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Microsomal Epoxide Hydrolase 1 (EPHX1): Gene, Structure, Function, and Role in Human Disease

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Abstract

Microsomal epoxide hydrolase (EPHX1) is an evolutionarily highly conserved biotransformation enzyme for converting epoxides to diols. Notably, the enzyme is able to either detoxify or bioactivate a wide range of substrates. Mutations and polymorphic variants in the *EPHX1* gene have been associated with susceptibility to several human diseases including cancer. This review summarizes the key knowledge concerning EPHX1 gene and protein structure, expression pattern and regulation, and substrate specificity. The relevance of EPHX1 for human pathology is especially discussed.

Keywords

EPHX1; gene; structure; function; genotype; disease

1. Introduction

Microsomal epoxide hydrolase 1 (EPHX1, EC 3.3.2.9) also known as MEH, EPHX, EPOX, or HYL1 was first purified from rabbit liver by Watabe and Kanehira (1970). Human EPHX1 was then characterized by Oesch et al. (1974) in human liver. EPHX1 is an evolutionarily highly conserved biotransformation enzyme expressed in nearly all tissues and localized mainly in the microsomal fraction of the endoplasmic reticulum of eukaryotic cells. Human *EPHX1* gene orthologues have been found in 127 organisms. Humans possess two EPHX enzymes, namely microsomal EPHX1 (OMIM: 132810) and soluble EPHX2 (reviewed by Harris and Hammock 2013). The prototypical EPHX1 reaction involves conversion of epoxides to *trans*-dihydrodiols (Oesch et al. 1971a). The role of *EPHX1*

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Conflict of interest statement

The authors declare no conflict of interest.

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genetic variability in individual susceptibility to cancer has frequently been studied. Regarding the role of the *EPHX1* gene in human disease, mutations in *EPHX1* likely contribute to the development of several hereditary disorders, e.g., preeclampsia (OMIM: 189800, MedGen UID: 334689) or hypercholanemia (OMIM: 607748, MedGen UID: 18608). Common genetic variability in *EPHX1* has also been suggested to affect individual susceptibility risk to cancer in several studies (see Section 5).

2. *EPHX1* gene, its variability, and expression

Human *EPHX1* (Gene ID: 2052) is located on chromosome 1 (1q42.12), consists of nine exons, and spans about 35 kb (Jackson et al. 1987, Skoda et al. 1988, Hassett et al. 1994a, Hartsfield et al. 1998). Exons 2–9 of *EPHX1* encode three transcription variants differing in the 5'-untranslated region, while each translated protein product has 455 amino acids.

The complex regulation of *EPHX1* gene expression was originally attributed to the presence of alternative promoters (Gaedigk et al. 1997), while later the contribution of various post-transcriptional mechanisms, e.g., upstream open reading frames was demonstrated (Liang et al. 2005, Nguyen et al. 2013). Human *EPHX1* expression in the liver is selectively driven by the proximal E1 promoter, but an alternative promoter region (E1-b promoter) drives expression in other tissues from both adult and fetal sources (Liang et al. 2005).

In contrast to the high conservation of the E1-b sequence among human, chimp, and rhesus monkeys, E1-b was not identified in other vertebrate species (Yang et al. 2009), suggesting a more recent evolution in higher primates. It contains several Sp1/Sp3 binding sites (Su and Omiecinski 2014) and there are two DNaseI hypersensitive sites (HS-1 and HS-2) in the intronic region between E-1b and E1 (Su et al. 2014). These elements take part in cell- and tissue-specific transcriptional regulation of *EPHX1*. Recently, a novel human *EPHX1* transcript (E1-b') generated from the upstream promoter was reported to be expressed in a tissue-selective manner with the highest level in human ovary (Nguyen et al. 2013). Based on these findings, the authors suggested an *EPHX1* transcription-independent (both *cis* and *trans*) regulatory role of E1-b' (Nguyen et al. 2013). Dysregulation of *EPHX1* expression has been linked to several human pathologies including cancer (see Section 5). Delineating the regulatory mechanisms of *EPHX1* gene expression (Figure 1) is key to understanding the role of *EPHX1* in human disease pathology and for predicting organ-specific toxicities.

The complexity of *EPHX1* gene regulation also derives from a diverse array of transcriptional factors binding to regulatory sequences. GATA4 (OMIM: 600576) is the major activator of *EPHX1* expression while HNF3 (FOXA1, 602294 and FOXB1, 600288) was shown to act as a co-repressor in HepG2 cells (Liang et al. 2005). Furthermore, CEBPA (116897) interacts with DNA-bound NFY (A subunit, 189903; B subunit, 189904; C subunit, 605344) as an additional regulator of *EPHX1* expression (Zhu et al. 2004). Recently, it was shown that other nuclear receptors (HNF4A (600281), CAR (NR1H3, 603881), and RXR (RXRA, 180245 and RXRB, 180246)) also bind to the proximal *EPHX1* promoter region and regulate its expression in human hepatocytes (Peng et al. 2013).

Interestingly, in regard to systemic hormonal regulation, a study in primary cultured rat hepatocytes demonstrated that insulin positively and glucagon negatively regulate *EPHX1*

expression (Kim et al. 2003) while progesterone was later shown to regulate EPHX1 expression in the endometrium during menstrual cycle (Popp et al. 2010). However, the relevance of these observations for human physiology remains to be elucidated.

Gene expression of EPHX1 in most tissues and anatomical compartments was demonstrated by microarray, SAGE (Serial Analysis of Gene Expression), and RNASeq (www.genecards.org). Human liver and skin express the highest EPHX1 transcript and protein levels. EPHX1 expression is tissue-, age-, and sex-specific (see Hammock et al. 1997 for review), but also shows high inter-individual variation among humans (Mertes et al. 1985, Hassett et al. 1997). EPHX1 transcripts were found in human primary bronchial epithelial cells, but not in alveolar macrophages (Willey et al. 1996). Strong to moderate immunohistochemical EPHX1 protein staining was observed in synovial blood vessels and lining cells (Backman et al. 1999). Moreover, it was shown that the topology of EPHX1 on the cell surface (Alves et al. 1993) greatly varies between different cell types (Duan et al. 2012).

Deleterious mutations and more common gene sequence variations such as single nucleotide polymorphisms (SNPs) can affect the physiological function of the protein product, which may have consequences for disease development or progression. There are 142 *EPHX1* gene variations currently listed in the National Cancer Institute dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP>), of which several may have clinical significance (18 copy number variations and four SNPs, ClinVar, <http://www.ncbi.nlm.nih.gov/clinvar/>).

Taken together, the complexity of EPHX1 gene regulation, its expression pattern, and presence of functional genetic variability suggest that individuals may considerably differ in the capacity of EPHX1 to metabolize diverse substrates with potential consequences for disease pathophysiology.

3. EPHX1 protein structure

EPHX1 belongs to the family of α/β hydrolases (Ollis et al. 1992). Comparisons of homologies among microsomal epoxide hydrolases from phylogenetically different organisms suggest their origin from a common ancestor (Arand et al. 1994, Beetham et al. 1995, van Loo et al. 2006). The N-terminal part anchors the EPHX1 protein into the membrane (Craft et al. 1990), while the C-terminus contains catalytic residues (Zou et al. 2000). Although the three dimensional structure of human EPHX1 has not been characterized so far, the crystal structure of its orthologue from *Aspergillus niger* is available (Zou et al. 2000). Subsequent quantitative structure–activity relationship (QSAR) model suggests this structure is relatively useful to predict the binding of small organic molecules such as styrene epoxide to human EPHX1. However, congeners with bulky side groups probably disrupt the charge-relay part of the catalytic mechanism (Lewis et al. 2005). A schematic representation of the major structural motifs of epoxide hydrolases is detailed in Figure 2.

Deciphering the crystal structure of human microsomal EPHX1 is very important for further elucidation of structure-function relationships and prediction of mechanistic implications of *EPHX1* gene variants.

4. EPHX1 enzyme function

The EPHX1 catalytic cycle comprises a so-called catalytic triad. This triad consists of fast nucleophilic attack of the substrate by the EPHX1-Asp²²⁶ residue forming an enzyme-substrate ester intermediate and subsequent hydrolysis of this complex by activated water (Amstrong et al. 1981). Water activation is fuelled by proton abstraction from the EPHX1-His⁴³¹-Glu⁴⁰⁴ charge relay system (Oesch et al. 2000). Moreover, Yamada et al. (2000) showed that Tyr³⁷⁴ of human EPHX1 also performs a significant mechanistic role in substrate activation.

While EPHX1 appears to play an important role in organ-specific human physiology, it is generally accepted that unlike EPHX2, EPHX1 more readily converts xenobiotics than endogenous substrates and has mostly a detoxifying function. However, there is some evidence that EPHX1 plays an important role in organ-specific human physiology. EPHX1 was first shown to convert epoxides such as styrene oxide, 1-methyl-1-phenyloxirane, indene 1,2-oxide, and cyclohexene oxide into the respective diols (Oesch 1974). Later it became apparent that EPHX1 has a broader substrate specificity (Lu et al. 1979, Fretland and Omiecinski 2000) and a marked substrate-dependent variation in EPHX1 enzymatic activity among different species has been reported (Kitteringham et al. 1995).

Substituted imidazole, metyrapone, and ethanol have been shown to activate EPHX1 *in vitro*. Overall, microsomal EPHX1 plays a dual role in the biotransformation of xenobiotics. While it detoxifies certain carcinogenic compounds, e.g., butadiene, benzene, styrene, etc. (Decker et al. 2009), it can also activate procarcinogens such as polycyclic aromatic hydrocarbons on the other hand (Shou et al. 1996, Casson et al. 2006, El-Sherbeni and El-Kadi 2014).

EPHX1 is also expressed on the sinusoidal plasma membrane where it mediates the sodium-dependent transport of bile acids into hepatocytes (Ananthanarayanan et al. 1988). Androstene oxide and epoxyestratrienol were shown to be endogenous EPHX1 substrates (Vogel-Bindel et al. 1982, Newman et al. 2005). Recently, it was reported that EPHX1 metabolizes endocannabinoid 2-arachidonoylglycerol to free arachidonic acid and glycerol (Nithipatikom et al. 2014). Thus, EPHX1 may play an important role in the endocannabinoid signaling pathway and modulate, through its dysregulation, energy metabolism and immunity. Examples of reactions catalyzed by EPHX1 are shown in Figure 3 (for more information on EPHX1 substrates see recent review by El-Sherbeni and El-Kadi 2014).

Enzyme induction and inhibition by both xenobiotics and endogenous substrates is an important phenomenon that may influence drug-drug interactions or disrupt important physiological processes. Mouse and rat EPHX1 were shown to be readily inducible by xenobiotics in several animal studies (Hardwick et al. 1983; Honscha et al. 1991; Cho and Kim, 1998, Abdull Razis et al. 2011). EPHX1 expression was induced by phenobarbital, β -naphthoflavone, benzanthrane, and *trans*-stilbene oxide in human fetal hepatocytes *in vitro* (Peng et al. 1984). However, a subsequent study exposing human hepatocytes to prototypical chemicals indicated only modest EPHX1 induction (Hassett et al. 1998).

Nevertheless, several polycyclic aromatic hydrocarbons (Pushparajah et al. 2008) were been shown to induce EPHX1 in precision-cut liver slices prepared from fresh human liver. Recently, it was demonstrated that methyl-2-cyano-3,12-dioxooleana-1,9(11)dien-28-oate, a potent inducer of transcription factor Nrf2 (nuclear factor, erythroid derived 2, GeneID: 18024) significantly alters the expression of EPHX1 protein in mice (Walsh et al. 2014) implicating a potential role of redox homeostasis in EPHX1 expression. Besides routinely used 1,1,1-trichloropropene-2,3-oxide and cyclohexene oxide as EPHX1 inhibitors (Oesch et al. 1971b), fatty amides such as elaidamide or 2-nonylsulfanyl-propionamide, were also proposed as potent inhibitors of EPHX1 (Morisseau et al. 2008).

Although recent studies suggest the ability of EPHX1 to convert certain physiological substrates, (e.g., arachidonic acid derivatives), there is no convincing evidence that its inhibition *in vivo* has any therapeutic potential.

5. EPHX1 role in human disease

As discussed above, the current knowledge indicates that EPHX1 plays an important role in both cellular defense against toxicity of xenobiotics and in general physiological maintenance of some organs. The presence of inherited genetic variability affecting EPHX1 activity or dysregulation of its expression may contribute to the development of human diseases.

Mutations in *EPHX1* may cause preeclampsia (Zusterzeel et al. 2001, Laasanen et al. 2002), hypercholanemia (Zhu et al. 2003), and are suspected to contribute to fetal hydantoin syndrome (Buehler et al. 1990) and diphenylhydantoin toxicity. Despite one study which subsequently presented evidence to argue against a functional role of EPHX1 in etiology of anticonvulsant adverse reactions such as diphenylhydantoin toxicity (Gaedigk et al. 1994), two maternally transmitted *EPHX1* SNPs (rs1051740 and rs2234922) were later associated with risk of craniofacial abnormalities in children of women taking phenytoin during the first trimester of pregnancy (Azzato et al. 2010).

The most frequently studied SNPs Y113H (rs1051740, T337>C) and H139R (rs2234922, A416>G) were previously used as markers to predict EPHX1 activity (Benhamou et al. 1998). However, their effect on enzyme activity *in vitro* is modest towards *cis*-stilbene oxide, none towards benzo[a]pyrene-4,5-epoxide, and was not confirmed in human liver microsomes (Hassett et al. 1994b, Hosagrahara et al. 2004). Additionally, the E1-b promoter region harbors several functionally important polymorphisms including a double Alu insertion, which may influence interindividual susceptibility to toxicity of xenobiotics (Yang et al. 2009).

The role of EPHX1 in neurological disorders and cancers is currently among the most emerging issues in the area of EPHX1-linked pathophysiology.

EPHX1 transcripts have been detected in various areas of the brain, e.g., cerebellum, frontal, occipital, pons, red nucleus, and substantia nigra regions. The observed presence of the EPHX1 protein in neurons and astrocytes was suggested to have potential implications for neurotoxicity (Farin and Omiecinski 1993). Subsequently, EPHX1 protein expression was

identified in human brain tumor cells (Kessler et al. 2000). A role of EPHX1 in pathogenesis of neurodegeneration was further supported by the discovery of its differential expression in patients with Alzheimer's disease (Liu et al. 2006), possibly providing one mechanistic route underlining the previously observed link between the disease and environmental exposure (Heininger 2000). In animal studies, the differential subcellular localization of microsomal and soluble epoxide hydrolases in rat brain cortical astrocytes suggested their involvement in cerebrovascular functions (Rawal et al. 2009). Epoxide intermediates mediate methamphetamine-induced drug dependence, and as EPHX1 was reported to be an endogenous modulator of drug dependence in mice this may offer a novel therapeutic target for drug addiction treatment (Shin et al. 2009). A detailed study of mouse brains has also shown that EPHX1 contributes to the cerebral metabolism of epoxyeicosatrienoic acids which could interfere with neuronal signal transmission (Marowsky et al. 2009), vasodilation, cardiovascular homeostasis, and inflammation (reviewed by Tacconelli, Patrignani 2014).

Pharmacological interventions based on EPHX1 biochemical function have marked potential for clinical interventions. An example in regards to neurological disease derives from studies of Japanese epilepsy patients carrying an *EPHX1* diplotype consisting of at least two His alleles in both rs1051740 and rs2234922. These subjects showed increased plasma carbamazepine-diol/carbamazepine-epoxide ratios providing a rationale for future therapeutic interventions (Nakajima et al. 2005, Puranik et al. 2013). However, Clinical Annotation for rs1051740 and carbamazepine has level 2B evidence according to PharmGKB (www.pharmgkb.org) and therefore would not be recommended for clinical use yet. Moreover, a recent study failed to confirm the previously observed effect of rs1051740 and rs2234922 SNPs on carbamazepine metabolism in epilepsy patients of Caucasian ancestry (Caruso et al. 2014). Further investigations involving larger and well-defined patients cohorts are needed to answer the question of EPHX1 involvement in adverse effects of anti-epileptic drugs. A recent meta-analysis has suggested that the rs2292566 SNP in *EPHX1* may affect the maintenance dosage of the widely used anticoagulant warfarin (Liu et al. 2015). Although the exact mechanism behind this association is currently unknown, patients with this SNP may require a lower maintenance dose of warfarin.

A significant association of the low EPHX1 activity diplotype harboring the rs1051740 and rs2234922 SNPs with alcohol dependence was recently found (Bhaskar et al. 2013) which supports the previously suggested role of *EPHX1* genotype in the risk of alcoholic liver disease (Wong et al. 2000).

Despite considerable research on EPHX1-related physiological and pharmacological consequences for human health, most investigations involving EPHX1 have focused on its contribution to gene-environmental susceptibility to genotoxicity and carcinogenesis using both human and animal model studies. Gene knockout mice models (Ephx1-null; mEH^{-/-}) were shown by Miyata et al. (1999) to be fertile and to have no phenotypic abnormalities. The lack of bioactivation of 7,12-dimethylbenz[a]anthracene to the carcinogenic metabolite 3,4-diol-1,2-oxide by the mEH^{-/-} mice supports the role of mEH in bioactivation of certain polycyclic aromatic hydrocarbons (Miyata et al. 1999). Moreover, Bauer et al. (2003) observed the disappearance of benzene-induced hematotoxicity and myelotoxicity in

mEH^{-/-} mice compared with the wild type ones. This evidence was further corroborated by the observed modification of benzene metabolism in the human liver in subjects differing in their *EPHX1* rs1051740 or rs2234922 SNP status (Kim et al. 2007). These *EPHX1* SNPs were also shown to have an important role in epigenetic changes and hematotoxicity in benzene-exposed Chinese workers (Xing et al. 2013). A significantly reduced excretion of styrene metabolites (mandelic and phenylglyoxylic acids) in occupationally exposed individuals carrying the His allele in *EPHX1* rs1051740 has recently been observed (Carbonari et al. 2015). This study confirmed the previously suggested *EPHX1* role in the styrene detoxification pathway and genetic susceptibility to DNA damage in exposed subjects (Vodicka et al. 2001, Laffon et al. 2003, Vodicka et al. 2004, Costa et al. 2012). Healthy individuals carrying high *EPHX1* activity genotypes were recently found to have a decreased frequency of nonspecific chromosomal aberrations (Hemminki et al. 2015). As an increased frequency of chromosomal aberrations predicts cancer risk (Vodicka et al. 2010) this study provides a biological link between genetic susceptibility and genotoxicity with potential implication for carcinogenesis.

The importance of *EPHX1* for cancer development and progression was further supported by comparing its expression in tumor tissues with disease progression and clinical outcomes of patients. *EPHX1* protein expression was demonstrated in 89% of tumor tissues from breast cancer patients (Murray et al. 1993) and correlated with poor disease outcome in patients receiving tamoxifen (Fritz et al. 2001). Furthermore, the differential *EPHX1* protein expression observed in normal liver compared with hepatocellular and liver metastases specimens suggests a potential role in tumor progression (Fritz et al. 1996). Importantly for cancer biomarkers, Collier et al. (2001) suggested that immunohistochemical detection of *EPHX1* may be a useful diagnostic tool for hepatocellular carcinoma. Localization of *EPHX1* in the membrane changes during liver pathogenesis, e.g., neoplasia (Gill et al. 1983) or hepatitis infection (Akatsuka et al. 1986) and is often accompanied by aberrations of *EPHX1* structure (Akatsuka et al. 2007). *EPHX1* specifically binds hepatitis B spliced protein (HBSP) and enhances the carcinogenic effect of benzo[a]pyrene *in vitro* (Chen et al. 2010 and 2014). *EPHX1* protein expression in several human malignancies (e.g., breast, lung, ovarian, and colorectal carcinomas) except melanomas, lymphomas, and renal carcinomas was also observed (Collier et al. 2001) suggesting a potential biological relevance of *EPHX1* for these cancers.

Given the known *EPHX1* role in metabolism of pro-carcinogens and the complex nature of its regulation, the relevance of *EPHX1* gene variation for susceptibility to cancers has been addressed by more than 200 association studies (<http://www.cancerindex.org/geneweb/EPHX1.htm>). These studies suggested that the presence of *EPHX1* SNPs may significantly affect the risk of lung, upper aerodigestive tract, breast, bladder, and ovarian carcinomas (Jourenkova-Mironova et al. 2000, Sarmanova et al. 2004, Spurdle et al. 2007, Khedaier et al. 2008, Andrew et al. 2009, Goode et al. 2011, Tan et al. 2014, Perez-Morales 2014). Two non-synonymous *EPHX1* SNPs (rs72549341 and rs148240980) were recently predicted by *in silico* models as breast cancer susceptibility modifiers (Masoodi et al. 2012) providing and intriguing hypothesis for further study. Low activity *EPHX1* alleles harboring the rs1051740 SNP increased the risk of localized, but not advanced, prostate carcinoma

(Catsburg et al. 2012). However, recent meta-analyses have demonstrated the lack of association of rs1051740 or rs2234922 SNPs with the risk of breast, hepatocellular, and esophageal carcinomas (Hu et al. 2013, Duan et al. 2014). Lack of detailed investigation in sufficient sample sizes of gene-gene and gene-environment interactions presents the most probable reason for the reported inconsistencies.

The most convincing evidence is currently available for lung and colorectal cancers, for which environmental exposures and gene-environment interactions are proposed to play major etiological roles. Several independent studies in diverse populations have all observed no significant associations of *EPHX1* rs1051740 or rs2234922 SNPs with risk of colorectal carcinoma or adenoma development (Landi et al. 2005, van der Logt et al. 2006, Mitrou et al. 2007, Hlavata et al. 2010, Gilsing et al. 2012, Zhao et al. 2012). Nevertheless, a meta-analysis showed a trend towards a protective effect of SNP rs2234922 against colorectal cancer risk (Liu et al. 2012). Interestingly, a novel c.293G>A (p.R98Q) mutation in the *N*-terminus of *EPHX1*, located by exome sequencing, was recently proposed as a putative colorectal cancer predisposition variant (Esteban-Jurado et al. 2015). Thus, the relevance of *EPHX1* genetic variability for susceptibility to colorectal carcinoma remains an open question.

More convincing evidence exists in respect to exposure-related lung carcinoma risk. A recent meta-analysis on rs1051740 SNP has suggested an association of the His allele with increased lung carcinoma risk in Asian, but not Caucasian populations (Wang et al. 2013). Another meta-analysis confirmed that this SNP may be a risk factor for lung carcinoma in Asians, but observed its protective effect in Caucasians as well (Tan et al. 2014). Two haplotypes constructed using eight *EPHX1* SNPs were significantly associated with lung carcinoma risk in a population-based case-control study involving more than 4000 participants (Rotunno 2009). Most interestingly, genetically predicted (estimated using rs1051740 or rs2234922 SNPs) low *EPHX1* activity was associated with an increased risk of developing tobacco-related cancer in smokers among 47,089 individuals from the Danish general population (Lee et al. 2011). A further study reported that subjects exposed to environmental tobacco smoke and carrying low *EPHX1* activity alleles had a significantly increased lung carcinoma risk (Fathy et al. 2014). Thus, the evidence for a link of tobacco exposure, *EPHX1* genetic variability, genetic damage (Agudo et al. 2009, Peluso et al. 2013) and lung carcinoma risk seems particularly strong.

Considering the nature of biotransformation enzymes like *EPHX1*, then it could reasonably be expected that *EPHX1* gene variants play a significant role in the development of lymphoid malignancies. This hypothesis was supported by a case-control study in a Czech population, where the rs1051740 His allele was significantly underrepresented in males with non-Hodgkin's lymphomas compared to healthy control individuals (Sarmanova et al. 2001). Subsequently, the number of carriers of the predicted low *EPHX1* activity diplotype were found to be significantly higher among controls compared with Brazilian patients with acute lymphoblastic leukemia (ALL), suggesting a protective effect of low *EPHX1* activity against childhood ALL (Silveira et al. 2010). However, this was contradicted by a Turkish study where a higher ALL risk was associated with low *EPHX1* activity alleles (Tumer et al. 2012). It seems an interesting topic for further investigations to explore whether population-

specific EPHX1 haplotypes with potentially functional effects exist that could explain contradictory results of these studies.

Besides cancers, there are interesting parallels with non-malignant diseases. Similarly to lung cancer, the low activity EPHX1 phenotype based on the rs1051740 and rs2234922 SNPs was found to be a risk factor for chronic obstructive pulmonary disease (COPD) in Caucasian, but not in Asian populations (Li et al. 2013). COPD is more frequent in smokers and together with the above discussion of EPHX1 and lung carcinogenesis, it seems that EPHX1 plays a pivotal role in protecting the lung against environmental exposures.

From the reviewed information it can be concluded that there is a convincing link between EPHX1 dysregulation and neurological pathologies, including both degenerative disorders such as Alzheimer's disease and various forms of drug-dependence. Regarding the interplay with environmental exposure, *EPHX1* gene variation is a putative susceptibility factor for lung carcinogenesis while its role in colorectal and liver cancers requires further observational and mechanistic studies.

6. Conclusions

Taken together, human EPHX1 presents an example of an evolutionarily highly conserved metabolizing enzyme with unusually broad substrate selectivity. Current evidence suggests that EPHX1 is an important part of microsomal defense mechanisms against toxicity of xenobiotics and accumulating knowledge suggests that the enzyme also has essential physiological roles. Genetic variability of *EPHX1* is associated with several pathological phenotypes and may in concert with environmental exposures contribute to the development of malignancies, especially in the lungs. Transformation of this information into clinical application is, however, hindered by the lack of the crystal structure of human EPHX1 and by the complexity of unexplored relationships between its genotype and phenotype.

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Abbreviations

EPHX1	epoxide hydrolase 1
SNP	single nucleotide polymorphism

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Figure 1.

EPHX1 gene sequence and regulatory elements on chromosome 1 at position 1q42.12
 E=exons, HS=DNaseI hypersensitivity sites, kb=kilobases, rs=reference SNP ID number.
 Coding exons are marked in grey. The figure was prepared using data from Hassett et al. 1994, Liang et al. 2005, Nguyen et al. 2013, Su & Omiecinski 2014, and Su et al. 2014.

Figure 2.

Schematic representation of structure of homologous EPHX enzymes

Domains of cytosolic (white) and microsomal (black) EPHX enzymes based on three resolved structures (*Mus musculus*, *Aspergillus niger* and *Agrobacterium radiobacter*) shown. N-terminal and C-terminal catalytic domains and caps (grey) are highly conserved. The NC-loop has a variable length from 16 to 57 residues and the cap-loop has a variable length from five to 59 residues. The information was adopted from Barth et al. 2004.

Figure 3.
Examples of reactions catalyzed by human EPHX1