

## CONTEMPORARY REVIEW

# Polycyclic Aromatic Hydrocarbons: From Metabolism to Lung Cancer

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## ABSTRACT

Excessive exposure to polycyclic aromatic hydrocarbons (PAHs) often results in lung cancer, a disease with the highest cancer mortality in the United States. After entry into the lung, PAHs induce phase I metabolic enzymes such as cytochrome P450 (CYP) monooxygenases, i.e. CYP1A1/2 and 1B1, and phase II enzymes such as glutathione S-transferases, UDP glucuronyl transferases, NADPH quinone oxidoreductases (NQOs), aldo-keto reductases (AKRs), and epoxide hydrolases (EHs), via the aryl hydrocarbon receptor (AhR)-dependent and independent pathways. Humans can also be exposed to PAHs through diet, via consumption of charcoal broiled foods. Metabolism of PAHs through the CYP1A1/1B1/EH pathway, CYP peroxidase pathway, and AKR pathway leads to the formation of the active carcinogens diol-epoxides, radical cations, and o-quinones. These reactive metabolites produce DNA adducts, resulting in DNA mutations, alteration of gene expression profiles, and tumorigenesis. Mutations in xenobiotic metabolic enzymes, as well as polymorphisms of tumor suppressor genes (e.g. p53) and/or genes involved in gene expression (e.g. X-ray repair cross-complementing proteins), are associated with lung cancer susceptibility in human populations from different ethnicities, gender, and age groups. Although various metabolic activation/inactivation pathways, AhR signaling, and genetic susceptibilities contribute to lung cancer, the precise points at which PAHs induce tumor initiation remain unknown. The goal of this review is to provide a current state-of-the-science of the mechanisms of human lung carcinogenesis mediated by PAHs, the experimental approaches used to study this complex class of compounds, and future directions for research of these compounds.

**Key words:** polycyclic aromatic hydrocarbons; PAH; lung cancer; Ah receptor; carcinogenesis; genetic susceptibility; metabolism; mixtures

Lung cancer is the leading cause of cancer-related death in both men and women (Horn *et al.*, 2012), responsible for 1.59 million deaths around the world in 2012 (IARC, 2012). Approximately 90% of lung cancer cases are related to tobacco smoking and 1–2% are accounted for by outdoor air pollution and secondhand smoke. Inhalation of incomplete indoor/outdoor combustion of coal and wood may induce lung cancer as well (Reid *et al.*, 2012). Among the many components in tobacco smoke and outdoor and indoor air pollution are polycyclic aromatic hydrocarbons (PAHs), which are defined as a group of chemicals containing 2 or more fused benzene rings but no heteroatoms (Agency for Toxic Substances and Disease Registry (ATSDR) December, 1990). The PAHs are considered to be the most important

carcinogens in these complex mixtures (Hecht, 2002, 2011; Rubin, 2001). Although PAHs can exist in hundreds of different combinations, the National Waste Minimization Program defines this group using the Toxic Release Inventory reporting category for PAHs, which include 20 compounds, such as benzo[a]pyrene (BaP), dibenzo[a,h]anthracene, 3-methylcholanthrene, 5-methylchrysene, and 7,12-dimethylbenz[a]anthracene (Agency for Toxic Substances and Disease Registry (ATSDR) December, 1990).

Inhalation exposure to PAH-containing substances increases the risk of lung cancer in humans (DeMarini, 2004; Eom *et al.*, 2013; Osgood *et al.*, 2013; Tsay *et al.*, 2013). It is estimated that there is 100 ng or more of total PAHs per gram of tobacco,

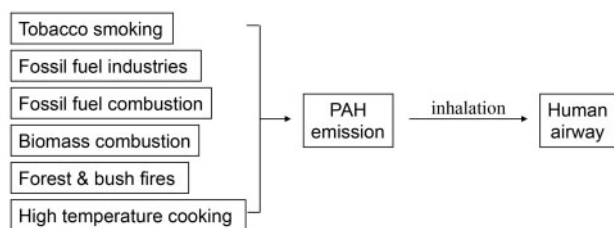


FIG. 1. Major sources through which PAH exposure occurs in humans.

regardless of the manufacturers and brands (Grimmer *et al.*, 1988), and smokers inhale  $\sim 0.26 \mu\text{g}$  of BaP per pack of 20 cigarettes (Piccardo *et al.*, 2010). Domestic wood burning and road traffic are also major sources of PAHs (Bostrom *et al.*, 2002). For example, in Stockholm, Sweden, the sum of 14 different PAHs was  $100\text{--}200 \text{ ng/m}^3$  (taken from road samples), with the most abundant being phenanthrene and BaP, both of which varied between 1 and  $2 \text{ ng/m}^3$ .

In addition to inhalation, it is well established that, in cigarette and cigar smokers, considerably greater amounts of PAHs are swallowed and enter the gastrointestinal tract than those that enter through the lung (Jarup, 2003; Nebert *et al.*, 2013; Rozman and Klaassen, 2007). Significant amounts ( $0.1\text{--}20 \mu\text{g/kg}$ ; up to  $100 \mu\text{g/kg}$ ) of PAHs are also detected in grilled, barbecued, or smoked meat products (Hansen *et al.*, 1992; Larsson *et al.*, 1983; Masuda *et al.*, 1966; Mottier *et al.*, 2000; Simko, 2002; Sinha *et al.*, 1994) (Fig. 1). Consumption of fried chicken and smoked dried beef translates to BaP concentrations of  $5.4\text{--}5.5 \mu\text{g/kg}$ , and charcoal-broiled steak contains BaP level of  $\sim 9.0 \mu\text{g/kg}$ . This amounts to an extrapolated environmental dose of  $\sim 40\text{--}50 \text{ ng/kg/day}$ . However, BaP concentrations have been reported to be as high as  $19 \mu\text{g/kg}$  in smoked meat in Austria (Tiefenbacher *et al.*, 1982) and  $69 \mu\text{g/kg}$  in rape seed oil (Pupin and Toledo, 1996). Ingestion of these foods would increase BaP amounts to  $80\text{--}380 \text{ ng/kg/day}$  (Pupin and Toledo, 1996). Researchers have not found a direct link between dietary PAH exposure and lung cancer incidence, except for one recent study from China (Cai *et al.*, 2012).

Although much research has been conducted on PAHs and the various components that occur within environmental PAH-containing mixtures, a comprehensive review of the mechanisms by which PAHs contribute to lung cancer has not been written. This review will provide an overview of the current state-of-the-science of the metabolism of PAHs and how these processes contribute to lung cancer, information on mode of action to inform current health assessments, and knowledge gaps to determine the next steps for research on these compounds. Specifically, this review will evaluate the most commonly studied PAHs including BaP and the novel approaches used to study the mechanisms by which these compounds may cause lung cancer. Moreover, a thorough understanding of these mechanisms by which PAHs induce cancer may lead to more targeted treatments for lung cancer, which often has a poor prognosis and results in billions of dollars expended on health care costs associated with this disease.

## THE PAHs—A COMPLEX GROUP OF COMPOUNDS

The carcinogenicity of PAHs is associated with the complexity of the molecule (i.e. increasing number of benzenoid rings). According to the United States Environmental Protection

Agency, there are at least 11 carcinogenic or mutagenic PAHs. The International Agency for Research on Cancer (IARC) lists the following PAHs as human carcinogens or potential carcinogens: benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, BaP, dibenz[a,h]anthracene, 7H-dibenzo[c,g]carbazole, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, indeno[1,2,3-cd]pyrene, benzo[k]fluoranthene, dibenzo[a,e]pyrene, dibenzo[a,l]pyrene, and 5-methylchrysene (Fig. 2) (IARC, 2010). The PAHs have been classified as belonging to different groups (IARC, 2010), based on their carcinogenicities. BaP is carcinogenic to humans (Group 1). Cyclopenta[cd]pyrene (Fig. 2), dibenz[a,h]anthracene, and dibenzo[a,l]pyrene are probably carcinogenic to humans (Group 2A). Benz[j]aceanthrylene, benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[c]phenanthrene, chrysene, dibenzo[a,h]pyrene, dibenzo[a,i]-pyrene, indeno[1,2,3-cd]pyrene, and 5-methylchrysene are possibly carcinogenic to humans (Group 2B) (Fig. 2).

BaP has often served as a reference for the carcinogenicity of other PAHs (Bostrom *et al.*, 2002) and most studies have been conducted using BaP because of its known carcinogenic effects. It is important to note, however, that other PAHs need to be studied as well, in addition to BaP.

## MECHANISMS OF ACTIVATION OF CARCINOGENIC PAHs

In general, PAHs are lipophilic compounds that can easily cross cell membranes through passive diffusion after inhalation. The parental PAH molecules that enter pulmonary cells are considered procarcinogens because they do not directly induce DNA damage (Alexandrov *et al.*, 2010; Miller and Ramos, 2001; Ramos and Moorthy, 2005; Rybicki *et al.*, 2006). Rather, it is the transformation of a single PAH into its carcinogenic metabolites that contribute to cancer etiology. Transformation of these compounds involves multiple metabolic enzymes and 3 known major pathways: the CYP1A1/1B1 and epoxide hydrolase pathway (CYP/EH pathway), CYP peroxidase pathway, and aldo-keto reductases pathway (AKR pathway). In general, PAHs are metabolized by CYPs and other metabolic enzymes into phenols, catechols, and quinones, resulting in the formation of diol-epoxides, radical cations, or reactive and redox-active o-quinones, which may all react with DNA to produce DNA adducts. For example, quinones react with the N-7 of guanine and N-3 of adenine in DNA (Liu *et al.*, 2002). This formation of DNA adducts can cause mismatch in DNA replication, as well as altered promoter methylation and/or promoter binding (Yang *et al.*, 2012), leading to an inheritable DNA mutation or abnormal gene expression, and ultimately tumorigenesis. Although PAHs are not considered liver carcinogens, they do become metabolized to DNA-reactive metabolites in liver following oral exposure (Kondraganti *et al.*, 2003).

The reactive metabolites of PAHs may also induce the formation of protein adducts in cells (Berge *et al.*, 2004; Kafferlein *et al.*, 2010), which may affect the normal activities of these proteins. PAH metabolites may also trigger an elevation in reactive oxygen species (ROS), which can directly affect DNA, lipids (Kwack and Lee, 2000), or proteins and initiate carcinogenesis.

The most commonly studied PAH, BaP, is transformed *in vivo* into BP-7,8-epoxide by CYP1A1 via the CYP/EH pathway. BP-7,8-epoxide is further oxidized by EH to form BP-7,8-dihydrodiol, followed by the final step of CYP1A1-catalyzed hydroxylation to form BP-7,8-dihydrodiol-9,10-epoxide (BPDE), the ultimate carcinogen (Beresford, 1993) (Fig. 3). BPDE reacts with



FIG. 2. Chemical structure of PAHs.

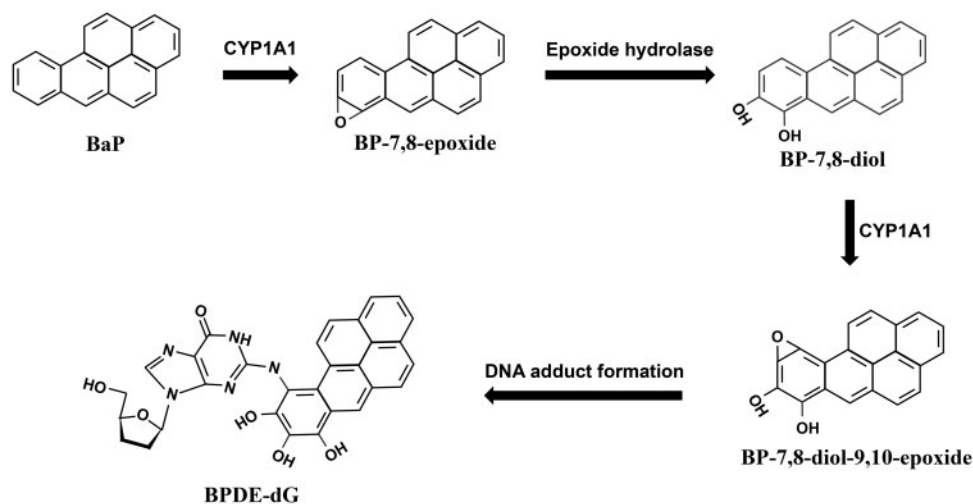


FIG. 3. Major pathways of metabolic activation of the PAH BaP to DNA-binding metabolites.

DNA to produce adducts that have been identified in lung tissues of smokers, and may cause mutations that are observed in p53 tumor suppressor gene and KRAS oncogene (Conney, 1982; Denissenko *et al.*, 1996; Geacintov *et al.*, 1997; Pfeifer and Besaratinia, 2009; Rojas *et al.*, 1998). Denissenko *et al.* (1996) reported that adduct formation was gene sequence-specific, with adducts being formed at codons 157, 248, and 273. Mutations of these sequences on the p53 gene lead to carcinogenesis and have been linked to human lung cancers (Denissenko *et al.*, 1996). Thus, targeted adduct formation rather than phenotypic selection appears to shape the p53 mutational spectrum in

lung cancer. These results provide a direct etiological link between a defined chemical carcinogen and human cancer.

CYPs also contain a 'peroxidase-like' activity (Hrycaj and Bandiera, 2012), which catalyze one-electron oxidation of BaP at the C6 position to produce radical cations (Cavalieri and Rogan, 1985). These PAH-derived radical cations, though short-lived, can react with DNA and cause mutations (Devanesan *et al.*, 1992).

In the AKR pathway, dihydrodiol dehydrogenase, a member of the aldo-keto reductase superfamily, catalyzes dehydrogenation of BP-7,8-diol, a BaP metabolite, to form a BP catechol

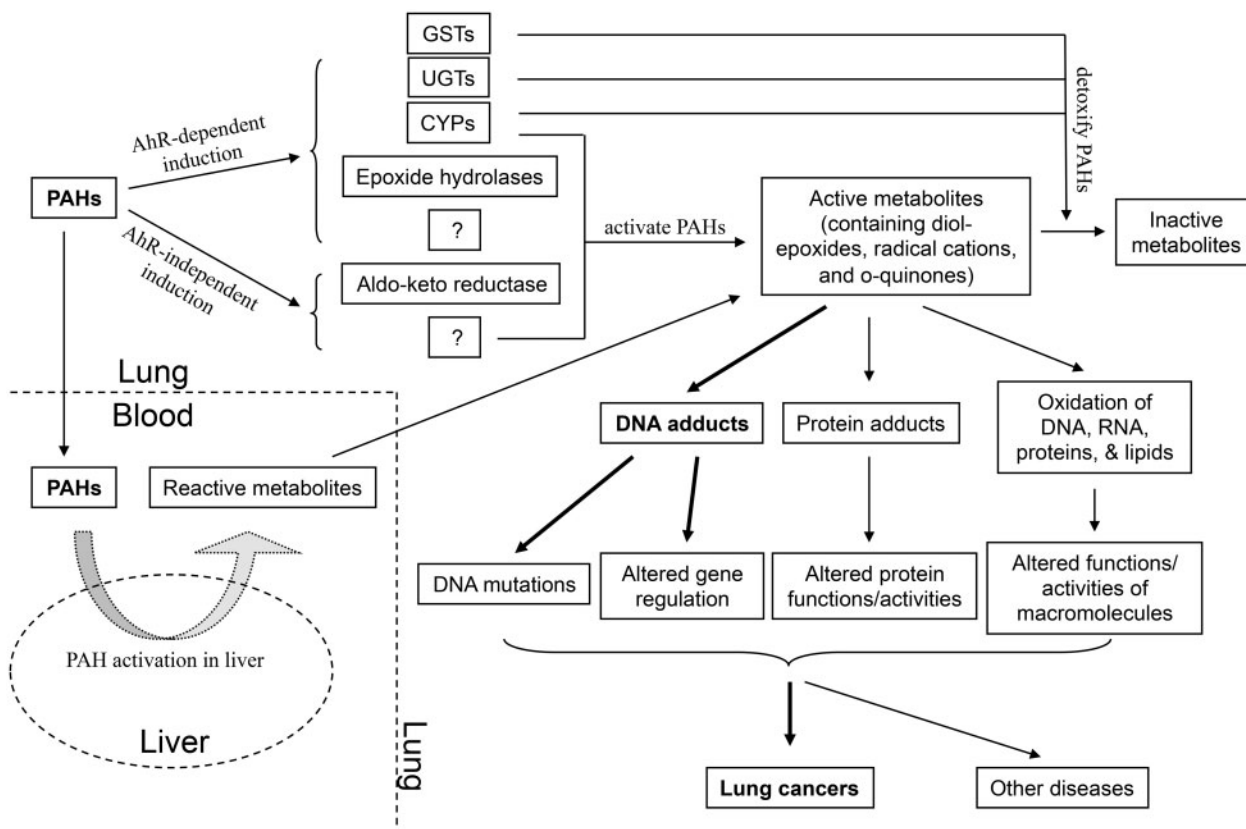


FIG. 4. Multiple mechanisms by which PAHs cause lung cancer.

(Buczynski *et al.*, 1999). Further oxidations of catechols generate *o*-quinones. Redox cycling of quinones could lead to formation of ROS, which could also lead to carcinogenesis via oxidative DNA damage (Moorthy *et al.*, 2002). In 2004, Lan *et al.* (2004) reported that AKR1C3-Gln/Gln genotype was associated with a 1.84-fold increased risk of lung cancer in a Chinese population with high coal smoke exposure, indicating a significant role of the AKR pathway in PAH activation in lung carcinogenesis.

Thus, the following pathways operate in the metabolic activation of PAHs (Borza *et al.*, 2008): (i) formation of dihydrodiol epoxides requiring 2 CYP-catalyzed oxidations and epoxide hydrolase; (ii) formation of phenols via radical cations by 1-electron oxidation (Cavalieri and Rogan, 1985); and (iii) formation of *o*-quinones via catechols by involvement of aldo-keto reductases with formation of ROS (Fig. 4).

## CURRENT RESEARCH ON PAH-INDUCED AHR ACTIVATION

The mechanism by which PAHs contribute to AhR-dependent induction is well understood (Marlowe and Puga, 2005; Nebert *et al.*, 2004; Ramadoss *et al.*, 2005) (Fig. 4). AhR is expressed in almost all tissues and is highly expressed in liver, adipose tissue, and bronchial epithelial cells (Tsay *et al.*, 2013). AhR is a transcription factor normally wrapped within an inactive protein complex in the cytosol. PAH binding to the receptor releases a key component, hepatitis B virus X-associated protein 2, from the AhR complex, resulting in the translocation of the AhR complex into the nucleus. Once in the nucleus, a dimer of heat

shock protein 90 (Hsp90) is released from the complex, allowing dimerization of AhR with the AhR nuclear translocator (ARNT). This AhR/ARNT heterodimer is the active form of the AhR transcription factor. This transcription factor is then able to recognize AhR-responsive elements (AHREs) in promoter regions of AhR responsive genes and regulate their transcription to alter expression levels of a battery of AhR-regulated genes, including CYP1 isoforms.

Carcinogenic PAHs such as BaP or 3-methylcholanthrene are ligands of AhR (Nebert *et al.*, 2004). Activation of the AhR has a variety of other downstream effects that include formation of DNA adducts (via CYP1A/1B1-dependent metabolic activation), tumorigenesis, inflammation, cell proliferation, and loss of cell-cell adhesion (Tsay *et al.*, 2013). Because of its high level of expression in human bronchial epithelial cells, AhR has many physiological consequences in the lung such as its effects on cell proliferation and differentiation, cell-cell adhesion interaction, cytokine expression, mucin production, and xenobiotic metabolism (Chiba *et al.*, 2011). Numerous studies have shown that the AhR also plays an important role in the development of lung cancer. Matsumoto *et al.* (2007) have shown that urban particulate matter induced lung cancer in wild type (AhR<sup>+/+</sup>), but not in AhR-null mice, suggesting that AhR plays a mechanistic role in the development of lung tumorigenesis by urban particulate matter, and this occurred through CYP1A1 induction (Matsumoto *et al.*, 2007). Therefore, the AhR plays an important role in lung tumorigenesis mediated by PAHs, and understanding the role of the AhR in lung tumorigenesis may lead to the identification of novel biomarkers for early diagnosis/prognosis and new targets for therapy. For example, tanespimycin (17-Allylamino-17-demethoxygeldanamycin), an Hsp90

inhibitor, is a drug candidate to treat lung cancer. Although tanespimycin decreases AhR levels and AhR-regulated gene expression in multiple lung adenocarcinoma cells, AhR expression is associated with increased anticancer activity of tanespimycin (Chen et al., 2013), thereby suggesting both the significance and complexity of the AhR in regard to PAH activation, metabolism, and lung cancer therapy.

## PAH ACTIVATION BY CYTOCHROME P450s

CYPs account for ~75% of the total metabolic enzymes for the various xenobiotics humans are exposed to everyday (Guengerich, 2008), and they are the major metabolic enzymes that catalyze the oxidation of organic substances such as PAHs. The CYP1 family, including CYP1A1, CYP1A2, and CYP1B1, plays a pivotal role in PAH activation (Bowes et al., 1996; Moorthy, 2008; Shimada et al., 1996, 2001; Walsh et al., 2013). This has been demonstrated by studies from Shimada et al. (2010), in which CYP1A1 catalyzed PAHs faster than other isoforms like CYP1A2, 2C9, 3A4, and 2C19. Other isoforms such as CYP2A6, 2B6, 2C8, 2D6, 2E1, 3A5, 3A7, and 4A11 may not play a significant role in PAH metabolism (Shimada et al., 2001).

CYP1 isoforms are all monooxygenases, which incorporate 1 oxygen atom into a substrate, often generating oxidative epoxides (Nebert et al., 2004). Endogenous CYP1A1, but not CYP1A2, is expressed in lung, and pulmonary CYP1A1 is highly inducible by PAHs (Choudhary et al., 2003; Jiang et al., 2004) (Fig. 4). Such induction may last weeks and months after exposure to PAHs is discontinued (Fazili et al., 2010; Jiang et al., 2009; Moorthy, 2000; Moorthy et al., 1993), a phenomenon that may have important implications for carcinogenesis (Moorthy, 2008).

CYP1B1, which is expressed in lung and other extra-hepatic tissues (Dey et al., 1999), is also known to play an important role in PAH metabolism in relation to carcinogenesis. Human CYP1B1 plays an important role in the activation of diverse pro-carcinogens such as BaP, BaP-7-8-diol, dibenzo[a]pyrene, benz[a]anthracene, 7,12-dimethylbenz[a]anthracene, 7,12-dimethylbenz[a]anthracene-3,4-diol, 5-methylchrysene, 2-nitropyrene, 3-methoxy-4-aminoazobenzene, etc.

CYP1B1 is also over-expressed in various tumor tissues, and therefore could be considered a histopathological tumor marker (Liehr et al., 1995; Murray et al., 1997; Spencer et al., 1999; Spink et al., 1997). Uppstad et al. (2010), using RNA interference studies, compared the individual role(s) of CYP1A1 and CYP1B1 in the metabolic activation of BaP to its carcinogenic metabolites in human lung cells, and showed a major role for CYP1A1 in the formation of carcinogenic BaP diol-epoxides, whereas both CYP1A1 and 1B1 contribute significantly to the formation of BaP-cis and trans-7,8-dihydrodiol isomers. On the other hand, Shimada et al. (1999) showed that CYP1B1, together with epoxide hydrolase, catalyzes the conversion of BaP to BaP-7-8-diol at much higher (~10-fold) higher than CYP1A1.

## DETOXIFICATION OF PAHS

Recent studies also suggest an important role for CYPs in the detoxification of PAHs (Arlt et al., 2012; Joubert et al., 2012). Nebert et al. (2013) showed that CYP1A1 is an absolute requirement for detoxification of oral BaP (Uno et al., 2006). Preliminary studies from one of our laboratories have suggested that CYP1A1 and 1A2 have reciprocal roles in lung cancer, with CYP1A1 playing a role in PAH activation, and 1A2 in their detoxification (Jiang, Zhou, Maturu, Wang, and Moorthy, unpublished data). Phase II enzymes in the liver are also responsible for the

clearance of PAHs. Beside UDP glucuronyl transferases (Saengtienchai et al., 2014), glutathione S-transferase Mu 1 (GSTM1) detoxifies PAHs (Fig. 4). Furthermore, the importance of this enzyme for detoxification has been indicated in GSTM null women, who exhibit a higher risk of lung cancer (Bennett et al., 1999).

## MODELS AND APPROACHES TO STUDY PAH METABOLISM

Due to the complexity of PAH metabolic pathways and metabolic enzymes (Fig. 4), in vitro cell culture may not seem suitable to simulate the PAH metabolism in human body, nor to study PAH carcinogenesis in the lung. However, the benefit of utilizing cell culture models cannot be ignored. Cell cultures continue to be applied in investigations of single metabolic pathway, cross-talking of signaling pathways, gene regulation such as promoter methylation, and cell biology in PAH carcinogenesis. We have recently identified the significant role of the AHRE<sub>974</sub> of CYP1A1 5-flanking sequence in PAH induction of CYP1A1 promoter in pulmonary cell culture systems (Chu, Wang, Basu, Maturu, Couroucli, Jiang, and Moorthy, unpublished data). In fact, because more than 1 cell type is involved in the development of pulmonary carcinogenesis, new 3-dimensional models using multiple cell types would be desirable.

Knock-out mice have emerged as important tools to investigate the role of a particular gene in PAH activation or carcinogenesis (Chavan and Krishnamurthy, 2012; Dragin et al., 2008; Jiang et al., 2009, 2010; Kondraganti et al., 2003; Shimada et al., 2002). For example, studies in AhR knockout mice indicate the existence of an AhR-independent pathway for PAH activation in mouse liver (Kondraganti et al., 2003), and the regulation of hepatic and pulmonary CYP1A1 by PAHs is altered in *Cyp1a2*-null mice (Jiang et al., 2010). Carcinogenicities of PAHs are also lost in AhR knockout mice (Nakatsuru et al., 2004; Shimizu et al., 2000), thereby suggesting that AhR is involved in pulmonary carcinogenesis. Knock-out animal models will continue to be used to determine many of the mechanisms associated with PAH toxicity; however, 1 potential disadvantage to the use of knock-out animal models is that the various animal genes encoding the metabolic enzymes may not be similar to those in the human.

To better study the roles of CYPs in PAH toxicities to humans, humanized mice have also been developed. To produce these animals, the endogenous rodent genes are replaced with their human homologues or equivalents (Dragin et al., 2007; Gonzalez, 2007; Kazuki et al., 2012; Moriguchi et al., 2003). Corchero et al. (2001) used a BAC clone containing both CYP1A1 and 1A2 genes to generate a transgenic mouse line that was bred into either *Cyp1a1*-null or *Cyp1a2*-null background, thereby creating functional humanized CYP1A1 (Cheung et al., 2005) and humanized 1A2 mice (Jiang et al., 2005), respectively. The humanized CYP1A2 mice has shown preferential N<sup>2</sup>-hydroxylation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, a pathway that is predominant in human, based on in vitro studies (Cheung et al., 2005). Dragin et al. (2007) have also developed humanized mice that express both human CYP1A1 and 1A2, but lack their mouse orthologues, making these models ideal for studying human metabolism of PAHs in mice (Uno et al., 2009). Recently, Li et al. (2014) reported the establishment of a humanized CYP1B1 mouse line. The use of humanized mouse models will be essential in the future elucidation of the health effects of PAHs in humans, provide a link between the in vitro and human

studies, and assist in addressing many of the data gaps listed in this review.

## GENETIC SUSCEPTIBILITY TO PAH-ASSOCIATED LUNG CANCERS

Less than one-fourth of tobacco smokers are diagnosed with lung cancer (Ezzati and Lopez, 2004), and yet, ~1 out of 10 of lung cancer deaths is among non-smokers (Alberg et al., 2013), indicating that many genes and gene families affect the activation and elimination of PAHs and genetic susceptibility plays a role in lung cancer. Mutations, including single nucleotide polymorphisms (SNPs), of these genes alter their biological functions such as catalytic activities, hence, affect the toxicity of PAHs and have implications for studying the mechanisms of PAHs by adding to their complex mechanism(s) of action. These mutations have been demonstrated in CYPs, GSTs, and p53. For example, polymorphisms of CYP1A1, GSTM1, and GSTT1 affect the susceptibility of lung cancer induced by PAH exposure (Bennett et al., 1999; Ji et al., 2012; Ketterer et al., 1992; Przygodzki et al., 1998).

The discovery of SNPs associated with PAHs and lung cancer susceptibility has also increased due to the fast development of highly efficient and economic gene sequencing technologies. These genes include GSTP1, AKR, microsomal epoxide hydrolase (EPHX1), X-ray repair cross-complementing protein 1, and excision repair cross-complementing protein EERC2 (Ada et al., 2012; Lan et al., 2004; Penning and Drury, 2007; Sun et al., 2010; Timofeeva et al., 2010; Wang et al., 2013; Zhou et al., 2011).

Moreover, a mutation that increases the susceptibility of any human disease should be critically evaluated. Such studies should consider sample size, gender, age, ethnicity, smoking habit, occupational and environmental PAH exposure, and sub-categories of lung cancers. For example, Ada et al. (2012) surveyed 213 lung cancer patients and 231 controls in a Turkish population and found that GSTP1 exon 6 variant genotypes exhibited an overall increase in lung cancer risk. However, GSTM1-null, GSTT1-null, and GSTP1 exon 5 variant genotypes were not associated with a significant risk for developing lung cancer. Similar observations were also reported in a study of Caucasians (Timofeeva et al., 2010). Timofeeva et al. (2010) surveyed 17 SNPs and 2 deletion polymorphisms in 638 patients and 1300 controls under the age of 51. They found that the mutations in myeloperoxidase, EPHX1, GSTT1, GSTM1, GSTP1, and NQO1 genes showed no significant overall increase in lung cancer risk. Subgroup analysis revealed gender- and/or smoking-specific effects of EPHX1, GSTT1 deletion, GSTP1, and NQO1 polymorphisms.

Current research also suggests that multiple mutation sites in multiple genes are involved in the PAH-associated lung cancer susceptibilities. A genome-wide association study, which is based on genotyping arrays (Carvalho et al., 2013; Lange et al., 2014), has also found several SNPs that are associated with lung cancer incidences, in different nations and ethnic groups (Dong et al., 2012; Lee et al., 2013; Spitz et al., 2013). Cross-sectional investigation with PAH-induced genetic damage in 1557 Chinese coke oven workers has identified that 13q12.12-rs753955C is associated with elevated urinary 8-hydroxydeoxyguanosine level (Dai et al., 2014), a biomarker of oxidative DNA damage. Future studies of these multiple mutation sites will require increased sample size along with novel genetic analyses from next-generation sequencing to determine the various genetic susceptibilities which contribute to cancer as a result of exposure to PAHs.

## IMPLICATIONS FOR HUMAN HEALTH

There is great variability among different PAHs with respect to carcinogenic potency and their dose-response relationships (Deutsch-Wenzel et al., 1983; Grimmer et al., 1988). Moreover, in reality, the environmental PAHs often exist as mixtures. Tarantini et al. (2011) reported that the components in PAH mixtures can affect the carcinogenicity of each PAH, by exhibiting synergistic and antagonistic effects, often simultaneously. This finding complicates the evaluation of cancer risk for PAHs. Recently Cioroiu et al. (2013) assessed PAHs in the lungs of 31 patients with lung cancer in Romania. Fifteen PAHs were detected, of which benz[a]anthracene, anthracene, fluoranthene, BaP, benzo[b]fluoranthrene, benzo[k]fluoranthrene were considered the major components of the mixture (Fig. 2). This study is the first to record PAH concentrations in human lung cancer tissue, and indicates that lung cancer patients present high concentrations of carcinogenic (0.33–31.94 ng/g wet tissue, mean = 6.12 ± 7.31 ng/g wet tissue) and noncarcinogenic (2.46–218.19 ng/g wet tissue, mean = 45.57 ± 54.83 ng/g wet tissue) PAHs in lung tissue, thereby providing strong evidence that PAHs are etiologic factors in lung cancer in humans. Further mechanistic studies of the relevant components of these mixtures, as well as the mixtures themselves, are needed to determine which component(s) (and which when combined), play a role in the etiology of lung cancer.

In conjunction with studying mixtures, quantitative cancer risk estimates of PAHs are highly uncertain because of the lack of good-quality data. According to the World Health Organization Air Quality Guidelines for Europe, the unit risk is  $9 \times 10^{-5}$  per ng/m<sup>3</sup> of BaP as an indicator of the total PAH content, namely, lifetime exposure to 0.1 ng/m<sup>3</sup> would theoretically lead to 1 extra cancer case in 100 000 exposed individuals. This concentration of 0.1 ng/m<sup>3</sup> of BaP is suggested as a health-based guideline. Because the carcinogenic potency of fluoranthene has been estimated to be ~20 times less than that of BaP, a tentative guideline value of 2 ng/m<sup>3</sup> is suggested for fluoranthene. Guidelines still need to be determined for other significant PAHs such as phenanthrene, methylated phenanthrenes/anthracenes and pyrene, and large-molecule PAHs such as dibenz[a,h]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-cd]pyrene. Thus, it is only through careful mechanistic studies that recommendations can be provided in support of these guidelines.

## KNOWLEDGE GAPS IN MECHANISTIC RESEARCH OF PAHS

Although there is a great amount of literature on PAHs, there are still several knowledge gaps that need to be fulfilled in order to fully understand the complex nature of these compounds, and the gaps increase exponentially when considering that PAHs often exist in mixtures. For example, most mechanistic studies have been conducted using BaP, suggesting there is need for investigations with other human carcinogenic PAHs and mixtures of these PAHs. Most work has focused on parent PAHs, but alkylated and oxygenated PAHs are also present in the environment. Also, more structure-activity relationship work is needed to predict which PAHs might be lung carcinogens. Other areas of needed research include: AhR versus AhR-independent pathways of PAH-induced carcinogenesis; identification of susceptible individuals and the mechanisms that play a role in susceptibility; immune-related mechanisms of PAH-mediated lung cancer; and the role of epigenetics in

PAH-induced lung cancer. For example, Pavanello *et al.* (2010) recently showed that shorter telomere length in peripheral blood lymphocytes of workers exposed to PAHs is predictive of lung cancer risk. Finally, treatments to prevent or reverse the mechanisms that induce carcinogenesis induced by PAHs are necessary to reduce the incidence of lung cancer.

## CONCLUSIONS

In conclusion, this review focuses on the mechanisms of toxicity of PAHs, in relation to pulmonary carcinogenesis in humans. To tease the mechanistic effects of multiple PAHs will require an inter-disciplinary approach with systems biologists, epidemiologists, pathologists, omics researchers, mechanistic researchers, and biostatisticians who can analyze complex data sets. PAHs are a complex mixture, often with over 100 components, making these compounds difficult to study. The most well-known PAH, BaP, is just the beginning of our understanding of the components of these mixtures. Further research is needed on how individual or binary and higher order mixtures of PAHs induce genetic and molecular alterations. To add to the complexity, these mixtures should be investigated in susceptible populations such as those with genetic polymorphisms as well as in sensitive populations such as children. This research will lead to novel strategies for the prevention and/or treatment of human lung carcinogenesis mediated by environmental PAHs.

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