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Nuclear Receptors in Drug Metabolism, Drug Response and Drug Interactions

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Abstract

Orally delivered small-molecule therapeutics are metabolized in the liver and intestine by phase I and phase II drug-metabolizing enzymes (DMEs), and transport proteins coordinate drug influx (phase 0) and drug/drug-metabolite efflux (phase III). Genes involved in drug metabolism and disposition are induced by xenobiotic-activated nuclear receptors (NRs), i.e. PXR (pregnane X receptor) and CAR (constitutive androstane receptor), and by the 1α, 25-dihydroxy vitamin D_3-activated vitamin D receptor (VDR), due to transactivation of xenobiotic-response elements (XREs) present in phase 0-III genes. Additional NRs, like HNF4-α, FXR, LXR-α play important roles in drug metabolism in certain settings, such as in relation to cholesterol and bile acid metabolism. The phase I enzymes CYP3A4/A5, CYP2D6, CYP2B6, CYP2C9, CYP2C19, CYP1A2, CYP2C8, CYP2A6, CYP2J2, and CYP2E1 metabolize >90% of all prescription drugs, and phase II conjugation of hydrophilic functional groups (with/without phase I modification) facilitates drug clearance. The conjugation step is mediated by broad-specificity transferases like UGTs, SULTs, GSTs. This review delves into our current understanding of PXR/CAR/VDR-mediated regulation of DME and transporter expression, as well as effects of single nucleotide polymorphism (SNP) and epigenome (specified by promoter methylation, histone modification, microRNAs, long non coding RNAs) on the expression of PXR/CAR/VDR and phase 0-III mediators, and their impacts on variable drug response. Therapeutic agents that target epigenetic regulation and the molecular basis and consequences (overdosing, underdosing, or beneficial

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Disclosure

Chandra Prakash, Baltazar Zuniga, and Chung Seog Song are joint first authors.

Conflict of Interest

No conflict of interest exists for any of the authors.

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outcome) of drug-drug/drug-food/drug-herb interactions are also discussed. Precision medicine requires understanding of a drug's impact on DME and transporter activity and their NR-regulated expression in order to achieve optimal drug efficacy without adverse drug reactions. In future drug screening, new tools such as humanized mouse models and microfluidic organs-on-chips, which mimic the physiology of a multicellular environment, will likely replace the current cell-based workflow.

Keywords

Nuclear receptors; PXR; CAR; Xenobiotic-response element; Gene induction; Phase 0-III mediators; Genetic polymorphism; Epigenetics; Drug interactions; Drug screening

1 Introduction

Drug metabolism, which occurs primarily in the liver and intestine, refers to the enzymatic modification and subsequent disposal of medicinally active compounds, originating either endogenously (as steroids, neurotransmitters, metabolic products like bile acids) or exogenously (as natural products or synthetic/semi-synthetic chemicals). Upon conversion to hydrophilic metabolites, drugs are eliminated from the body following biliary excretion and renal clearance by glomerular filtration and tubular secretion. Drug metabolism is also integral to the biotransformation of pro-drugs to pharmaco-active agents. Drug metabolism and disposition is coordinated by an array of liver- and intestine-expressed drug-metabolizing enzymes (DMEs) and drug-transporting proteins whose tissue abundance is transcriptionally regulated by specific nuclear receptors (NRs), which are ligand-activated transcription factors [1].

Of the 48 distinct receptors comprising the NR superfamily in humans, pregnane X receptor (PXR, NR1I2) and constitutive androstane receptor (CAR, NR1I3) are primary transcriptional regulators of the genes involved in the metabolism and elimination of drugs/drug metabolites [4, 2, 3]. PXR and CAR are generically referred as xenobiotic NRs, since structurally diverse drugs and environmental xenobiotics activate these two NRs. PXR and CAR are also activated by a number of endobiotics (steroids, sterols, retinoids, thyroid hormones, bile acids). In addition, PXR and CAR activation can result from receptor phosphorylation by various kinases that are activated in response to drug-mediated induction of specific intracellular signal cascades; in this case, drugs may not directly interact with the xenobiotic NRs [5]. Vitamin D receptor (VDR, NR1I1), beyond its classic role in calcium and phosphate homeostasis, has the ability to transcriptionally induce drug transporters and DMEs, especially in the enterocytes of intestinal tissue [6, 7]. In certain contexts, additional NRs, such as the bile acid-activated farnesoid X receptor (FXR, NR1H4); oxysterol-activated liver X receptor (LXR-α, NR1H3); fatty acid/eicosanoid-activated peroxisome proliferator activated receptor (PPAR-α, NR1C1), and retinoid-related orphan receptors (ROR-α, ROR-γ) regulate genes linked to drug absorption, distribution, metabolism and excretion (ADME) [8]. Hepatocyte nuclear factor (HNF4-α, NR2A1), a member of the NR superfamily, plays a synergizing role in the PXR- and CAR-mediated transactivation of DME- and transporter-encoding genes [11, 9, 10] .

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Altered activities of polymorphic variants of NRs and ADME-related gene products account for variable response to prescription medicine between individuals. Amino acid changes due to nucleotide polymorphisms in the coding region can influence protein stability or activity, while polymorphism at upstream, downstream or intragenic regulatory loci can alter NR-mediated ADME gene transactivation. Epigenetic, transcriptional and posttranslational regulation of xenobiotic NRs can further impact drug metabolism and clearance. Evaluation of drug-drug interactions (DDI), which result from changes in the level or activity of DMEs and/or transporters due to a second drug, is an essential component of drug development workflow.

In this review, we describe various classes of DMEs and transporters, present an overview of the molecular underpinnings for NR-mediated genetic and epigenetic regulation of ADME genes and consider roles for various NRs (especially PXR/CAR/VDR) and their target genes in differential drug response. Illustrative examples highlighting critical roles of xenosensing NRs, DMEs and transporters in DDI are also examined. Finally, we discuss current drug-screening platforms and their potential future improvements.

2 Drug Metabolizing Enzymes (DMEs) and Drug Transporters

The four phases of drug metabolism entail cellular uptake of therapeutic molecules (phase 0); their enzymatic oxidation (phase I) and conjugation (phase II), and efflux of drug metabolites for clearance (phase III). PXR and CAR activate genes involved in all four phases. General steps in drug metabolism and elimination are shown in Figure 1.2.

2.1 Phase 0 uptake proteins

Basolaterally located uptake proteins guide cellular entry of drugs from circulation; drug influx can be a rate-limiting step for drug metabolism and clearance [15, 12]. All uptake proteins are members of the solute carrier (SLC) protein family of which there are more than 300 members grouped under 52 subfamilies. Liver, intestine and kidneys are major sites of SLC expression. Most SLC proteins localize to cell membrane, although some may localize to mitochondria and other organelles. Proteins from nineteen *SLC* gene subfamilies have drug uptake activity. Most significant gene families of uptake transporters are *SLC15* (oligopeptide transporter), *SLC22* (organic anion/cation/zwitterion transporter), *SLCO* (organic anion transporting polypeptide) and *SLC47* (organic cation transporter) [13, 14]. For example, OCT1 is a *SLC22A1* encoded organic cation uniporter involved in the influx of the antiviral agent acyclovir, ganciclovir and the anti-diabetic drug metformin. Drug substrates for proteins encoded by *SLC15*, *SLC22*, *SLCO,* and *SLC47* families have been described [13, 14, 15]. SLCs either serve as channels (uniporter) to guide drug diffusion down an electrochemical gradient, or drive drug transport against a diffusion gradient that is coupled to the symport or antiport of small inorganic or organic ions.

2.2 Phase I DMEs

Heme-containing cytochrome P450s (CYPs), flavin-containing monooxygenases, monoamine oxidases and xanthine oxidase/aldehyde oxidases are examples of phase I DMEs, which generally localize to the endoplasmic reticulum of cells. CYP enzymes play

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the most prominent role in phase I metabolism. Liver is the first pass and primary site of phase I metabolism, along with the gastrointestinal tract, kidneys, skin, and lung serving as additional sites; most tissues, however, express phase I DMEs. Addition or exposure of polar functional groups (e.g., –OH, hydroxyl; –COOH, carboxyl; –NH2, amine; –SH, sulfhydryl) to drug substrates enhances their bioavailability and solubility and promotes pro-drug biotransformation. Polar groups also arise by reduction of a ketone or aldehyde group to an alcohol; oxidation of an alcohol to an acidic group; hydrolysis of esters and amides; reduction of azo and nitro groups; oxidative dealkylation of N-alkyl, O-alkyl, and S-alkyl groups. When sufficiently polar, phase I metabolites can be pumped out of cells without additional modification.

CYPs are products of a multigene family, which for humans include 57 *CYP* genes [16]. Individual CYP is specified by the family (with an Arabic numeral), then the subfamily (with a letter) followed by the isozyme within the subfamily (with another Arabic numeral) and the allele number (with a preceding asterisk) of an individual gene within the subfamily. As an example, for CYP2D6*1, the *1 allele is wild type CYP2D6 with normal activity. Additional alleles of CYP2D6, marked with higher numbers preceded by *, exhibit aberrant functions [17]. CYPs are expressed in practically all tissues, with liver exhibiting the highest abundance and expressing largest number of individual CYPs. Enzymes of the CYP-1, -2, and -3 families metabolize majority of drugs and nondrug xenobiotics. The fraction of clinical drugs that are substrates for individual CYPs is schematically presented as Figure 2.2. CYP3A4, the most abundant CYP enzyme in human liver, acts on the greatest number of drugs and other xenobiotics. CYP2D6, although present at lower abundance, metabolizes numerous drugs. Substrate specificity is narrower for other members of the CYP family that are expressed in hepatic and extrahepatic tissues. They target endogenous substrates like sterols, fatty acids, eicosanoids and vitamins. A comprehensive list of drug substrates for CYPs has been reported ([18]; http://www.pharmacologyweekly.com/cytochrome-cyp-p450-enzyme-medication-herbs-substrates, *updated May, 2015*).

2.3 Phase II DMEs

Broad-specificity phase II transferases catalyze conjugative reactions. Common phase II modifications are glucuronidation by UDP-glucuronyltransferase (UGT), sulfonation by sulfotransferase (SULT), glutathionylation by glutathione S-transferase (GST), acetylation by N-acetyl transferase (NAT) and methylation by methyltransferase (MT). For any given transferase family, individual family members show predilection for a distinct set of substrates. Cofactors of phase II transferases react with functional groups that are either part of the parent drug or generated by phase I modification. In contrast to the enhanced potency of many phase I metabolites, phase II modified drug metabolites normally exhibit diminished function. PXR- and CAR-mediated gene regulation for a number of phase II transferases has been studied [9, 19].

2.4 Phase III efflux proteins

Members of the ATP binding cassette (ABC) superfamily, encoded by the *ABCB*, *ABCC*, and *ABCG* gene subfamilies, are broad-specificity exporters that pump drugs out of cells using energy from ATP hydrolysis. In hepatocytes, efflux proteins reside either in

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canalicular/apical membranes or in blood-facing basolateral/sinusoidal membranes, guiding drugs, endobiotics and their metabolites for biliary excretion and efflux into systemic circulation. Multidrug-resistance associated proteins MRP2 (ABCC2), the bile salt export pump BSEP (ABCB11), the breast cancer resistance protein BCRP (ABCG2) are examples of ABC cassette family transporters which mediate apical efflux of drugs, steroids, bile acids and their conjugates. P-glycoprotein (MDR1, ABCB1) is an apical membrane transporter in hepatocytes [13]. Basolateral efflux of unconjugated and phase II-conjugated drugs, steroids, prostaglandin and bile acids from hepatocytes into sinusoidal blood is assisted by ABC transporters such as the multi-drug resistance associated proteins MRP3, MRP4, MRP5 and, also, by the ATP-independent OSTα/OSTβ complex that functions as an organic solute and steroid transporter. OST α contains seven transmembrane domains and OST β has a single transmembrane domain [20]; neither is part of the ABC transporter superfamily. MATE (multidrug and toxin extrusion) efflux transporters are H^+-coupled antiporters, which transport structurally unrelated organic cations out of cells. They are members of the solute carrier subfamily SLC47, expressed primarily in the liver and kidney, and they localize at apical membranes of renal tubular epithelia and bile canaliculi. MATE1 (product of *SLC47A1*) mediates extrusion of organic cations into urine and bile. In the human kidney, the uptake transporter OCT2 (organic cation transporter, *SLC22A2* encoded) promotes the import of cationic drugs (such as metformin, cisplatin, imatinib) from the blood at the basolateral membrane of the proximal tubule epithelial cells. MATE-1 and the isoform MATE-2K mediate secretion of cationic drugs across the brush-border membrane into the proximal tubule lumen [21].

3 Nuclear Receptors (NRs), Response Elements, Gene Regulation by PXR/CAR/VDR

NRs, upon association with DNA response elements, induce a cascade of protein-protein interactions that lead to the assembly of multiple classes of regulatory proteins (coactivators, corepressors, histone modifiers, chromatin remodeling complex) at the NR-bound chromatin region. Signal transmission from the coregulator assembly to the basal transcription machinery via a multi-protein mediator complex culminates in altered RNA polymerase II activity and transcriptional response of NR-regulated genes.

The NR superfamily of ligand-activated transcription factors in humans is defined by 48 receptors grouped into four classes (Type I–IV) based on the nature of activating ligands, preferred sequence organization of NR-binding DNA response elements in target genes and dimerization partner of the activated NR [22, 23]. Type I NRs reside in the cytoplasm in an inactive state in association with chaperone proteins. They are activated upon binding cognate steroid hormone ligands, translocated to the nuclear compartment and bind target gene response elements as homodimers to mediate gene regulation. Type II receptors, such as the vitamin D receptor (VDR), are activated by nonsteroid endocrine ligands (1α,25-dihydroxy vitamin D_3 (1,25-D_3 , in short) for VDR; retinoic acid-all trans, for RAR-α/-β/-γ; thyroid hormone for TR-α/-β). Several Type II receptors are activated by intracrine ligands (e.g. bile acids activating FXR-α; oxysterols activating LXR-α/-β; fatty acids/eicosanoids activating PPAR- α/-γ/-δ). Type II NRs in an inactive state remain tethered to corepressors

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as heterodimers with the obligate partner retinoid X receptor (RXR). Exchange of corepressors for coactivators initiates activation of ligand-bound Type II NRs. PXR and CAR, comprising the Type III subgroup, are transported from cytoplasm to the nucleus upon activation by chemical inducers. Nuclear PXR and CAR bind to DNA response elements as dimers with RXR to set the stage for subsequent regulation of target gene transcription. Activation of CAR in most cases entails a ligand-binding independent mechanism, as reported for phenobarbital-like chemicals, which induce dephosphorylation of CAR at threonine-38, thereby activating CAR and promoting its nuclear translocation. Direct ligand binding and activation of CAR has also been reported for some xenobiotic compounds [24, 25]. Type IV NRs (e.g., LRH1, NGF1-B/NUR77, RORs) bind to DNA elements as a monomer, homodimer, or even as a heterodimer, partnering with RXR or another member of the same subfamily [26]. Although PXR, CAR and, to a significant extent VDR, are primary regulators of drug metabolism and disposition, NRs from all four classes are known to influence drug/xenobiotic response, as discussed under Section 3.3.

For all NRs, the primary structure specifies a common generalized organization based on functional domains [23, 2]. The highly variable amino-terminal A/B domain harbors constitutively active transactivation function (AF-1) and multiple autonomous transactivation domains. This is followed by a DNA-binding domain (DBD, C domain), through which an activated NR binds to a DNA response element. The ligand-binding domain (LBD, E domain) at the carboxyl end encompasses the activation function-2 region (AF-2). A less conserved flexible hinge domain (D) connects DBD and LBD. The hinge region contains a nuclear localization signal (NLS) sequence, which extends to the 3’ end of DBD. A variable F domain follows the LBD E domain in some but not all NRs. X-ray crystallography, cryo electron microscopy and solution structure determination by various methods including small-angle X-ray scattering and hydrogen-deuterium exchange, revealed DBD and LBD structures of several NRs, such as the first and second zinc finger modules and DNA-binding specificity motif of DBD; receptor dimerization motif; twelve α-helices of LBD and ligand-induced helix-12 repositioning that creates an interaction surface for coactivator or corepressor recruitment [29, 28, 27].

DNA elements cognate to Type I-III NRs constitute repeats of the half-site consensus sequence RG(G/T)TCA (R: purine), configured as a direct repeat (DR), inverted repeat (IR), or everted repeat (ER) and separated by a varying number of nucleotides. Type I NRs recognize IR3-type palindromic elements; Type II and III NRs recognize specific repeat motifs of the consensus half site. Type IV NRs bind to a single hexamer consensus RG(G/T)TCA, which may contain a short preferred sequence 5’ to the hexameric site [26].

Preferred response elements for PXR, CAR and VDR are 3- or 4- nucleotide spaced direct repeats (DR3, DR4), as concluded from *in vitro* DNA-binding studies and response element-induced promoter activity in transfected cells. Numerous PXR/CAR/VDR target genes are also found to contain ER or IR motifs as response elements. Nevertheless, genome-wide chromatin immunoprecipitation (ChIP) and deep sequencing of immunoprecipitated DNAs (ChIP-Seq) identified DR4 as the most frequent PXR- associated recruitment sites in mouse liver [30]. DR4 in the human genome is a preferred DNA-binding site for the CAR/RXR heterodimer as well, as recently observed in a modified yeast one- hybrid assay [31]. DR3 is

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the prevalent VDR-binding site at genomic regions that contain primary VDR target genes. These genomic regions are induced for chromatin opening in response to 1,25-D_3 signaling [32].

3.1 Regulation of PXR, CAR, VDR expression

PXR and CAR are the primary mediators of transcription regulation of ADME relevant genes. Pathological conditions negatively impact drug metabolism due to reduction of PXR and CAR activity. As an example, CYP3A4 expression is suppressed by inflammation in part due to interference of inflammation-activated NF-κB with PXR's transactivation function, since the p65 subunit of NF-κB was found to disrupt DNA binding of the PXR/RXRα complex in the *CYP3A4* gene [33]. Reduced PXR and CAR activity impairs drug metabolism under conditions of hepatic steatosis as well, since SREBP-1 (sterol regulatory element binding protein-1), activated in hepatocytes by lipogenesis- stimulated LXR-α, prevented p160 coactivator interaction with CAR or PXR, which curtailed phenobarbital-induced, PXR/CAR-mediated *CYP3A4/CYP2B6* gene transactivation [34].

PXR and *CAR* gene expression is regulated by many transcription factors including various NRs [35, 25, 36]. Cholic acid-activated FXR robustly induced the mouse *Pxr* gene in the liver via four FXR-binding elements in the *Pxr* promoter [37]. HNF4α regulates xenobiotic response in mice during fetal liver development through *Pxr* gene activation [38]. GR regulated rat *Pxr* promoter in transfected primary hepatocytes and in hepatoma cells [39]. Human PXR expression in liver is transcriptionally regulated by PPARα [35] and HNF4-α [40]. Expression of CAR is induced by agonist ligands for GR, PPARα and functional binding sites for these NRs as well as a binding site for HNF4α were identified in the upstream sequence of the CAR promoter [25, 41]. Furthermore, in animal studies, CAR mRNA expression was induced by fasting and calorie restriction [41]. Additional mechanisms entailing genetic polymorphism, changes in the epigenetic landscape, post-transcriptional regulation by micro RNAs, and functional modulation through posttranslational modification (PTM) can have major impacts on the expression and activity of PXR and CAR. These examples are discussed under Sections 4.1 and 4.2.

VDR, upon activation by cognate ligands (i.e., 1,25-D_3 and lithocholic acid, LCA), can also induce ADME relevant genes, especially in the intestine. Examples for VDR-mediated induction of DMEs and drug transporters in 1,25-D_3 - or LCA-treated cells include *CYP3A4*, *CYP2B6*, *CYP2C9* [6, 42], *SULT2A1* [43], *OATP1A2* [44], *ABCA1* [45], and *MRP3*, *MRP2* [46]. Crystal structures of VDR bound to LCA- and 3-ketoLCA have been determined [47]. Seasonal differences in intestinal CYP3A4 levels are attributed to season-related fluctuations in sunlight exposure that lead to variations in serum levels of 25-hydroxy-D_3 and 1,25-D_3 [48].

Transcription of the *VDR* gene is under auto-regulation; 1,25-D_3 can increase *VDR* gene expression. Various endocrine factors including parathyroid hormone, retinoic acid, and glucocorticoids also regulate *VDR* expression [49]. Like PXR/CAR, VDR expression/activity is influenced by gene polymorphism, micro RNAs and by post-translational modification, as discussed in Section 4.

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3.2 Xenobiotic response element (XRE)

At the chromatin level, XREs serve as sensors of xenobiotic (or endobiotic) signals by recruiting activated PXR/RXR and CAR/RXR to target genes. XRE activation is demonstrated by its activity in cis to induce promoter-directed reporter gene expression in transfected cells. Screening for XRE activation by synthetic or semi-synthetic chemicals is an integral part of the workflow for drug development. XREs also help identify other regulatory factors, which modulate PXR- and CAR-mediated expression of phase 0-III mediators. XREs in the phase II DME genes *UGT1A1* (for the glucuronidating enzyme UDP glucuronosyltransferase, isoform A1, subfamily-1) and *SULT2A1* (for the sulfotransferase enzyme, isoform A1, subfamily-2) are briefly described.

The phenobarbital responsive enhancer module (PBREM) in the human *UGT1A1* includes three CAR-responsive XREs that are required for the optimal induction of *UGT1A1* by phenobarbital (PB) [51, 50]. Protein-DNA interaction, analyzed by electrophoretic gel mobility shift assay (EMSA), revealed that CAR binds as a monomer to one of the functional XREs in the *UGT1A1* PBREM, and similar to the CAR/RXR dimer, the DNA-bound CAR monomer can interact with coactivators and corepressors. Furthermore, binding of the monomeric CAR or CAR/RXR dimer to XRE is most favored when the hexamer repeat of the response element is preceded at the 5' end by the dinucleotide AG. Arginine residues at positions 90 and 91, located within the carboxy-terminal extension of CAR's DBD, mediate the dinucleotide-dependent binding preference [50].

XRE-dependent and PXR- and CAR-mediated induction of human *SULT2A1* was investigated in our laboratory [9]. Preferred substrates for SULT2A1 are bile acids and dehydroepiandrosterone (DHEA) – the latter is the steroid precursor for testosterone and dihydrotestosterone. A major role for SULT2A1 in the enterohepatic tissue is to promote bile acid clearance as the sulfate conjugate. Notably, the prostate cancer drug Zytiga (abiraterone acetate) is hydrolyzed *in vivo* to the therapeutic metabolite abiraterone, which is cleared from the body after conversion by SUL2A1 to the inactive abiraterone sulfate, and by CYP3A4 to the inactive N-oxide abiraterone, which is then converted to a sulfated derivative (*PubChem database, CID 132971*). *SULT2A1* expression is induced by VDR and the bile acid receptor FXR as well, which is in keeping with its role in bile acid homeostasis [52, 43]. A PXR/CAR- responsive composite XRE in the human *SULT2A1* promoter and a synergizing role of HNF4-α in XRE- induced *SULT2A1* expression is described below and is summarized schematically in Figure 3.2.

3.2.1 A composite XRE and HNF4-α-responsive DR1 element in the human SULT2A1 promoter—Induction of the *SULT2A1* promoter by ligand-activated PXR and CAR in transfected liver and intestinal cells was shown to be mediated by an upstream xenobiotic-responsive composite element (XRE). Specific interaction of XRE with PXR/RXRα and CAR/RXRα was demonstrated by DNAse1 footprinting and EMSA. The XRE from −190 to −131 positions, was defined by an inverted repeat and a direct repeat of the AG(G/T)TCA element, which are configured as IR2 ($^{-190}$AACGCA**AGCTCA**-GA**TGACCC**CTAA^{-167}) and DR4 ($^{-55}$GATA**AGTTCA**TGAT**TGCTCA**ACATC^{-131}) [9]. XRE-mediated stimulation required both IR2 and DR4 elements; neither by itself was

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sufficient to cause robust *SULT2A1* promoter induction. Thus XRE is a composite element. The composite XRE spanning −190 to −131 positions stimulated a heterologous promoter. Point mutations in the XRE prevented its interaction with PXR and CAR and abrogated induction of the *SULT2A1* and the heterologous thymidine kinase promoter.

HNF4-*α* plays a modifying role in the PXR- and CAR-mediated target gene transcription, since HNF4-*α* potentiated PXR- and CAR-mediated transactivation of the *SULT2A1* promoter. A DR1 element ($^{-63}$GTGACATGC**TGGGAC**A**AGGTTA**AAGATCG^{-35}) in the *SULT2A1* gene promoter, located upstream of −30 nucleotide position, serves as an HNF4-*α*-binding element. A schema on the regulation of *SULT2A1* by PXR and CAR via the composite XRE, and the synergizing influence of DR1-bound HNF4-*α* on xenobiotic-induced *SULT2A1* expression is presented as schema in Figure 3.2.

3.2.2 Sult2A1 induction by FXR via an IR0 element—Apart from xenobiotic chemicals, bile acid overload induces *SULT2A1* expression. For example, Sult2A1 mRNAs were induced in the mouse liver when animals were fed a cholic acid containing diet (Figure 4.3.2), and bile acid activated FXR robustly induced the *Sult2A1* promoter through an FXR-bound IR0 element [52]. However, IR1 is the most abundantly encountered FXR-responsive element. An IR1 element drives FXR-mediated transactivation of *ABCB11*, the gene for the human bile salt export pump [53]. A number of other repeat motifs of the half site RG(G/T)TCA including DR1, ER6, ER8 are known to be FXR-responsive functional elements in FXR target genes. Assessment of genome-wide FXR binding in the mouse hepatic chromatin showed an IR1-type sequence as the preferred chromatin occupancy site for FXR *in vivo* [54]. FXR-occupied IR1 sites are frequently juxtaposed to a hexameric half-site consensus sequence, which binds a monomeric NR such as LRH-1 (liver receptor homolog-1). Positive interplay between FXR and LRH-1 for the gene encoding the small heterodimer partner (SHP), which is an atypical NR devoid of a DBD, as well as several other FXR target genes has been demonstrated [55]. The FXR/LRH-1/SHP axis plays a key role in bile acid homeostasis, as discussed in the next section.

In summary, above examples of XREs demonstrate that PXR, CAR, FXR bind a variety of repeat motifs of the consensus half site RG(G/T)TCA to induce genes involved in drug metabolism and disposition.

3.3 NR, a drug target for diseases from disrupted bile acid/cholesterol homeostasis

Bile acid synthesis is the primary pathway for cholesterol catabolism in liver, accounting for ~50% of daily cholesterol turnover. Cholesterol overload, the underlying cause for cholesterol stone, results from insufficient bile acid synthesis when bile acid saturation with cholesterol leads to the formation of cholesterol stone. On the other hand, bile acid accumulation leads to cholestasis due to reduction or stoppage of bile flow. Oral bile acid therapy is given to patients with cholesterol stones, and ursodeoxycholic acid is used to treat cholestasis of pregnancy and primary biliary cirrhosis (PBC), the autoimmune disease causing bile duct destruction. FXR and other NRs, such as LRH-1, HNF4-*α*, LXR-*α*, SHP, PXR, and VDR maintain bile acid/cholesterol homeostasis [56, 55].

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CYP7A1 (cholesterol 7 α hydroxylase) is the rate-limiting enzyme for bile acid production from the catabolic breakdown of cholesterol. HNF4-α and LRH-1 are positive regulators of *CYP7A1* expression. Some aspects of CYP7A1 regulation are, however, species-specific—a prominent example being the positive regulation of the basal expression of *CYP7A1* by oxysterol-activated LXR-α in the rodent liver but not in human liver, since the LXR-binding site in the human promoter is mutated [57]. Toxic accumulation of bile acids, on the other hand, is prevented by FXR-imposed negative feedback regulation of *CYP7A1*. In this case, bile acid activated FXR induces SHP [58], and interference from SHP due to protein-protein interaction inhibits positive regulation of the *CYP7A1* promoter by LRH-1 (an NR activated by phospholipids) [56]. SHP also interferes with the stimulatory interaction between HNF4-α and the coactivator PGC1- α (peroxisome proliferator activated receptor γ coactivator 1-α) on the *CYP7A1* promoter [55]. In the ileum part of intestine, SHP plays a role in the *CYP7A1* repression by the fibroblast growth factor-19 which, like SHP, is induced by FXR [56]. PXR blocks *CYP7A1* expression by disrupting the PGC1α $\rightarrow$ HNF4 stimulatory axis [59]. Thus, like FXR, PXR also regulates bile acid homeostasis upon activation by drugs and certain bile acids. Drugs targeting FXR, SHP, LRH-1, PXR and HNF4-α have therapeutic potential against liver and biliary disorders. Small molecules that augment SHP activity may robustly reduce *CYP7A1* expression to prevent bile acid overload. Small molecules, which elevate LRH-1 activity or increase PGC1-α $\leftrightarrow$ HNF4-α interaction, would be useful in enhancing CYP7A1 expression, which then would promote cholesterol breakdown and reduce cholesterol build up.

Apart from FXR, TGR5, a transmembrane G protein coupled receptor, mediates bile acid signaling. TGR5 is located in intestinal epithelium, Kupffer cells, sinusoidal endothelium and bile duct cells. Both TGR5 and FXR are hotly pursued drug targets for diseases of errant bile acid and cholesterol metabolism [55]. The athero-protective effect of LXR-α arises in part from the LXR-α-mediated induction of efflux transporters in resident macrophages of the arterial wall, and this in turn promotes cholesterol efflux and reverse cholesterol transport to the liver and intestinal tissue and subsequent removal of cholesterol as part of excreta. Therefore, small molecule activators of LXR-α may normalize cholesterol homeostasis. Finally, VDR can regulate bile acid and cholesterol homeostasis, since agonist-activated VDR promotes cholesterol catabolism by repressing SHP and increasing CYP7A1 expression [60].

4 Genetics, Epigenetics and Interindividual Differences in Drug Response

4.1 Gene polymorphism and NR-regulated variable DME/drug transporter activity

Single nucleotide polymorphism (SNP) at regulatory loci of ADME related genes, or non synonymous SNPs in the coding region of NR itself, alter NR-mediated DME/transporter expression. A non-coding SNP at an HNF4-α binding site in the *CYP2B6* promoter contributes to the interindividual variations in *CYP2B6* expression [61], and a common African haplotype for an SNP at a PXR-binding enhancer in *GSTA* (encoding glutathione S-transferase A) causes hypersensitivity for *GSTA* induction by the human PXR ligand rifampin [62]. CYP2D6, which metabolizes a large number of drugs including antidepressants and β blockers, shows wide interindividual differences in expression and

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activity. An HNF4-α variant having reduced binding to the *CYP2D6* promoter and causing decreased *CYP2D6* expression has been identified. The variant HNF4-α arises from a non synonymous SNP, which yields glycine → aspartic acid substitution at the position 60 (G60D). Compared to this variant, the wild-type HNF4α genotype is associated with higher CYP2D6 activity in the human liver [63, 10]. The G60D HNF4-α appears at low frequency in Asian populations; it has not been detected in Africans or Caucasians [63]. Variable *CYP2D6* expression also results from gene amplification that ranges from 3 to 13 gene copies. CYP2D6 deficiency is an autosomal recessive trait in ~7% Caucasians and ~1% Orientals, making these individuals poor metabolizers of CYP2D6 drug substrates [64]. Pharmacogenomic tests for CYP2D6 variants are common practice for assessing the appropriateness and efficacy of a CYP2D6 drug substrate. Interindividual differences in drug response are managed by dosage adjustment based on the patient's pharmacogenetic profile.

The basal level of CYP3A4 in the liver varies up to 60-fold between individuals, although SNPs in coding sequences and regulatory loci of *CYP3A4* do not explain this variability [65]. Association analysis suggests that nonsynonymous SNPs of PXR and FOXA2 (aka HNF3-β, a liver-enriched transcription factor) contribute to CYP3A4 variation in the human liver, since the mRNA expression level for CYP3A4 in the human liver significantly relates to SNPs of PXR and FOXA2, and PXR expression itself is regulated by FOXA2. Binding sites for FOXA2 and PXR in the human *CYP3A4* distal promoter were identified [66]. VDR polymorphism accounts for disparate intestinal CYP3A4 levels and variable first pass intestinal absorption and metabolism of CYP3A4-targeted drugs [48].

FXR, which regulates the expression of many uptake and efflux transporters, shows a common non-coding -1G>T polymorphism, where T replaces G at the -1 position of the translation start site causing reduced FXR expression. The FXR-1G>T SNP is associated with increased efficacy of the statin drug rosuvastatin in lowering hepatic cholesterol biosynthesis, thus affording greater LDL-cholesterol response [67]. Rosuvastain remains un-metabolized in hepatocytes and ABCG2 (BCRP), an apical ABC cassette efflux transporter, plays a major role in the biliary clearance of rosuvastatin. ABCC2 (MRP2) and possibly ABCC11 (BSEP) also contribute to rosuvastatin disposition from human liver. Mechanistically, low expression of the variant FXR accounts for reduced expression of the transporters ABCG2, ABCC2, ABCC11, which leads to a blockade in the biliary clearance of rosuvastatin and longer residency of the drug in hepatocytes – hence a more potent effect of this statin on hypercholesterolemic patients who carry the FXR-1G>T SNP [67].

A large number of SNPs for PXR (NR1I2)- and CAR (NR1I3)- encoding genes are known, several of which are associated with altered expression and/or function of these receptors [69, 68]. For the *NR1I2* SNP 63396C>T, located in a putative transcription factor binding site, the 63396T variant associates with elevated PXR expression, increased *CYP3A4* expression and decreased plasma levels of the CYP3A4 substrate atazanavir (an anti-retroviral drug). Natural PXR variants, which harbor single amino acid changes, confer altered transactivation response of the *CYP3A4* promoter [65]. Among the 22 naturally occurring splice variants of CAR, some are nonfunctional due to nonsense mutations. For the *CAR* (*NR1I3*) SNP rs2307424C>T, the T allele is associated with a low plasma level of the anti-retroviral drug efavirenz, which is a CYP2B6 and CYP3A4 substrate [70]. Extensive

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VDR gene polymorphism has been reported [71], and it has been reported that intestinal CYP3A4 expression levels are functions of *VDR* polymorphisms [48].

4.2 Epigenetic machinery and drug response

Roles for DNA methylation, histone modification and microRNAs in the regulation of a large number of mediators of phase 0-III processes and their NR regulators (PXR, VDR, HNF4-α) have been reported [73, 72]. Epigenetic factors confer heritable changes in chromatin structure and function, caused by mechanisms other than DNA sequence alteration at the coding or non-coding region of a gene. An integral role of epigenetics in health and disease is revealed by the tragic history of the synthetic estrogen diethyl stilbesterol (DES) as a birth control pill. *In utero* DES exposure caused vaginal tumors and breast cancer in adult females. In mice, DES altered gene-specific DNA methylation, expression of epigenetic enzymes (DNMT3A, MBD2, HDAC2, EZH2), and the abundance of HOTAIR, a lncRNA [74]. Epigenetic systems are briefly discussed and current information on their roles in drug metabolism and drug response is presented.

4.2.1 DNA methylation, ADME gene activity, interindividual differences—DNA methylation at the 5' cytosine of the CpG sequence (5mC) is an epigenetic mark for gene activity [75]. Gene repression is linked to hypermethylated promoters when 5mC methylation occurs within long stretches of CpG repeats (CpG island) at proximal promoters, although 5C-methylation at low CpG density (CpG shores) or even at single CpG sites can mark reduced gene expression. Of 3 major DNA methyltransferases (DNMTs) in mammals, DNMT1 is the maintenance methyltransferase; DNMT-3a and -3b are *de novo* enzymes, essential for the genome-wide methylation of DNA following embryo implantation. Gene repression by DNA hypermethylation is aided by the interaction of DNMTs with the polycomb repressor complex (PRC2), especially with EZH2 (Enhancer of Zeste homolog 2), the histone methyltransferase component of PRC2 [75]. Cancer development is associated with genome-wide DNA hypomethylation, which activates proto-oncogenes. For many tumor suppressors, site-specific hypermethylation contributes to gene silencing [75]. 5-hydroxymethylcytosine (5hmc) modification of DNA, on the other hand, is an activation mark, linked to active gene transcription [76]. Notably, the paternal sperm DNA methylation pattern has been linked to autism risks in an autism-dense cohort [77]. Extensive interindividual differences in the genome-wide DNA methylation pattern have been reported [78].

Acquired drug resistance has been linked to altered DNA methylation of NRs and NR-regulated ADME genes, as observed in i) drug-induced demethylation of *MDR1* and *BCRP*, which leads to their overexpression causing multidrug resistance (MDR) of cancer cells [79, 80]; ii) drug-induced methylation of the estrogen receptor (ER-α) encoding *ESR1* gene promoter, causing reduced ER-α expression and tamoxifen resistance in breast cancer [81]; and iii) resistance to progesterone therapy in endometrial cancer due to reduced expression caused by enhanced methylation of the gene encoding progesterone receptor isoform A (PR-A) [82]. Methylation of the *PXR* gene promoter attenuated PXR expression and reduced *CYP3A4* expression in colon cancer cells [83]. In colon and endometrial cancers, the *VDR* gene is aberrantly methylated [83]; differential methylation of *PXR* and *FXR* at CpG

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promoter sites has been reported in cholestatic pregnancy versus normal healthy pregnancy [84].

A role for DNA methylation in the expression of a number of DMEs and drug transporters has been reviewed [73, 85]. A few representative examples are discussed here. 1) *CYP3A4/5/7* expression is dependent upon the methylation status of these genes, since their expression was altered when human hepatoma cells were treated with 5-aza-2′-deoxycytidine (a DNA demethylating agent). *CYP3A4* induction is associated with reduced 5mC at CpG-rich regions located at or near the binding sites for PXR, CAR, and VDR, which are well-known regulators of *CYP3A4* [73]. 2) Altered *CYP1A1* expression in response to cigarette smoking is associated with changes in the methylation status of *CYP1A1*. 3) Development stage-dependent *CYP2E1* expression is influenced by the methylation status of this gene. 4) Phase II genes including *UGT1A1*, *GSTP1*, *SULT1A1* and genes for efflux transporters like MDR1, BCRP and members of the OATP family of uptake transporters are epigenetically regulated due to DNA methylation [86, 87, 89, 88].

4.2.2 Histone marks; impact on NR-regulated ADME genes—Post-translational modification (PTM) of histones (methylation, acetylation, phosphorylation, ubiquitinylation, sumoylation, ADP- ribophosphorylation and several other modifications), especially acetylation and methylation at the amino-terminal histone tails for histone H3 and H4, are well-characterized epigenetic signatures that influence gene activity. PTMs are also known for histone H2A and H2B and the linker histone H1. More than 10 different PTMs at ~80 sites on histone tails, histone core domains and on the H1 linker histone have been identified [90]. Gene-activating histone marks include H4 lysine-16 acetylation (H4K16ac); H3 trimethylation at lysine-4 (H3K4me3) and lysine 36 (H3K36me3), and H3 phosphorylation at serine-10. Among repression marks, trimethylated histone H3 at lysine-9, lysine-27 and lysine-20 are most well characterized [91]. Histone deacetylases (HDACs, subgrouped as class I to IV), histone acetyltransferases (HATs), histone methyltransferases (HMTs) and histone demethylases (HDMs) are important drug targets. Numerous lysine-specific HMTs (SET7/9, MLL, EZ2H2) and lysine-specific HDMs (LSD1/KDM-1, JMJD2A/KDM4A, JARID1A/KDM5A) have been characterized. Arginine methylation of histones mediated by protein arginine methyltransferases (PRMTs) also regulates gene activity, as seen for histone H3 Arg-17, H4 Arg-3. Histone marks are the "codes" that are "read" by chromatin remodelers (such as SWI/SNF containing complexes) and histone- modifying enzyme complexes (such as PRC2) in order to prepare chromatin for positive or negative transcriptional response. Enzymes that mark histones through PTM are "writers"; those involved in removing histone marks are "erasers"; and protein/enzyme complexes, which recognize histone codes, are "readers". Crosstalk of HMTs with DNMT1 influences epigenetic regulation [75].

Among the ADMEs whose genes are known to be regulated by histone modification include the phase I DMEs CYP3A4, CYP2E1, phase II DMEs SULT2B1, UGT1A1, the efflux transporters MDR1, BCRP, the OATP family of uptake transporters and the *SLC5A5* encoded iodine uptake transporter sodium/iodide symporter [86, 73]. Histone modification can have long- lasting effects on ADME genes. For example, the CAR target genes *Cyp2b10* and *Cyp2c37*, when neonatally exposed to the CAR ligand TCPOBOP, remained induced in

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adult mouse livers; as a result, adult mice were much less sensitive to the Cyp2b10 substrate zoxazolamine [92]. H3K4 methylation and H3K9 demethylation at CAR-responsive elements in the *Cyp* genes, detected in the neonatal liver, persisted in the adult mouse liver. It was concluded that the early-life methylation status of histone H3 played a role in the *Cyp* gene induction in the adult liver, since hepatocytes isolated from the livers of mice receiving neonatal CAR activation were significantly more sensitive to low TCPOBOP concentrations for *Cyp* gene induction than hepatocytes from control mice lacking neonatal exposure to this inducer [92]. Also, the possibility remains that the long-lasting stability and biological potency of TCPOBOP *in vivo*, with activity persisting in mouse liver for 6 months or more [93], contributed to the hepatic *Cyp* induction in adult mice when receiving a single dose of TCPOBOP as neonates. PXR-mediated *CYP3A4* induction was regulated by PRMT1, which methylated histone H4 at arginine-3 that is located within a PXR-responsive chromatin region in the CYP3A4 gene [94]. In a rat model of chronic kidney disease, reduced *Cyp2C* and *Cyp3A* expression, and associated reduction in PXR and HNF4-α binding to cognate sites in the *Cyp2C11* and *Cyp3A2* promoters, was accompanied by reduced histone H4 acetylation at the *Cyp3A2* promoter regulatory region and reduced histone H3 acetylation at the PXR- and HNF4-α-bound regulatory loci of *Cyp2C11* and *Cyp3A2* promoters [95].

Given the reversible nature of chromatin modifications by DNA methylation and histone PTM, drugs targeting epigenetic enzymes ("epi drugs"), especially DNMT, HAT, HDAC, HMT and HDM, are being developed. 5-azacytidine (Azacitidine) and 5-aza-2'-deoxycytidine (Decitabine) are nucleoside analogs and DNA demethylating agents that are clinically used against myelodysplastic syndromes, chronic myelomonocytic leukaemia and acute myeloid leukaemia. Second-generation DNA de-methylating agents (SGI-110, CP-4200) are under development [96].

Valproic acid, a class I HDAC inhibitor and an anticonvulsant, activates CAR and PXR to induce *CYP3A4*, *CYP2B6* and *MDR1* expression [97, 99, 98]. Valproic acid also enhances tissue sensitivity to estrogen and progesterone by potentiating estrogen receptor (ER) and progesterone receptor (PR) activity due to HDAC1 inhibition [100]. Vorinostat (a hydroxamate) and romidepsine (a depsipeptide) are orally administered pan-HDAC inhibitors, which are used in combination therapy with chemotherapeutics like paclitaxel, doxorubicin. Vorinostat resistance is thought to develop from increased expression of efflux transporters, since MDR1, BCRP, and MRPs were detected at elevated levels in vorinostat-treated cells [73]. Inhibition of ABC transporters in this case may improve vorinostat's therapeutic efficacy. Epi drugs, which target histone methylation, are at various stages of development [101].

4.2.3 Non-coding RNA-mediated regulation of PXR, CAR, VDR and ADME gene expression—

Non-coding RNAs (ncRNAs), best characterized for micro RNAs, are integrally linked to epigenetic machinery. Transcripts of more than 90% of the human genome represent ncRNAs, many of which regulate gene expression at transcriptional and posttranscriptional levels. Short (<30 nucleotides) ncRNAs, best characterized for micro RNAs (miRNAs), and long ncRNAs (lnc RNAs) with >200-nucleotide lengths are two major categories of ncRNAs. The list for micro RNAs, which influence ADME gene expression, is steadily growing [83, 103, 102].

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The miRbase lists as many as 2555 unique mature human miRNAs (database *version 20; June 2013 release*). Base pairing of the miRNA nucleotide sequence with a cognate sequence in the 3' untranslated region (3'-UTR) of a target messenger RNA (mRNA) within the RNA-induced silencing complex leads to either mRNA degradation (in the case of a perfectly complemented base pairing), or translational suppression of the target mRNA when base pairing is not 100% complementary. A single miRNA can target 3'UTRs of multiple messenger RNAs.

Studies in cell culture show that the messenger RNAs for ADME related genes are targeted directly or, via upstream regulatory NR and other transcription factors, by one or more miRNAs. Regulation of CYP1B1 and CYP3A4 mRNAs by miR-27b; CYP2E1 mRNA by miR-378; the MDR1 transporter by miR-451; and the BCRP transporter by miR-328, miR-519C, miR-520h underscores the impact that miRNAs may have on drug metabolism and disposition, provided these miRNA-dependent regulations are upheld *in vivo*. While miR-27b directly regulates CYP3A4 expression, the VDR level is regulated by this micro RNA as well, so that miR-27b can both directly and indirectly influence CYP3A4 expression. PXR expression is regulated also by miR-148a. The miRNA-dependent regulation of several epigenetic enzymes including DNMTs, HDACs, EZH2, and epigenetic enzymes that regulate miRNA-specifying genes (which produce miRNA precursor transcripts) have been reported [104, 105]. Such cross talks provide a miRNA-dependent additional regulatory cascades that may alter DME/transporter expression. The abundance of specific miRNAs may predict drug response, since miR-21 levels in pancreatic cancer biopsies correlated with gemcitabine responsiveness, and ectopic miR-21 expression caused gemcitabine resistance in pancreatic cancer cells [106].

A lncRNA known as AIR is indirectly involved in the inactivation of the mouse organic cation transporter (OCT) genes *Slc22a2* and *Slc22a3*, since AIR plays a role in silencing the *Igf2R* gene cluster and *Slc22a2* and *Slc22a3* are located within this cluster [107]. LncLSTR, a recently reported liver-enriched lncRNA, is a regulator of *Cyp8b1*, which is involved in bile acid biosynthesis [108]. The lncRNAs PCA3 and PCGEM1 are elevated in human prostate cancer. PCGEM1 coactivates activities of the androgen receptor and cMYC oncoprotein. [109]. Whether lncRNAs directly regulate ADME-relevant genes remains to be determined.

5 Drug Interactions: A Role for Xenosensing NRs

5.1 Drug-drug, drug-food, drug-herb interaction

Drug-drug interaction (DDI) reflects changes in target drug pharmacokinetics or bioavailability in the presence of a co-administered drug. By activating PXR and CAR, the interfering drug renders changes in one or more components of the drug metabolizing and disposition machinery. DDI is assessed quantitatively by the pharmacokinetic parameters C_{max}, which refers to the peak plasma drug concentration at post-dosing; and AUC (area under the time-plasma drug concentration curve), which defines total serum drug levels over time. DDI has three possible outcomes: i) overdosing and potential toxicity due to increased half-life of a target drug caused by one or more of the following – excessive pro-drug bioactivation; attenuated DME activity; increased uptake activity and reduced efflux activity

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of transporters; ii) underdosing resulting in low drug efficacy, which is due to reduced drug uptake and/or reduced bioactivation; enhanced metabolism and/or accelerated drug efflux; iii) a boost in medicinal potency. CYP-mediated DDI led to the withdrawal of numerous drugs from clinical use, such as terfenadine (the antihistamine Seldane) and cerivastatin (a cholesterol-lowering statin). Dietary ingredients (e.g., furanocoumarins in grapefruit juice) or phytochemicals in medicinal herbs (e.g., hyperforin in St John's Wort) can modulate a drug's efficacy and engender potentially fatal drug-food and drug-herb interactions. CYP3A4/3A5 and CYP2D6 are most frequent participants in DDI [18, 110].

Desirable outcomes may also result from drug interactions, as seen in the hepato-protective effect of ginger extracts against diverse drugs including high-dose acetaminophen [111]. DDI is not a concern for peptide or antibody based therapeutics, since they do not activate PXR and CAR. Recently approved PCSK9 inhibitors are antibody-based drugs, which aid in LDL-cholesterol clearance from circulation by preventing PCSK9-mediated degradation of the LDL receptor [112].

5.2 Linking xenobiotic NRs to drug interactions

Association of the xenobiotic NR activity with clinical DDI has been reported [1, 113, 114]. Orally delivered drugs, which are CYP3A4 and/or MDR1 transporter substrates, can exhibit markedly altered pharmacokinetics in response to rifampicin co-administration. For example, increased enterohepatic expression of CYP3A4, triggered by the long-term treatment with the human PXR agonist rifampicin caused a 96% decrease in the oral bioavailability of the CYP3A4 substrate (S)-verapamil and loss of the anti-hypertensive effect of this drug in patients [115]. *Cyp2C9* induction by rifampicin-activated PXR reduced plasma concentrations of CYP2C9 substrates such as warfarin (anticoagulant) and sulfonylurea (antidiabetic) [116]. Binding and activation of PXR by hyperforin, a bioactive component of St. John's Wort, leads to the transcriptional induction of CYP3A4 and widely prevalent clinical DDI due to increased metabolism and hence decreased efficacy of numerous drugs including oral contraceptives, the immune suppressant drug cyclosporine and the anti-HIV protease inhibitor indinavir [117].

Apart from acting as direct ligands, certain drugs induce phosphorylation of PXR and CAR by activating signal pathways that lead to activation of kinases such as PKA, PKC, CDK2, CDK5, and p70S6K [5]. One such PXR activator is forskolin, a diterpene constituent of the Indian plant *C. forskohlii,* which is used for the treatment of glaucoma, asthma and various other diseases. Forskolin induces PXR phosphorylation through PKA activation, and enhances PXR-coactivator interaction upon its direct binding to the PXR LBD [118]. Additionally, forskolin is a constituent of an herbal mixture marketed over-the-counter for weight loss. DDI/drug-herb interaction may interfere with forskolin's therapeutic value.

In the case of CAR, metformin induces phosphorylation of this xenosensing NR at threonine-38, mediated by activated AMPK and the MAP kinase ERK1/2. Thr-38 phosphorylation restricts nuclear translocation of CAR and disruption of coactivator-CAR interaction, thereby preventing CAR-mediated induction of target genes such as *CYP2B6* [25]. As a result, co-administration of metformin is known to cause altered pharmacokinetics for CYP2B6 drug substrates [119]. Reciprocally, reduced CYP2B6 and CAR activity may

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render a negative impact on the renal clearance of metformin as a result of reduced expression of the renal OCT2/MATE transporter system. Pronounced DDI may be expected as a result of such negative interplays.

Additional examples below underscore how PXR and CAR may play roles in clinical DDI events. Several reviews on NR-regulated drug interactions provide further elaborations on this subject [120, 121, 15, 114].

5.3. Drug-drug interaction

5.3.1 Statin-induced myopathy, DDI: a likely role for NRs—Uptake transporters of the OATP family are predominantly involved in the hepatic import of statins [15], and common variants in *SLCO1B1*, which encodes OATP1B1, are strongly linked to an increased risk for statin-induced myopathy [122]. As an example of adverse DDI, cyclosporine A, which competitively inhibits OATP-mediated hepatic statin uptake, caused skeletal muscle statin overload and muscle damage upon co-administration with pitavastatin or rosuvastatin. Extreme statin overload is linked to the fatal condition of rhabdomyolysis [123].

Various statins, however, differ significantly in pharmacokinetic characteristics due to differences in ADME. It is, therefore, conceivable that additional to altered uptake activity, the PXR-/CAR-regulated expression of uptake transporters may influence the hepatic uptake of some form of statins. Indeed, long-term treatment of rifampin reduced atorvastatin bioavailability due to induced expression of CYP3A4 and efflux transporters by rifampin-activated PXR [124], whereas short-term rifampicin administration caused inhibition of OATP-mediated hepatic uptake of atorvastatin and caused elevated AUC for this statin [125]. PXR- and CAR-responsive functional XREs are present in genes for many drug transporters including OATPs and various efflux transporters [126].

5.3.2 Prostate cancer, ZYTIGA®, DDI with rifampicin—Zytiga (abiraterone acetate), the anti-androgen drug against recurrent metastatic prostate cancer, is a CYP3A4 and SULT2A1 substrate. In a DDI trial, serum Zytiga exposure decreased by 55% in the presence of rifampin, indicating a need for higher dosage of this drug when a PXR activator is co-administered [127]. DDI is likely caused by increased CYP3A4 and SULT2A1 expression by rifampicin-activated PXR, since inhibition of CYP3A4 activity by co-administered ketoconazole (a strong CYP3A4 inhibitor) did not significantly alter Zytiga pharmacokinetics (*Clinical Pharmacol 12.3; FDA Drug Safety Reporting, 2015*).

5.4. Drug-food, drug-herb interactions

5.4.1 Grapefruit juice, CYP3A4, drug transporters, PXR/CAR—Grapefruit and several other citrus fruits contain furanocoumarins in addition to other phytochemicals. Furanocoumarins, which inhibit OATPBs and CYP3A4, elevate the bioavailability of CYP3A4/OATPB substrates including cyclosporine, midazolam, calcium channel blockers and certain statins [128]. Although in humans furanocoumarins predominantly inhibit CYP3A4 activity, *Cyp* 1, 2, 3 expression and activity in mice was induced by isopimpinellin (a furanocoumarin) in a Pxr- and Car-dependent manner [129]. To settle whether species

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specificity explains such differences, effects of furanocoumarins on PXR and CAR activity should be re-assessed in humanized PXR-CAR-CYP3A4/5 mice where human counterparts replace rodent *Pxr*, *Car* and *Cyp* genes [131, 130].

5.4.2 Pomegranate juice, SULT2A1, Zytiga® activity—Punicalagin, a polyphenol constituent of pomegranate, impairs sulfoconjugation of drugs in the intestine [132], which leads to reduced clearance and thus overdosing of orally delivered Zytiga (abiraterone acetate) which, as a CYP3A4 and SULT2A1 substrate, is normally metabolized to abiraterone sulfate and *N*-oxide abiraterone sulfate. Inhibition of CYP2C9 by punicalagin has also been reported. It is not known whether this polyphenol influences PXR or CAR activity.

5.4.3 St. John's Wort, PXR, CAR, CYP3A4—Hyperforin, which confers the anti-depressant activity of St. John's wort, is a ligand for human PXR and CAR [117, 133]. Hyperforin-activated PXR/CAR induces *CYP3A4*, other *CYP* genes (*CYP2B6*, *CYP2C9*, *CYP2C19*), as well as *MDR1*. Acute rejection of transplanted hearts in patients due to self-medication with St. John's Wort is an example of serious drug-herb interactions. Rejection was caused by a drop in plasma levels of cyclosporine, which is a CYP3A4 and MDR1 substrate [134].

5.4.4 Garlic, CYP2C9, Warfarin—Garlic extracts suppressed CYP2C9 mRNA expression and activity in the human hepatocyte-derived Fa2N-4 cell line; furthermore, garlic extract can competitively inhibit CYP2C9 activity [135]. Increased systemic exposure of CYP2C9 drug substrates such as warfarin in the presence of garlic extract has been reported. Reduced warfarin metabolism may enhance the possibility for uncontrolled bleeding. Since the diallyl sulfide in garlic extracts can activate CAR [136], *CYP2C9* gene suppression may be driven by a CAR-dependent mechanism.

5.4.5 Protection from acetaminophen toxicity by garlic extracts: a role for CAR-induced SULT—The hepato-protective effect of organo-sulfers in garlic extracts against acetaminophen-induced liver injury is due to two mechanisms: 1) reduction of hepatic CYP2E1 expression and inhibition of CYP2E1- mediated acetaminophen biotransformation to a toxic metabolite [137]; 2) increased acetaminophen clearance as a sulfate metabolite by SULT activity. It has been reported that CAR, activated by diallyl sulfide (a garlic constituent), promotes acetaminophen conversion to a sulfated metabolite by inducing SULT2A1 and other SULTs (SULT1A1, SULT1A3/4, SULT1E1) [139, 138]. Reduced build up of acetaminophen prevents GSTpi induction by acetaminophen-activated CAR. The net result is diminished oxidative stress from glutathione depletion and consequent reduction of oxidant-induced liver injury [140].

Additional NRs can potentially generate drug interactions. VDR-mediated regulation of DMEs and transporters and a modifier role of HNF4 in the expression of ADME-relevant genes have been reported [43, 9, 126, 6, 141]. Whether long-term use of vitamin D supplements would cause adverse drug interactions should be evaluated. Drug interaction from activated glucocorticoid receptor (GR) is a distinct possibility, since ligand-activated GR induces CAR and PXR expression; a GR-responsive element has been identified in the

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CAR gene promoter [142]. Dexamethasone, a synthetic glucocorticoid, promotes nuclear translocation of CAR and PXR and induces PXR/CAR target genes [142, 143]. Ketoconazole, an anti-fungal agent and GR antagonist, prevented rifampin- and phenobarbital-mediated PXR/CAR activation and induction of their target genes [144]. Thus, under ketoconazole co-medication, a primary drug may respond with altered pharmacokinetics.

5.5 Platforms for screening drug candidates

Early assessment of drug candidates can avoid late-stage failure of clinical trials due to DDI and help minimize costs for developing and marketing a new drug. Candidate drugs are routinely screened in a cell based workflow for their impact on DME activity and PXR/CAR-mediated transactivation of XREs. Humanized mouse models, where *Pxr*, *Car* and *Cyp* rodent genes are replaced by corresponding human genes, are better suited for drug testing since these models provide *in vivo* relevance and they approximate as human surrogates. The humanized *PXR-CAR-CYP3A4/3A7* mouse strain is commercially available. A new hPXR-hCAR-hCYP3A4/3A7-hCYP2C9-hCYP2D6 mouse strain, with human PXR and CAR genes substituted for the rodent *Pxr* and *Car* genes and the gene clusters *Cyp3a*, *Cyp2c* and *Cyp2d* replaced by counterpart human genes, has been reported [145].

In the not-to-distant future, microfluidic organs-on-chips may be adopted as a preferred platform for drug testing, replacing animal models. In a microfluidic device, live cells on chips, organized in continuously perfused chambers, mimic the complex multicellular environment so that bioavailability, efficacy and toxicity of test molecules could be assessed in a context which, in part, recapitulates human tissue and organ physiology [146, 147]. The future drug discovery pipeline may also include a workflow that assesses drug-induced PTM profiles of PXR and CAR determined through liquid chromatography-coupled-tandem mass spectrometry, and examines how PTM alters PXR/CAR activity using an approach similar to that reported recently for PXR [148].

6 Summary and Perspectives

PXR and CAR, the two nuclear receptors that are activated by drugs and other xenobiotics, coordinate both metabolism of orally administered drugs in the liver and intestine and excretion of drug metabolites by mediating transcriptional induction of genes encoding phase I/phase II drug-metabolizing enzymes (DMEs) and transporters which regulate drug influx (phase 0) and efflux (phase III) of drug metabolites. Phase 0-III mediators are also induced by ligand-activated VDR, especially in the enterocytes of intestine. Additional nuclear receptors, especially FXR, HNF4-α, LRH-1 and SHP regulate expression of the enzymes and transporters involved in cholesterol and bile acid homeostasis. More than 90% of all known drugs are metabolized by a subset of cytochrome P450s (CYPs) – CYP3A4/3A5, CYP2D6, CYP2B6, CYP2C9, CYP2C19, CYP1A2, CYP2C8, CYP2A6, CYP2J2, and CYP2E1. In the human liver and intestinal epithelium, CYP3A4 and its functionally indistinguishable isoform CYP3A5 are the most abundant CYP enzymes and together, they metabolize more than half of all prescription medicines. Overdosing or underdosing leading to drug toxicity or reduced drug efficacy, respectively, is the

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consequence of interference from a co-administered second drug (DDI, i.e. drug-drug interaction) or from a dietary or herbal agent (drug-food/drug-herb interaction). Adverse (or beneficial) drug interaction results from i) enhanced gene transactivation for DMEs or transporters due to PXR/CAR activation by the interfering drug or other agent; and/or ii) altered DME or transporter activity. In order to minimize late-stage failure of clinical trials, an essential routine at early stages of drug development is to evaluate candidate molecules for effects on the activities and expression of a select set of CYP isozymes; for PXR and CAR activation and for DDI. Humanized mouse strains, as in hPXR-hCAR-hCYP3A4-hCYP3A7 mice (available commercially), or recently reported hPXR-hCAR-hCYP3A4/3A7-hCYP2C9-hCYP2D6 mice, may replace a cell-based workflow for screening candidate drugs. A humanized mouse model provides human-like drug metabolism machinery and *in vivo* relevance. A microfluidic organ-on-a chip platform, which mimics human physiology at tissue and organ levels, may be used in the near future as a preferred alternative to animal models for screening drug candidates (Figure 5.6).

Disparate drug response among individuals results from altered activity or expression of DMEs/transporters due to single nucleotide polymorphisms (SNPs) in coding regions or in PXR-/CAR-/VDR/HNF4- α -regulated genomic loci; it can also be due to SNPs of PXR/CAR/VDR/HNF4-α that lead to variable expression or activity of these nuclear receptors. An epigenome signature is specified by DNA methylation, chromatin histone marks for transcription activation/repression (largely defined by lysine acetylation and lysine/arginine methylation of the amino-terminal tails of H3 and H4 histones), and by non-coding regulatory RNAs (microRNAs, long non-coding RNAs). The signature can have a profound impact on drug metabolism and disposition due to changes in PXR/CAR/VDR mediated transactivation of phase 0-III genes. The epigenome landscape also contributes to interindividual variations in drug response, since such a landscape is shaped by endogenous regulatory molecules and exogenous factors that are as varied as lifestyle, food habits, pollution and psychological disposition.

An integrated scheme linking genetic and epigenetic factors to drug metabolism/disposition, and interindividual variations in drug response is presented in Figure 6.6. In the era of personalized medicine, all of these regulatory factors must be taken into consideration before deciding on a medicinal regimen that provides optimal therapeutic efficacy and minimal toxicity, while preventing adverse drug reactions.

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Abbreviations

NR nuclear receptor

DBD DNA-binding domain

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LBD	ligand-binding domain
XRE	xenobiotic response element
PXR	pregnane X receptor
CAR	constitutive androstane receptor
VDR	vitamin D receptor
FXR	farnesoid X receptor
LXR	liver X receptor
CYP	cytochrome P450
DME	drug-metabolizing enzyme
ADME	absorption, distribution, metabolism, excretion
DDI	drug-drug interaction
PTM	post-translational modification
MDR	multi-drug resistance
ABC	ATP-binding cassette
HDAC	histone deacetylase
HAT	histone acetyltransferase
HMT	histone methyltansferase
HMD	histone demethylase
DNMT	DNA methyltransferase
SNP	single nucleotide polymorphism

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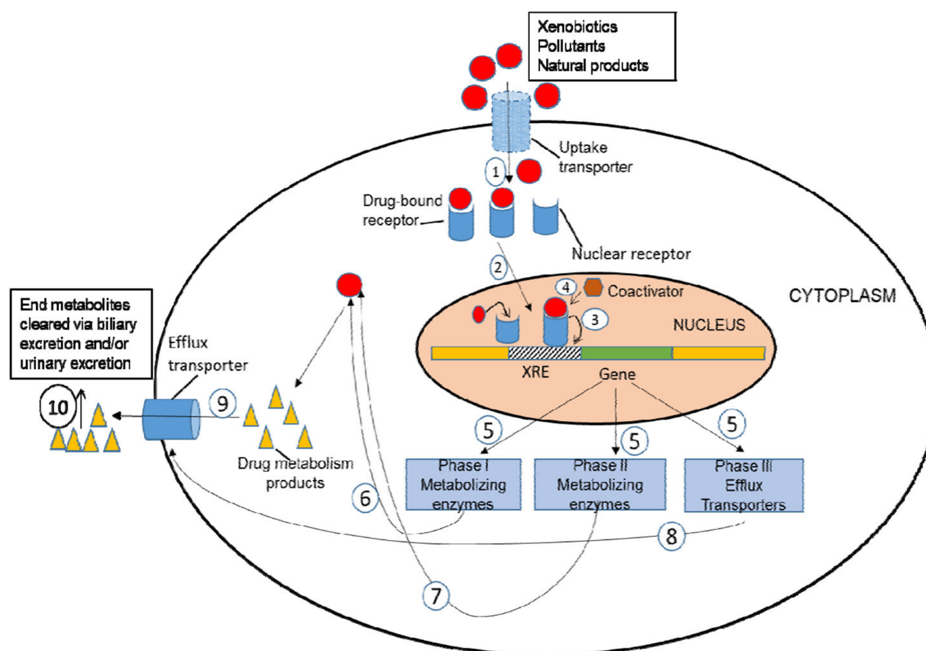

Figure 1. Steps involved in drug metabolism and disposition

(1) Uptake transporter mediates drug entry into the cell. (2) Drug activates xeno-sensing NR in the cytoplasm or nucleus. (3) NR binds to XREs in target genes that are involved in drug metabolism and clearance. (4) Coactivator association with the DNA-bound NR and a cascade of activating steps, which culminate in gene transcription for DMEs, transporters. (5) Expression of phase 0-III mediators. (6) Phase I enzyme adds water-soluble functional groups to the drug structure. (7) **A** phase II conjugative transferase adds hydrophilic groups to drug/drug metabolite. (8) Phase III efflux transporter moves to plasma membrane. (9) Transporter- assisted drug efflux. (10) Drug clearance through biliary and urinary excretion.

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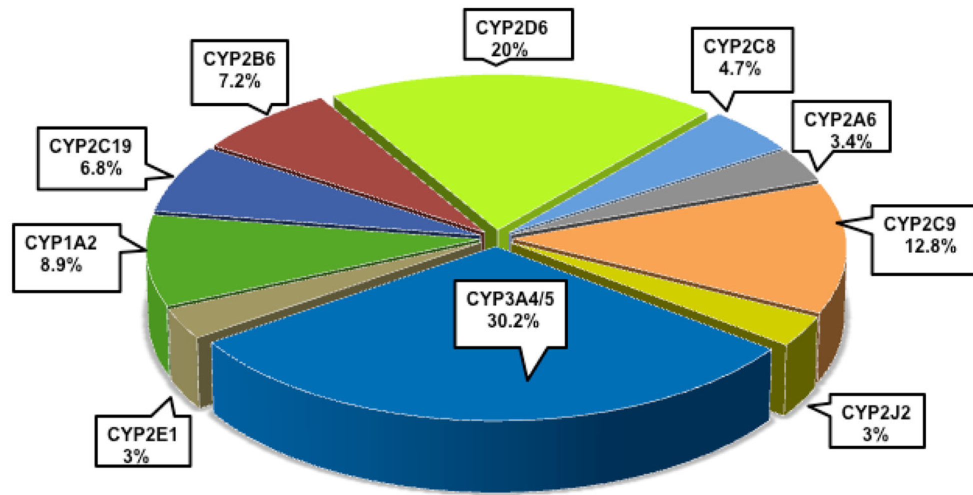

Figure 2. Percentage of all prescription drugs metabolized in human liver by a particular CYP enzyme

(adapted from [16]).

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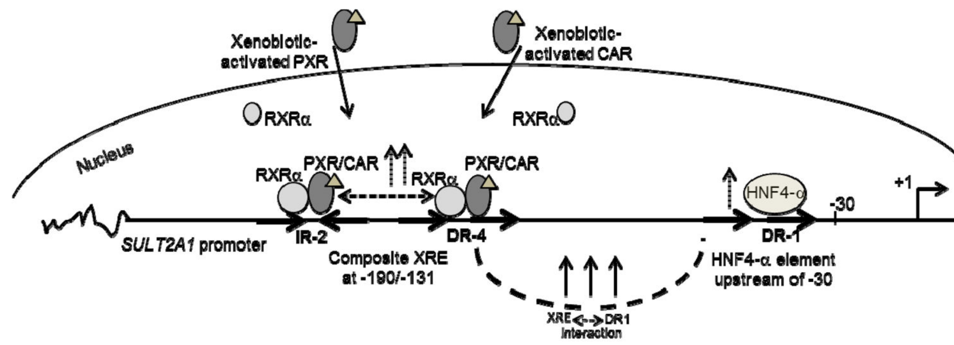

Figure 3. Induction of the human SULT2A1 promoter by PXR, CAR and a synergizing effect of HNF4-α

Schema showing a PXR- and CAR-binding composite XRE comprised of IR2 and DR4 elements, and an HNF4-α-binding DR1 element located downstream of XRE. Dotted, upward arrows signify promoter induction. Interaction between DR1-bound HNF4-α and XRE-bound PXR/RXR, CAR/RXR has an synergistic effect (triple upward arrows) on SULT2A1 induction. (based on results described in [9]).

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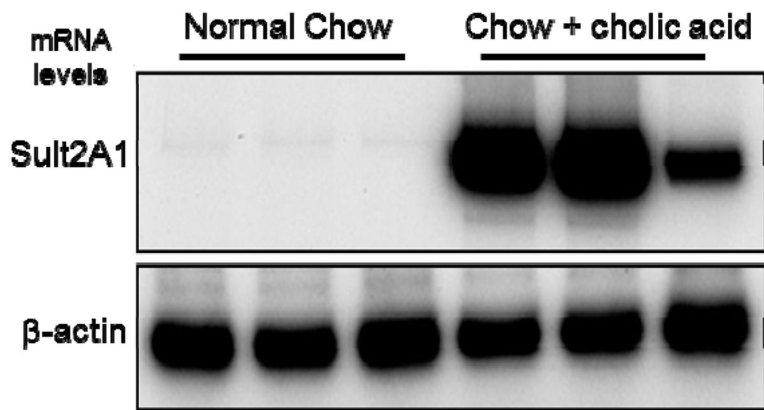

Figure 4. SULT2A1 mRNA induction by cholic acid in mouse liver

Sult2A1 mRNAs in mouse livers were assayed by semi-quantitative RT-PCR. Cholic acid, a primary bile acid, was added to diet at 1% w/w. Data are for 3 individual mice (6-month-old, male) from the control and experimental group. Levels of β-actin mRNAs served as the normalization control (*B. Chatterjee & CS Song, unpublished*).

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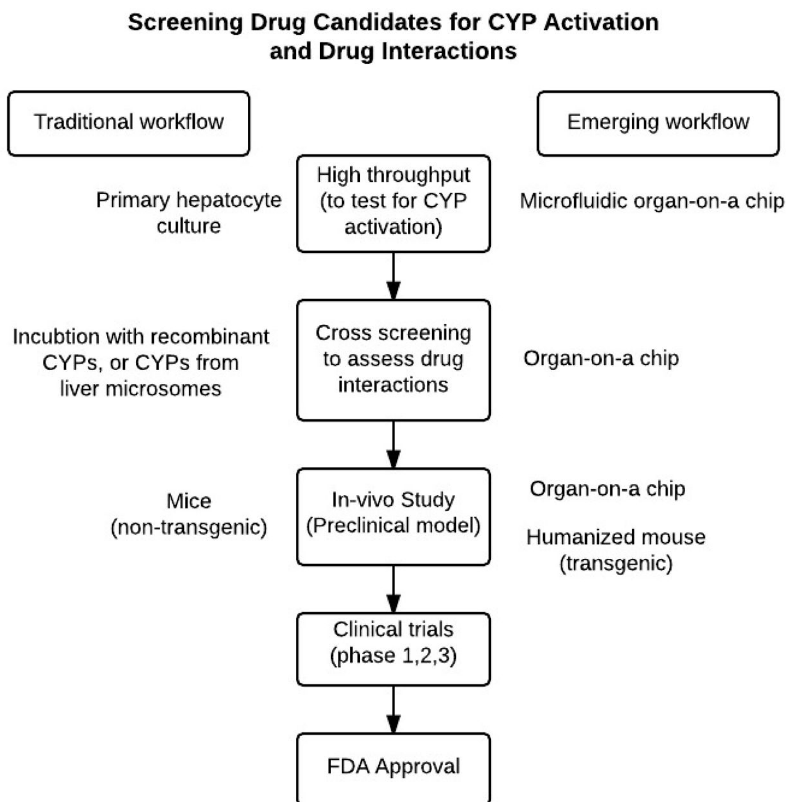

Figure 5. Overview of drug-screening platforms

Candidate drugs are screened for effects on the activity and expression of a select set of CYPs (e.g., CYP3A4, and several other CYPs). Workflow for traditional screening (shown at left) relies on cell-based high throughput assay to identify and narrow down candidates with potential for optimal drug activity. Microfluidic organ-on-a chip constitutes an emerging technology that may replace cell-based screening as the primary assay platform. In cross screening, cells are co-administered with a test drug and a second drug or a non-drug xenobiotic agent (such as a medicinal herb or a foodstuff) in order to reveal drug-drug or drug-herb or drug-food interactions. Subsequently, drugs are tested in mice. A humanized mouse model (transgenic mice with human PXR, CAR and CYP genes replacing the counterpart rodent genes) can serve as a human surrogate for the examination of drug interactions in the preclinical stage of drug screening.

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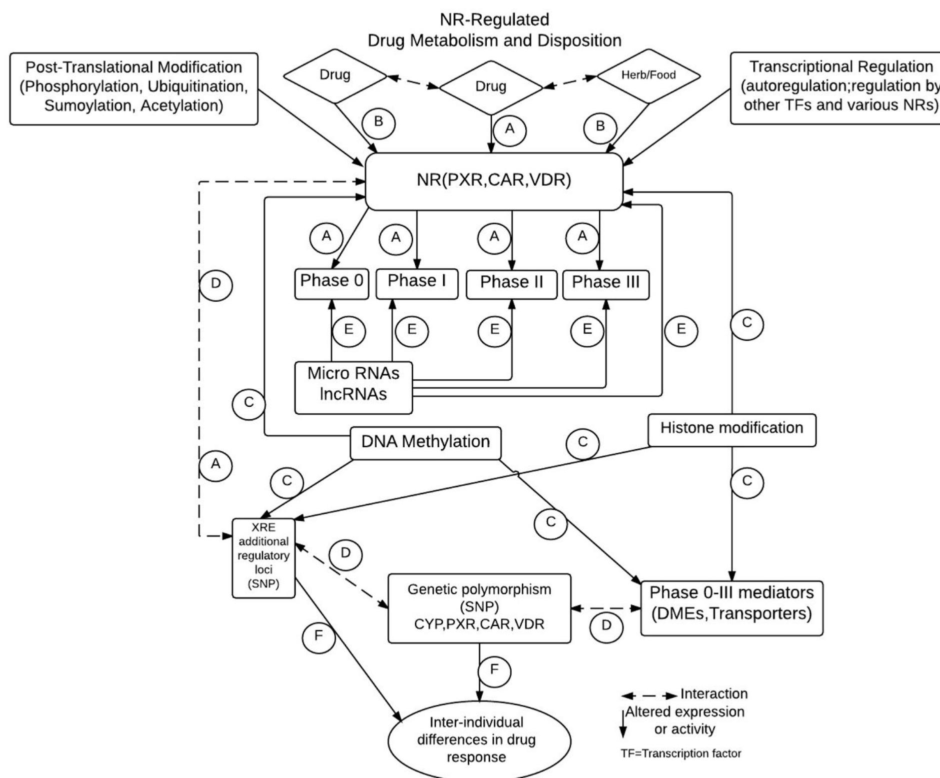

Figure 6. NR-mediated regulation of drug metabolism, drug disposition: control at multiple steps

Transcriptional regulation primarily dictates NR expression and its cellular abundance (box at the upper right corner). Post-translational modification modulates NR stability and NR activity (box at upper left corner). (A) Drugs activate xenobiotic NRs (PXR, CAR), which in turn modulate the expression of phase 0-III mediators via induction of XREs. Ligand-activated VDR also induces DME and transporter expression. (B) Drug-drug, drug-herb, drug-food interactions cause altered NR expression/activity leading to altered expression of DME/transporter. An interfering agent (such as a second drug or a dietary constituent) may also modulate DME/transporter activity via competitive or allosteric regulation. (C) Histone modification and DNA methylation modulate NR expression; they also modulate NR-regulated DME/transporter expression due to epigenetic changes at or near XREs. (D) SNP at an XRE or at an alternate regulatory locus of phase 0-III genes leads to a change in the NR interaction with the response element, which alters DME and transporter expression. SNP in coding regions of PXR/CAR/VDR/HNF4-α, DMEs or transporters can alter the activity or cellular abundance of these proteins/enzymes. (E) Micro RNAs and long non-coding RNAs (lncRNAs) regulate the cellular abundance of NRs and mediators of phase 0-III processes. (F) Interindividual differences in drug response stem from SNP at an XRE, at another NR-interacting regulatory locus of the target gene, or at the coding region of NRs (PXR/CAR/VDR/HNF4-α) or phase 0-III mediators.