Xenobiotic-Induced Transcriptional Regulation of Xenobiotic Metabolizing Enzymes of the Cytochrome P450 Superfamily in Human Extrahepatic Tissues

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Abstract: Numerous members of the cytochrome P450 (CYP) superfamily are induced after exposure to a variety of xenobiotics in human liver. We have gained considerable mechanistic insights into these processes in hepatocytes and multiple ligand-activated transcription factors have been identified over the past two decades. Families CYP1, CYP2 and CYP3 involved in xenobiotic metabolism are also expressed in a range of extrahepatic tissues (e.g. intestine, brain, kidney, placenta, lung, adrenal gland, pancreas, skin, mammary gland, uterus, ovary, testes and prostate). Since the expression of the majority of the isoforms appears to be very low in the extrahepatic tissues in comparison with predominant expression in adult liver, the role of the enzymes in overall biotransformation and total body clearance is minor. However, basal expression and up-regulation of extrahepatic CYP enzymes can significantly affect local disposition of xenobiotics or endogenous compounds in peripheral tissues and thus modify their pharmacological/toxicological effects or affect absorption of xenobiotics into systemic circulation.

The goal of this review is to critically examine our current understanding of molecular mechanisms involved in induction of xenobiotic metabolizing CYP genes of human families CYP1, CYP2 and CYP3 by exogenous chemicals in extrahepatic tissues. We concentrate on organs such as the intestine, kidney, lung, placenta and skin, which are involved in drug distribution and clearance or are in direct contact with environmental xenobiotics. We also discuss single nucleotide polymorphisms (SNPs) of key CYPs, which at the level of transcription affect expression of the genes in the extrahepatic tissues or are associated with some pathophysiological stages or disorders in the organs.

Keywords: Transcriptional regulation, cytochrome P450, extrahepatic, xenobiotics, metabolism, intestine, lung, kidney.

1. INTRODUCTION

Hepatic xenobiotic metabolizing enzymes of cytochrome P450 (CYP) family play central roles in the overall metabolism and disposition of endogenous substrates and xenobiotics in humans. Expression of the enzymes in a number of extrahepatic tissues contributes to overall drug biotransformation, prodrug or procarcinogen activation, and co-determines systemic exposure, although magnitude of metabolic capacity and significance in total body clearance is much lower in comparison to hepatic biotransformation. Nevertheless, extrahepatic metabolism may determine local exposure to drugs and xenobiotics and thus influence their resultant pharmacological and toxicological effects in peripheral tissues.

Interindividual variation in drug response in terms of loss of drug efficacy or increase of drug toxicity is often related to variability in expression of genes involved in the drug disposition processes. Significant progress has been made over the past few years in unraveling the induction mechanisms of most CYPs in the liver. Less attention was, however, paid to study of xenobiotic-mediated induction processes for these genes in extrahepatic tissues. It appears that basal, induced, and repressed expression of drug disposition genes is largely under transcriptional control. Recent studies indicate that most CYP genes are transactivated via ligand-activated nuclear receptors (NRs) or transcriptional factors. Cytochrome P450 gene families 2 and 3 share similar mechanisms of xenobiotic-mediated gene activation through ligand-activated nuclear receptors Pregnane X receptor (PXR, NR1I2), Constitutive Androstane receptor (CAR, NR1I3), Glucocorticoid receptor (GR,

NR3C1), and Vitamin D receptor (VDR, NR1I1) in connection with some additional transcriptional factors such as Hepatocyte Nuclear Factor 4α (HNF4α, NR2A1) or Short/small Heterodimer partner (SHR, NR0B2). PXR, CAR and VDR nuclear receptors belong to the same subfamily of nuclear receptors (NR1I) and interact with a common heterodimerization partner, retinoid X receptor (RXR) [1-9]. Although the key nuclear receptors, including PXR, CAR, and HNF4α, have been identified for more than a decade, their functional role in the induction of CYP2B, CYP2C, and CYP3A genes has not been fully explained. Moreover, recent studies illuminated networks of the xenobiotic-activated nuclear receptors with other nuclear receptors or transcription factors, in particular the cholesterol-sensing liver X receptor (LXR), the bile-acid-activated farnesoid X receptor (FXR) and NF-kappaB, that regulate the homeostasis of bile acids, lipids, hormones, glucose, vitamins and inflammation. These findings provide novel insights into connection of intermediate metabolism with xenobiotic/drug metabolism [5, 10].

In contrast to CYP2 and CYP3 families, xenobiotic/drug-mediated inducible expression of CYP1 family genes is controlled by Aryl hydrocarbon receptor (AhR). AhR belongs to the basic helix-loop-helix/ Per-Arnt-Sim (bHLH/PAS) family of transcription factors and require AhR nuclear translocator (ARNT) as its heterodimerization partner [6, 11].

In this review, we will summarize recent findings regarding xenobiotic-mediated transcriptional regulation of genes of human families CYP1, CYP2 and CYP3 in the extrahepatic tissues. In detail, we will concentrate on major extrahepatic tissues and organs involved in drug distribution and clearance such as intestine, kidney, and placenta. In addition, we summarize data about transcriptional regulation of the genes in the lung and skin. These organs are in direct contact with environmental contaminants or are important sites of local application of medicines. We focus on transcriptional regulation mechanisms that determine basal tissue-specific expression or that control ligand/xenobiotic-induced expression of the genes in humans. We also discuss single nucleotide polymorphisms (SNPs) of key CYPs, which affect transactivation of the genes in the extrahepatic tissues.

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2. NUCLEAR RECEPTORS AND TRANSCRIPTIONAL FACTORS INVOLVED IN INDUCIBLE CYPs GENE REGULATION

There are 49 known members of the nuclear receptor (NR) superfamily. However, for many NRs, ligands are still unknown (so-called *orphan* receptors) or may not exist ("*true orphans*"). With respect to transactivation of xenobiotic-metabolizing CYP enzymes, ligand-activated NRs of subfamily NR1I play a key role. Members of the NR family share a common structure that includes a variable amino-terminal domain, a highly conserved central DNA binding domain (DBD) of about 70 amino acids and a carboxyterminal ligand-binding domain (LBD) of about 250 amino acids. Typically, there are two transcriptional activation domains in a nuclear receptor: the activation function 1 (AF-1), which resides in the N-terminal domain, and the AF-2, which is present in the C-terminal portion of the LBD [4, 12]. Ligands for the nuclear receptors are all small and lipophilic in nature, which permits them to diffuse into cells. The activation of nuclear receptors mostly takes place in the cytoplasm, where unliganded nuclear receptors typically reside. However, some nuclear receptors are localized in the nucleus, where they accept their ligands (e.g. LXRα). The schematic of PXR and CAR nuclear receptors activation is shown in Fig. **1**. The binding of a ligand to the LBD results in a conformational change in the AF-2 that disrupts interactions with transcriptional corepressor proteins such as NCoR and SMRT and permits formation of homodimers or, in many cases, heterodimers with RXR and interactions with transcriptional coactivator proteins such as members of the p160/steroid receptor coactivator (SRC) family

[12-14]. These heterodimer complexes bind short DNA sequence motifs, termed response elements (RE), which are located in the regulatory regions of target genes. Cognate REs for NRs are repeats of single hexamers in a distinct arrangement toward each other in terms of relative orientation and spacing. The hexamer half-sites have a canonical consensus sequence of AG(G/T)TCA. REs are categorized into direct repeats (DRs) and palindromic inverted repeats (IRs) or everted repeats (ERs)[4, 8, 9].

AhR is a ligand-activated transcription factor involved in the regulation of biological responses to many xenobiotics, pollutants, drugs and environmental contaminants. Classical exogenous AhR ligands are hydrophobic, planar or co-planar molecules of polycyclic structure such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin), 3-methylcholanthrene (3-MC), benzo[*a*]pyrene (BP) or β-naphthoflavone [15]. The AhR battery of controlled genes includes phase I xenobiotic metabolizing enzymes (CYP1A1, 1A2 and 1B1) and phase II enzymes (NQO1, GSTA2, UGT1A1 and UGT1A6)[11, 16]. The activated AhR/ARNT heterodimer complex binds to its cognate DNA sequences termed xenobiotic response elements (XREs) or digoxin response elements (DREs) [17]. The XRE sequence 5'-T/GnGCGTG-3' is not symmetrical suggesting that AhR and ARNT bind to different parts of the sequence.

3. CYTOCHROME P450

Humans have 57 genes and more than 58 pseudogenes divided among 18 families of cytochrome P450 genes and 43 subfamilies (David Nelson's P450 Homepage, http://drnelson.utmem.edu/ Cy-

Coactivators
PXR: NCOA1, NCOA2, FOXO-1, PGC-1
CAR: NCOA1, PGC-1, PPARPB

Corepressor
PXR: SMRT

Target genes
PXR:
CYP2A6
CYP2B6
CYP2C9, CYP2C19
CYP3A4, CYP3A5, CYP3A7
CAR:
CYP2B6
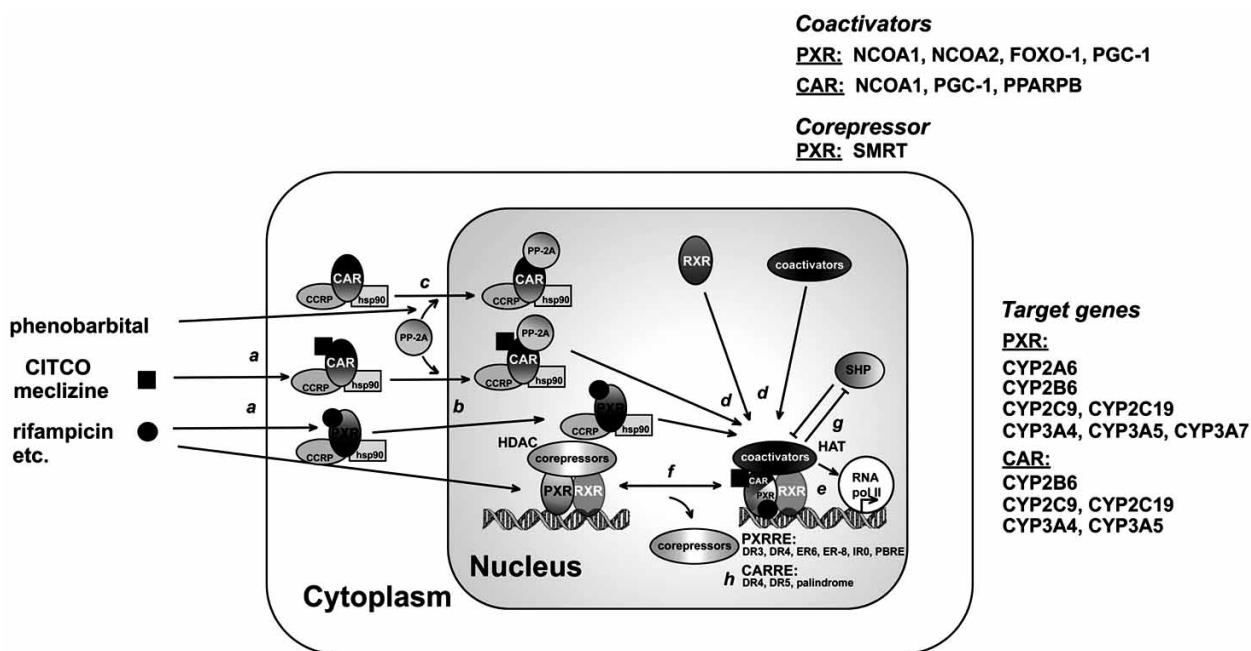CYP2C9, CYP2C19
CYP3A4, CYP3A5

Fig. (1). Schematic of PXR and CAR nuclear receptor transactivation. A ligand enters the cell either by passive diffusion or by an uptake transporter and binds to a nuclear receptor in the cytosol (*a*). CAR and PXR are retained in the cytoplasm in a complex with chaperones such as heat shock protein 90 (hsp90) or cytoplasmic CAR retention protein (CCRP) (a). Liganded nuclear receptor translocates to the nucleus (*b*). Phenobarbital triggers cytoplasmic-nuclear translocation of unliganded CAR complex indirectly by promoting the recruitment of protein phosphatase 2A (PP-2A) to the CAR/CCRP/hsp90 complex (*c*). The liganded nuclear receptor binds to RXR nuclear receptors forming PXR/RXR or CAR/RXR heterodimers, which recruits coactivators and bind to regulatory regions (PXRRE or CARRE) of a subset of target genes listed (*d*). The nuclear receptor heterodimer/coactivators complex promotes general transcriptional machinery of gene expression with RNA polymerase II (RNA pol II) (*e*). Coactivators possess histone acetyltransferase activity (HAT) that allows chromatin decompactation, which promotes gene activation. PXR is also supposed to be associated in a corepressor complex in the nucleus in the absence of ligand. The complex containing SMRT (NCOR2) corepressor recruits histone deacetylases (HDACs). Deacetylation of histones leads to chromatin compaction and transcriptional repression of a gene. Ligand binding to PXR nuclear receptor causes release of the corepressors, recruitment of a coactivator complex and transcriptional activation of gene expression (*f*). Feedback regulation of PXR-mediated regulation of CYP3A4 expression (*g*). SHR inhibits PXR-mediated transactivation of CYP3A4. Activated PXR inhibits SHP expression. As a consequence, SHP expression is reduced and CYP3A4 transcription is activated. Crosstalk of PXR and CAR – these nuclear receptors share responsive elements of their target genes (*h*) [223-226].

tochromeP450.html). Fifteen human CYPs are primarily involved in xenobiotic meablism, all of them being from CYP1, CYP2 and CYP3 families [18]. Genes encoding CYP enzymes, and the enzymes themselves, are designated with the abbreviation "CYP", followed by an Arabic numeral indicating the gene family, a capital letter indicating the subfamily, and other numerals for the individual gene. See also the homepage of the Cytochrome P450 Nomenclature Committee for the most detailed information (Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee, Karolinska Institute, http://www.cypalleles.ki.se). Members of the superfamily of heme-containing monooxygenases are involved in phase I of xenobiotic biotransformation, endogenous compound and steroid hormone metabolism, cholesterol biosynthesis, and steroidogenesis in eukaryotic organisms. They are localized mainly in the endoplasmic reticulum and inner mitochondrial membrane of cells.

3.1. Brief Overview of Families CYP1, CYP2 and CYP3 and Mechanisms of their Xenobiotic/Drug-Mediated Transcriptional Regulation

Families CYP1, CYP2 and CYP3 play a central role in phase I of xenobiotic biotransformation. The principal members of human CYP1A family are **CYP1A1, CYP1A2** and **CYP1B1** [19]. Human cytochrome CYP1A1 is located primarily in extrahepatic tissues, where its constitutive expression is virtually undetectable [20-22], but the enzyme is highly inducible in many organs by polycyclic aromatic hydrocarbons, such as 3-MC and BP, and halogenated aromatic hydrocarbons, such as TCDD [23]. CYP1A1 is one of the most important detoxification enzymes due to its broad substrate specificity and wide distribution throughout the body. Nevertheless, CYP1A1 can also produce highly carcinogenic intermediate metabolites through oxidation of polycyclic aromatic hydrocarbons [11, 16].

CYP1B1 gene is differentially expressed between tissues, with the highest constitutive levels of mRNA detected in extrahepatic tissues such as uterus, heart, brain, lung, skeletal muscle and kidney (see Table **1**), although its expression does not always correlates with protein expression. CYP1B metabolizes a range of polycyclic aromatic hydrocarbons [24] and is involved in the metabolism of endogenous steroids, retinol and retinal, arachidonate, and melatonin [25]. Moreover, estradiol 4-hydroxylation appears to be a characteristic reaction catalyzed by human CYP1B1. In humans CYP1B1 is overexpressed in some tumor cells and metabolic activation of estrogens has been postulated to be a major factor in endometrial carcinogenesis and breast tumors [25, 26].

CYP1A2 is a hepatic enzyme, it is expressed constitutively and is inducible by the same compounds as CYP1A1 and CYP1B1 [23]. Importantly, CYP1A2 metabolizes several clinically relevant drugs such as caffeine, tizanidine, zolmitriptan, and tacrine, which are not at the same time substrates of CYP1A1 [27].

The most important genes of CYP2 family contributing to xenobiotic metabolism of clinically relevant drugs and alcohol are **CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1,** and **CYP2F1**. CYP2C9 is the second most abundantly expressed CYP in human liver and intestine. It has been estimated that CYP2C9 metabolizes approximately 16% of clinically prescribed drugs [28]. Like in case of CYP3A4, PXR, CAR, VDR, HNF4α and GR have been implicated in regulating CYP2C9 expression [6, 29, 30]. A functional GR (GRE) and CAR/PXR response elements have been defined in CYP2C19 promoter [31].

Table 1.   **Relative Expression of Human Xenobiotic Metabolizing Enzymes of CYP1, 2 and 3 Subfamilies in Normal Extrahepatic Tissues at the Level of mRNA in Comparison with the Liver**

Enzyme	Small intestine	Kidney	Lung	Placenta	Liver
CYP1A1	+	+	+++ [c] +/-	++/+	++
CYP1A2	-	-	+/-	+ [a] /-	+++
CYP1B1	+	++/+	++/+	+	+
CYP2A6	-	-	++/+	+/-	+++
CYP2A13	+/-	+/-	+	-	+++
CYP2B6	++/+	++/+	+++	+/-	+++
CYP2C8	+	+/-	+/-	+/- [ab]	+++
CYP2C9	++	+/-	+/-	+/- [ab]	+++
CYP2C19	++	+/-	+/-	+/- [ab]	+++
CYP2D6	++/+	+	+	++/+ [a]	+++
CYP2E1	++/+	+	+++/++/+	+	+++
CYP2F1	-	-	+++/++	+/-	-
CYP2J2	++/+	+	+	++	++
CYP2R1	+	+	+	+	+
CYP2S1	++/+	+/-	+	+	-
CYP3A4	+++	+	+/-	+/-	+++
CYP3A5	+++/++	++	+++/++	+	+++/++
CYP3A7	+/-	+	+/-	+ [a] /-	+ [d]
CYP3A43	-	+/-	-	-	+++

+++ = organ with high expression; ++ = organ with moderate expression; + = low expression; - = undetectable expression; +/- = controversial expression or reports.
[a] expressed in the first trimester
[b] CYP2C expression reported in the first trimester [190]
[c] smokers, +/- mRNA expression in non-smokers
[d] the fetal liver expresses the highest level
Data of the table were adapted from papers [22, 58, 59, 133, 146, 151, 189, 190, 232].

Induction of human CYP2A6 is mediated by PXR with peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) [32]. It was recently demonstrated that GR controls expression of CYP2A6 in primary human hepatocytes despite the absence of functional GRE in the promoter. Cycloheximide had no effects on CYP2A6 induction by glucocorticoids implying that no downstream transcriptional factor, such as PXR, is needed for CYP2A6 induction. GR has been evidenced that transcriptionally controls CYP2A6 in complex with HNF4α1 without direct interaction between GR protein and DNA [33]. CYP2B6 is dominantly regulated by CAR and to the lesser extend via PXR and VDR nuclear receptor [34-36]. Induction of CYP2E1 by alcohol or acetone is thought to be caused by a posttranslational mechanism through stabilization of the enzyme protein, not involving a receptor-mediated mechanism [37, 38]. However, induction of CYP2E1 mRNA by ethanol was also reported in human hepatocytes [39]. HNF1α control in large part the hepatocyte-specific expression of CYP2E1 gene [38].

CYP3 family comprises only four members in humans: **CYP3A4, CYP3A5, CYP3A7** and **CYP3A43**. CYP3A4 is the most important isoform of the CYP3 family, which has been implicated in the metabolism of more than 50% of xenobiotics. Consistently, CYP3A4 is highly expressed in key sites of drug disposition such as in the liver and small intestine. CYP3A4 expression is regulated by a number of nuclear receptors, including PXR, CAR, VDR, GR,
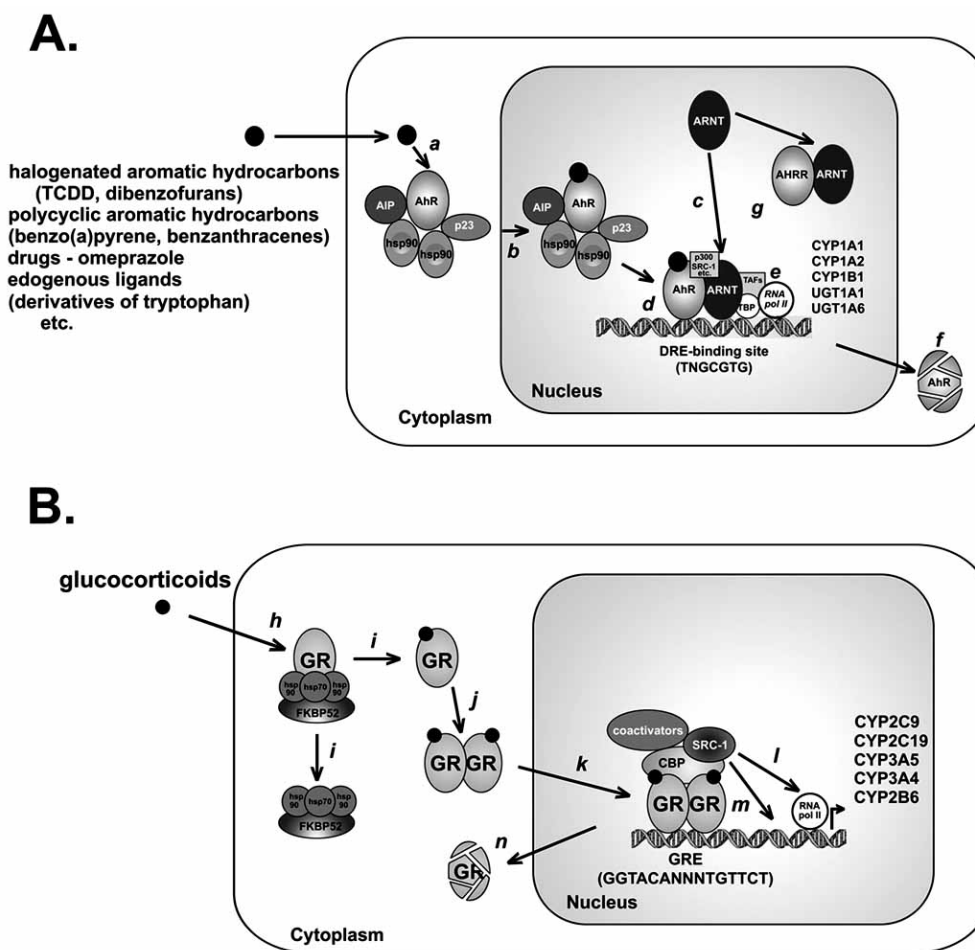

Fig. (2). A. Schematic of the ligand-activated AhR signal transduction pathway. (*a*) A ligand enters the cell and binds to the cytosolic complex of AhR, chaperones hsp90, co-chaperone p23 and aryl hydrocarbon receptor interacting protein (AIP, *syn.* ARA9). (*b*) Liganded AhR complex translocates into the nucleus. (*c*) The AhR forms heterodimer with the aryl hydrocarbon receptor nuclear translocator (ARNT). (*d*) The heterodimer than binds „digoxin responsive elements" (DREs, *syn.* xenobiotic response element -XRE). (*e*) AhR/ARNT heterocomplex recruits coactivator (such as SRC-1, NCOA2). AHR/ARNT binds with TATA-binding protein (TBP) and several TBP-associated factors (TAFs) leading to general transcriptional machinery with RNA polymerase II (RNA pol II) and transcriptional activation of target genes. (*f*) The AhR is then exported to the cytosol and degraded by 26S proteasome pathway. (*g*) Mechanism of negative feedback regulation of AhR function by the aryl hydrocarbon receptor repressor (AhRR). Ligand-activated AhR/ARNT heterodimer transactivates the expression of target genes including AHRR gene. AHRR suppresses AhR transcriptional activity by competing with AhR for dimerizing with ARNT and binding to the DRE sentence of target genes. [227-229]. **B. Schematic of transcriptional regulation through the glucocorticoid receptor (GR).** GR predominantly resides in the cytoplasm in a complex with several proteins and partners including chaperones hsp90 and hsp70, FK506 binding protein 4 (FKBP4, FKBP52) etc. (*h*). Binding of glucocorticoids induces conformational changes in the receptor, dissociation from chaperone proteins, dimerization of the receptor (*i*), nuclear translocation and DNA binding (*k*). Activated GR selectively recruits cofactors including SRC-1, TIF-2, p300/CBP, general transcription-factor-bridging factors, which activate general transcriptional machinery and recruitment of RNA polymerase II (*l*) or factors with intrinsic histone acetyltransferase and methyltransferase activities (*m*), which alter chromatin structure and facilitate access of transcription machinery components to DNA. GR is depredated by proteasone in cytoplasm (*n*).

GR regulates target gene expression through specific DNA sequences that bind activated GR and are termed glucocorticoid response elements (GREs)(CYP2C9, CYP2C19) or through another transcriptional factors (e.g. CYP3A4). GR has a "hit-and-run" mechanism of action rather than a stable association with GRE [230, 231].

HNF4α and FXR [6, 40]. CYP3A5 and CYP3A7 are transactivated by PXR and CAR NRs [41-43]. The constitutive intestinal and hepatic expression of CYP3A4 is highly dependent on the expression of PXR and HNF4α [6, 44, 45]. Similarly, employing antisense or small interfering RNA technologies, PXR and HNF4α were found to determine basal hepatic expression of CYP3A4, CYP3A5, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2B6 and CYP2D6 [46-48]. Moreover, it has been shown that HNF4α is a critical determinant of maximal PXR- and CAR-mediated induction of CYP3A4 through its binding to a DR1 site in the distal xenobiotic-responsive enhancer module (XREM) [49]. In contrast, more recently it was suggested that PXR strongly induces CYP3A4 gene transcription by interacting with HNF4α, SRC-1, and PGC-1α functioning as cofactors and concomitant inhibition of SHP [50]. Thus the latter study and recent paper by Tegude and coworkers [44] questioned the role of XREM DR1 HNF4α-binding site in inducible PXR-mediated transactivation of CYP3A4 in hepatoma HepG2 cells. Moreover, the authors suggested tissue-specific (intestine *versus* hepatocyte) interactions of HNF4α with its binding sites within XREM and constitutive liver enhancer module (CLEM) in basal expression of CYP3A4. Thus additional mechanistic studies should resolve discrepancies in the proposed mechanisms of HNF4α coregulation in PXR-controlled induction of CYP3A4 gene [44, 46, 49, 50]. Gnerre and coworkers showed that FXR agonist chenodeoxycholate (CDCA) and syntetic agonist GW4064 transactivated expression of CYP3A4 through two functional FXR recognition sites located in a 345-bp element within the 5'-flanking region of CYP3A4, and this inductive effect was independent of PXR [51].

In addition to this, CYP3A4 and CYP2B6 genes were shown to be regulated synergistically through GR and PXR nuclear receptors. The mechanism underlying the synergism has been proposed either through GR-mediated up-regulation of PXR and CAR receptors (in case of CYP3A4) or via a GR-dependent mechanism that does not require up-regulation of the nuclear receptors (in case of CYP2B6) [35, 45, 52].

Extrahepatic expression of major xenobiotic metabolizing CYPs in intestine, kidney, lung and placenta is summarized in Table **1**. Relative expression of major nuclear receptors and transcriptional factors controlling transcriptional regulation of the CYPs in intestine, kidney, lung and placenta is summarized in Table **2**.

3.2. Single Nucleotide Polymorphisms Affecting Transcriptional Regulation of CYPs in Extrahepatic Tissues

Polymorphisms affecting transcriptional regulation or gene expression at the level of mRNA play an important role in CYP phenotype variability. Several mechanisms of altered gene expression caused by SNPs have been proposed. SNP in *cis*-acting regulatory sequences can affect magnitude of transcriptional regulation and gene expression. Altered mRNA processing, pre-mRNA splicing, mRNA stability, mRNA trafficking, or affected regulatory RNAs are another mechanisms, which can be involved in variability of CYP genes expression [53]. Nevertheless, also polymorphisms in *trans*-acting factors should be considered since interindividual variability in target gene expression appears to be largely under control of *trans*-acting factors [54]. Thus interidividual variability in gene expression is determined by SNPs in *cis*- and *trans*-acting factors as well as by environmental and epigenetic factors.

The best known polymorphisms affecting gene regulation of major CYPs are CYP1A2*1C, CYP2A6*9, CYP2A6*1D, CYP2 A6*1H, CYP2B6*1B, CYP2B6*1G, CYP2D6*41, CYP2E1*1D, CYP2J2*7, CYP3A4*1B and CYP3A7*1C, although many more SNPs in promoter regions of almost all xenobiotic metabolizing CYPs have been identified [53](see also home page of the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee, http://www.cypalleles.ki.se). Strikingly, there is minimal data in

literature on the effect of the SNPs on CYP extrahepatic expression and we mention only a few reports on the issue.

4. INTESTINE

4.1. Constitutive Expression of CYP1, CYP2 and CYP3 Subfamilies Genes in the Intestine and their Function

Human small intestine epithelial cells (enterocytes) are the first site for CYP-catalyzed metabolism of orally ingested xenobiotics. On average, CYP3A and CYP2C9 represents the major parts of the intestinal cytochrome P450, accounting for 80 and 15%, respectively, of total immunoquantified P450s [55]. Largest interindividual variation in the expression levels was reported especially in case of CYP1A1, CYP1A2, CYP2A6 and CYP2E1 [56, 57]. Along the human gastrointestinal tract, CYP3A4 and CYP3A5 have their highest mRNA expression in the duodenum and the jejunum and than tend to decrease [58-60]. CYP2E1 has the highest expression in the stomach and duodenum [58]. Interestingly, the expression of CYP2E1 and CYP3A5 was reported to vary in different parts of the colon [61]. CYP1A1 and CYP1A2 were not or only faintly detected in duodenum and jejunum of some donors at the level of protein, although low levels of CYP1A1 mRNA were detected in the duodenum and jejunum [55, 62]. On the other hand, the expression of CYP1A1 and CYP1A2 mRNAs was observed in human colon and rectum [63]. For more detailed review on CYP expression in different parts of the gastrointestinal tract, we refer to the review by Ding and Kaminsky [64].

The small intestine metabolism by **CYP3A4**, either constitutive or induced, contributes to overall first-pass metabolism of many drugs (cyclosporin, simvastatin, nifedipine) which are administered per os [65-71]. Some studies have even suggested that the role of intestinal metabolism mediated by CYP3A4 is as important as hepatic metabolism, or even more important than hepatic metabolism in the overall first-pass effect, both under constitutive or induced conditions [65-68, 72-74], see review [74]. Similarly, intestinal CYP3A5, together with hepatic CYP3A5, was shown to play an important role in the first-pass effect of orally administered tacrolimus [68].

However another data deny any significant role of small intestine in first-pass metabolism and raise the question as to whether the role of intestinal metabolism is generally overemphasized [74, 75]. Clinical studies in patients with a portocaval shunt have demonstrated that the intestinal metabolism of nifedipine and verapamil was absent [76, 77]. Although recent reports suggest much higher content of CYP3A4 protein in human enterocytes isolated from human duodenal or jejunal mucosa than in paired specimens of liver tissue, the total CYP3A4 in the whole intestine is expected to be 30 times lower than that in the whole liver [78-80]. In addition, most literature reports suggest that the degree of CYP3A4 induction in the intestine is generally lower than the degree of hepatic CYP3A4 induction [74, 81, 82]. Similarly, weak induction of another PXR target genes, CYP2C8, and CYP2C9, by rifampicin in preparations of human enterocytes have been reported [82]. These results are consistent with the fact that the expression levels of PXR and HNF4α are substantially lower in the small intestine than those in the liver (Table **2**). Based on the data, we can hypothesize that small intestine possesses substantial CYP3A4 metabolic capacity; however, which could be clinically relevant only in case of excellent substrates with slow penetration through the intestinal epithelium.

The expression of CYP1 enzymes in alimentary tract is believed to be associated with chemically-induced carcinogenesis, in particular with colorectal carcinoma. For instance, the food constituents such as polyaromatic hydrocarbons contained in grilled or smoked meat are capable to induce CYP1 genes, which in turn convert these procarcinogens into active mutagens. Conversely, the compounds capable to inhibit CYP1 enzymes activities such as constituents contained in cruciferous vegetables (broccoli, cauli-

Table 2. Relative Expression of Major Human Nuclear Receptors and Transcriptional Factors in Normal Extrahepatic Tissues at the Level of mRNA in Comparison with the Liver

Gene	Small intestine	Kidney	Lung	Placenta	Liver
PXR (NR1I2)	++	+/-	-	-	+++*
CAR (NR1I3)	+/-	++	-	+/-	+++*
FXR (NR1H4)	+++/++	++	+/-	++/+	+++*
LXRα (NR1H3)	++	++	+	++	+++*
HNF4α (NR2A1)	+++/++	+++	+/-	-	+++/++
AhR (AHR)	+	++	+++/++	+++*	++
GRα (NR3C1)	++	++	++	+++/++	++
SHR (NR0B2)	+++	+	NA	++/+	+++/++
RXRα (NR2B1)	++	+++/++	++	++	+++
VDR (NR1I1)	+++*	+++/++	++	++	+
PPARα (NR1C1)	++	++	+	+	+++/++

+++ = organ with high expression; ++ = organ with moderate expression; + = low expression; - = undetectable expression; +/- = controversial expression or reports; * = the organ with the highest expression; NA = data not available.

Data of the table were adapted from papers [233-237].

flower etc.) [83, 84] or AhR antagonist resveratrol contained in red wine [85] were reported as anti-cancer nutrition agents [86].

4.2. Transcriptional Regulation of Xenobiotic Metabolizing CYPs in the Intestine

Accumulating evidence suggests differential modulation of hepatic and intestinal cytochrome P450 gene expression by PXR ligands both in mice [87-89] and in humans [66]. Moreover, the basal expression of the PXR target genes, CYP3A4 and MDR1, does not appear to be co-regulated in the liver and intestine [78]. It has been suggested in mice that CAR plays a greater role than PXR in hepatic drug disposition gene expression, whereas the converse may be the case in the intestine [89]. In addition, sex-related differences exist in the extent of intestinal and hepatic CYP3A induction by rifampin [66]. This indicates that other factors are involved in controlling gene expression in response to a PXR agonist in these tissues [87]. The large interindividual variation in the extent of induction is explained in part by the variation in baseline expression of CYP3A4 both in the intestine and liver [66, 90], which, in turn, is determined by HNF4α expression [44, 46].

Recently, Burk's group has discovered novel aspects concerning intestine-specific transcriptional regulation of CYP3A genes. The group demonstrated contribution of HNF4α in direct regulation of basal CYP3A4 expression in the intestine (and in liver) and found correlation between intestinal expression of CYP3A4 and HNF4α ($r=0.47$, $n=21$) [44]. Importantly, the authors also demonstrated intestine-specific molecular mechanism of HNF4α-mediated constitutive transactivation of CYP3A4. Moreover, they indicate that CYP3A4 and CYP3A7 are differently transactivated by HNF4α in intestinal cells although the DR1(II) element is present in promoters of both genes. This discrepancy is due to base -189T localized close to DR1(II) site in CYP3A4 promoter and corresponding base -188T in CYP3A7, which binds unknown intestine-specific protein. Finally, good correlation between CYP3A7 and HNF4α intestinal expression ($r=0.995$, $p=0.0004$) was demonstrated in CYP3A7*1C heterozygotes with -188G>T [91]. The SNP is associated with high expression of CYP3A7 in adult liver and intestine [91]. The authors also suggest that the ratio of HNF4α and COUP-TFII may contribute to the interindividual variability of CYP3A4 expression in the intestinal cells as these two transcriptional factors compete for the DR1 (III) element in distal CYP3A4 promoter region.

Interesting data have been recently published by Dr. Erin Schuetz's group opening new dimension of complexity in basal expression of CYP3A4 both in liver and intestine [92]. Authors show that MDR1 genotype 2677T is associated with higher CYP3A4 expression in the liver and intestine suggesting that the intracellular concentration of an endogenous low-molecular regulator of CYP3A4 expression is controlled by P-glycoprotein efflux transporter [92].

In agreement with PXR-mediated induction of CYP3A4 in the intestine, the target gene is inducible is some intestinal cell lines. LS180 and its variant LS174T are human colon adenocarcinoma epithelial cell lines, which were repeatedly shown to express inducible CYP3A4 and MDR1 genes. [44, 93-98]. In contrast, Caco-2 human colon adenocarcinoma cell line is PXR-deficient and CYP3A4 is not inducible in this cell line by PXR ligands [94].

Recently, Ma and coworkers reported rifaximin to be a gut-specific human PXR activator and inducer of *Cyp3a11* in PXR-humanized mice due to its poor absorption and consequent high concentration of the compound in the intestine [99]. This study thus show important aspect of intestinal CYPs transcriptional regulation via PXR and suggest that pharmacokinetic profile of a ligand/drug may determine tissue-specific effect on transactivation of CYPs.

Research of the mechanisms involved in **CYP3A5** induction revealed that PXR and CAR transactivate CYP3A5 through an everted repeat separated by 6 bp (ER6) [43]. Induction of CYP3A5 in intestinal biopsies by prototypical PXR ligand rifampicin was found to be CYP3A5*1 allele-specific [43]. Similarly, polymorphic CYP3A7 expression in adult human liver and intestine has been reported [91].

$1\alpha,25$-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$) and VDR were demonstrated to induce expression of CYP3A4 by binding of the activated VDR-RXR heterodimer to the CYP3A4 DR3 and ER6 response elements in intestinal cells *in vitro* [100, 101]. Correspondingly, rodent orthologues *Cyp3a11* and *CYP3A23* are up-regulated by $1\alpha,25(OH)_2D_3$ in the itestine *in vivo* [102, 103]. In addition, it was suggested that $1\alpha,25(OH)_2D_3$ strongly induce *Cyp3a23* in rat intestine, but not in liver, which corresponds with about 366-fold higher expression of rat intestinal VDR in comparison with the liver [103]. Consistently, 77-fold higher level of VDR mRNA expression was reported in the human jejunal mucosa compared to the liver [103].

The metabolism of $1\alpha,25(OH)_2D_3$ by intestinal CYP3A4 suggests that the enzyme may exert negative feedback control of

$1\alpha,25(OH)_2D_3$ transcriptional effects. Thus, increased expression of intestinal CYP3A4 through VDR, in response to $1\alpha,25(OH)_2D_3$ would result in enhanced metabolic elimination of $1\alpha,25(OH)_2D_3$ leading directly to impaired calcium absorption [104]. Importantly, VDR also functions as a receptor for the secondary bile acid lithocholic acid (LCA) [102]. It was suggested that activation of VDR by lithocholic acid, a hepatotoxic metabolite and potential enteric carcinogen, induced expression and activity of mouse *Cyp3a11* and CYP3A in general, which detoxifies LCA in the liver and intestine [102].

Very recently, Kosuge and coworkers demonstrated that physiological isotonic conditions may regulate the basal expression of CYP3A4, CYP3A5 and CYP3A47 genes in intestinal cell lines and human primary colonic cells and proposed the role of the nuclear factor of activated T-cells 5 (NFAT5) in tonicity-dependent expression of the genes [105].

Function and both basal and inducible expression of *Cyp1a1* throughout the mouse gastrointestinal tract has been comprehensively reported in the recent paper by Dr. Nebert's laboratory [106]. *Cyp1a1* has been shown as a detoxifying barrier to orally ingested BP. Moreover, *Cyp1a1* up-regulation compensated ablation of either *Cyp1a2* or *Cyp1b1* gene in the intestine of knockout mice [106].

Several clinical studies have been conducted to study inducibility of intestinal CYP1A1 gene. McDonnell *et al.* used model AhR activator omeprazole [62]. The authors found that omeprazole induced CYP1A1 mRNA and enzymatic activity in the duodenum. However, 35% of individuals had lower levels of CYP1A1 mRNA in several other alimentary tissues as well as low levels of CYP1A2 mRNA in the duodenum after treatment with omeprazole [62]. In a subsequent study, CYP1A1 protein and activity was found to be significantly induced in the human duodenum of smokers and omeprazole-treated patients [107]. Finally, in a recent clinical study, healthy adults were fed a diet enriched with chargrilled meat for 7 days [108]. The chargrilled meat diet resulted in significant induction of CYP1A enzymes in the liver and small intestine of each subject [108].

4.3. Influence of Polymorphism and Some Diseases on Intestinal CYPs Expression

Polymorphism is another important factor that determines expression of CYPs in different tissues. In case of CYP3A4, however, high variations (>20-fold) found in hepatic and intestinal CYP3A4 activity and expression cannot be explained on the basis of genetic polymorphisms of either 5'-flanking region, coding regions or introns as none of the SNPs influences CYP3A4 expression [40, 90]. Moreover, no 'null' allele has been found for CYP3A4 gene. Hence, variations in the expression of transcription factors controlling CYP3A4 transactivation (e.g. PXR and HNF4α) or their polymorphism could contribute to a variable CYP3A4 expression in the human intestine and liver [40, 44, 46]. Indeed, PXR -566C polymorphism was found to be significantly associated with increased CYP3A4 mRNA expression in colon tumor (P = 0.04)[109].

On the other hand, CYP3A5 is polymorphically expressed in human small intestine, where the isoform can contribute significantly to the total CYP3A activity [90, 110-112]. Variability of CYP3A5 expression in the liver and intestinal mucosa is associated with intronic SNP (CYP3A5*3 allele) resulting in a frameshift and truncation of the translated protein. For CYP3A7, the CYP3A7*1C allele was reported to be associated with high expression of CYP3A7 in adult intestine (and liver). The allele contains the replacement of a 60-bp promoter segment homologous to the CYP3A4 with ER-6 PXR binding site into the CYP3A7 promoter [91].

Incomplete intestinal metaplasia (IM) and increased risk of stomach cancer have been associated with CYP2E1 c1/c1 genotype

(CYP2E1*5), which has been linked to a higher transcription level, but with decreased enzyme activity [113-115]. CYP1A1 polymorphism was associated with colorectal cancer in numerous studies, although contradictive data have also been published (see reviews [116, 117]). In contrast, CYP2A6 polymorphism is well documented to be associated with colorectal cancer risk [118-120]. Similarly, polymorphism of CYP2C9 and CYP2C19 is linked with colorectal cancer risk, although no protective CYP2C9 genotype, which enhances the effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on the prevention of colorectal cancer, have been conclusively identified [118, 121-125]. Recent studies also found association between CYP1A2 and CYP1B1 and colorectal cancer susceptibility [121, 126-128]. The associations of the CYP polymorphisms to colorectal cancer risk could be related to CYP-mediated metabolic activation of dietary carcinogens such as polycyclic aromatic hydrocarbons and heterocyclic aromatic amines [125, 128].

Expression of intestinal CYPs was also analyzed under pathological conditions and has been related to some diseases. Dysregulation of CYP3A4, CYP2C9 and CYP3A7, and PXR activity in the colon was suggested to contribute to the pathophysiology of ulcerative colitis [129]. Decreased intestinal immunoreactive CYP3A has been also reported in celiac disease [130]. On the other hand, CYP3A4, CYP3A5, and P-glycoprotein levels were reported to be significantly higher in children with Crohn's disease in comparison with healthy children [131]. Under *in vitro* conditions, pro-inflammatory cytokines were reported to suppress expression of CYP3A4 in Caco-2 cells [132].

5. KIDNEY

CYP3A5 is the prevailing CYP3A isoform in the kidney [133], see Table **1**. CYP3A5 expression is strongly polymorphic and variable in the kidney. It was reported that individuals with CYP3A5 *1/*3 had 8-fold higher kidney microsomal CYP3A5 content and 18-fold higher CYP3A catalytic activity than did those from *3/*3 individuals [134]. Significant association has also been reported between the A6986G polymorphism of CYP3A5 and systolic blood pressure, mean arterial pressure, and creatinine clearance in healthy African-American adults [134]. Importantly, CYP3A5 is suggested to have important physiological function in metabolism of cortisol in the kidney, which regulates Na^+ transport in the nephron [90]. Therefore, variable expression of CYP3A5 in the kidney may have important effect on endocrine and paracrine functions of steroids and could play an etiological role in salt-selective hypertension in CYP3A5*1 allele [90]. The allele, but not CYP3A4*1B, has been also associated with increased risk for Balkan endemic nephropathy (BEN) [135].

While CYP2B6 mRNA expression is detectable in the kidney [21, 22], the immunoreactive CYP2B6 protein was not detected in renal microsomes [136]. In contrast to the latter report, Aleksa and coworkers showed enzymatic activity of both CYP2B6 and CYP3A4/5 in kidney microsomes [137]. Inducibility of *Cyp2b* genes has been studied in C57BL/6NCrj mice employing prototypical inducers phenobarbital, PCN (pregnenolone 16α-carbonitrile), DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) and dexamethasone; however, only dexamethasone up-regulated *Cyp2b9* mRNA [138].

CYP1B1, but not CYP1A1, is inducible in human renal adenocarcinoma cells by the typical AhR ligand TCDD [139, 140]. In contrast, both CYP1A1 and CYP1B1 were reported to be inducible in glomerular mesangial cells at the mRNA level by AhR ligands BP and TCDD [141].

CYP1B1 mRNA is expressed in normal and neoplastic kidney [21, 142]. However, its activity was not detected in normal tissue, but in most renal cell carcinoma samples [143]. CYP1B1 activates various environmental carcinogens in human tissues including the kidney. In addition, activities of CYP1B1 with SNP on codon 119 and 432 are 2- to 4-fold higher in comparison with the wild-type

enzyme. Therefore, polymorphism of CYP1B1 gene has been hypothesized as risk factor for pathogenesis of renal cell cancer. Indeed, Sasaki *et al.* found that 119T/T and 432G/G genotypes occurred with higher frequency in renal cell cancer patients, especially in males [144]. In addition, CYP1A1 polymorphism was associated with 2-fold increase in renal cell carcinoma (RCC)[145].

Other isoforms of CYP1, CYP2 and CYP3 families are not or at very low levels expressed in the kidney [22, 146].

6. LUNG AND RESPIRATORY TRACT

6.1. Constitutive Expression of CYP1, CYP2 and CYP3 Subfamilies Genes in the Respiratory Tract

The lung serves as a primary site for xenobiotic metabolism of environmental toxicants and airborne pollutants. The lung is composed of more than 40 different cell types [64]. Xenobiotic metabolizing CYP enzymes are expressed in bronchial and bronchiolar epithelium, Clara cells, type II pneumocytes, and alveolar macrophages [147]. Lung tissue is known to activate pro-carcinogens (e.g. polycyclic aromatic hydrocarbons or N-nitrosamines) into more reactive intermediates that easily form DNA adducts [148]. Essential role of pulmonary P450-mediated metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco smoke-derived nitrosamine, resulting in lung carcinogenesis has been clearly demonstrated in a recent paper employing lung-Cpr cytochrome P450 reductase-null mice [149].

Predominantly expressed CYP mRNAs in the lung and trachea are CYP1A1, CYP1B1, CYP2B6, CYP2F1 and to lesser extent CYP3A5 (Table **1**). The following drug-metabolizing CYP enzymes have been detected at the protein level employing specific antibodies: CYP1A1, CYP1B1, CYP1A2, CYP2A, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5 [147, 150, 151]. Conflicting results have been published for some CYP enzymes concerning their expression in human lung tissue at the mRNA, protein and activity levels [22, 146, 150]. In addition, there are also CYP enzymes which have been detected at the mRNA level, but not at the protein level (such as CYP2F1 in bronchial epithelium [152]) or which have been detected at the protein, but not at the activity level (e.g. CYP3A4)[153].

In the human larynx, mRNAs for the CYP1A1, CYP2A6, CYP2B6, CYP2C, CYP2D6, CYP2E1 and CYP3A5 were detected [154] and mRNAs of CYP2B6/7, CYP2C, CYP2E1, CYP2F1 and CYP3A5 were detected in human bronchoalveolar macrophages [155].

The CYP2F1 is a human cytochrome P450 that is selectively expressed in lung tissue, where it is propossed to be involved in the metabolism of various xenobiotics with potential carcinogenic effects. However, there is no evidence now that a CYP2F1 polymorphism has implication in the pathogenesis of lung cancer [156]. The tissue-selective expression of CYP2F1 is due to Sp1-dependent proximal promoter *cis* elements and Sp1 and Sp3 transcriptional factors, which control constitutive expression of CYP2F1 in lung cells [157]. In addition, a lung-specific binding motif in the 5'-upstream region of the CYP2F1 promoter binding a lung-specific factor (LSF) was identified [158].

CYP2S1 is also predominantly expressed in human lung and trachea [159, 160]. CYP2S1 is inducible by the typical AhR ligand TCDD and metabolizes naphthalene, although the enzyme does not activate/metabolize cigarette smoke carcinogens [159-161].

CYP1A1 and CYP1A2 mRNAs, proteins and catalytic activities were detected in fresh, small-sized lung biopsy specimens from human subjects [150, 162]. CYP1B1 mRNA (>95% of individuals) and protein (>75% of individuals) is commonly expressed in the normal human lung, however, the expression in tumor lung tissues is higher than that demonstrated in normal tissue [163].

Members of CYP2A subfamily (CYP2A6 and CYP2A13) are highly expressed in nasal and olfactory mucosa, but less in the lung [22, 64, 146]. CYP2A enzymes metabolize a variety of carcinogens including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), aflatoxin B1 or 1,3-butadiene, which can lead to the development of lung cancer [64, 164]. The mechanism of the tissue-specific expression of the human CYP2A genes as well as their rodent orthologues CYP2A3 and *Cyp2a5* involve a conversed binding site in their promoter regions, named the NPTA element. The regulatory element was found to interact with a unique protein of oftalmory mucosa termed NFI-A2 [64, 164].

CYP2E1 is expressed at the protein level in human lung or bronchial epithelial cells [150, 152] and CYP2E1 activities (oxidation of butadiene, demethylation of N-nitrosodimethylamine, 6-hydroxylation of chlorzoxazone and hydroxylation of *p*-nitrophenol) have been determined in human lung microsomes [165, 166] and bronchial epithelial cells [152]. Moreover, ethanol treatment increased CYP2E1 activity in short-term cultures of isolated bronchial epithelium [152]. CYP2E1 expression is controlled by methylation of dinucleotide CpG residues in the 5' end of the CYP2E1 gene in the lung [167]. Hypomethylation of the CYP2E1 gene was associated with low expression of the CYP2E1 gene in lung tumors [166].

The CYP3A family of monooxygenases plays a key role in pulmonary drug metabolism. Inhaled drugs, such as salmeterol, theophylline, or glucocorticoids (e.g., budesonide) are substrates for CYP3A enzymes. Both CYP3A5 and CYP3A4 were detected in the human lung tissue [150]. CYP3A5, a predominant isoform of CYP3A subfamily in the respiratory tract and lung, is localized in the ciliated and mucous cells of the bronchial wall, bronchial glands, bronchiolar ciliated epithelium, bronchiolar columnar and terminal cuboidal epithelium, vascular and capillary endothelium, alveolar macrophages and in type I and type II alveolar epithelium [147, 153, 168]. On the other hand, CYP3A4 was found in bronchial glands, bronchiolar columnar and terminal epithelium, type II alveolar epithelium, and alveolar macrophages, although only about 20% of individuals was positive in the immunostaining [153]. Recently, a putative double E-box repressor motif in the 5'-upstream region of the CYP3A4, but not CYP3A5 promoter, has been discovered, which attenuates CYP3A4 expression in the human lung cell line A549 cells derived from type II alveolar pneumocyte [169]. Induction of CYP3A5 and CYP3A4 genes was also examined in A549 adenocarcinoma cell line. CYP3A5 mRNA was markedly induced in the cell line by dexamethasone, but not by other prototype CYP3A4 inducers rifampicin, clotrimazole or nifedipine [168, 170]. More detailed study confirmed GR-mediated up-regulation of CYP3A5 in the cell line and show no expression of PXR and CAR [170].

6.2. Effect of Smoking on CYPs expression in the respiratory tract

Regulation of CYP1 genes in human lung is mediated by AhR/ARNT as in other tissues. Cigarette smoke contains agonists of AhR, such as dioxins and dioxin-like chemicals including PCDDs and PCDFs, and polycyclic aromatic hydrocarbons (PAHs) including benzo[*a*]anthracene, chrysene, benzo[*a*]pyrene, benzo[*b*] fluoranthene, benzo[*k*]fluoranthene, benzo[*g,h,i*]perylene and dibenzo[*a,h*]anthracene) [171]. Consistently, CYP1A1 and CYP1A2 mRNAs were induced by various model inducers including TCDD, benzo[*a*]pyrene, pyridine, nicotine, and omeprazole in explant cultures obtained from human lung specimen [172]. It was shown that Clara bronchiolar epithelial cells may be more sensitive to AhR ligands in induction of CYP1A1 and CYP1B1 than other cell types in the lung [173].

In another study, the expression of CYP1A1 and CYP1B1 proteins in normal human alveolar type I and II cells, ciliated columnar epithelial cells lining bronchoalveolar airways, and alveolar macro-

phages was demonstrated. The levels of CYP1A1 and CYP1B1 were elevated in smokers, and showed high interindividual variation [174]. Interestingly, it was found that women smokers exhibited a significantly higher expression level of lung CYP1A1 in comparison with men [175].

Levels of CYP1B1 mRNA were also up-regulated in bronchial mucosa of tobacco smokers [176]. Of interest, short-term exposure of mice to tobacco smoke induced *Cyp1b1* in the tongue and esophagus [176].

In lung tissue, complete or partial methylation of CpG sites up to 1.4 kb upstream of CYP1A1 gene promoter occurred in 33% of heavy smokers, 71% of light smokers, and in 98% of nonsmokers [177]. The methylation was found to increase in 1-7 days after quitting smoking. In active smokers the lack of methylation of CYP1A1 promoter was associated with a slightly higher pulmonary 7-ethoxyresorufin O-deethylase activity (EROD) activity. Thus it is believed that promoter methylation associated with tobacco smoking is involved in the regulation of CYP1A1 induction *in vivo* in the lung [177].

In another study, an association between AhR, CYP1B1, and CYP1A1 expression in noninvasive bronchioloalveolar carcinoma and lung adenocarcinoma was examined [178]. Expression of AhR and CYP1A1 was found to be associated in smoking adenocarcinoma patients. In contrast, expression of AhR and CYP1B1 was associated in adenocarcinoma patients regardless of smoking status [178]. The level of CYP1B1, but not CYP1A1, was positively associated with AhR overexpression in noninvasive bronchioloalveolar carcinomas. Authors also concluded that AhR overexpression up-regulates the expression of CYP1B1 in the early stage of lung adenocarcinoma [178].

Similarly as in liver, functional cross-talk between AhR and ERα was discovered in regulation of CYP1A1 and CYP1B1 genes in the bronchial epithelial cells [179]. ERα modulated both basal and cigarette smoke extract-induced expression of CYP1B1 mRNA, but not protein. Chromatin immunoprecipitation assay (ChIP) assay confirmed ERα binding to CYP1B1 promoter near the transcription start site. On the other hand, ERα did not alter the CYP1A1 mRNA level, but increased protein expression after cigarette smoke extract and/or 17β-estradiol exposure. Hence, ERα modulates the CYP1B1 expression at a transcriptional level, and CYP1A1 expression at a translational level. These data raise the possibility that inter-gender differences in expression of ERα in human lung may influence expression of CYP1A1 and CYP1B1, resulting in differences in carcinogen metabolism and cancerogenesis [175, 179].

Gene expression of major xenobiotic metabolizing CYPs and several nuclear receptors was also examined in bronchoalveolar lavage cells and bronchial biopsies derived from both smokers and nonsmokers [180]. Gene expression of CYP1A1, CYP1B1, CYP2S1, the liver X receptor (LXR) and the glucocorticoid receptor was induced in bronchoalveolar lavage cells of smokers, whereas expression of CYP2B6/7, and CYP3A5 was repressed. In bronchial biopsies of smokers, CYP1A1, CYP1B1 and CYP2C9 were induced, but CYP2J2 was repressed [180]. The authors of the study suggested lung tissue–specific responses in CYPs expression to tobacco smoke. In the latter study, expression of AhR in bronchial cells was not affected by cigarette smoke, which contrasts to recently reported observation in human lung fibroblasts that cigarette smoke extract induced nuclear translocation and activation of the AhR [181]. Interestingly, the up-regulation of GR reported in bronchial cells [180] correlates well with the increased expression of CYP2C9, a target CYP gene transcriptionally regulated by GR [29].

Consistently with the report by Thum *et al.* [180], both mouse and human CYP2S1 were demonstrated to be inducible by dioxin (TCDD) in mouse lung after systemic administration or in human

lung carcinoma A549 cell line, which might indicate AhR-dependent induction of the isoenzyme [160].

Previously, Hukkanen and coworkers [170] demonstrated decreased CYP3A5 expression in alveolar macrophages of smokers in comparison with nonsmokers.

6.3. Influence of CYP Polymorphism on Lung Cancer Risk

The expression and regulation of CYP1 genes in human respiratory tract was studied in particular with respect to the involvement of these genes in pathogenesis and susceptibility to tobacco-induced lung carcinoma. Myriad studies and several meta-analyses were performed to study the association between CYP1 polymorphisms and incidence of lung carcinoma in non-smokers and smokers (see reviews [116, 117, 151]. Despite the fact that many contradictory studies have been reported on the proposed association, CYP1A1*2 alleles (*MspI* polymorphism) are now believed to play a role in lung cancer susceptibility and etiology [116, 117]. Similarly, inconsistent data have been published regarding CYP1B1 polymorphism [182-184]. Finally, inconclusive reports exist on association of CYP2D6, CYP2C19 and CYP2E1 genes polymorphisms with lung cancer [116]. In case of CYP3A4, a significantly increased risk of small cell lung cancer (SCLC) for CYP3A4*1B allele carriers in a gender-specific manner was found [185]. The CYP3A4*1B allele is related with a two-fold higher promoter activity [186]. Comprehensive discussion of all studies on the topic is impossible in the review and we therefore refer to the reviews by Agundez [116], Zhang and coworkers [151] and Gresner and coworkers [117].

Association of AhR polymorphism with lung cancer incidence was also studied [187]. Two AhR alleles with one amino acid replacement of Arg by Lys at codon 554 did not show any significant association with aryl hydrocarbon hydroxylase (AHH) inducibility or with lung cancer incidence in Japanese population [187]. Recently, the role of genetic polymorphism of AhR on lung cancer risk was evaluated in extended study [188]. Any of three tested AhR alleles did not show association with lung cancer risk. When haplotypes were composed of the AhR SNP sites, smokers with GGG haplotype showed the highest risk. Similarly, non-smokers with GGG haplotype and smokers without GGG haplotype showed significantly increased risk of lung cancer compared to non-smokers without GGG haplotype. This result suggests that haplotypes of AhR gene play an important role in lung cancer and that there is a synergistic interaction between AhR gene polymorphism and smoking for lung cancer risk [188].

7. PLACENTA

The placental trophoblast expresses several CYP enzymes at mRNA level, although only a few of them have detectable enzymatic activity (Table **1**). More CYP enzymes are expressed in the first trimester of pregnancy in comparison with full-term placenta [189, 190]. It is believed that feto-protective xenobiotic metabolism in the placenta is critical in particular in embryogenic and organogenic stages of the first trimester of pregnancy. In the second and third trimesters activities of xenobiotic metabolizing CYPs decline [191, 192].

CYP1A1 is the only placental xenobiotic metabolizing enzyme for which expression and inducibility have been convincingly demonstrated in the placental trophoblast throughout of pregnancy. In human first-trimester placenta, CYP1A2 mRNAs was identified, however, CYP1A2 mRNA was not detected in human full-term placenta [189]. AhR and ARNT are abundant in the placental trophoblast and CYP1A1, but not CYP1B1, are highly inducible in the placenta or placental trophoblast cell lines with AhR ligands [193-195]. In agreement, many papers reported significant induction of placental CYP1A1 activity in women exposed to cigarette smoke [196-198]. Importantly, placental CYP1A1 is also involved in the bioactivation of carcinogenic and promutagenic polycyclic aromatic hydrocarbons (PAHs), such as benzo[*a*]pyrene, to DNA-reactive

species forming DNA adducts both in the placenta and fetal tissues [199]. In addition to bioactivation/metabolism of xenobiotics, placental CYP1A1 is involved in formation of 2-, 4-, 6α-, and 15α-hydroxylated metabolites of 17β-estradiol, which is synthesized in a considerable quantity in the placenta to maintain gestation [198, 200]. Consistently, elevated CYP1A1 activity in the placenta from smokers has been associated with adverse birth outcomes (such as premature birth, intrauterine growth retardation, structural abnormality) [201].

Most studies using specific antibodies and substrates to identify the presence of CYP2A, 2B, 2C, 2D and 2E isoenzymes and their catalytic activities have yielded negative results in the placenta [202-204]. Moreover, CYP2A and 2B mRNAs have not been detected in human placenta at any stage of pregnancy [189, 190]. CYP2E1 has been detected at the level of mRNA and protein from the first trimester onwards and it has been demonstrated that ethanol is metabolized to acetaldehyde in the human placenta [190, 203, 205]. Like CYP1A1, CYP2E1 levels in the placenta exhibit considerable individual variation [167].

Even though CYP3A mRNAs and proteins have been detected in human placenta, no relevant activities for CYP3A enzymes (e.g. 6β-hydroxylation) have been reported so far [190, 202, 204].

Glucocorticoid receptor (GR) and VDR, but not PXR, are expressed in both placental syncytiotrophoblast and cytotrophoblast [22, 206], see Table **2**. Recently, we examined expression and activity of GR in several placental trophoblast cell lines in transcriptional regulation of CYP3A4 and CYP2C9, which are known target genes regulated by GR in the liver. We demonstrated that CYP3A4 and CYP2C9 genes are not significantly inducible by glucocorticoids in placental cell lines due to the absence of HNF4α and probably other hepatocyte-specific transcriptional factors [206].

8. SKIN

Mammalian skin is composed of the dermis and epidermis. The major cell type in the epidermis is the keratinocyte. Numerous CYPs have been detected in the skin at the level of mRNA (CYP1A1, CYP1A2, CYP1B1, CYP2A6/7, CYP2B6/7, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2S1, CYP3A4/7, and CYP3A5). However, only CYP1A1, CYP2B6/7, CYP2E1, CYP3A4/7 and CYP3A5 were immunodetected and their catalytic activities demonstrated [207].

The comprehensive paper on CYP1 genes regulation through AhR signaling pathway in human skin and keratinocytes was published by Swanson [207]. The highest amount of AhR protein is found in *Granular layer* and *Spinous layer*, and the lowest amount of AhR is found in the *Basal layer* of keratinocytes in the epidermis. Interestingly, VDR and GR nuclear receptor have opposite patterns of expression in epidermis [207]. In normal human keratinocytes, CYP1A1, CYP1A2 and CYP1B1 mRNAs were detected; however, only CYP1A1 protein was found in these cells [207]. Another authors described CYP1A1 protein in *Basal layer* and CYP1B1 protein in the epidermal cells other than the basal cell layer. They concluded that localizations of CYP1A1 and CYP1B1 in human skin are different and may be related to keratinocyte differentiation [208]. The AhR activators benz[*a*]anthracene and β-naphtoflavone were shown to induce CYP1A1 mRNA and catalytic activity in human epidermal keratinocytes [209]. Similarly, induction of CYP1B1 in human dermal fibroblasts by TCDD was observed [210]. The cell-type specific up-regulation of CYP1A1 mRNA (>100 fold) and protein was observed in normal human keratinocyte cultures but not in dermal fibroblast cultures. This up-regulation occurred in the absence of xenobiotics and the change in CYP1A1 levels depended on the cell shape and adhesion. These data indicate possible physiological role of CYP1A1 in the human skin [211].

An intriguing phenomenon is the induction of CYP1 genes in human skin by ultraviolet radiation UVB. Following the exposure of human keratinocytes and HaCaT cultures to UVB, the induction of CYP1A1 and CYP1B1 mRNAs and proteins was observed. This induction was of transcriptional origin and involved AhR receptor [208, 212, 213]. The process probably includes AhR activation by photoreactive products of tryptophan [214, 215]. UVB-mediated induction of cytochromes CYP1A1 and CYP1B1 in human skin will probably result in enhanced bioactivation of polycyclic aromatic hydrocarbons and other environmental pollutants to which humans are exposed, which in turn could make the human skin more susceptible to UVB-induced skin cancers or allergic and irritant contact dermatitis [208]. A novel strategy in protection of skin against UV light might thus involve the use of inhibitors of CYP1A1/1B1 enzymes in skin care products. An example is flavonolignan silybin and its dehydroderivative which we described to inhibit CYP1A1 in HaCaT and HepG2 cells [216].

CYP2S1 expression was investigated by quantitative real-time RT-PCR in skin of healthy volunteers and patients with psoriasis [217]. CYP2S1 expression showed pronounced individuality in constitutive expression of the enzyme and its induction after ultraviolet irradiation or topical drug treatment. Cutaneous expression of CYP2S1 was induced by ultraviolet radiation, psoralen-ultraviolet A (PUVA) treatment, coal tar exposure, and all-trans retinoic acid treatment. Moreover, it was demonstrated that all-trans retinoic acid is metabolized by CYP2S1 in the skin [217].

Expression of several other CYPs at the level mRNA, protein and activity was studied in proliferating human epidermal keratinocytes under constitutive conditions and after induction with various inducers [218]. In addition to genes of CYP1 family, CYP2B6, CYP2E1, and CYP3A5 were found in keratinocytes. Moreover, CYP3A4 was inducible after exposure to dexamethasone [218].

The CYP genotypes of CYP1A1 (in combination with the GSTT1 null allele), CYP1B1 and CYP2D6 have been reported to be associated with an increase in the incidence of skin tumors in humans [219, 220] or in mice [221].

CONCLUSION

There is a great progress in unraveling of the transcriptional mechanisms that regulate both basal and inducible expression of drug disposition genes. In contrast to the intensive research of xenobiotic-induced transcriptional regulation of CYPs in the liver, less attention was paid to study of the induction processes in extrahepatic tissues. Although the discovery of key nuclear receptors and ligand-activated transcriptional factors in CYP transactivation represents a major breakthrough in our understanding of the molecular mechanisms of CYP induction, much remains to be elucidated.

For example, all target genes regulated by nuclear receptors have not been identified so far. With respect to transcriptional regulation of CYPs in extrahepatic tissue, our understanding of the both basal and inducible tissue-specific gene regulation has just begun and identification of tissue-specific transcriptional factors and their involvement in transactivation of CYP genes in extrahepatic tissues is being intensively studied. It appears now that some nuclear receptors and transcription factors may be important determinants of basal expression of CYPs in liver and intestine and thus play an important role in interindividual variability of CYP enzyme activity in population. However, these findings come from several pilot studies examining transactivation of several genes via some nuclear receptors and considering only some SNPs. Little is also known about the effect of SNPs in regulatory sequences of CYP genes on their expression and inducibility in extrahepatic tissues. Other tasks in research of CYPs induction in extrahepatic tissues are the impacts of tissue-dependent splice variants of nuclear receptors and transcriptional factors [222] and their SNPs affecting transcriptional regulation of CYPs. Moreover, recent discoveries suggest that interaction of co-activators and co-repressors with nuclear receptors

could determine gene-, ligand- and tissue-specific transcriptional activation of CYPs. Intriguing connections have been recently discovered in terms of crosstalk of xenobiotic-activated nuclear receptors with other nuclear receptors or transcription factors that regulate the homeostasis of bile acids, lipids, hormones, glucose, vitamins and inflammation. These findings provide novel insight into connection of intermediate metabolism with xenobiotic metabolism.

However, there are still several challenging issues that remain to be elucidated regarding the general aspects of extrahepatic xenobiotic metabolism. For example, the contribution of CYPs in the gut and other extrahepatic tissues to first-past and overall metabolism is still unresolved. In context with the relevance of extrahepatic metabolism in total drug clearance, drug-drug interactions in extrahepatic tissues should be also considered. Other controversial issue is whether CYP-mediated metabolism of some toxic compounds is beneficial due to detoxification or is detrimental because of metabolic activation of procarcinogens. Comprehensive elucidation of these issues would encourage our research of CYP induction mechanisms in extrahepatic tissues. While the answers to these questions are not apparent, drug-mediated induction of CYPs in extrahepatic tissues has potential risk of unintended drug-drug interactions or adverse toxic effects of pharmacotherapy.

We can therefore conclude that comprehensive understanding of the extent and relevance of nuclear receptor-mediated drug disposition gene regulation in extrahepatic tissues may result in more effective and safer drug therapy.

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ABBREVIATIONS

AhR	=	Aryl hydrocarbon receptor
ARE/EpRE	=	Antioxidant response/electrophile response element
ARNT	=	AhR receptor nuclear translocator
CAR	=	Constitutive androstane receptor
CCRP	=	Cytoplasmic CAR retention protein, a mouse ortholog of human TPR2 co-chaperone
Cyp	=	Cytochrome P450
ChIP	=	Chromatin immunoprecipitation assay
DBD	=	DNA binding domain
DR	=	Direct repeat
ER	=	Everted repeat
FOXO-1	=	Forkhead box O1
FXR	=	Farnesoid X receptor
GR	=	Glucocorticoid receptor α
GSTA2	=	Glutathione S-transferase A2
bHLH/PAS	=	Basic helix-loop-helix/ Per-Arnt-Sim family of transcription factors
HNF4α	=	Hepatocyte nuclear factor 4α
HNF1α	=	Hepatocyte nuclear factor 1α
LBD	=	Ligand-binding domain
LXRα	=	Liver X receptor α
MDR1	=	Multidrug resistance gen-1, P-glycoprotein
NQO1	=	NAD(P)H dehydrogenase, quinone 1
NR	=	Nuclear receptor
NSAID	=	Nonsteroidal anti-inflammatory drugs

PAS	=	PER-ARNT-SIM homology domain
PCDD	=	Polychlorinated dibenzo-p-dioxins
PCDF	=	Polychlorinated dibenzofurans
PCN	=	Pregnenolone 16α-carbonitrile
PGC-1α	=	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
PPARα	=	Peroxisome proliferator activated receptor alpha
PPARBP	=	PPAR binding protein, *syn.* mediator complex subunit 1 (MED1)
PXR	=	Pregnane X receptor
PXRE	=	PXR response element
RXRα	=	Retinoid X receptor alpha
SHP	=	Short/Small heterodimer partner; small heterodimer partner (NR0B2)
SMRT	=	Nuclear receptor corepressor 2 (NCOR2); silencing mediator for retinoid and thyroid hormone receptors
SNP	=	Single nucleotide polymorphism
SRC-1	=	Steroid receptor coactivator-1 (NCOA1)
TCDD	=	2, 3, 7, 8-tetrachloro-dibenzo-*p*-dioxin
TonEBP	=	Tonicity-responsive enhancer binding protein, *syn.* Nuclear factor of activated T-cells 5 (NFAT5)
UGT1A1	=	UDP glucuronosyltransferase 1 family, polypeptide A1
VDR	=	Vitamin D receptor
XRE	=	Xenobiotic response element

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