptor-mediated transcription process known as the "AhR/DRE paradigm" (see Figure 1) (*11*). An inducer (TCDD, for instance) diffuses into cells and binds to the Ah receptor, a member of the bHLH−PAS family of transcription factors, present in the cytoplasm in a complex with hsp90 and an immunophilin chaperone protein AIP. Activated AhR dissociates from the complex, translocates into the nucleus, and dimerizes with another bHLH−PAS factor Arnt. The dimer of AhR/Arnt binds to DNA response elements (termed DRE) located in the enhancer region of the P4501A genes, leading to transcription of the genes. The mRNAs are translated into P4501A enzymes, which localize in the smooth endoplasmic reticulum for xenobiotic metabolism.

It is conceivable from this scheme of P4501A induction that variations in several steps can potentially influence the induction. Indeed, research over the years has provided evidence that underlies the importance of a number of factors in individual variability of P4501A induction. Major determinants that affect individual variability of P4501A induction in humans are summarized in Table 1. In addition, mechanistic studies of P4501A induction have revealed that several protein factors interact with AhR and modulate AhR function (summarized in Table 2). These factors regulate AhR activity at different steps of AhR signal transduction such as receptor activation, nuclear targeting, dimerization with Arnt, protein turnover, and transcription activation;

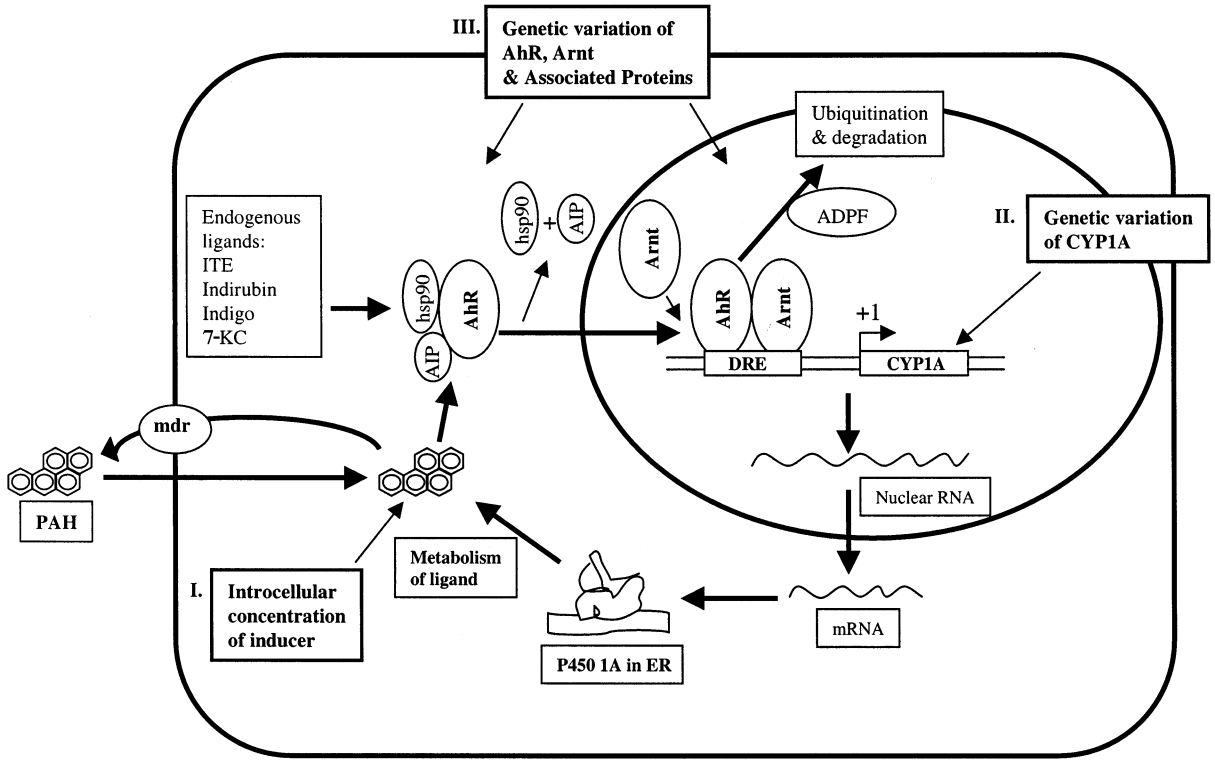

Figure 1. Scheme of P4501A1 induction. The cytoplasmic AhR is in complex with hsp90 and AIP. Agonist binding activates AhR, which dissociates from the cytoplasmic complex and translocates into the nucleus. Nuclear AhR dimerizes with Arnt and binds to DRE to mediate transcription of P4501A. P4501A is synthesized and localized in endoplasmic reticulum where it metabolizes inducers. Nuclear AhR is degraded through the ubiquitin-26S proteasome-mediated proteolysis, which is regulated by ADPF. Three major genetic determinants of variability of the induction are indicated (i.e., intracellular concentration of inducers, genetic variation of P4501A, and genetic variation of AhR and associated proteins). Examples of endogenous agonists and antagonists are listed. Physiological/pathophysiological factors and environmental variables modulate various steps of the induction and contribute to variability of P4501A induction.

Table 1. Individual Variability in P4501A Induction: Major Determinants

- •Variability in intracellular concentration of inducers
  - −modulation by P-glycoprotein and other transporters
  - −metabolism of inducers by P450 and other enzymes
- •Genetic variations of P4501A
  - −variable induction due to alteration in the regulatory region of P4501A genes
  - −variable induction due to different P4501A1/2 phenotypes
- •Genetic variations of AhR and Arnt
  - −variable expression
    - ◆mutation in the regulatory region of the gene
    - ◆altered function of regulatory proteins
  - −structural polymorphism with altered function
- •Physiological factors interfering with induction process
  - −endogenous agonist and antagonist of AhR
  - −disease states: inflammation, infection, and liver diseases
  - −gender
  - −hormonal homeostasis
- •Environmental factors
  - −dietary components
  - −environmental contaminants

examples of the factors include hsp90 (*24*), AIP (also termed Ara9 and XAP2) (*25*−*27*), p23 (*28*), AhRR (*29*), ADPF (*30*), Mybbp1a (*31*), and CBP/p300 (*32*). Alternatively, AhR cross-interacts with a number of signaling pathways of gene regulation, such as HIF1α (*33*), pRB (*34*), and NF-κB (*35*). Variations in the activity or expression of these AhR-associated proteins can, in principle, influence the induction of P4501A and contribute to individual variations in humans under certain circumstances. In the following sections, we will analyze the evidence supporting the involvement of these determinants in P4501A induction variability with focus on evidence obtained from human subjects. Animal data will be used only when human studies are not available. In addition, the mechanism by which these gene−chemical interactions influence the induction variability and directions for future research will be discussed.

3. Individual Variability in Intracellular Concentration of Inducer

Induction of P4501A by chemicals is dose-dependent. Therefore, variability in the intracellular concentration of an inducer among individuals would result in different extents of induction. Intracellular concentration of inducers is modulated by the action of transporters, such as P-glycoprotein, and through the metabolism by P450 and other drug-metabolizing enzymes. Individual differences

Table 2. Protein Factors Affecting AhR Signal Transduction

factor	effect on AhR function	ref
cytoplasmic protein	receptivity, protein stability, cytoplasmic retention, and nuclear targeting	
Hsp90	cytoplasmic AhR complex	*24*
AIP	cytoplasmic AhR complex	*25*−*27*
p23	cytoplamsic AhR complex	*28*
cytoskeleton protein	cytoplasmic retention of AhR	*91*
nuclear protein	DNA binding, transactivation, and nuclear receptor turnover	
Arnt	form DNA-binding AhR/Arnt dimer	*67*
AhRR	form non-DNA binding AhRR/Arnt dimer; negative regulation of AhR	*29*
Mybbp1a	transcription activation by AhR acidic transactivation domain	*31*
CBP/p300	transcription activation by Arnt transactivation domain	*32*
ADPF	control nuclear AhR turnover; negative regulation of AhR	*30*
cross-signaling	cross-interact with other pathways of gene regulation	
HIF1a	form HIF1a/Arnt dimer to hypoxia; compete with AhR for Arnt	*33*
pRB	tumor suppressor/cell cycle regulation; enhance AhR activity	*34*
NF-κB	control cytokine expression and apoptosis; repress AhR activity	*35*

in the activities of P-glycoprotein (*36*) and drug-metabolizing enzymes (*37*) are well-established, both qualitatively and quantitatively.

P-glycoprotein is an ATP-dependent drug efflux transporter encoded by the multidrug resistance gene mdr. Two forms of mdr genes are found in humans: mdr1 is mostly responsible for multidrug resistance, while the function of mdr3 remains to be established. Substrates of P-glycoprotein include chemotherapeutic agents (*38*) and environmental carcinogens such as B(a)P (*39*) and 3-MC (*40*), which are AhR ligands. P-glycoprotein is expressed in tissues that are active in drug metabolism such as liver and intestine; this coexistence of P-glycoprotein and drug-metabolizing enzymes in cells provides an effective cellular defense against foreign chemicals. While overexpression of P-glycoprotein in tumor cells is associated with multidrug resistance to anticancer drugs, large variations of P-glycoprotein expression were found in normal human tissues (*41*). For instance, P-glycoprotein in human liver preparations was found to vary ~5-fold in male and ~10-fold in female; the expression of P-glycoprotein is 2-fold higher in male than in female (*36*). In addition, P-glycoprotein is inducible via a number of inducer−receptor systems including the Ah receptor (*36*). Induction of P-glycoprotein by B(a)P or TCDD is mediated through p53 and requires AhR/Arnt (*42*). Moreover, the induction is only observed in about half of the human liver preparations (*36*). These studies established that individual variations in mdr expression and its induction by AhR ligands exist in human populations and thus represent a determinant of the variation in P4501A inducibility phenotype. The avalaibility of mdr knock-out mice may be exploited to quantify contributions of mdr to P4501A induction variability in mice in future studies.

P4501A can be induced by substrates of P450 enzymes other than P4501A. Omeprazole, an acid pump inhibitor, suppresses gastric acid secretion by inhibiting H+, K+-ATPase in gastric parietal cells. A major pathway of omeprazole metabolism is through P4502C19 to 5-hydroxy-omeprazole; metabolism to 5-O-desmethyl-, 3-hydroxy-, and sulfone metabolites by P4502C19 and 3A constitutes the minor pathways of omeprazole metabolism (*43*). The 5-hydroxylation of omeprazole in human liver microsomal preparations and the in vivo clearance of omeprazole exhibit large individual variations (*44*, *45*), which correlate with polymorphic phenotypes of S-mephenytoin hydroxylase (catalyzed by P4502C19). Thus, PMs of mephenytoin metabolize omeprazole slowly as compared with EMs in humans. Because omeprazole is an inducer of P4501A, the metabolic rate of omeprazole influences the induction of the enzymes. For instance, induction of P4501A2 (measured by caffeine *N*-3-demethylation-dependent breath test) was observed in individuals with a P4502C19 PM phenotype but not with the EM phenotype at a therapeutic dose of omeprazole (40 mg) (*46*). However, induction in EMs can be demonstrated at a higher dose (120 mg) (*47*). Thus, individual variations in the metabolic rate of omeprazole clearly affect the intracellular concentration of the inducer, contributing to variability of P4501A induction. In addition, omeprazole is both a substrate and an inhibitor of P-glycoprotein (*48*). Thus, induction of P4501A by omeprizole represents an example in which the concentration of an inducer is affected by both its metabolism through P450 reactions and efflux transport via P-glycoprotein.

4. Genetic Polymorphism of P4501A

The regulatory regions of P4501A genes contain DREs and several other binding sites, to which the AhR/Arnt heterodimer and other regulatory proteins bind to mediate gene induction. Several copies of DREs are found in the enhancer region upstream of P4501A1 promoter and mediate the induction by AhR ligands (*23*). In addition, negative response elements were identified in the 5′ regulatory region of P4501A1 and were implicated in the regulation of human P4501A1 induction (*49*, *50*). Expression and induction of P4501A2 appear to be regulated by multiple elements. A 3-MC responsive region in human P4501A2 gene was identified between nucleotides −2532 to −2423 (*51*). Several other positive and negative cis elements were reported to exist from −2352 to −2094 upstream of human P4501A2 gene (*52*). Mutations in these regulatory sequences can affect the expression and/or induction of P4501A genes. A polymorphism was identified at position −2964 in the 5′-flanking region of human P4501A2 gene by using *Dde*I or *Bsl*I restriction digestion (*53*). The polymorphism in a Japanese population showed 0.77 and 0.23 for wild-type and variant allele frequencies, respectively. The point mutation caused a significant decrease in P4501A2 activities (measured as caffeine 3-demethylase by breath test) in smokers ($p < 0.05$) but not in nonsmokers; the difference in the activities between wild-type and variant alleles is similar to that between smokers and nonsmokers in the tested

population. These results demonstrate that the mutation at −2964 does not affect the constitutive expression of the gene but primarily influences the in vivo induction of P4501A2 by smoking. Reduced induction of P4501A2 in the variant allele was attributed to differential binding of a protein factor(s) to the wild-type and variant allele sequences.

Variation in the activity of a P450 enzyme due to a genetic polymorphism can affect the induction of the enzyme itself. However, the influence of P450 phenotypes on the induction varies depending on the inducer and the phenotype. Many inducers of P4501A are substrates of the enzymes (with the exception of TCDD and a few related chemicals, which are highly resistant to metabolic breakdown due to symmetric distribution of chlorine atoms on aromatic rings of the molecules). P4501A phenotypes with a low enzyme activity metabolize inducers slowly, resulting in the accumulation of the inducers in cells and consequently increased induction of P4501A mRNA. For instance, Hankinson and associates used a single step selection procedure to select variant cells that are resistant to cytotoxicity of B(a)P due to the lack of AHH. The complementation group A variants correspond to a defective P4501A1 gene and were found to express higher levels of P4501A1 mRNA than wild-type cells in the absence of exogenous inducers (*54*). A simple explanation of the observation is that an endogenous agonist(s) of AhR, which is normally metabolized by P4501A1 in wild-type cells, is accumulated in P4501A1 deficient variant cells, leading to an increased expression of P4501A1 mRNA. In this scenario, induction of P4501A mRNA/protein is not accompanied by an increase in AHH activity, since the P4501A phenotype is associated with "little AHH activity".

Sachse et al. found that a single nucleotide polymorphism in intron 1 of the P4501A2 gene at position 734 (C→A) downstream of the first transcribed nucleotide was associated with high inducibility of P4501A2 (measured by caffeine demethylation) in Caucasian smokers but not in nonsmokers (*55*). The A/A genotype may be either a direct cause of increased P4501A2 activity or genetically linked to a polymorphism conferring high inducibility. Further studies are needed to distinguish these possibilities.

5. Genetic Variations of Ah Receptor and Other Regulatory Proteins

5.1. A Historical Background. Genetic studies using inbred mouse strains that exhibit a genetic polymorphism for AHH induction provided the first indirect evidence for a receptor in the induction of P4501A (*56*, *57*). Some mouse strains, such as DBA/2, are resistant to induction of AHH by 3-MC (i.e., the "nonresponsive" phenotype); other strains, such as C57BL/6, are about 10 times more sensitive (*58*−*60*). The sensitive phenotype segregates as an autosomal dominant trait and thus defines a genetic locus (designated as Ah locus) for the induction. Subsequent studies by comparing ligand binding affinities of liver preparations from the inbred strains revealed a cytoplasmic receptor for AHH inducers (*61*). Moreover, these studies suggested that a mutation(s) in the receptor reduces the binding affinity for inducers, resulting in the nonresponsive phenotype in DBA mice (*58*). Cell genetic studies took the advantage of metabolic activation of B(a)P in cells expressing high, inducible AHH activity that leads to cell death in the presence of B(a)P. Variant cells defective in AHH induction are resistant to B(a)P toxicity and therefore were selected (*62*, *63*). At least two complementation groups of variants were isolated. The class I variants (B group) exhibit diminished binding to inducers and are defective in AhR. The class II variants (C group) define a second protein in the induction; the variant cells exhibit abnormal nuclear localization of ligand-activated AhR; hence, the protein was designated as Arnt.

Cloning of AhR was facilitated by biochemical purification of photoaffinity labeled AhR from mouse liver cytosolic preparations (*64*). Greater than 150 000-fold purification of AhR was achieved using this procedure. A peptide fragment at the amino terminus of the receptor was obtained; the sequence of the peptide was used to raise antibodies against AhR, which made it possible to clone the mouse AhR cDNA. The cDNA sequence of AhR contains a structural organization that is now recognized as representative of bHLH−PAS transcription factors (*65*, *66*). The bHLH domain is located near the amino terminus and is involved in DNA binding, heterodimerization with Arnt, and nuclear cytoplasmic transport. Adjacent to bHLH is an imperfect inverted repeat of about 300 amino acid residues, known as the PAS region because of homology of the region in Per, Arnt, and Sim (*67*). The PAS region in AhR contributes to dimerization with Arnt, ligand binding, and interaction with hsp90. The C-terminal region consists of modular transcription activation domains, which are regulated by an inhibitory domain located between the PAS region and the TA domains (*11*).

The human AhR cDNA has the same modular structures as its murine homologue (*68*). However, human AhR is structurally more similar to AhR from DBA mice (nonresponsive) than from C57BL/6 mice ("sensitive"). Both human and DBA AhRs have elongated carboxyl-terminal sequences (848 amino acid residues), which result from a mutation at the stop codon of C57 AhR (805 residues). The elongated C terminus and an alanine to valine mutation in the ligand binding domain (i.e., Val-381 in human AhR and Val-375 in DBA AhR) appear to be responsible for diminished binding affinity of human and DBA AhRs for AhR ligands. The dissociation constants (K_d) for TCDD are 0.27 nM for C57 AhR, 1.66 nM for DBA AhR, and 1.58 nM for human AhR, respectively (*68*). These structure−function analyses not only revealed new insights into the structure and mechanism of action of AhR but also provided a blueprint for analyzing genetic polymorphisms and phenotypes of AhR in humans (*69*).

5.2. Variable Induction Due to AhR Polymorphism. Because AhR is the major protein factor mediating the induction of P4501A and defective AhR functions are responsible for nonresponsiveness in DBA mice and class I variant cells, the role of AhR polymorphism in individual variability of P4501A induction in human populations has long been suggested. Evidence supporting this notion, however, is just beginning to unveil. Polymorphisms of human AhR can be manifested as variable receptor expression or structural mutations.

Ah receptor is expressed in most tissues and during development. However, expression of AhR in humans exhibits individual variation. By using quantitative RT-PCR, Hayashi et al. (*70*) observed a 3.3-fold variation in AhR mRNA expression in peripheral blood cells among 20 healthy Japanese individuals. A 6.3-fold variation was

found for Arnt mRNA in the same population. Furthermore, expression of P4501A1 in the same samples was variable and correlated with the expression of AhR and Arnt. In addition, an association between cigarette smoking and expression of AhR and Arnt was observed. These results demonstrated that variable expression of AhR in a human population can influence the inducibility of P4501A genes and suggested induction of AhR and Arnt by smoking as a mechanism of variable expression of the proteins.

The class I variant cells derived from mouse hepatoma cells exhibit reduced expression of AhR mRNA. Mechanistic analyses of the variants suggest that the clones are defective in a regulatory protein(s) required for AhR expression, because a fusion of the mutant clone with a rat hepatoma cell line restores expression of the mouse AhR gene (*71*). By analogy with this finding, it can be postulated that a polymorphism in the regulatory mechanisms governing AhR expression (for instance, a mutation in the 5′-flanking region of human AhR gene or a regulatory protein of AhR transcription) causes variable expression of AhR and contributes to variable inducibility of human P4501A.

A number of structural polymorphisms of human AhR have been described. Analyses of AhR functions in human placenta samples revealed more than 20-fold differences in AhR affinity for ligand binding between the "high" and the "low" P4501A1 inducibility phenotypes (*72*). The correlation between P4501A1 inducibility and AhR affinity phenotype is analogous to that observed in sensitive and nonresponsive mouse strains, suggesting that a polymorphism(s) affecting ligand binding is responsible for the phenotype. In a separate study (*73*), a ~6.6-fold variation in P4501A1 induction was observed in 13 families; genetic linkage analyses in a three generation family revealed a segregation of high P4501A1 inducibility with the 7p15 chromosomal region where human AhR is located. The P4501A1 inducibility appears to exhibit bimodality with a wide range of variation, suggesting a polyallelic mode of inheritance for the P4501A1 inducibility/AhR affinity phenotype, in which one or two amino acids play a major role and several additional amino acids contribute to the trait. The study also revealed that although an A381V mutation in human AhR, which corresponds to A375V mutation in DBA AhR, is shown to reduce ligand binding in in vitro experiments, the polymorphism is seen in both high and low inducibility individuals, implying that the A381V polymorphism itself is not sufficient to confer nonresponsiveness and cause the low inducibility in humans. However, this conclusion does not exclude the possibility that a second polymorphism of AhR in the individuals examined compensates the A381V mutation, allowing high inducibility in A381V polymorphic individuals.

By using single strand conformation polymorphism (SSCP), Kawajiri et al. detected an Arg to Lys mutation at codon 554 of AhR (R554K for peptide sequence or G1721A for nucleotide sequence) in Japanese populations with a frequency of 0.43 (*74*). The R554K polymorphism is also present with a high frequency (0.41) in African-American populations (*75*) and with low frequencies in Caucasian populations (0.09 and 0.11) (*17*, *75*). The R554K polymorphism did not show a significant association with AHH inducibility in the French and Japanese studies (*17*, *74*) but exhibited a significantly higher level of induced P4501A1 activity in individuals with at least one copy of the mutant allele as compared with individuals negative for the polymorphism in the study by Smart and Daly (*75*). Differences in the methods used to measure 1A1 inducibility and the gender populations included in the studies may contribute to the discrepancies between the studies. A recent study by Harper and associates examined AhR polymorphisms in several populations of different ethnic backgrounds by using RFLP (*76*, *77*). The study identified three variants of AhR (R554K, V570I, and P571S). V570I was present in Africans at frequencies of 0.03−0.07 similarly to a previous report of an allele frequency of 0.05 in African-Americans, while P571S was found in Africans at frequencies of 0.035−0.05. Furthermore, the results revealed apparent linkage disequilibrium of V570I and P571S with R554K. The combined polymorphism of R554K and V570I exhibited normal ligand and DNA binding capacities but failed to support induction of P4501A1 by potent AhR ligand TCDD. These observations, together with the notion that the mutations are located in the acidic activation domain of AhR (*78*), suggest that the combined mutations at 554 and 570 residues inhibit the transcription activation activity of AhR, thereby blocking the induction of P4501A1. In addition, an allelic variant was found in the 5′-untranslated region at position 157 (G157A) with a frequency of 0.25; no significant relationship was found between this allelic variant and P4501A1 inducibility (*17*). A rare allele was reported in a French population at codon 786 (M786V, A2417G) with a frequency of 0.005. The allele appears to coexist with high P4501A1 inducibility. However, a study with a larger population is needed to establish the correlation, due to the rareness of the variant allele (*17*).

Several recent studies using AhR null mice generated by targeted gene knockout provided additional genetic evidence supporting a central role of AhR in the induction of P4501A and carcinogenicity by PAH. The findings reveal that AhR is required for constitutive expression of P4501A2 in liver (*79*), induction of mouse P4501A1 and P4501A2 by AhR agonists (*79*, *80*), malignancy in skin induced by BaP (*81*), teratogenicity, and other toxic effects of AhR ligands in mice (*82*, *83*). Moreover, a mouse strain that expresses a human form of AhR in mouse AhR null genetic background was generated by using the "knock-out/knock-in" approach.[2] This "humanized" mouse model of AhR is potentially useful for analyzing similarities and differences in the regulation of P4501A expression and pharmacokinetics of P4501A substrates by murine and human AhR in whole animals with similar genetic backgrounds. Expression of polymorphic forms of human AhR in mice using the knock-in approach can facilitate establishing causal relationships between human AhR polymorphisms and phenotypes, as well as dissecting mechanisms by which AhR polymorphisms affect P4501A induction in future studies.

5.3. Variable Induction Due to Variations in Associated Proteins. The class II variant cells are incapable of P4501A induction by AhR ligands, due to the lack of functional Arnt. The mutant Arnt cDNA contains a single mutation, leading to replacement of

[2]Moriguchi, T., Motohashi, H., Aoki, Y., Ohasako, S., Nakajima, O., Fujii-Kuriyama, Y., Toyama, C., and Yamamoto, M. *Decreased Sensitivity to Xenobiotics in a Humanized Mouse Model*, Presented at the 14th International Symposium on Microsomes and Drug Oxidations (MDO2002), Sapporo, Japan, July 22−26, 2002.

Gly326 with Asp in the conserved PAS regions (*84*). The mutation does not affect the capacities of Arnt for binding with AhR and nuclear localization but reduces the DNA binding affinity of AhR/Arnt dimer and accelerates the turn over of Arnt. Several polymorphisms of human Arnt due to point mutations exist in the literature; the functional impact of the polymorphisms on human P4501A induction, if any, has not been established (*85*−*87*). While total knock-out of Arnt in mice is embryonic lethal (*88*), conditional disruption of the gene provided evidence supporting a critical role of Arnt in AhR-mediated induction of P4501A1, P4501A2, and UGT*06 in liver (*89*). However, the study also revealed that Arnt is not limiting to AhR signaling in certain tissues, since maximum induction of P4501A1 was observed in lung despite more than 80% loss of Arnt in the tissue. Arnt2, which shares high similarity in sequence to Arnt, is expressed in mouse brain and kidney and capable of forming functional dimer with AhR similarly to Arnt (*90*). Targeted mutation of mouse Arnt2 reveals partial functional redundancy of Arnt2 with Arnt. Whether Arnt2 is expressed in human tissues and contributes to P4501A induction in humans remains to be determined.

A number of protein factors interact with and control the activity of AhR (Table 2). These factors include hsp90 (*24*), AIP (*25*), p23 (*28*), and certain cytoskeleton proteins (*91*), which regulate cytoplasmic AhR; AhRR, which represses nuclear AhR by forming non-DNA binding AhRR/Arnt dimer (*29*); ADPF that promotes agonist-induced degradation of nuclear AhR through the ubiquitin-26S proteasome pathway (*30*); and Mybbp1a (*31*) and CBP/p300 (*32*), which function as coactivators of AhR and Arnt, respectively (*32*). In addition, AhR cross-interacts with several signaling pathways of gene regulation including HIF1α (*33*), NF-κB (*35*), and tumor suppressor pRB (*34*) (Table 2). In principle, variations in the expression or structural alteration of these regulatory or interacting proteins can influence the induction of P4501A by AhR and thus represent potential target molecules for analyzing human polymorphism of P4501A inducibility phenotypes in future.

6. Variable Induction Due to Physiological/ Pathophysiological Factors Interfering with the Induction Process

6.1. Endogenous Ligands of AhR. Analyses of AhR null phenotype in AhR knock-out mice reveal that AhR is required for constitutive expression of P4501A2 in liver (*79*), development of liver and lymphoid organs (*79*, *80*), and formation of vasculature in sinusoidal tissues (*92*). AhR is also linked to cell growth and differentiation in rat and mouse hepatoma cells (*93*, *94*). In addition, suspension of hepa1c1c7 cells for 4 h leads to a rapid induction of P4501A1 in an AhR/Arnt-dependent manner (*91*). Because these observations were made in the absence of known exogenous ligands of AhR, the findings suggest that AhR is activated by a mechanism independent of a ligand(s) under these circumstances; alternatively, an endogenous agonist(s) of AhR exists in cells and mediates AhR functions under physiological conditions. Indeed, several recent studies have uncovered a number of endogenous chemicals with high affinities for AhR from animal tissues. By using an HPLC chromatography-based approach, Song et al. isolated 20 μg of an AhR ligand from 35 kg of porcine lung tissue (*95*). The ligand was identified as ITE; ITE was found to bind AhR directly and induce a DRE-driven reporter expression with a potency five times greater than BNF. By using a yeast AhR signaling assay (reporter assay), Adachi et al. identified and isolated two AhR ligands, indirubin and indigo, from human urine collected from healthy individuals (*96*). Both chemicals are present at average concentrations of ∼0.2 nM in urine samples of normal donors. The activities of indirubin and indigo are comparable with or more than TCDD in yeast reporter assay. In search of endogenous modulators against AhR-mediated toxicity of TCDD, Savoret and associates identified 7-KC as an endogenous antagonist of AhR, which displaces TCDD for AhR binding and blocks TCDD-induced P4501A1 and AhR reporter gene expression (*97*). Because differential sensitivities of mammalian species to toxicity of AhR ligands are closely correlated with species specific expression of 7-hydroxycholesterol dehydrogenase, which synthesizes 7-KC, the authors suggest that endogenous 7-KC serves as a protective modulator for AhR-mediated chemical toxicity. Taken together, these findings imply that endogenous agonists and antagonists of AhR modulate AhR functions in tissues; variations in the concentrations of the endogenous AhR ligands can potentially contribute to individual variability of P4501A induction by AhR ligands.

6.2. Disease States. Induction of P4501A can be profoundly altered in disease states, causing potential impairment of drug/chemical clearance and therapeutic/toxic effects. However, care should be taken in the interpretation of the in vivo effects of various diseases on drug metabolism, since these effects are often compounded by factors such as use of drugs, changes in hepatic blood flow, coexistence of other diseases, and nutritional status, as compared with healthy individuals.

Cumulated data indicate that infections and inflammatory diseases affect the expression and induction of P4501A proteins (*98*). For instance, the metabolic clearance of theophylline, a substrate of 1A2, is impaired by infection with influenza or adenovirus (*99*, *100*) and influenza or BCG vaccination (*101*, *102*), by IFNα or IFNβ therapy (*103*, *104*), and by low doses of bacterial endotoxin (*105*). Analyses of liver biopsies in patients undergoing treatment with IFNα or IFNβ revealed decreases in microsomal 7-methoxycoumarin O-demethylase and 7-ethoxycoumarin O-deethylase activities, providing direct evidence for suppression of 1A2 by interferons in humans (*104*, *106*). Inflammatory cytokines are mediators of many disease processes and are produced by inflammatory cells in a tissue and disease stage-dependent manner. The effects and mechanism of action of cytokines on P4501A induction have been extensively studied in human primary hepatocyte cultures. For example, induction of P4501A by BNF was shown to be inhibited by IL6 and TNFα (*107*), whereas induction of the P450 by 3-MC was repressed by TGFβ (*108*). Oncostatin M, IL-6, IFNγ, TNFα, and TGFβ inhibit the expression of P4501A2 (*98*). Inhibition of P450 by inflammatory cytokines is often accompanied by changes in mRNA expression of P450 enzymes; however, both transcriptional and posttranscriptional mechanisms have been suggested for the effects. Liver diseases such as hepatitis with severe liver failure or cirrhosis impair P450 activities by 20−80%, with P4501A2 being the most sensitive enzyme (*109*). On the other hand, clearance of theophylline or caffeine can be increased in diabetic

patients, suggesting that P4501A2 may be induced in diabetic conditions under certain circumstances in humans (*98*).

6.3. Gender. Gender-related differences can affect the expression and induction of P4501A. For instance, a study of 30 Caucasian volunteers revealed that the induced EROD activity (P4501A1) of freshly isolated lymphocytes was significantly lower in female individuals than in male (4.50 vs 9.01 pmol/min/mg protein, p value = 0.006); the difference between female and male is also seen in the level of induced 1A1 protein (*75*). Several studies on P4501A2 phenotypes using caffeine have also found evidence for lower enzyme activities in women (*110*, *111*). Differences in endocrine homeostasis and factors that influence pharmacokinetics of inducers such as P-glycoprotein (*36*) between male and female may contribute to the differential induction and activities of P4501A between the genders.

6.4. Hormonal Homeostasis. The effect of endocrine changes on the induction of drug-metabolizing enzymes is mostly manifested through sex, age, and disease processes. Direct evidence for hormonal influence on drug metabolism largely comes from experiments on animal subjects and cultured cells. By using glucocorticoid deficient animal models and rat hepatocytes, it was shown that glucocorticoid hormones modulate the induction of P4501A and other AhR-regulated genes such as glutathione *S*-transferase Ya1 through a glucocorticoid receptor-dependent process (*112*). The effect of the hormones on AhR gene expression can be biphasic: at physiological concentrations, the hormones suppress the gene induction/expression, whereas at supraphysiological or therapeutic concentrations, they potentiate PAH induction of AhR-regulated genes (*112*).

7. Variable Induction Due to Environmental Factors

Dietary components, either as natural ingredients, food additives, or produced during food processing, can directly or indirectly affect AhR-mediated P4501A induction (*113*). Individuals with different diets exhibit marked variations in the induction and activities of P4501A enzymes. For instance, feeding a diet that contained charcoal-broiled beef to nine normal individuals for 4 days prior to the administration of phenacetin (metabolized by 1A2) markedly decreased the plasma levels of the drug without appreciably changing the plasma concentration of the metabolite, APAP, or the plasma half-life of phenacetin, in comparison with feeding a control diet (*114*). Furthermore, the average peak concentration of phenacetin rose after the subjects were subsequently fed the control diet for 7 days. Cigarette smoking showed a similar effect on phenacetin metabolism in humans (*115*). These findings suggest that a diet containing charcoal-broiled beef or cigarette smoking enhances the metabolism of phenacetin in the gastrointestinal tract and/or during its first pass through the liver by inducing P4501A2.

The inhibitory property of many dietary chemicals on P4501A induction was exploited for the development of chemopreventive agents against cancer. Resveratrol is present in mulberries, peanuts, grapes, red wines, and other edible plant products. Resveratrol exhibits potent anticancer effects against aromatic hydrocarbon-induced malignancy in animal models. Mechanistic studies revealed that it inhibits TCDD-induced increases of P4501A1 mRNA and enzyme activity. Resveratrol inhibits the binding of activated AhR to promoter sequences that regulate P4501A1 transcription (*116*). Other studies suggest that resveratrol is a competitive antagonist of TCDD and other AhR ligands (*117*).

Dietary flavones and flavonols exhibit both antagonistic and agonistic activities for the induction of P4501A in animals and in vitro. The IC_{50} values of the antagonistic action are in the range of 0.14−10 μM, whereas higher concentrations are required to induce P4501A (agonism) (*118*). The order of potency of antagonism is flavones = favonols > flavanones > catechins. Structure−activity analyses of the flavonoid binding to AhR revealed that the flavonoids bind to the ligand binding pocket of AhR, which overlaps with the binding region for full agonists such as TCDD. In addition, a similarity between flavonoid binding to AhR and to P4501A1 was observed, suggesting a structural overlap between the binding pockets of the receptor and the enzyme. From these studies, it is clear that dietary components affect the induction of P4501A. However, evidence for such effects in humans is currently lacking.

Assessing the effect of man-made environmental chemicals such as pesticides, air pollutants, and occupational chemicals on drug metabolism can be complex, due, in part, to the fact that environmental chemicals are often widespread and exist in mixtures. Moreover, human exposure to environmental chemicals is often for long terms, at low dosages, and through multiple routes of exposure. Consequently, epidemiological data of human exposure to the environmental contaminants are difficult to obtain. Many AhR ligands are environmental pollutants including halogenated aromatic hydrocarbons (i.e., polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenols) and PAHs (B[a]P and 3-MC). These environmental AhR ligands are often partial agonists (*119*, *120*). These partial agonists can stimulate the induction of P4501A through their agonistic action on AhR. However, when present at high concentrations, partial agonists can inhibit the induction of P4501A by a full agonist at a low concentration. In addition, certain AhR ligands, including some polybrominated biphenyls, are strong inhibitors of 1A2 enzyme; in this scenario, induction of P4501A proteins and activities may not always match.

TCDD and certain PCB type AhR ligands cause various toxicities in humans ranging from chloracne to neurocoganitive symptoms (*121*, *122*). These toxic phenotypes exhibit large individual variations. Because the toxicities are mostly mediated through AhR, it will be intriguing to examine whether variations in P4501A induction by AhR ligands can be correlated with the variations of the toxic phenotypes in human populations exposed to environmental AhR ligands in future studies.

8. Conclusion

Individual susceptibility to carcinogenesis, chemical toxicity, and adverse and therapeutic drug response constitutes a major challenge in understanding host−xenobiotic interactions in the postgenome era. The induction of P4501A enzymes by xenobiotics, which is an adaptive cellular response to chemical exposure, represents a unique model for analyzing such interactions, due to its role in the metabolic activation of PAH and other chemicals to carcinogens or toxic species, its high vari-

ability in humans, and a receptor-dependent mechanism. The remarkable progress in the mechanistic understating of the induction at molecular levels, the molecular dissecting of variant phenotypes of the induction in mammalian cell and animal models, and the accumulation of clinical data on the induction in human populations over several decades have provided information that permits the identification and analysis of major determinants of individual variability in P4501A induction in humans.

Drug transporters, P4501A phenotypes, and protein factors mediating signal transduction of 1A inducers (in particular, the Ah receptor) form the main cellular components governing the induction. Variations in these genetic factors can profoundly influence the induction and thus impact on human health in certain populations. With the rapid accumulation of genomic information from various ethnic groups and the feasibility of safe tests of P4501A induction in humans (e.g., caffeine breath test) and large scale epidemiological studies, it is conceivable that much progress will be made soon in identifying genotypes in which altered P4501A expression and its health effects are identified. Environmental factors such as pathophysiological abnormalities, dietary components, and environmental pollutants can modify various steps of P4501A induction. Future studies should focus on the confirmation of many of the in vitro or animal findings that dietary components and environmental chemicals can indeed modulate P4501A1/2 induction in humans by using in vivo probes such as the caffeine breath test. Studies on these environmental factor−gene induction interactions can contribute to human health through promoting healthy lifestyles, effective chemoprevention strategies, and environmental protection.

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