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REVIEW

Applications of CYP-450 expression for biomonitoring in environmental health

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Abstract Cytochrome P450s (CYPs) are one of the first steps in the metabolism of xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs), which are bioactivated into carcinogens. As such, changes in CYP expression are potential biomarkers in human biomonitoring applications. For the proper biomonitoring of environmental toxicants, it is important to understand the biological relevance of each biomarker and the associations among the biomarkers for uses as exposure, effects, and susceptibility biomarkers. Here, we have reviewed various aspects of CYPs for biomonitoring environmental health in terms of the CYP substrates, such as PAHs, aromatic amines, benzene/toluene, and tobacco smoking-related nitrosamines. This review also includes association studies between CYP phenotypical alterations and other exposure, susceptibility, and effect biomarkers. The association studies were mainly performed in CYP gene-transfected cells and noninvasive human biospecies, such as urine and peripheral blood. In conclusion, we suggest that phenotypical alterations in CYPs with exposure to environmental toxicants are useful as susceptibility or effect biomarkers, particularly when the phenotype-related genotypes are unknown.

Keywords Association · Biomonitoring · Biomarker · Cytochrome P450s · Exposure · Genotype · Phenotype

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Introduction

During the past 30 years, biomarker-based approaches have been used in the area of human biomonitoring with the expectation of refining exposure assessment, providing tools for the detection of disease-related changes and their association with environmental and genetic factors and, thereby, facilitating an improved understanding of the etiology of human disease [1, 2]. The National Institute of Environmental Health Sciences (NIEHS) in the USA, for example, has prioritized research focusing on the development of markers sensitive to environmental exposure, early (preclinical) biological response, and genetic susceptibility as one of its strategic plans for 2006–2011 [3].

In biomonitoring environmental toxicants, each biomarker and the associations among the biomarkers of exposure, effect, response and susceptibility have the potential to provide a better understanding of the biological relevance of the markers themselves. Exposure biomarkers, such as hydroxylated metabolites of toxicants in urine, are used to indicate the internal dose received and to help estimate the exposure amounts of toxicants that have gained entry into the body [4]. Effect biomarkers are measured as the forms that interact with critical targets, such as DNA- and hemoglobin-adducts or cytogenic alterations [for example, chromosomal aberrations (CAs), micronuclei (MN), sister chromatid exchange (SCE), comet/single-cell gel electrophoresis assay, among others]. Susceptibility biomarkers include genetic variations on metabolic enzymes, such as cytochrome P450s (CYPs). Figure 1 summarizes the relations among these biomarkers in terms of risk assessment, which is the ultimate goal of biomonitoring.

CYPs, which are localized in endoplasmic reticulum, are a hemoprotein super-gene family. They are one of the first

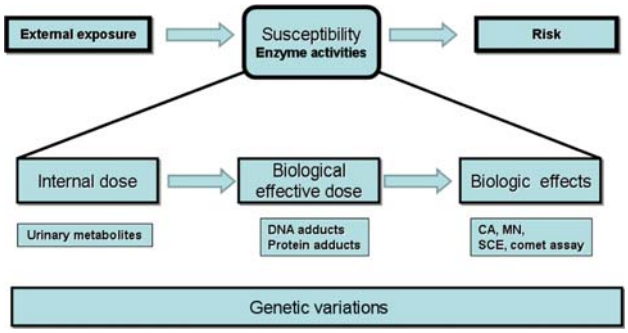

Fig. 1 The procedures of risk assessment with various biomarkers

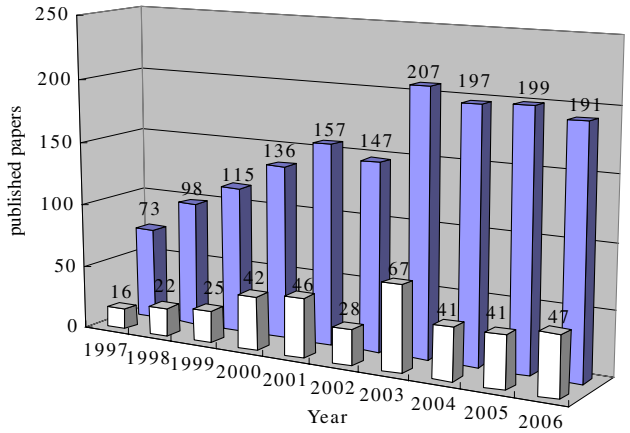

Fig. 2 Published papers dealing with applications of cytochrome P450s (CYPs) in human biomonitoring (1997–2006). *Dark bars* All applications, *light bars* applications of *CYP* polymorphisms in biomonitoring

steps in the metabolism of xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs) and aromatic amines (AAs) that are bioactivated to carcinogens [5, 6]. In addition, expressions of the various CYPs have been broadly used as biomarkers in environmental pollution, drug metabolism, and remedies for pollutants. The increasing number of articles published yearly on web sites dealing with the applications of CYPs in biomonitoring – from 73 in 1997 to 191 in 2006 – are an indication of the increased interest of the scientific community in this super-gene family (Fig. 2).

Various methods, including enzyme assays, immunoassays, and reverse transcriptase (RT)-PCR, have been developed to determine CYP expression. One recent development is a 96-well plate filtration technique for the quick removal of precipitated proteins, followed by fast liquid chromatography/mass spectrometry (LC-MS/MS) analyses for inhibition assays for CYPs [7].

CYPs are known to consist of 99 isozymes in 18 families; there are 583 polymorphic forms in humans, and these polymorphisms have been focused upon due to their induced susceptibility to environmental toxicants [8].

Here, we have categorized four groups of CYP substrates, based on the most widely accepted categorizations in the literature, and reviewed the various applications of CYPs, with special emphasis on their phenotypical alterations, in terms of biomonitoring.

Polycyclic aromatic hydrocarbons

Although coal processing wastes, petroleum sludge, asphalt, creosote, and other wood preservative wastes contain high levels of PAHs, food, water, air, and tobacco smoke are known as the main or most frequent sources of human exposure to PAHs [9, 10]. A number of epidemiological studies carried out on PAHs have shown that highly exposed workers have an increased cancer risk [11]. The current understanding of the carcinogeneses of PAHs is based predominantly on two complementary mechanisms – the bioproduction of bioactive metabolites, such as diol epoxides, and reactive oxygen species [12, 13].

The interaction of PAH ligands with the arylhydrocarbon receptor (AhR) can explain the pathway of CYP induction [14]. The AhR is a ligand-activated transcription factor that acts in concert with the AhR nuclear translocator (ARNT). The nuclear AhR complex, ligand–AhR–ARNT, interacts with consensus dioxin or xenobiotic response elements (DREs/XREs) in the CYP 1 promoter and in promoters of other Ah-responsive genes, and subsequent recruitment of coactivators and general transcription of coactivators and general transcription factors results in the expression of target genes, such as *CYP1A1, 1A2*, and *1B1*, as well as the genes of other biotransformation enzymes, including NAD(P)H:quinoneoxidoreductase, glutathione *S*-transferase, and UDP-glucuronosyltransferase [15].

In the human lymphocytes, mRNA levels of *AhR* were found to be positively correlated with those of *CYP1B1* and *CYP1A1* [16, 17], while mRNA levels of *ARNT* levels were found to be correlated with only those of *CYP1B1* [18]. On the other hand, Hayashi et al. [19] reported that mRNA expression levels of *CYP1A1* were associated with those of *AhR* and *ARNT* in smoker's lymphocytes. Protein levels of CYP1A2 were also associated with those of AhR/ARNT in the human LS180 cell lines [20].

Biomonitoring of PAHs has been mainly accomplished by measuring urine metabolites, such as 1-hydroxypyrene (1-OHP), and PAH-related DNA- and protein-adducts. Urinary 1-OHP, a metabolite of pyrene, has been used as a biomarker for determining broad environmental PAH exposure. An in vitro study showed that CYP1A1, CYP1B1, and CYP1A2 are major metabolizing enzymes involved in the bioproduction of 1-OHP from pyrene [21].

Urinary levels of 1-OHP in smokers were correlated to CYP1A2 activity in plasma [22].

Urinary 1-hydroxypyrene-*O*-glucuronide (1-OHPG) is approximately five fold more fluorescent than 1-OHP, leading Strickland et al. [23] to propose that 1-OHPG has the potential to be a sensitive exposure biomarker of PAHs. Pyrene is metabolized into 1-OHPG by CYPs and UDP-glucuronyltransferase (UGT); however, the effects of CYP expression on the bioproduction of 1-OHPG has not yet been thoroughly studied.

Urinary naphthols [2-naphthol (2-NT) more than 1-NT] reflect route-specific exposure to PAHs via air, as compared to 1-OHP, which reflects PAH-total exposure from diet, air, among others [24]. Cho et al. [25] reported that CYP1A2 and CYP3A4 were their most effective in the bioproduction of 1- and 2-NT, respectively, with both CYP2E1 and CYP2A6 being slightly involved in these metabolisms. We previously reported that the *CYP2E1*5* polymorphism with $-1019C > T$ affects the levels of urinary naphthols [24]. Compared to the number of 1-OHP studies, biomonitoring studies on 2-NT are relatively rare; consequently, further biomonitoring studies are needed to determine whether the phenotype or genotype of *CYP2E1* are susceptibility biomarkers for biomonitoring with 2-NT.

Benzo(a)pyrene (B*a*P), a representative carcinogen of PAHs, is oxidized into the chemically reactive diolepoxide (BPDE), which subsequently interacts with DNA to form both stable and unstable adducts of DNA (Fig. 3; [26]). CYP1A1 predominantly mediates BPDE-DNA adducts as a rate-limiting step [27]. In addition, Alexandrov et al. [28] observed a positive correlation between BPDE-DNA adduct levels and microsomal aryl hydrocarbon hydroxylase (AHH) activity in lung tissues. Mollerup et al. [29] reported that *CYP1A1* and *CYP1B1* mRNA expression levels were positively associated with bulky-DNA adducts. Furthermore, Piipari et al. [30] found higher levels of PAH-DNA adducts in lung tissues of active smokers who had higher protein levels of CYP3A4, and CYP3A5 than others. Degawa et al. [31] also reported positive associations between total DNA adduct levels and immunoreactive amounts of CYP1A1, -2C, and -3A4 in the larynx tissues of smokers.

In contrast to PAHs, metabolites of nitro-PAHs and their DNA-adducts have been proposed as biomarkers for exposure to petroleum products, such diesel exhaust [32]. In addition, 3-nitrobenzanthrone (3-NBA), a nitro-PAH, is known as an extremely potent mutagen [33–35]. In a study of CYP-expressing V79 cells, Bieler et al. [35] observed that 3-NBA-DNA adduct levels increased with increasing activities of CYP3A4 and CYP oxidoreductase, but not with increasing levels of CYP1A1.

The activity of CYPs, particularly that of the CYP1A subfamily, is known to be involved in the metabolism of aromatic compounds and may modify the effects of the aromatic compounds in terms of DNA oxidation [36]. The

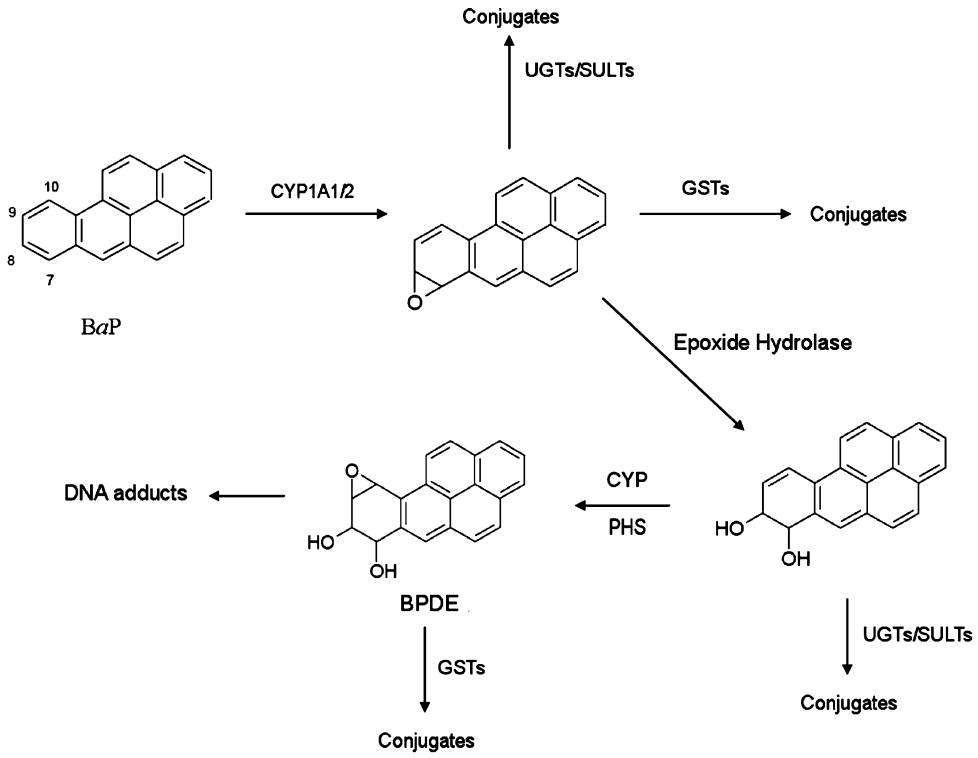

Fig. 3 Metabolic pathway of *BaP* benzo[*a*]pyrene (*BaP*), benzo[*a*]pyrene-7, 8-dihydrodiol-9,10-epoxide (*BPDE*), glutathione *S*-transferases (*GSTs*), UDP-glucuronosyltransferases (*UGTs*), sulfotransferases (*SULTs*), prostaglandin H synthase (*PHS*)

oxidative damage to both DNA and DNA-adducts may be involved in the increased risk of cancer in individuals exposed to PAHs [37, 38]. Quantification of 8-hydroxydeguanosine (8-OHdG) in the urine and blood has been used as a biomarker of oxidative DNA damage [39]. After evaluating three different studies, Poulsen et al. [40] concluded that 8-OHdG was influenced by CYP1A2 activity and that CYP1A2 activity was significantly correlated with 24-h urinary 8-OHdG excretion. In addition, Kim et al. [41] reported correlations between the levels of urinary 1-OHP and 8-OHdG in human leukocytes.

In terms of effect biomarkers, since the 1970s, the conventional cytogenic analysis of peripheral blood lymphocytes has been accepted as a technique suitable for the monitoring of genetic damage in somatic cells [42]. Chromosomal aberrations (CAs), a cytogenic biomarker, are abnormalities in chromosome number or structure. Brandt and Watson [43] proposed that they can function as surrogate endpoints in human peripheral lymphocytes. In a tire plant workers' study, Vodicka et al. [44] found that CAs were somewhat higher in individuals with higher *CYP2E1* expression levels in peripheral lymphocytes than in those with relatively lower *CYP2E1* expression levels. However, the relationships between CYP expressions and cytogenic alterations have not yet been clarified.

Various biomarkers, or combinations of them, have been used to assess exposure to PAHs. In the case of occupational exposure, urinary excretion of hydroxylated PAH is considered to be the standard for exposure assessment. However, hydroxylated PAH-metabolites in urine can only be used to assess the internal dose following recent exposure to PAHs [45]. Therefore, biomarkers such as DNA-, protein-, and hemoglobin-adducts are preferable for assessing long-term exposure to PAHs. In addition, these biomarkers are subject to a greater variability due to differences in metabolic capacity and genetic polymorphisms in CYPs. The susceptibility towards the induction of CYPs may play an important role in the bioactivation and detoxification of PAHs.

Benzene/toluene

Benzene is a volatile aromatic hydrocarbon solvent that is widely used in industrial processes. It is absorbed by all routes of exposure, rapidly distributed throughout the body and metabolized to a variety of intermediate compounds, such as benzene oxide, catechol, phenol, hydroquinone, and benzoquinone in several organs, including the liver and bone marrow [46]. The toxicity of benzene in occupational populations has been characterized as either hematotoxicity, including anemia leukopenia, and thrombocytopenia with prolonged exposure to high doses, and irreversible bone marrow damage [47]. Therefore, many attempts have been made to monitor benzene exposure by analyzing blood benzene levels and urinary metabolites, such as urinary *trans, trans*-muconic acid (*t, t*-MA) [48].

CYP2E1 has been suggested to be responsible for the metabolism of benzene [49–52]. Sheets et al. [52] also reported that CYP2E1 and CYP2F1 had important roles in the metabolism of benzene in the BEAS2B and A549 human lung cell lines, respectively. In an in vitro study, Tassaneeyakul et al. [53] showed correlations between CYP2E1 activity and the oxidation rates of different benzene metabolites.

Due to the hematotoxicity of benzene, toluene has widely replaced benzene in many industrial processes. Therefore, the biomonitoring of toluene is an important issue in various aspects of occupational health. The major product of the metabolism of toluene by CYPs is benzyl alcohol, which is easily converted to benzoic acid via benzylaldehyde and excreted into the urine as hippuric acid (HA) [53]. *O*-cresol and *p*-cresol are also minor metabolic products of the catalytic action of CYPs on toluene [54]. Nakajima et al. [54] reported that CYP2E1 is the most active enzyme in the formation of benzyl alcohol, followed by CYP2B6, CYP2C8, CYP1A2, and CYP1A1. Epidemiologic studies have shown that the *CYP2E1*5* polymorphism is associated with toluene metabolism [55, 56]. The most active isozymes in the formation of benzyl alcohol and minor phenolic metabolites are CYP2E1 and CYP1A2, respectively [57]. In a cross-sectional study in the print industry workers, levels of environmental toluene were positively associated with *CYP2E1* mRNA levels, while urinary HA levels were negatively correlated with *CYP2E1* mRNA levels in peripheral lymphocytes [58]. This dissociation may be due to the capacity of toluene to induce *CYP2E1* mRNA expression, whereas HA reflects toluene disposition: Therefore, *CYP2E1* mRNA level and the toluene exposure ratio (environmental toluene concentration:urinary HA concentration) showed a statistically significant relationship.

In addition, genetic variations in CYPs, particularly the *CYP2E1*5* genetic polymorphism, have been emphasized as susceptibility biomarkers for benzene or toluene exposure [57, 59]. Therefore, we suggest that both CYP phenotypes and genotypes should be considered for the proper biomonitoring of CYP-substrates. However, phenotypes of CYPs should be used, when phenotype-associated genetic polymorphisms are unavailable.

Heterocyclic amines and aromatic amines

Heterocyclic amines (HCAs) originate from the cooking of meats, fish, and poultry and from tobacco smoke, diesel

exhaust, etc. [60]. A number of epidemiological studies have shown an association between the frequent consumption of well-done cooked meats containing HCAs and elevated risks for colon, prostate, and mammary cancers [61–63]. There are two classes of HCAs formed in cooked meats. One class consists of the *N*-methyl-2-aminoimidazole moiety, possibly formed through the reaction of pyridine or pyrazines, which are heat-catalyzed degradation products of amino acids, with sugars and creatine. These compounds form in meats heated at 150°C or higher, and their formation has been characterized in model systems. The second class of HCAs includes 2-amino-9H-pyridine[2,3-b]indole, 2-amino-3-methyl-9H-pyridole[2,3-b]indole, and the glutamic acid and tryptophan pyrolysate mutagens. They are formed in proteins or produced directly from the pyrolysis of these latter two amino acids generated at high temperature (>250°C) [64]. HCAs are not intrinsically genotoxic; they require metabolic activation, as do most chemical carcinogens. Therefore, HCAs are oxidized to hydroxyamino derivatives by CYPs and then further converted to ester forms. They may eventually produce DNA-adducts through the formation of N–C bonds at guanine bases by initially producing highly reactive ester derivates that are known to be bioproduced by four enzyme systems: *N*-acetyltransferase (NAT), sulfotransferase, prolyl tRNA synthetase, and kinases [65]. As HCAs are known to be mainly metabolized by CYP1A2, the rapid CYP1A2 phenotype confers an increased risk of colorectal cancer when combined with the rapid NAT2 phenotype and consumption of well-done meat [66, 67]. Representative carcinogens of HCAs, 2-amino-3, 8-dimethylimidazo [*4, 5-f*] quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo [*4, 5-b*] pyridine (PhIP), require metabolic activation via *N*-oxidation to convert them into reactive species with genotoxic activity. The *N*-oxidation of HCAs is catalyzed primarily by hepatic CYP1A2 [68].

Other CYPs, such as CYP1A1, -1B1, and -3A4, are also responsible, to some extent, for the oxidation of the exocyclic primary amino group to a hydroxyamino group [69, 70]. For example, the induction of PhIP-DNA adducts in human microsomes has been related to the activities of CYP1A1, CYP1A2, and CYP3A4 [71].

Aminobiphenyls (ABPs) are one of the groups of aromatic AAs. Of the various ABPs, 4-ABP is classified by IARC [13] as a group 1 carcinogen. ABPs are known to be metabolized into the *N*-hydroxy–ABP intermediates principally in the liver, and these intermediates are precursors to the formation of ABP–DNA adducts in the liver and bladder as well as ABP–hemoglobin (Hb) adducts in blood [72]. A significant linear relationship was observed between 4-ABP–Hb adducts and CYP1A2 activity [73]. Therefore, phenotypes with rapid CYP1A2 and slow acetylraters have been implicated in the activation (*N*-oxidation) and detoxification (*N*-acethylation) of AAs for human bladder carcinogenesis [73, 74]. Landi et al. [75] reported that a combination of rapid CYP1A2 and slow acetylraters increased the level of ABP–Hb adducts.

Stillwell et al. [76] reported that CYP1A2 activity showed a notable influence on total MeIQx excreted in urine (0–12 h). Sinha et al. [77] also reported a relationship between unconjugated MeIQx in 12-h urine samples and CYP1A2 activity, indicating that more MeIQx is converted to the bioactive *N*-hydroxy derivative with higher CYP1A2 activity.

In addition to HCAs and ABPs, nitrosamines are also important chemicals for biomonitoring. Due to the importance of tobacco-specific and carcinogenic nitrosamines, the biomonitoring issues in terms of nitrosamines are reviewed in the following section on tobacco smoking.

Tobacco smoking

Both active tobacco smoking and passive smoking, such as environmental tobacco smoking (ETS), have been emphasized as major public health factors [78]. Of the 4000 identified chemicals in tobacco smoke, more than 60 are established carcinogens based on IARC assessments [79].

Various biomarkers, such as nicotine, cotinine, 1-OHP, naphthols, have been used as indicators of exposure to tobacco smoking. Compared to nicotine in tobacco, cotinine, a primarily metabolite of nicotine, is stable in body fluids and has a relatively long half-life of approximately 17 h. Therefore, the detection of cotinine is less dependent on the time of sampling than nicotine and other metabolites and has been used an exposure biomarker for active and passive tobacco smoking [78].

CYP2B6, CYP 2C9, CYP 2D6, CYP 2E1, and CYP2A6 are known to biotransfer nicotine into cotinine [80–82]. Extensive empirical evidence indicates that CYP2A6 is the major enzyme involved in nicotine C-oxidation. In addition, numerous epidemiology studies have been carried out with the aim of clarifying whether CYP2A6 is functionally associated with individual variations in the biomonitoring of tobacco smoking [83–85]. We previously reported that *CYP2A6*4*, the CYP2A6 gene-deleted type, showed lower urinary cotinine levels [86].

The tobacco-specific nitrosamines, *N*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), have been known to play a role in human tobacco-related cancers. Fujita and Kamataki [87] reported the involvement of CYPs in the metabolic activation of tobacco-related *N*-nitrosamines in *CYP*-transfected cells. CYP3A4 and CYP2E1 were linked to the activation of

Table 1 Alterations in cytochrome P450s (CYPs) due to exposure to xenobiotics

Xenobiotics	Exposure biomarker[a]	CYPs	Associations[b]	Biospecimens[a]	References
Polycyclic aromatic hydrocarbons (PAHs)	B*a*P-DNA adducts	CYP1A1*- and CYP1B1** – total RNA	↑	NHMECs	Keshava et al. [89]
		CYP1A2 activity	-	Human lymphocytes	Funck-Brentano et al. [22]
	BPDE-DNA adducts	CYP1A1 activity**	↑	Human lung tissue	Alexandrov et al. [28]
	3-NBA-DNA adducts	CYP3A4** and CYP1A1 activity	↑ and -, respectively	V79 cell lines	Bieler et al. [35]
		CYP2C9 activity**	↑	Human B-lymphoblastoid MCL-5	Arlt et al. [33]
	3-ABA-DNA adducts	CYP1A1**, CYP1A2** activity	↑	Human B-lymphoblastoid MCL-5	Arlt et al. [34]
	4-ABP-Hb adducts	CYP1A2 activity**	↑	Human Peripheral blood	Sarkar et al. [73]
		CYP1A2 activity	↑	Urine	Landi et al. [75]
	Bulky-DNA-adducts	CYP1A1 mRNA	↓	Human placenta tissue	Whyatt et al. [27]
		CYP1A1, CYP2C and CYP3A4 activity**	All ↑	Human larynx tissue	Kap-Soon et al. [10]
		CYP1B1 and CYP3** protein	- and ↑, respectively	Human BAL sample	Piipari et al. [30]
		CYP1A1 total RNA**	↑	Human lung tissue in NSCLC	Mollerup et al. [29]
	AhR/ ARNT	CYP1B1 total RNA**	↑	Human lymphocytes	Lin et al. [18]
		CYP1A2 protein	↑	LS180 cell lines	Li et al. [20]
	AhR	CYP1B1 mRNA	↑	MOG-G-CCM cells	McFadyen et al. [16]
		CYP1A1** and 1B1** protein	All ↑	Lung tissues in adenocarcinoma	Chang et al. [17]
		CYP1A1** mRNA	↑	Human pheripheral blood	Hayashi et al. [19]
	1-OHP	CYP1B1 mRNA	↑	Human pheripheral blood	Hanaoka et al. [90]
		CYP1A1, CYP1B1, CYP1A2 activity	all ↑	BTI-TN-5B1-4 cells	Kim et al. [21]
		CYP1A2 activity**	↑	Human lymphocyte	Funck-Brentano et al. [22]
	CAs	CYP2E1 mRNA*	↑	Human peripheral blood	Vodicka et al. [44]
	8-OHdG	CYP1A1 activity	↑	Hepa1c1c7 cell	Park et al. [91]
		CYP1A2 activity**	↑	Urine	Loft et al. [36], Poulsen et al. [40]
	1-naphthol and dihydrodiol	CYP1A2 activity	↑	BTI-TN-5B1-4 cells	Cho et al. [25]
	2-NT	CYP3A4 activity	↑	BTI-TN-5B1-4 cells	Cho et al. [25]
		CYP2E1 mRNA**	↑	Human blood sample	Nan et al. [92]
Benzene	Total hydroxylated benzene metabolites	CYP2E1 and CYP2F1 activity**	All ↑	Human BEAS-2B cells and Human A549 cells	Sheets et al. [52]
		CYP2E1 activity**	↑	Human liver sample	Nedelcheva et al. [49]
	2-nitrophenol	CYP2E1 activity**	All ↑	Human hepatic micromosome	Miksanova et al. [93]
Toluene	HA	CYP2E1 mRNA**	↑	Human lymphocytes and urine	Mendoza-Cantu et al. [58]
	Toluene metabolites	CYP2E1 and CYP1A2 activity**	↑	Human liver microsomes	Tassaneeyakul et al. [53]
Heterocyclic amines (HCAs)	Urinary unconjugated MeIQx	CYP1A2 activity**	↑	Urine	Sinha et al. [77]
	PhIP metabolites	CYP1A1 activity	↑	Sf9 insect cells	Crofts et al. [94]
	HONH-PhIP	CYP1A2 activity	↑	Human liver sample	Langouet et al. [95]

Table 1 continued

Xenobiotics	Exposure biomarker[a]	CYPs	Associations[b]	Biospecimens[a]	References
Tobacco	Cotinine	CYP2A6 content**	↑	Human B-lymphoblastoid cells	Nakajima et al. [84]
		CYP2E1** activity	↓	Human microsomes	Van Vleet et al. [96]
		CYP1B1 mRNA**	↑	Human peripheral blood	Hanaoka et al. [90]
		CYP1A2 activity**	↑	Human lymphocyte and urine	Funck-Brentano et al. [22]
Nitro-samines	Induced revertants	CYP1A1, 2A6 and 2E1 activity	All ↑	*S. typhimurium* YG7108 cells	Fujita and Kamataki [87, 88], Smith et al. [97], Kushida et al. [98]
	NNK and NNAL metabolites	CYP3A4 activity**	↑	Human hepatic microsomes	Staretz et al. [99]

*Borderline significant, $0.05 < P < 0.1$; **significant, $P < 0.05$

[a] NHMEC, normal human mammary epithelial cell; V79, Chinese hamster cell line; LS-180, human colon adenoma cell line; NSCLC, nonsmall cell lung cancer; BTI-TN-5B1-4, haculovirous-infected insect (*Autographa californica*) cell; Sf9, insect cell; BEAS-2B, bronchiolar derived human cell line; MOG-G-CCM, human cell line; AaC, 2-amino-9*H*-pyrido[2,3-*b*]indole; HONH-PhIP, 2-(hydroxyamino)-1-methyl-6-phenylimidazo[4,5-b]pyridine

[b] ↑, Positive association; ↓, negative association; −, no association

NNN and *N*-nitrosodemetylamine (NDMA) in human fibroblasts [87], while CYP1A1 showed tobacco smoking-inducible expression in the human placenta [88].

Therefore, levels of urinary cotinine and nitrosamines are related to the expression of CYPs, particularly CYP2A6. When the aim is to perform proper exposure monitoring of active and passive tobacco smoking, alterations of CYP2A6 expression should be considered.

Conclusion

In this review, we have summarized phenotypical alterations to CYPs resulting from the exposure to xenobiotics (see Table 1). The capacity in inducing CYPs may play an important role between activation of the xenobiotics and subsequent risk for developing chemically induced diseases. In addition, assessment of the set of putative CYPs will allow the identification of susceptible individuals or subgroups. However, there remains some discrepancies in reports on the associations between CYP expression and exposure or effect biomarkers. In addition, various characteristics of CYPs, such as their broad spectrum or the overlapping of substrates, induce combined effects of CYP isozymes during biomonitoring.

In conclusion, we suggest that phenotypical alterations in CYPs with exposure to environmental toxicants are preferable as susceptibility or effect biomarkers, particularly when phenotype-related genotypes are unknown.

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