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Current knowledge of microRNA-mediated regulation of drug metabolism in humans

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Abstract

Introduction: Understanding the factors causing inter- and intra-individual differences in drug metabolism potencies is required for the practice of personalized or precision medicine, as well as for the promotion of efficient drug development. The expression of drug-metabolizing enzymes is controlled by transcriptional regulation by nuclear receptors and transcriptional factors, epigenetic regulation, such as DNA methylation and histone acetylation, and post-translational modification. In addition to such regulation mechanisms, recent studies revealed that microRNAs (miRNAs), endogenous ~22-nucleotide non-coding RNAs that regulate gene expression through the translational repression and degradation of mRNAs, significantly contribute to post-transcriptional regulation of drug-metabolizing enzymes.

Areas covered: This review summarizes the current knowledge regarding miRNAs-dependent regulation of drug-metabolizing enzymes and transcriptional factors and its physiological and clinical significance. We also describe recent advances in miRNA-dependent regulation research, showing that the presence of pseudogenes, single-nucleotide polymorphisms, and RNA editing affects miRNA targeting.

Expert opinion: It is unwavering fact that miRNAs are critical factors causing inter- and intra-individual differences in the expression of drug-metabolizing enzymes. Consideration of miRNA-dependent regulation would be a helpful tool for optimizing personalized and precision medicine.

Keywords

drug metabolism; CYP; UGT; SULT; microRNA; post-transcriptional regulation

1. Introduction

Variability in expression and activity of drug-metabolizing enzymes, such as cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT), is a major factor in the inter- and intra-individual differences observed in pharmacokinetics and drug responses [1]. Therefore, understanding the causes of the variation of drug metabolism potencies is essential for efficient drug development and promotion of safe use of medicines. Expression of drug-metabolizing enzymes is influenced by intrinsic factors, such as genetic polymorphisms, disease states, sex, and age, as well as by extrinsic factors, such as exposure to drugs and environmental chemicals, alcohol, smoking, and diet [2]. The knowledge of the mechanisms of the transcriptional regulation of drug-metabolizing enzymes has greatly matured. Most CYP and UGT isoforms expressed in the human liver are regulated by nuclear receptors such as hepatocyte nuclear factors (HNF) 4α, pregnane X receptor (PXR), constitutive androstane receptor (CAR), and transcriptional factors such as aryl hydrocarbon receptor (AhR), as well as epigenetic regulators, such as DNA methylation and histone modification. In addition, there is growing evidence that microRNAs (miRNAs) modulate the expression of drug metabolism-related genes via post-transcriptional regulation. More recently, it has been clarified that pseudogenes, single nucleotide polymorphisms (SNPs) and RNA editing affect miRNA-dependent regulatory mechanisms. In this review, we summarize the current knowledge of miRNA-mediated regulation of drug metabolism and discuss its physiological and clinical significance.

2. Biogenesis and function of miRNAs

miRNAs are endogenous, small (~22 nt) non-coding RNAs, which down-regulate gene expression through translational repression or degradation of mRNA via imperfect base pairing with the target mRNA [3]. In 1993, the first miRNA, lin-4, was discovered in *Caenorhabditis elegans* [4,5]. In 2000, the presence of miRNAs was observed in several species, including humans [6]. To date, more than 2,500 miRNAs have been identified in humans. It has been estimated that almost all human genes are regulated by miRNAs [7].

Growing evidence shows that miRNAs play role in fundamental cellular functions, such as development, differentiation, cell proliferation, apoptosis, immune responses, and metabolism. Therefore, miRNA dysregulation is associated with the incidence and progression of various diseases, such as cancer, diabetes, and cardiovascular diseases.

miRNA genes are located throughout the genome. In humans, 52% of miRNAs are located in intergenic regions, whereas the other miRNAs are located in intragenic regions (43% of miRNAs are in introns; 5% of miRNAs are in exons) [8]. miRNAs are produced via a multi-step process. The miRNA genes are transcribed by RNA polymerase II to generate long primary transcripts (200 - 5000 nt, on average), known as primary microRNAs (pri-miRNAs), containing a stem-loop structure. Intergenic pri-miRNAs have an original promoter, whereas intragenic pri-miRNA may use the promoter of the host gene. In nucleus, Drosha and DiGeorge syndrome critical region 8 (DGCR8) bind to pri-miRNA to cleave into 70-100 nt precursor miRNAs (pre-miRNAs). The pre-miRNAs are exported into cytosol by exportin 5. Subsequently, cytoplasmic Dicer TAR RNA binding protein (TRBP) cleaves pre-miRNAs to produce miRNA duplexes. Next, the miRNA duplexes are unwound into the guide strand, which is loaded into RNA-induced silencing complex (RISC), composed of Dicer, TRBP and argonaute protein Ago2. The other passenger strand is usually degraded. The guide and passenger strands were previously named miRNA and miRNA*, respectively. However, passenger strands are sometimes substantially expressed and functional. Therefore, miRNA/miRNA* nomenclature was replaced with miR-##-5p and miR-##-3p (## represents sequential numerical identifier) to indicate the location in 5' arm or 3' arm of the pre-miRNA. In addition to the canonical pathway, miRNAs can be generated from short hairpin introns by splicing machinery and lariat-debranching enzyme, which is called a mirtron pathway [9].

The mature miRNAs are loaded onto the RISC guides to miRNA recognition elements (MREs) on target transcript via imperfect base pairing and cause translational repression or mRNA degradation [10]. MREs are primarily in the 3'-untranslated region (UTR) and occasionally in the coding region [11,12]. Nucleotides 2-7 at the 5'-end of the miRNA, known as the seed sequence, are critical for recognizing the targets [13]. Since miRNAs bind to their

target by partial complementarity, each miRNA can regulate the expression of hundreds of transcripts. A number of computational programs to predict miRNA-target pairs have been established, considering complementarity to seed sequence, accessibility to target mRNA, and evolutionary conservation of MRE. Although the computational prediction is helpful to find potential miRNA-target pairs, the false-positive rate is thought to be 30%-50% [14]. In any case, experimental proof is required to clarify true miRNA targets.

3. Regulation of human CYPs expression by miRNAs

CYP consists of a superfamily of heme-containing monooxygenases responsible for the oxidative metabolism of many drugs and environmental chemicals, as well as of endogenous substances, including steroids. Since we first reported (2006) that CYP1B1 is regulated by miR-27b [15], accumulating evidence has revealed that many CYP isoforms are regulated by miRNAs (Table 1). More recently, it has been uncovered that the miRNA-mediated regulation of CYPs is affected by the presence of pseudogenes, SNPs, and RNA editing. Current knowledge regarding human CYPs regulated by miRNAs is summarized below.

3.1 CYP1 family and related transcription factors

The CYP1 family comprises three functional isoforms, CYP1A1, CYP1A2, and CYP1B1. In humans, CYP1A2 is constitutively expressed only in liver, whereas CYP1A1 and CYP1B1 are primarily expressed in extrahepatic tissues [2]. These isoforms are highly inducible by xenobiotics, such as 3-methylcholanthrene, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and omeprazole, which activate the aryl hydrocarbon receptor (AhR) as ligands [16].

3.1.1 CYP1A1

CYP1A1 is a cancer-related isoform, as it catalyzes the metabolic activation of procarcinogens, such as polycyclic aromatic hydrocarbon. The formed metabolite can bind to DNA, resulting in abnormal cell proliferation and tumorigenesis [17,18]. Choi et al. [19] found by luciferase assay that miR-892a binds to MRE in the 3'-UTR of CYP1A1 to down-

regulate expression (Fig. 1). In addition, these researchers observed that the overexpression or inhibition of miR-892a decreased or increased the expression of the CYP1A1 protein in human breast cancer-derived MCF-7 cells. These researchers demonstrated that benzo(*a*)pyrene (BaP) decreased the viability of MCF-7 cells, and miR-892a overexpression attenuated the BaP-dependent decrease in cell viability. Taken together, these results suggest that miR-892a down-regulates CYP1A1 expression, leading to suppression of BaP-mediated cytotoxicity.

3.1.2 CYP1A2

CYP1A2 is highly expressed in human liver, accounting for approximately 13% of the total cytochrome P450 content. CYP1A2 is responsible for metabolism of 9% of clinically used drugs [20]. Recently, Chen et al. [21] determined by luciferase assay and electrophoretic mobility shift assay that miR-132-5p binds to 3'-UTR of CYP1A2 to down-regulate expression (Fig. 1). Overexpression of miR-132-5p suppresses CYP1A2 expression in HepG2, Huh-7, and HepaRG cells. Notably, these researchers showed that miR-132-5p attenuates hepatic cell toxicity by flutamide because it has been known that flutamide is metabolized to 2-hydroxyflutaminde, a liver toxin, by CYP1A2 [22].

3.1.3. CYP1B1

CYP1B1 is the first CYP isoform that we demonstrated to be regulated by miRNA [15]. There are three reasons why we first focused on CYP1B1. First, in the early 2000s, global changes in miRNAs expression in various cancer tissues was demonstrated to be associated with the development of cancer [23, 24]. Second, CYP1B1 is a cancer-associated isoform, since it catalyzes the metabolic activation of a variety of procarcinogens and promutagens, including polycyclic aromatic hydrocarbons and aryl amines, and metabolizes 17β-estradiol to form a catechol that binds to DNA [25]. CYP1B1 expression is higher in cancer tissues than in normal tissues [26]. Third, CYP1B1 is highly expressed in breast, uterus, and ovaries at the mRNA level, but the protein level in normal tissue is low [27], suggesting the

involvement of post-transcriptional regulation. By our pioneering study, it was clearly demonstrated that CYP1B1 is directly regulated by miR-27b in human breast tissue and that lower expression of miR-27b in cancer tissues compared with normal tissues could explain why higher expression of CYP1B1 protein is observed in cancer tissues than in normal tissues (Fig. 1) [15].

After our study, Chang et al. [28] reported that miR-200c down-regulates CYP1B1 expression in renal cell carcinoma, and attenuates resistance of the cancer cells to docetaxel, which is detoxified by CYP1B1 (Fig. 1) [29]. Recently, Mao et al. [30] revealed that miR-187-5p directly down-regulates CYP1B1 expression, leading to suppression of non-small cell lung cancer progression (Fig. 1). These studies indicate miRNA-dependent regulation of CYP1B1 would be involved in progression of cancer as well as in modulation of drug resistance.

3.1.4 AhR and current knowledge of the effects of RNA-editing on miRNA-dependent regulation

AhR is a ligand-dependent transcription factor that regulates expression of xenobiotic-metabolizing enzymes, including CYP1A1, CYP1A2, CYP1B1, UGT, and glutathione *S*-transferase [16]. In addition to drug metabolism, AhR is involved in the regulation of the circadian rhythm, fatty acid synthesis, cholesterol biosynthesis, glucose metabolism, apoptosis, and cancer development [31]. It has been demonstrated that miR-29a directly down-regulates AhR, leading to promotion of cholesterol and fatty acid syntheses [32]. miR-124 directly down-regulates AhR, leading to suppression of neuroblastoma differentiation and the cell cycle (Fig. 1) [33], although the relevance to drug metabolism has not been determined.

Our recent study revealed that AhR is regulated by a miR-378 in an adenosine-to-inosine (A-to-I) RNA editing-dependent manner (Fig. 1) [34]. A-to-I RNA editing is the most frequent post-transcriptional process that alters the nucleotide sequence of transcripts in mammals [35,36]. Adenosines in double-stranded RNA structures are converted to inosines,

which behave as guanosines, by hydrolytic deamination catalyzed by adenosine deaminase acting on RNA (ADAR) enzymes, ADAR1 and ADAR2. We found that there are 38 edited sites in the 3'-UTR of AhR in the human liver. In Huh-7 cells, knockdown of ADAR1 (but not of ADAR2) decreased the RNA editing levels of AhR and resulted in an increase in AhR protein levels, indicating that ADAR1-mediated RNA editing negatively regulates AhR expression. The down-regulation of the AhR attenuated TCDD-mediated induction of CYP1A1, a downstream gene of AhR. We observed that ADAR1-dependent RNA editing creates an miR-378 recognition site to down-regulate AhR. The impact of A-to-I RNA editing-dependent regulation by miR-378 on the constitutive expression of AhR was revealed by a significant inverse correlation between miR-378 and AhR protein levels in a panel of human liver samples. It should be noted that miR-378 cannot be predicted by conventional computer analyses as a regulator of AhR because this miRNA binds to the edited sequence only. In other cases, RNA editing may destroy miRNA binding sites. Thus, it is of note that post-transcriptional regulation by miRNAs is modulated in a complex manner by a post-transcriptional process, that is, RNA editing.

3.1.5 ARNT

Aryl hydrocarbon receptor nuclear translocator (ARNT) is a heterodimer partner of AhR to transactivate target genes. We reported that human ARNT in HuH-7 and HepG2 is down-regulated by miR-24 (Fig. 1), although functional MRE was not identified [37]. The contribution of miR-24 to the constitutive expression of ARNT was demonstrated by a significant inverse correlation between the miR-24 and ARNT protein levels in a panel of human liver samples. In addition, down-regulation of ARNT by miR-24 decreased the expression of its downstream CYP1A1 expression. Thus, CYP1 isoforms are directly and indirectly regulated by several miRNAs.

3.2 CYP2 family

Among the CYP2 family members, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19,

CYP2D6, and CYP2E1 have the ability to metabolize clinically used drugs. It is well-known that CYP2A6, CYP2B6, and CYP2Cs are induced by such drugs as phenobarbital and rifampicin, which are activators of CAR and PXR, respectively, via transcriptional activation. CYP2E1 is also induced by compounds with small molecular weight, such as ethanol and acetone, in a nuclear receptor-independent manner, whereas CYP2D6 is not induced by compounds. Recent studies clarified that these CYP2 isoforms are post-transcriptionally regulated by miRNAs.

3.2.1 CYP2A6 and current knowledge of the effects of a pseudogene on miRNA-dependent regulation

The CYP2A subfamily consists of three isoforms, CYP2A6, CYP2A7, and CYP2A13. CYP2A6 and CYP2A13 are functional, whereas CYP2A7, showing 96.5% nucleotide identity with CYP2A6, is a pseudogene that produces nonfunctional protein [38,39]. CYP2A6 is primarily expressed in liver and is responsible for nicotine metabolism [40] and metabolic activation of tobacco-related nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [41]. CYP2A6 also catalyzes the metabolism of several pharmaceutical agents such as tegafur, losigamone, and letrozole [42]. We demonstrated that CYP2A6 is regulated by miR-126-5p, which was previously called miR-126*, in human hepatocytes, through binding to an MRE in the 3'-UTR (Fig. 2) [43]. We focused on the fact that CYP2A7 mRNA is substantially expressed in the human liver, and has the MRE for miR-126-5p because of high sequence identity with CYP2A6. Interestingly, it was demonstrated that CYP2A7 restores the miR-126-5p-dependent down-regulation of CYP2A6 by acting as a decoy for miR-126-5p. Thus, CYP2A7 has the ability to regulate CYP2A6 expression when CYP2A7 mRNA levels are higher than CYP2A6 mRNA levels in the human liver. We clearly demonstrate for CYPs that the regulation by miRNAs is modulated by pseudogenes.

3.2.2 CYP2B6

CYP2B6 is primarily expressed in the liver and is responsible for the metabolism of 25%

of drugs, including anticancer drugs, such as cyclophosphamide, ifosfamide, and efavirenz [44]. It is well-known that the expression of CYP2B6 is transcriptionally regulated by CAR and PXR [45]. Recently, Jin et al. [46] reported that CYP2B6 is post-transcriptionally regulated by miR-25-3p in human liver cells, and the miR-25-3p-mediated regulation attenuates rifampicin-dependent induction of CYP2B6. More recently, it was reported that a SNP in the 3'-UTR of CYP2B6 leads to a decrease in CYP2B6 activity in healthy subjects and human liver microsomes [47]. The authors demonstrated that the binding of miR-1275 was enhanced by the presence of the SNP, leading to a decrease in CYP2B6 expression. Thus, miRNAs and SNP in the 3'-UTR could be a causal factor affecting hepatic expression of CYP2B6.

3.2.3 CYP2C8, 2C9, 2C19

The CYP2C subfamily consists of four isoforms, CYP2C8, CYP2C9, CYP2C18, and CYP2C19. These isoforms are responsible for the metabolism of ~20% of therapeutic agents [48]. Zhang et al. [49] reported that three members (CYP2C8, CYP2C9, and CYP2C19) are all regulated by miR-103 and miR-107. An inverse correlation was observed between the translation efficiency (protein/mRNA ratio) of CYP2C8 and expression of miR-103 and miR-107. After that study, Yu et al. found that the CYP2C9 is regulated by miR-128-3p [50] and CYP2C19 is regulated by miR-23-3p and miR-29a-3p [51]. These researchers showed an inverse correlation between the CYP2C19 mRNA levels and miR-23a-3p or miR-29a-3p levels in human liver samples. Rieger et al. [52] reported that CYP2C9 is regulated by miR-130b. These researchers identified an MRE for miR-130b in the 3'-UTR of CYP2C9 and demonstrated that overexpression of miR-130b resulted in a decrease in CYP2C9 mRNA and protein levels, as well as tolbutamide hydroxylase activity in HepaRG cells. Thus, miRNAs would be a factor to convey the interindividual variability in CYP2C expression in the human liver.

3.2.4 CYP2D6

CYP2D6 is responsible for the metabolism of 20% to 25% of such drugs as tamoxifen, haloperidol, and antiarrhythmics [53], although it accounts for 2% of total hepatic CYP content [54]. There is a large (~100-fold) interindividual variability in the expression and activity of CYP2D6 in the human liver [54]. CYP2D6 is not inducible by xenobiotics [55], and genetic polymorphisms are the primary factors that cause interindividual variability [56]. A recent study revealed that miR-370-3p regulates CYP2D6 expression by facilitating mRNA degradation [57]. At present, this study is the first to present evidence indicating miRNA-mediated CYP2D6 regulation.

3.2.5 CYP2E1 and advanced knowledge of the effects of a SNP on miRNA-dependent regulation

CYP2E1 catalyzes the metabolism of numerous xenobiotics with low molecular weight, including acetaminophen, isoniazid, and bromobenzene [58]. CYP2E1 is the most abundant isoform among all CYPs in human liver (56% of total CYP) at the mRNA level [59], whereas it is the fourth most abundant isoform (7% of total CYP) at the protein level [53]. In addition, the involvement of the post-transcriptional regulation in the constitutive expression of CYP2E1 was suggested by the finding of no positive correlation between the CYP2E1 mRNA levels and chlorzoxazone 6-hydroxylase activity, a probe activity for CYP2E1 in a panel of human liver samples [60].

Employing a luciferase assay and overexpression study, we observed that miR-378 down-regulates CYP2E1 by binding to MRE in the 3'-UTR (Fig. 3) [61]. Since the miR-378 levels were inversely correlated with the protein levels and the translational efficiency of CYP2E1, it is highly possible that miR-378-dependent regulation would be a causal factor of the difference in the hepatic basal CYP2E1 expression. In a subsequent study, we found that SNPs in the 3'-UTR of CYP2E1 affect the binding of miR-570. We demonstrated, by an overexpression study of the miRNA into HEK293 cells stably expressing CYP2E1, including the 3'-UTR, that miR-570 regulates CYP2E1 haplotype I (1556T and 1561A) but not haplotype II (1556A and 1561G) (Fig. 3) [62]. This report was the first to show that SNPs in

the 3'-UTR affects the miRNAs-dependent regulation of CYPs. For many other CYP isoforms, Ramamoorthy and Skaar [63] revealed the possibility, by *in silico* analysis, that SNPs in 3'-UTR may create or destroy miRNA binding sites, although experimental proof is required. Not only SNPs in promoter region but also SNPs in the 3'-UTR are critical factors to modulate expression levels.

As described, mature miRNAs usually function in the cytoplasm, but they have also been found in the nucleus [64,65]. Recently, Miao et al. [66] reported that miR-552 exists in both cytosol (~80%) and nucleus (~20%), and down-regulates CYP2E1 in human hepatoma PLC/PRF/5 cells in a post-transcriptional and transcriptional manner, respectively (Fig. 3). A luciferase assay revealed that miR-552 binds to the 3'-UTR of CYP2E1 in a seed-dependent manner, and UV melting curve analysis revealed that miR-552 binds to the promoter of CYP2E1 in a non-seed region dependent manner. The negative regulation of CYP2E1 transcription is due to the competition of miR-552 with SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1 (SMARCE1) for binding to CYP2E1 promoter.

More recently, Wang et al. [67] reported that CYP2E1 is regulated by miR-214-3p (Fig. 3). Electrophoretic mobility shift assays revealed that there are two MREs for miR-214-3p in the coding region of CYP2E1. Interestingly, these researchers demonstrated that miR-214-3p suppressed the cytotoxicity of acetaminophen, which is metabolized to *N*-acetyl-benzoquinone imine, a potential hepatic toxicant, by CYP2E1, using HepG2 cells overexpressing CYP2E1. Collectively, miRNAs modulate hepatic CYP2E1 expression through post-transcriptional regulation via binding to the 3'-UTR or coding regions, as well as transcriptional regulation via binding to the promoter region.

3.3 CYP3A4 and related transcriptional factors

CYP3A4 is the most important CYP isoform, since it is responsible for the metabolism of more than 50% of clinically used drugs that are metabolized by the liver [68]. There is a large interindividual difference (~50-fold) in the CYP3A4 expression in the human livers. The

expression of CYP3A4 is primarily regulated by transcriptional factors, such as CCAAT/enhancer binding proteins α (C/EBPα), C/EBPβ, hepatocyte nuclear factor 4α (HNF4α) HNF3γ, PXR, CAR, and vitamin D receptor (VDR) [69]. For post-transcriptional regulation, accumulating evidence indicates that CYP3A4 is regulated by miRNA both directly and indirectly.

3.3.1 CYP3A4

In 2009, Pan et al. [70] reported that miR-27b directly down-regulates CYP3A4 expression. Luciferase assays revealed that miR-27b recognizes MRE in the 3'-UTR of CYP3A4 (Fig. 4). In 2015, Shi et al. [71] reported that miR-27a down-regulates CYP3A4 expression. Because miR-27a and miR-27b are highly homologous (20 nt out of 21 nt are identical) and have the same seed sequence, the suppressive effect of these miRNAs on CYP3A4 expression would be the same. However, the contribution of miR-27a and miR-27b to the interindividual difference in CYP3A4 levels in the human livers would be different depending on their expression levels. To solve the question, correlation analysis between miRNA and CYP3A4 levels using a panel of human liver samples may provide a hint.

There are several papers showing that miRNAs other than the miR-27 family regulate the expression of CYP3A4. Wei et al. [72] reported that miR-577, miR-1, miR-532-3p, and miR-627 down-regulate CYP3A4 by translational repression (Fig. 4). Yan et al. [73] reported that the miR-628-3p and miR-641 down-regulate CYP3A4 in HepaRG cells (Fig. 4). Liu et al. [74] reported that miR-206 directly down-regulates CYP3A4 in human primary hepatocytes (Fig. 4). Thus, multiple miRNAs could contribute to the variability in CYP3A4 expression in the human livers.

3.3.2 VDR

In a paper by Pan et al. [70], it was demonstrated that miR-27b down-regulate VDR, which transactivates CYP3A4 expression. Therefore, miR-27b has a role in down-regulation of CYP3A4 via direct and indirect mechanisms.

3.3.3 PXR

Before the studies described above were reported, we observed that miR-148a indirectly down-regulates CYP3A4 via suppression of PXR expression [75] (Fig. 4). Luciferase assay revealed that miR-148a binds to MRE in the 3'-UTR of PXR, and overexpression and inhibition studies revealed that miR-148a down-regulates endogenous PXR in HepG2 cells. Interestingly, the miR-148-dependent down-regulation of PXR attenuated the induction of endogenous CYP3A4. Contribution of miR-148a to regulation of PXR and CYP3A4 expression was demonstrated by an inverse correlation between miR-148a level and the translational efficiency of PXR, as well as a positive correlation between PXR protein and CYP3A4 mRNA or protein levels in a panel of human liver samples. Following our study, recent studies reported that miR-30c-1-3p [76] and miR-18-5p [77] down-regulate PXR expression, leading to suppression of CYP3A4 expression (Fig. 4).

3.3.4 RXRα

Retinoid X receptor α (RXRα) is a heterodimer partner of nuclear receptors, such as PXR, CAR, and VDR, to transactivate target genes. We observed that human RXRα is down-regulated by miR-34a [78]. Luciferase assay revealed that miR-34a binds to an MRE in the coding region but not to a potential MRE in the 3'-UTR, and an overexpression study revealed that miR-34a down-regulates endogenous RXRα in HepG2 cells (Fig. 4). The down-regulation mechanism by miR-34a involved facilitating the degradation of RXRα mRNA. Interestingly, it was demonstrated that miR-34a-dependent down-regulation of RXRα abolished the induction of CYP3A4 by rifampicin, a typical ligand of PXR in HepG2 cells.

3.3.5 HNF4α

HNF4α, which is highly expressed in the liver, regulates the expression of various genes involved in the synthesis/metabolism of fatty acid, cholesterol, glucose, and urea. We revealed

that miR-34a and miR-24 recognize MRE in the 3'-UTR and the coding region, respectively, to down-regulate HNF4α [12]. Because HNF4α is critically involved in the regulation of hepatic expression of CYP3A4 [69], these miRNAs might affect CYP3A4 expression although experimental proof is required.

3.4 Cytochrome b_5

Cytochrome b_5 (b_5) is a hemoprotein that transfers electrons to CYP enzymes to enhance their activity [79]. We found that miR-223 down-regulates b_5 expression by mRNA degradation [80]. The luciferase assay showed that miR-223 functionally binds to the 3'-UTR of b5 mRNA. Overexpression of miR-223 reduced b_5 protein level in HepG2 cells, resulting in decreases CYP3A4-catalyzed testosterone 6β-hydroxylation activity and CYP2E1-catalyzed chlorzoxazone 6-hydroxylase activity. miR-223-dependent regulation would be important as a modulating factor of CYPs activities.

4. Regulation of human UGTs expression by miRNAs

UGTs are major phase II drug-metabolizing enzymes that catalyze the transfer of glucuronic acid from UDP-glucuronic acid to various xenobiotics and endobiotics [81]. There are 19 functional UGT isoforms in humans, divided into three subfamilies, UGT1A, UGT2A, and UGT2B, based on evolutionary divergence and sequence homology [82]. It has become increasingly clear that human UGTs are regulated miRNAs (Table 1), as described below.

4.1. UGT1A

The UGT1A subfamily includes nine functional isoforms, UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10. Of these, UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 are expressed in the human liver and are responsible for the glucuronidation of clinical agents including ezetimibe, mycophenolic acid, and sorafenib (Fig. 5A) [81]. The *UGT1A* genes are located on chromosome 2q37 and share alternative first exons spliced to common exons 2-5. The common fifth exon contains a

shared 3'-UTR [82]. Therefore, miRNAs which bind to the 3'-UTR have potential to regulate all members of the UGT1A subfamily.

Dluzen et al. [83] clarified that miR-491-3p binds to the 3'-UTR of UGT1A (Fig. 5B). Overexpression of miR-491-3p in Huh-7 cells significantly repressed UGT1A1, UGT1A3, and UGT1A6 mRNA levels. Additionally, this repression resulted in decrease raloxifene glucuronidation. In contrast, inhibition of miR-491-3p expression resulted in increases in UGT1A mRNA and activity. Significant inverse correlations between miR-491-3p expression and UGT1A3 and UGT1A6 mRNA levels were observed in a panel of normal human liver specimens, suggesting that miR-491-3p is a causal factor in regulating the hepatic expression of UGT1A enzymes.

Recently, Papageorgiou and Court [84] found that miR-103b, miR-141-3p, miR-200a-3p, and miR-376b-3p bind to the 3'-UTR, regardless of SNPs rs10929303, rs1042640, and rs8330, which are in linkage disequilibrium, to down-regulate UGT1As. Interestingly, these researchers demonstrated that the binding of miR-1286 was disrupted by the presence of the SNP s8330, while the binding of miR-21-3p was enhanced by the presence of the SNP rs10929303 (Fig. 5B). Thus, these SNPs in the 3'-UTR would contribute to the interindividual differences in UGT1A expression via affecting miRNA recognition. For miR-141-3p, we found that it down-regulates endogenous UGT1A1, UGT1A4, UGT1A6, UGT1A9, and UGT1A10 mRNAs, as well as 4-methylumbelliferone glucuronidation in Huh-7 or Caco-2 cells [Tatsumi et al., submitted].

Papageorgiou et al. [85] found that miR-375 indirectly down-regulates UGT1A1 and UGT1A6 via binding to 3'-UTR of AhR (Fig. 1). Overexpression of miR-375 in LS180 cells resulted in a decrease in endogenous AhR expression, leading to decrease in expression levels of UGT1A1 and UGT1A6, which are regulated by AhR, as well as ezetimibe glucuronidation. Based on the fact that these UGT1As catalyze glucuronidation of acetaminophen, which may cause acute liver failure, the authors discussed the possibility that miR-375-dependent indirect down-regulation of UGT1A may predispose some individuals to increased risk for acetaminophen-induced liver failure.

4.2 UGT2B

The *UGT2B* genes are located on chromosome 4q13 and encode seven functional proteins including UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17, and UGT2B28 [81]. These genes are all expressed in the human livers at higher levels than UGT1As [86] and are responsible for the metabolism of numerous drugs, such as morphine, zidovudine and efavirenz [81].

Dluzen et al. [87] found that miR-216-5p down-regulates UGT2B4, UGT2B10 and UGT2B15 expression. Luciferase assay revealed that miR-216b-5p binds to MRE in the 3'-UTR of UGT2B7, UGT2B4, and UGT2B10. These researchers observed that the overexpression of miR-216b-5p decreased these UGT2B mRNA levels in human liver Huh-7 and Hep3B cells and decreased epirubicin glucuronidation, which is a marker activity of UGT2B7. Wijayakumara et al. [88] reported that miR-135a and miR-410 down-regulate UGT2B4 and that miR-3664 down-regulates UGT2B7 expression in HepG2 and Huh-7 cells via binding to the 3'-UTRs. They demonstrated that UGT2B4 levels were inversely correlated with miR-135a and miR-410 levels in a panel of human liver samples.

Papageorgiou and Court [89] observed, by luciferase assay using a library containing 2,048 miRNAs, that miR-1293, miR-3664-3p, miR-4317, miR-513c-3p, miR-4483, and miR-142-3p bind to the 3'-UTR of UGT2B7 and that miR-770-5p, miR-103b, miR-3924, miR-376b-3p, miR-455-5p, miR-605, miR-624-3p, miR-4712-5p, miR-3675-3p, miR-6500-5p, miR-548as-3p, and miR-4292 bind to the 3'-UTR of UGT2B15. Among these, the miRNA showing inverse correlation with UGT2B15-mediated *S*-oxazepam glucuronidation in a panel of human liver samples was only miR-455-5p. To prove whether these miRNAs are actually involved in the regulation of UGT2B7 and UGT2B15 in the human liver, overexpression and inhibition studies of these miRNAs using hematoma cell lines or primary human hepatocytes are required.

Two research groups, Wijayakumara et al. [90] and Margaillan et al. [91], independently reported a significance of miR-376c in the down-regulation of UGT2B15 and UGT2B17 in

prostate cancer. UGT2B15 and UGT2B17 inactivate androgens such as testosterone and dihydrotestosterone, which play a central role in prostate cancer progression. Wijayakumara et al. [90] reported that overexpression of miR-376c decreases the glucuronidation of testosterone and androsterone in androgen receptor-positive prostate adenocarcinoma LNCaP cells. Luciferase assay revealed that miR-367c directly binds to the 3'-UTRs of UGT2B15 and UGT2B17. After that step, Margaillan et al. [91] revealed that down-regulation of UGT2B15 and UGT2B17 by the overexpressed miR-376c led to decreased dihydrotestosterone glucuronidation and enhanced proliferation of prostate cancer cells. Interestingly, it was demonstrated that miR-376c levels were lower in prostate cancer tissues than in normal tissues, whereas UGT2B15/2B17 levels were higher in prostate cancer tissues than in normal tissues. Thus, miR-376c regulating UGT2B15 and UGT2B17 plays a role in control of steroid metabolism in prostate.

5. Regulation of human SULT expression by miRNA

Sulfotransferases (SULTs) are phase II drug-metabolizing enzymes which catalyze the transfer of sulfonyl group from 3'-phosphoadenosine 5'-phosphosulfate to nucleophilic groups of a variety of xenobiotic and endogenous compounds to increase the solubility and facilitate the excretion. There are 11 functional isoforms, SULT1A1, SULT1A2, SULT1A3, SULT1A4, SULT1B1, SULT1C2, SULT1C4, SULT1E1, SULT2A1, SULT2B1, and SULT4 in human. These isoforms are divided into three families, SULT1A, SULT2, SULT4 [92]. Among the isoforms, only for SULT1A1, which is responsible for metabolism of such xenobiotics as acetaminophen and minoxidil [93], was miRNA-dependent regulation reported. There is a large interindividual variability in SULT1A1 protein level and activity [94]. Yu et al. [95] focused on the fact that an SNP, 972C>T in the 3'-UTR SULT1A1, is related to the decreased SULT1A1 mRNA level and enzymatic activity. They found that miR-631 directly binds to the 3'-UTR to down-regulate SULT1A1 in the case of 972T type. Thus, an miRNA is responsible for the genotype-dependent variability of SULT1A1 expression.

6. Conclusions

In the past decade, a wealth of knowledge regarding miRNA-mediated regulation of drug metabolism has accumulated. It has become clear that the presence of pseudogenes, SNPs, and RNA editing should be considered in the regulation by miRNA. Studies employing current knowledge to uncover the significance of miRNAs in controlling the expression of genes related drug metabolism and pharmacokinetics would be useful to facilitate drug discovery and to realize precision medicine.

7. Expert opinion

In this review, we summarized the miRNA-dependent regulation of drug metabolism-related genes. In most cases, one or a few miRNAs were experimentally proven for their role in the regulation of a certain target. It is possible that other miRNAs may be underappreciated, because the studies to identify miRNAs/target mRNA pairs is time-, money-, and labor-consuming. In addition, it should be noticed that different miRNAs can act synergistically to regulate a gene expression. Therefore, a large number of miRNAs may be involved in fine-tuning of drug metabolizing enzymes. On the other hand, single miRNA can regulate the expression of multiple targets controlling related functions within a gene network. Therefore, some miRNAs may be master regulators of drug metabolism-related genes, as exemplified by miR-27b or miR-34a.

In addition to the evidence described above sections, other potential miRNAs/target mRNA pairs for several CYPs or UGTs have been identified [96,97], based on the inverse correlation between miRNA levels and concerned target mRNA levels in a panel of human liver samples. However, it should be noted that it is unclear whether such miRNAs directly regulate the CYPs or UGTs, because it is possible that the inverse correlation may occasionally be caused by third factors. Therefore, it is hasty to draw a conclusion for the role of miRNAs in regulation of target mRNA based on only the correlation analysis. To clarify the effects of miRNA on target gene expression and understanding biological function of miRNA, overexpression or inhibition of miRNA would be effective methods, although such

experiments cannot rule out the indirect regulation. Luciferase assay is the most commonly used to validate the potential interactions between miRNA and target genes. Even though, the luciferase assays using reporter plasmids including entire 3'-UTR might be insufficient to prove direct regulation by miRNA, because it is possible that the change in luciferase activity may be caused by third factors. To demonstrate direct regulation, using of reporter plasmids containing only MRE or 3'-UTR with mutated MRE would be useful. Additionally, electrophoretic mobility shift assay or crosslinking, ligation, and sequencing of hybrids (CLASH) would be beneficial method to investigate miRNA-mRNA binding.

miRNA expression, which is regulated at both a transcriptional level and a post-transcriptional level (i.e., processing), can be changed by intrinsic and extrinsic factors. For example, SNPs in pri-miRNA and pre-miRNAs affect the expression level of mature miRNAs [98,99]. miRNAs expression is readily changed by stimuli including oxidative stress, hormones, and exposure to xenobiotics such as rifampicin (a ligand of PXR) [100,101], phenobarbital (an activator of CAR) [102], and TCDD (a ligand of AhR) [103]. Since such studies are still immature, the information is limited as to how each miRNA is regulated. Elucidation of regulatory mechanisms of miRNA expression in the human livers, focusing on genetic and non-genetic factors, is the subject of future studies in order to determine the events causing changes in drug-metabolizing enzyme expression.

Another issue is that almost all of the studies demonstrated the significance of miRNAs in the regulation of drug-metabolizing enzymes by *in vitro* experiments. Therefore, it is uncertain whether the identified miRNAs may cause changes in pharmacokinetics in the whole body. To address this issue, Jilek et al. [104] intravenously administered miR-34a to mice and determined the pharmacokinetics of CYP substrates (midazolam, dextromethorphan, phenacetin, diclofenac, and chlorzoxazone), focusing on the fact that the development of miR-34a replacement therapy is ongoing as a new cancer treatment. In their study, although the hepatic miR-34a level was increased by 80-fold by administration of miR-34a, they observed only a marginal increase in systemic exposure to midazolam, phenacetin, and dextromethorphan. They concluded that miR-34a has minor or no effects on the

pharmacokinetics of CYP substrates. However, it must be emphasized that we have to pay attention to species differences in miRNA targeting, because the MRE may not be conserved though the sequences of miRNAs are almost the same across species. For example, although several miRNAs were identified to regulate human CYP2E1 [61,62,66,67], they are unlikely to be functional for mouse and rat CYP2E1 because of poor similarity of the sequence in the 3'-UTR. Therefore, extrapolation of the results of *in vivo* studies from rodent to human is not feasible. Studies using humanized mice are intriguing and helpful to know the impact of miRNA-dependent regulation of drug-metabolizing enzymes on pharmacokinetics *in vivo* in humans.

At present, miRNA-based medicines, such as miravirsen (miR-122 antisense oligo nucleotides), are being developed [105,106]. It is a concern that miRNA-based medicine may cause drug-drug interaction via changing the expression of drug-metabolizing enzymes. Recently, it has been reported that miR-122 protects human hepatocytes from acetaminophen toxicity by repressing CYP1A2 and CYP2E1 expression [107]. The study suggested that the miR-122 inhibitor has a risk to enhance susceptibility to acetaminophen toxicity. Although experimental animals are used in preclinical studies to examine toxicity of drug candidate, it is not suitable for miRNA-based medicines because of species differences of sequence of MRE as described above. Therefore, in addition to the humanized mouse as a useful tool for *in vivo* studies, establishment of *in vitro* systems to examine the potency of miRNA-based medicine to cause drug-drug interaction is an issue to be solved in the future.

In summary, since the first discovery that miR-27b regulates human CYP1B1 expression [15], abundant evidence has been obtained to show the significance of miRNAs in modulating drug metabolism potencies. There is no room for doubt that miRNA is one of the causal factors of interindividual difference in drug metabolism potency, in addition to well-known factors including genetic polymorphisms, disease state, sex, age, and exposure to xenobiotics. The day will come when miRNAs that regulate drug response and toxicity can be combined to create precision medicine.

Article highlights box

1. Variability in expression of drug-metabolizing enzymes, such as CYP and UGT, is a critical factor in interindividual differences in drug response and toxicity
2. Some miRNAs down-regulate CYP, UGT, SULT and related transcriptional factors through translational repression or mRNA degradation.
3. Presence of pseudogenes, SNPs, and RNA editing affects the miRNA-dependent regulation of drug metabolizing-enzymes.
4. Elucidation of regulatory mechanisms of miRNA expression in the human livers, focusing on genetic and non-genetic factors, will be useful to understand the causes of inter- or intra-individual variability of drug metabolism potencies.
5. The next challenge is to prove the impact of miRNA-dependent regulation of drug-metabolizing enzymes on pharmacokinetics *in vivo*.

Reference

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers

1. Lu AY. Drug-metabolism research challenges in the new millennium: individual variability in drug therapy and drug safety. Drug Metab Dispos. 1998;26(12):1217-1222.
2. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013;138(1):103-141
3. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-297.
4. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell. 1993;75(5):843-854.
5. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in C. elegans. Cell. 1993;75(5):855-862.
6. Pasquinelli AE, Reinhart BJ, Slack F, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature. 2000;408(6808):86-9.
7. Hammond SM. An overview of microRNAs. Adv Drug Deliv Rev. 2015;87:3-14.
8. Hinske LC, Galante PA, Kuo WP, et al. A potential role for intragenic miRNAs on their hosts' interactome. BMC Genomics. 2010;11:533.
9. Curtis HJ, Sibley CR, Wood MJ. Mirtrons, an emerging class of atypical miRNA. Wiley Interdiscip Rev RNA. 2012;3(5):617-632.
10. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010;11(9):597-610.
11. Duursma AM, Kedde M, Schrier M, et al. miR-148 targets human DNMT3b protein coding region. RNA. 2008;14(5):872-877.
12. Takagi S, Nakajima M, Kida K, et al. MicroRNAs regulate human hepatocyte nuclear

factor 4α, modulating the expression of metabolic enzymes and cell cycle. J Biol Chem. 2010;285(7):4415-4422.

13. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120(1):15-20.
14. Alexiou P, Maragkakis M, Papadopoulos GL, et al. Lost in translation: an assessment and perspective for computational microRNA target identification. Bioinformatics. 2009;25(23):3049-3055.
15. Tsuchiya Y, Nakajima M, Takagi S, et al. MicroRNA regulates the expression of human cytochrome P450 1B1. Cancer Res. 2006;66(18):9090-9098.

**** The first study demonstrating that miRNA regulates human CYP expression**

16. Ramadoss P, Marcus C, Perdew GH. Role of the aryl hydrocarbon receptor in drug metabolism. Expert Opin Drug Metab Toxicol. 2005;1(1):9-21.
17. Kondraganti SR, Fernandez-Salguero P, Gonzalez FJ, et al. Polycyclic aromatic hydrocarbon-inducible DNA adducts: evidence by 32P-postlabeling and use of knockout mice for Ah receptor-independent mechanisms of metabolic activation in vivo. Int J Cancer. 2003;103(1):5-11.
18. Moorthy B, Chu C, Carlin DJ. Polycyclic aromatic hydrocarbons: from metabolism to lung cancer. Toxicol Sci. 2015;145(1):5-15.
19. Choi YM, An S, Lee EM, et al. CYP1A1 is a target of miR-892a-mediated post-transcriptional repression. Int J Oncol. 2012;41(1):331-336.
20. Zhou SF, Yang LP, Zhou ZW, et al. Insights into the substrate specificity, inhibitors, regulation, and polymorphisms and the clinical impact of human cytochrome P450 1A2. AAPS J. 2009;11(3):481-494.
21. Chen Y, Zeng L, Wang Y, et al. The expression, induction and pharmacological activity of CYP1A2 are post-transcriptionally regulated by microRNA hsa-miR-132-5p. Biochem Pharmacol. 2017;145:178-191.
22. Shet MS, McPhaul M, Fisher CW, et al. Metabolism of the antiandrogenic drug (Flutamide) by human CYP1A2. Drug Metab Dispos. 1997;25(11):1298-1303.

23. Takamizawa J, Konishi H, Yanagisawa K, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res. 2004;64(11):3753-3756.
24. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA. 2002;99(24):15524-15529.
25. Hayes CL, Spink DC, Spink BC, et al. 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. Proc Natl Acad Sci USA. 1996;93(18):9776-9781.
26. Murray GI, Taylor MC, McFadyen MC, et al. Tumor-specific expression of cytochrome P450 CYP1B1. Cancer Res. 1997;57(14):3026-3031.
27. Ragavan N, Hewitt R, Cooper LJ, et al. CYP1B1 expression in prostate is higher in the peripheral than in the transition zone. Cancer Lett. 2004;215(1):69-78.
28. Chang I, Mitsui Y, Fukuhara S, et al. Loss of miR-200c up-regulates CYP1B1 and confers docetaxel resistance in renal cell carcinoma. Oncotarget. 2015;6(10):7774-7787.
29. McFadyen MC, McLeod HL, Jackson FC, et al. Cytochrome P450 CYP1B1 protein expression: a novel mechanism of anticancer drug resistance. Biochem Pharmacol. 2001;62(2):207-212.
30. Mao M, Wu Z, Chen J. MicroRNA-187-5p suppresses cancer cell progression in non-small cell lung cancer (NSCLC) through down-regulation of CYP1B1. Biochem Biophys Res Commun. 2016;478(2):649-655.
31. Sato S, Shirakawa H, Tomita S, et al. Low-dose dioxins alter gene expression related to cholesterol biosynthesis, lipogenesis, and glucose metabolism through the aryl hydrocarbon receptor-mediated pathway in mouse liver. Toxicol Appl Pharmacol. 2008;229(1):10-19.
32. Kurtz CL, Fannin EE, Toth CL, et al. Inhibition of miR-29 has a significant lipid-lowering benefit through suppression of lipogenic programs in liver. Sci Rep. 2015;5:12911.
33. Huang TC, Chang HY, Chen CY, et al. Silencing of miR-124 induces neuroblastoma SK-

N-SH cell differentiation, cell cycle arrest and apoptosis through promoting AHR. FEBS Lett. 2011;585(22):3582-3586.

34. Nakano M, Fukami T, Gotoh S, et al. RNA editing modulates human hepatic aryl hydrocarbon receptor expression by creating microRNA recognition sequence. J Biol Chem. 2016;291(2):894-903.

***An interesting study providing new insight that RNA editing affects miRNA-dependent down-regulation of gene associated with pharmacokinetics**

35. Nishikura K. A-to-I editing of coding and non-coding RNAs by ADARs. Nat Rev Mol Cell Biol. 2016;17(2):83-96.
36. Slotkin W, Nishikura K. Adenosine-to-inosine RNA editing and human disease. Genome Med. 2013;5(11):105.
37. Oda Y, Nakajima M, Mohri T, et al. Aryl hydrocarbon receptor nuclear translocator in human liver is regulated by miR-24. Toxicol Appl Pharmacol. 2012;260(3):222-231.
38. Yamano S, Tatsuno J, Gonzalez FJ. The CYP2A3 gene product catalyzes coumarin 7-hydroxylation in human liver microsomes. Biochemistry. 1990;29(5):1322-1329.
39. Ding S, Lake BG, Friedberg T, et al. Expression and alternative splicing of the cytochrome P-450 CYP2A7. Biochem J. 1995; 306:161-166.
40. Nakajima M, Yamamoto T, Nunoya K, et al. Role of human cytochrome P4502A6 in C-oxidation of nicotine. Drug Metab Dispos. 1996;24(11):1212-1217
41. Tiano HF, Hosokawa M, Chulada PC, Smith PB, et al. Retroviral mediated expression of human cytochrome P450 2A6 in C3H/10T1/2 cells confers transformability by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Carcinogenesis. 1993;14(7):1421-1427.
42. Nakajima M, Kuroiwa Y, Yokoi T. Interindividual differences in nicotine metabolism and genetic polymorphisms of human CYP2A6. Drug Metab Rev. 2002;34(4):865-877.
43. Nakano M, Fukushima Y, Yokota S, et al. CYP2A7 pseudogene transcript affects CYP2A6 expression in human liver by acting as a decoy for miR-126*. Drug Metab Dispos. 2015;43(5):703-712.

*** The first study demonstrating that a pseudogene affects miRNA-dependent regulation of CYP expression**

44. Lamba V, Lamba J, Yasuda K, et al. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther. 2003;307(3):906-922.
45. Sueyoshi T, Kawamoto T, Zelko I, et al. The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. J Biol Chem. 1999;274(10):6043-6046.
46. Jin Y, Yu D, Tolleson WH, et al. MicroRNA hsa-miR-25-3p suppresses the expression and drug induction of CYP2B6 in human hepatocytes. Biochem Pharmacol. 2016;113:88-96.
47. Burgess KS, Ipe J, Swart M et al. Variants in the CYP2B6 3'UTR alter *in vitro* and *in vivo* CYP2B6 activity: potential role of microRNAs. Clin Pharmacol Ther. in press.
48. Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol. 2001;52(4):349-355.
49. Zhang SY, Surapureddi S, Coulter S, et al. Human CYP2C8 is post-transcriptionally regulated by microRNAs 103 and 107 in human liver. Mol Pharmacol. 2012;82(3):529-540.
50. Yu D, Green B, Marrone A, et al. Suppression of CYP2C9 by microRNA hsa-miR-128-3p in human liver cells and association with hepatocellular carcinoma. Sci Rep. 2015;5:8534.
51. Yu D, Green B, Tolleson WH, MicroRNA hsa-miR-29a-3p modulates CYP2C19 in human liver cells. Biochem Pharmacol. 2015;98(1):215-223.
52. Rieger JK, Reutter S, Hofmann U, et al. Inflammation-associated microRNA-130b down-regulates cytochrome P450 activities and directly targets CYP2C9. Drug Metab Dispos. 2015;43(6):884-888.
53. Rendic S. Summary of information on human CYP enzymes: human P450 metabolism data. Drug Metab Rev. 2002;34(1-2):83-448.

54. Shimada T, Yamazaki H, Mimura M, et al. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther. 1994;270(1):414-423.
55. Hart SN, Wang S, Nakamoto K, et al. Genetic polymorphisms in cytochrome P450 oxidoreductase influence microsomal P450-catalyzed drug metabolism. Pharmacogenet Genomics. 2008;18(1):11-24.
56. Bock KW, Schrenk D, Forster A, et al. The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes. Pharmacogenetics. 1994;4(4):209-218.
57. Zeng L, Chen Y, Wang Y, et al. MicroRNA hsa-miR-370-3p suppresses the expression and induction of CYP2D6 by facilitating mRNA degradation. Biochem Pharmacol. 2017;140:139-149.
58. Bolt HM, Roos PH, Thier R. The cytochrome P-450 isoenzyme CYP2E1 in the biological processing of industrial chemicals: consequences for occupational and environmental medicine. Int Arch Occup Environ Health. 2003;76(3):174-185.
59. Bièche I, Narjoz C, Asselah T, et al. Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1, CYP2 and CYP3 families in 22 different human tissues. Pharmacogenet Genomics. 2007;17(9):731-742.
60. Sumida A, Kinoshita K, Fukuda T, et al. Relationship between mRNA levels quantified by reverse transcription-competitive PCR and metabolic activity of CYP3A4 and CYP2E1 in human liver. Biochem Biophys Res Commun. 1999;262(2):499-503.
61. Mohri T, Nakajima M, Fukami T, et al. Human CYP2E1 is regulated by miR-378. Biochem Pharmacol. 2010;79(7):1045-1052.
62. Nakano M, Mohri T, Fukami T, et al. Single-Nucleotide Polymorphisms in Cytochrome P450 2E1 (CYP2E1) 3'-Untranslated Region Affect the Regulation of CYP2E1 by miR-570. Drug Metab Dispos. 2015;43(10):1450-1457.

*** The first study demonstrating that a SNP affects miRNA-dependent regulation of CYP**

63. Ramamoorthy A, Skaar TC. In silico identification of microRNAs predicted to regulate the drug metabolizing cytochrome P450 genes. Drug Metab Lett 2011;5(2):126-131.
64. Jeffries CD, Fried HM, Perkins DO, et al. Nuclear and cytoplasmic localization of neural stem cell microRNAs. RNA. 2011;17(4):675-686.
65. Kim DH, Saetrom P, Snøve O Jr, et al. MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc Natl Acad Sci USA. 2008;105(42):16230-16235.
66. Miao L, Yao H, Li C1, et al. A dual inhibition: microRNA-552 suppresses both transcription and translation of cytochrome P450 2E1. Biochim Biophys Acta. 2016;1859(4):650-662.
67. Wang Y, Yu D, Tolleson WH, et al. A systematic evaluation of microRNAs in regulating human hepatic CYP2E1. Biochem Pharmacol. 2017;138:174-184.
68. Bertz RJ, Granneman GR. Use of *in vitro* and *in vivo* data to estimate the likelihood of metabolic pharmacokinetic interactions. Clin. Pharmacokinet. 1997;32(3):210-258.
69. Martínez-Jiménez CP, Jover R, Donato MT, et al. Transcriptional regulation and expression of CYP3A4 in hepatocytes. Curr Drug Metab. 2007;8(2):185-194.
70. Pan YZ, Gao W, Yu AM. MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. Drug Metab Dispos. 2009;37(10):2112-2117.
71. Shi Y, Liu Y, Wei Z, et al. Hsa-miR-27a is involved in the regulation of CYP3A4 expression in human livers from Chinese Han population. Pharmacogenomics. 2015;16(12):1379-1386.
72. Wei Z, Jiang S, Zhang Y, et al. The effect of microRNAs in the regulation of human CYP3A4: a systematic study using a mathematical model. Sci Rep. 2014;4:4283.
73. Yan L, Liu J, Zhao Y, et al. Suppression of miR-628-3p and miR-641 is involved in rifampin-mediated CYP3A4 induction in HepaRG cells. Pharmacogenomics. 2017;18(1):57-64.
74. Liu JE, Ren B, Tang L, et al. The independent contribution of miRNAs to the missing heritability in CYP3A4/5 functionality and the metabolism of atorvastatin. Sci Rep. 2016;6:26544.

75. Takagi S, Nakajima M, Mohri T, et al. Post-transcriptional regulation of human pregnane X receptor by micro-RNA affects the expression of cytochrome P450 3A4. J Biol Chem. 2008;283(15):9674-9680.
76. Vachirayonstien T, Yan B. MicroRNA-30c-1-3p is a silencer of the pregnane X receptor by targeting the 3'-untranslated region and alters the expression of its target gene cytochrome P450 3A4. Biochim Biophys Acta. 2016;1859(9):1238-1244.
77. Sharma D, Turkistani AA, Chang W, et al, Negative regulation of human pregnane X receptor by microRNA-18a-5p: evidence for suppression of microRNA-18a-5p expression by rifampin and rilpivirine. Mol Pharmacol. 2017;92(1):48-56.
78. Oda Y, Nakajima M, Tsuneyama K, et al. Retinoid X receptor α in human liver is regulated by miR-34a. Biochem Pharmacol. 2014;90(2):179-187.
79. Porter TD. The roles of cytochrome b5 in cytochrome P450 reactions. J Biochem Mol Toxicol. 2002;16(6):311-6.
80. Takahashi K, Oda Y, Toyoda Y, et al. Regulation of cytochrome b_5 expression by miR-223 in human liver: effects on cytochrome P450 activities. Pharm Res. 2014;31(3):780-794.
81. Oda S, Fukami T, Yokoi T, et al. A comprehensive review of UDP-glucuronosyltransferase and esterases for drug development. Drug Metab Pharmacokinet. 2015;30(1):30-51.
82. Mackenzie PI, Bock KW, Burchell B, et al. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. Pharmacogenet Genomics. 2005;15(10):677-685.
83. Dluzen DF, Sun D, Salzberg AC, et al. Regulation of UDP-glucuronosyltransferase 1A1 expression and activity by microRNA 491-3p. J Pharmacol Exp Ther. 2014;348(3):465-477.
84. Papageorgiou I, Court MH. Identification and validation of microRNAs directly regulating the UDP-glucuronosyltransferase 1A subfamily enzymes by a functional genomics approach. Biochem Pharmacol. 2017;137:93-106.
85. Papageorgiou I, Freytsis M, Court MH. Transcriptome association analysis identifies

miR-375 as a major determinant of variable acetaminophen glucuronidation by human liver. Biochem Pharmacol. 2016;117:78-87.

86. Izukawa T, Nakajima M, Fujiwara R, et al. Quantitative analysis of UDP-glucuronosyltransferase (UGT) 1A and UGT2B expression levels in human livers. Drug Metab Dispos. 2009;37(8):1759-1768.
87. Dluzen DF, Sutliff AK, Chen G, et al. Regulation of UGT2B expression and activity by miR-216b-5p in liver cancer cell lines. J Pharmacol Exp Ther. 2016;359(1):182-193.
88. Wijayakumara DD, Mackenzie PI, McKinnon RA, et al. Regulation of UDP-Glucuronosyltransferases UGT2B4 and UGT2B7 by MicroRNAs in Liver Cancer Cells. J Pharmacol Exp Ther. 2017;361(3):386-397.
89. Papageorgiou I, Court MH. Identification and validation of the microRNA response elements in the 3'-untranslated region of the UDP glucuronosyltransferase (UGT) 2B7 and 2B15 genes by a functional genomics approach. Biochem Pharmacol. 2017;146:199-213.
90. Wijayakumara DD, Hu DG, Meech R, et al. Regulation of human UGT2B15 and UGT2B17 by miR-376c in prostate cancer cell lines. J Pharmacol Exp Ther. 2015;354(3):417-425.
91. Margaillan G, Lévesque É, Guillemette C. Epigenetic regulation of steroid inactivating UDP-glucuronosyltransferases by microRNAs in prostate cancer. Biochem Mol Biol. 2016;155 (Part A) :85-93.
92. Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL, McManus ME. Human sulfotransferases and their role in chemical metabolism. Toxicol Sci. 2006;90(1):5-22.
93. Hildebrandt M, Adjei A, Weinshilboum R, et al. Very important pharmacogene summary: sulfotransferase 1A1. Pharmacogenet Genomics. 2009;19(6):404-406.
94. Jones AL, Roberts RC, Coughtrie MW. The human phenolsulphotransferase polymorphism is determined by the level of expression of the enzyme protein. Biochem J. 1993;296 (Pt 2):287-290.

95. Yu X, Dhakal IB, Beggs M, et al. Functional genetic variants in the 3'-untranslated region of sulfotransferase isoform 1A1 (SULT1A1) and their effect on enzymatic activity. Toxicol Sci. 2010;118(2):391-403.
96. Rieger JK, Klein K, Winter S, et al. Expression variability of absorption, distribution, metabolism, excretion-related microRNAs in human liver: influence of nongenetic factors and association with gene expression. Drug Metab Dispos. 2013;41(10):1752-1762.
97. Lamba V, Ghodke Y, Guan W et al. microRNA-34a is associated with expression of key hepatic transcription factors and cytochromes P450. Biochem Biophys Res Commun. 2014;445(2):404-411.
98. Iwai N, Naraba H. Polymorphisms in human pre-miRNAs. Biochem Biophys Res Commun. 2005;331(4):1439-44.
99. Duan R, Pak C, Jin P. Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-miRNA. Hum Mol Genet. 2007;16(9):1124-1131.
100. Takahashi K, Tatsumi N, Fukami T, et al. Integrated analysis of rifampicin-induced microRNA and gene expression changes in human hepatocytes. Drug Metab Pharmacokinet. 2014;29(4):333-340.
101. Ramamoorthy A, Liu Y, Philips S, et al. Regulation of microRNA expression by rifampin in human hepatocytes. Drug Metab Dispos. 2013;41(10):1763-1768.
102. Koufaris C, Wright J, Osborne M, et al. Time and dose-dependent effects of phenobarbital on the rat liver miRNAome. Toxicology. 2013;314(2-3):247-253.
103. Singh NP, Singh UP, Guan H, et al. Prenatal exposure to TCDD triggers significant modulation of microRNA expression profile in the thymus that affects consequent gene expression. PLoS One. 2012;7(9):e45054.
104. Jilek JL, Tian Y, Yu AM. Effects of microRNA-34a on the pharmacokinetics of cytochrome P450 probe drugs in mice. Drug Metab Dispos. 2017;45(5):512-522.

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105. Titze-de-Almeida R, David C, Titze-de-Almeida SS. The race of 10 synthetic RNAi-

based drugs to the pharmaceutical market. Pharm Res. 2017;34(7):1339-1363.
106. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov. 2014;13(8):622-638.
107. Chowdhary V, Teng KY, Thakral S, et al. miRNA-122 protects mice and human hepatocytes from acetaminophen toxicity by regulating cytochrome P450 family 1 subfamily A member 2 and family 2 subfamily E member 1 expression. Am J Pathol. 2017;187(12):2758-2774.

Figure legend

Figure 1

Regulation of CYP1 family and related transcription factors by miRNAs. miR-892a and miR-132-5p directly down-regulate CYP1A1 and CYP1A2 expression, respectively [19,21]. miR-27b, miR-187-5p, miR-200c directly down-regulate CYP1B1 expression [15]. miR-29, miR-124, miR-375 and miR-378 down-regulate AhR expression, leading to decreases in its downstream CYP1A1, CYP1A2 and CYP1B1 expression [32-34,85]. The miR-378-mediated regulation of AhR expression is dependent on RNA editing by ADAR1 [34]. miR-24 regulates ARNT expression, a heterodimer partner of AhR [37]. ADAR1, adenosine deaminase acting on RNA 1; AhR, arylhydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; CYP, cytochrome P450; MRE, microRNA recognition element; UTR, untranslated region.

Figure 2

Regulation of CYP2A6 by miR-126-5p and inhibitory effect of CYP2A7 pseudogene transcript on the miR-126-5p-dependent down-regulation in human liver cells. CYP2A6 and CYP2A7 are post-transcriptionally regulated by miR-126-5p. CYP2A7 can restore the miR-126-5p-dependent down-regulation of CYP2A6 by acting as a decoy for miR-126-5p [43]. CYP, cytochrome P450; MRE, microRNA recognition element; UTR, untranslated region.

Figure 3

Regulation of CYP2E1 expression by miRNAs. miR-214-3p down-regulates CYP2E1 expression by binding to the coding region [67]. miR-378 and miR-570 down-regulate CYP2E1 expression by binding to the 3'-UTR [61,62]. SNPs in MRE for miR-570 in the 3'-UTR enhance the binding of miR-570 [62]. miR-552 down-regulates CYP2E1 expression through post-transcriptional regulation via binding to the 3'-UTR and transcriptional repression via binding to the promoter region [66]. CYP, cytochrome P450; MRE, microRNA recognition element; SNP, single nucleotide polymorphism; UTR, untranslated region.

Figure 4

Regulation of CYP3A4 and related nuclear receptors by miRNAs. miR-1, miR-27a, miR-27b, miR-206, miR-532-3p, miR-577, miR-627, miR-628-3p and miR-641 directly down-regulate CYP3A4 expression by binding to the 3'-UTR [70-74]. miR-18-5p, miR-30c-1-3p and miR-148a down-regulate PXR expression, leading to decrease in its downstream CYP3A4 expression [75-77]. miR-34a and miR-27b down-regulate RXRα and VDR expression, respectively, leading to decrease in its downstream CYP3A4 expression [70,78]. CYP, cytochrome P450; MRE, microRNA recognition element; PXR, pregnane X receptor; RXRα, Retinoid X receptor α; UTR, untranslated region; VDR, vitamin D receptor.

Figure 5

Regulation of UGT1A expression by miRNAs. (A) *UGT1A* gene contains multiple unique exon 1s and common exons 2-5, which produce nine functional isoforms, UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10. In the human liver, UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 are expressed. (B) miR-21-3p, miR-103b, miR-141-3p, miR-200a-3p, miR-376b-3p, miR-491-3p, and miR-1286 down-regulate UGT1As expression by binding to the 3'-UTR [83,84] A SNP in MRE for miR-21-3p in the 3'-UTR enhance the binding of miR-21-3p and a SNP in MRE in the 3'-

UTR for miR-1286 disrupts the binding of miR-1286 [84]. MRE, microRNA recognition element; UGT, UDP-glucuronosyltransferase; UTR, untranslated region.

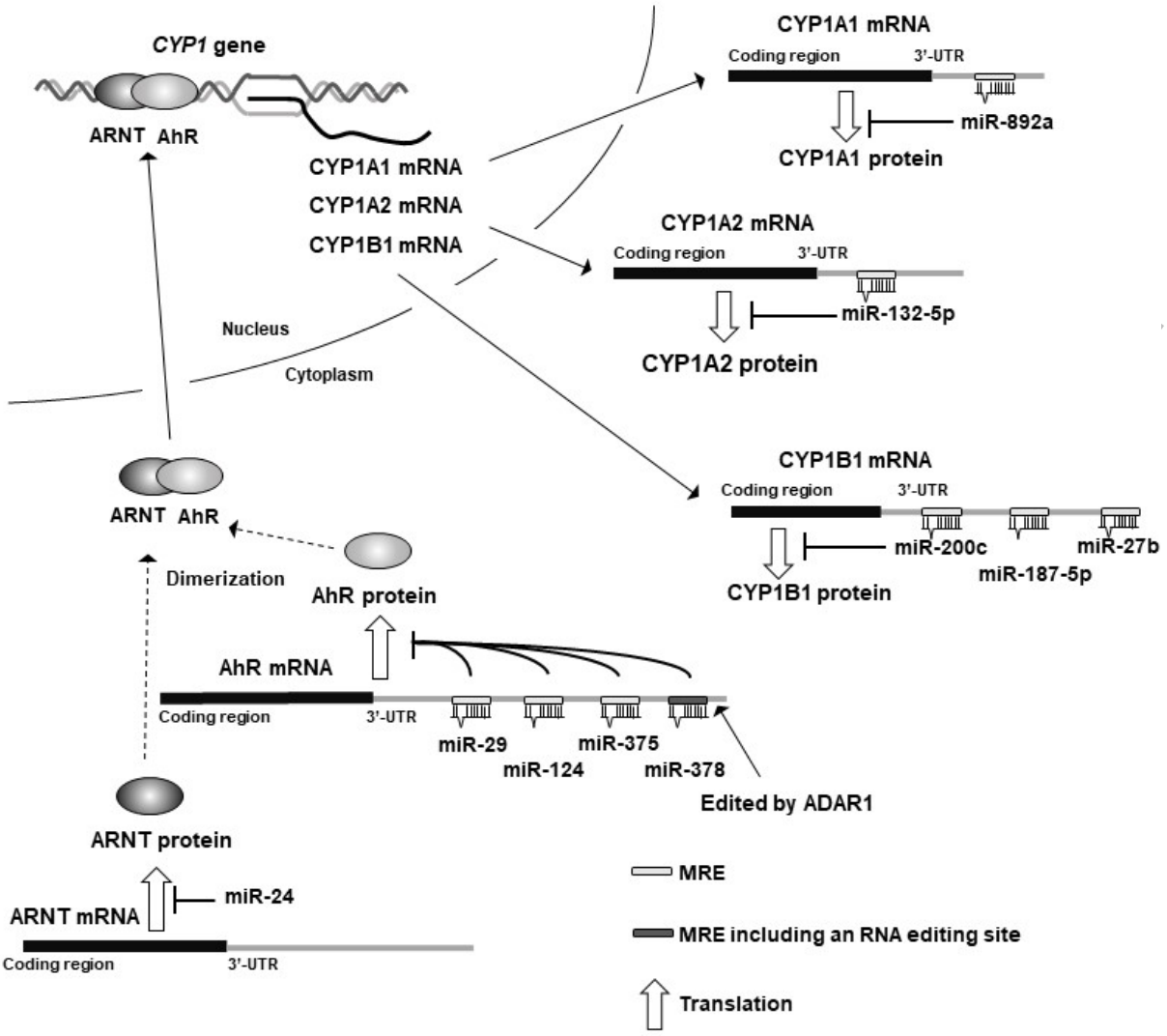
CYP1 gene
ARNT AhR
CYP1A1 mRNA
CYP1A2 mRNA
CYP1B1 mRNA
Nucleus
Cytoplasm
CYP1A1 mRNA
Coding region
3'-UTR
miR-892a
CYP1A1 protein
CYP1A2 mRNA
Coding region
3'-UTR
miR-132-5p
CYP1A2 protein
CYP1B1 mRNA
Coding region
3'-UTR
miR-200c
miR-27b
miR-187-5p
CYP1B1 protein
ARNT AhR
Dimerization
AhR protein
AhR mRNA
Coding region
3'-UTR
miR-29
miR-124
miR-375
miR-378
Edited by ADAR1
ARNT protein
ARNT mRNA
miR-24
Coding region
3'-UTR
MRE
MRE including an RNA editing site
Translation

Figure 1

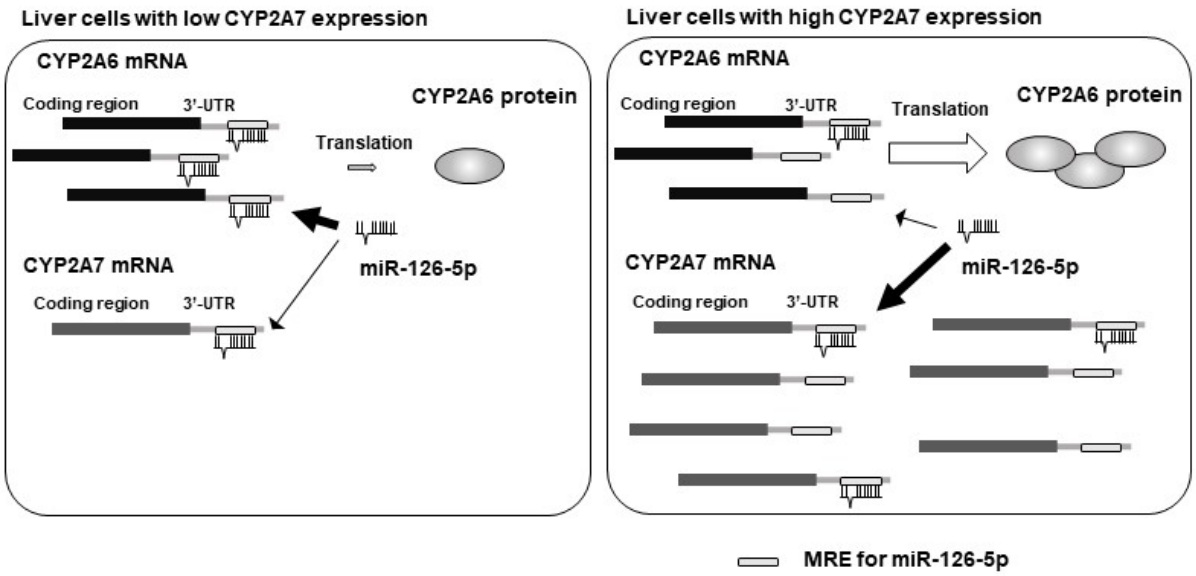
Liver cells with low CYP2A7 expression
CYP2A6 mRNA
Coding region
3'-UTR
CYP2A6 protein
Translation
CYP2A7 mRNA
miR-126-5p
Coding region
3'-UTR
Liver cells with high CYP2A7 expression
CYP2A6 mRNA
Coding region
3'-UTR
Translation
CYP2A6 protein
CYP2A7 mRNA
miR-126-5p
Coding region
3'-UTR
MRE for miR-126-5p

Figure 2

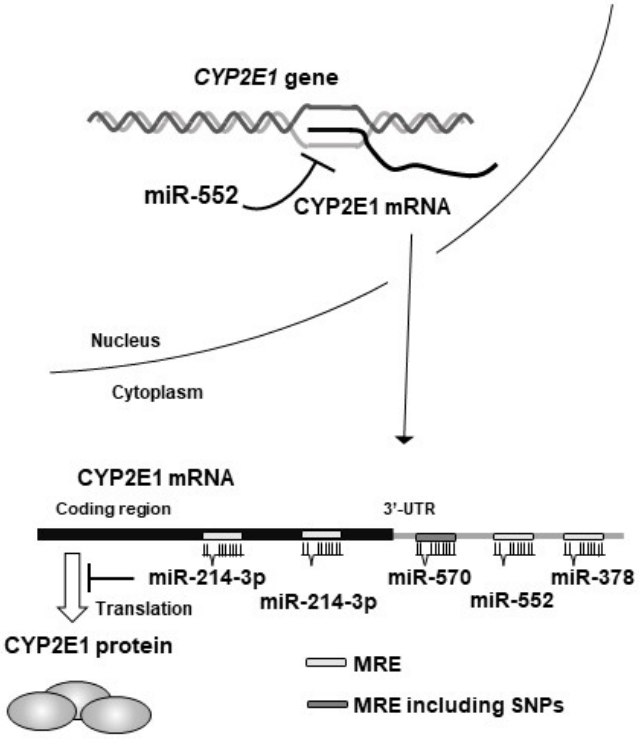
CYP2E1 gene
miR-552
CYP2E1 mRNA
Nucleus
Cytoplasm
CYP2E1 mRNA
Coding region
3'-UTR
miR-214-3p
miR-214-3p
miR-570
miR-552
miR-378
Translation
CYP2E1 protein
MRE
MRE including SNPs

Figure 3

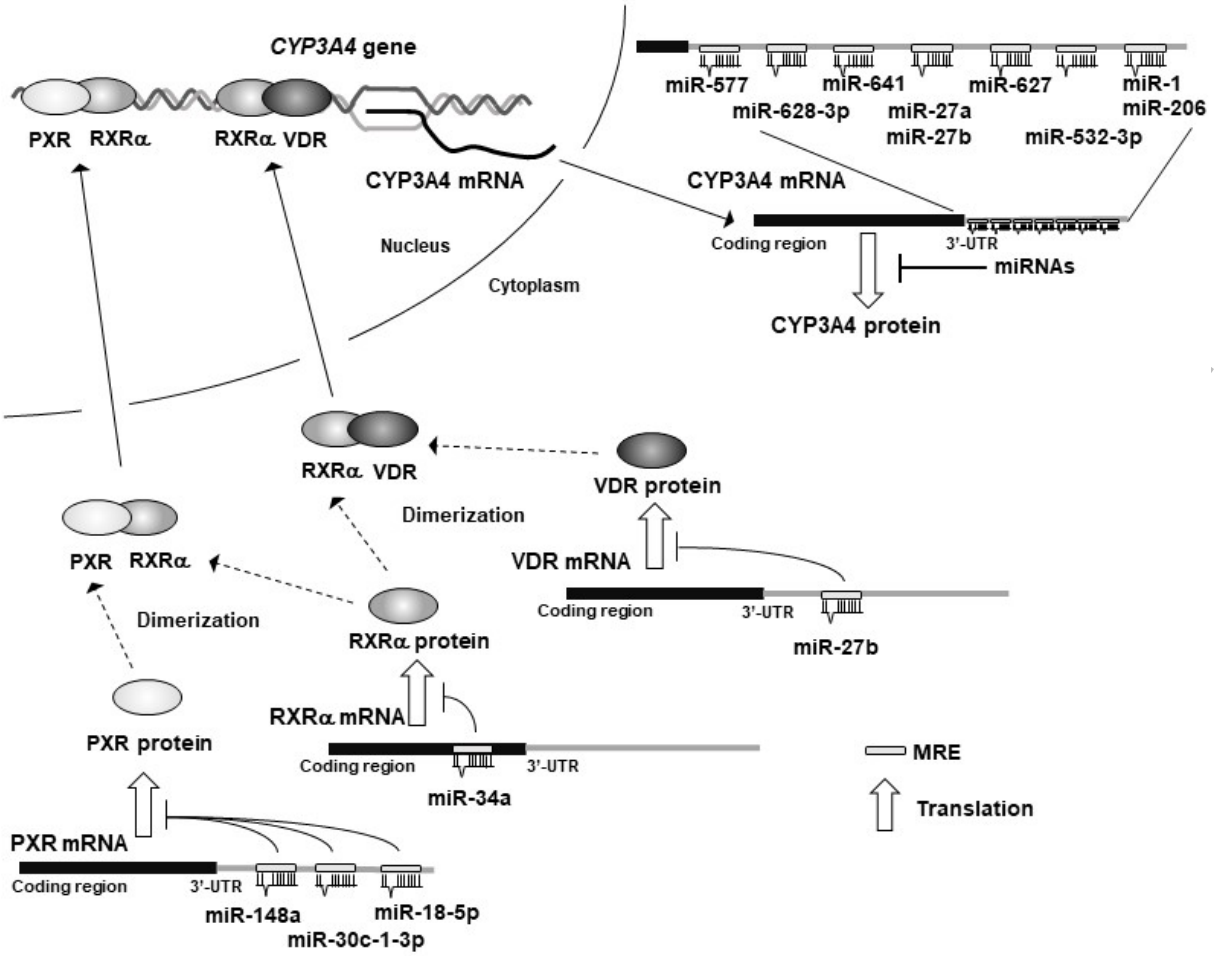
CYP3A4 gene
PXR RXRα
RXRα VDR
CYP3A4 mRNA
miR-577
miR-641
miR-627
miR-1
miR-206
miR-628-3p
miR-27a
miR-27b
miR-532-3p
CYP3A4 mRNA
Nucleus
Cytoplasm
Coding region
3'-UTR
miRNAs
CYP3A4 protein
RXRα VDR
VDR protein
Dimerization
PXR RXRα
VDR mRNA
Coding region
3'-UTR
miR-27b
Dimerization
RXRα protein
RXRα mRNA
PXR protein
Coding region
3'-UTR
miR-34a
MRE
Translation
PXR mRNA
Coding region
3'-UTR
miR-148a
miR-18-5p
miR-30c-1-3p

Figure 4

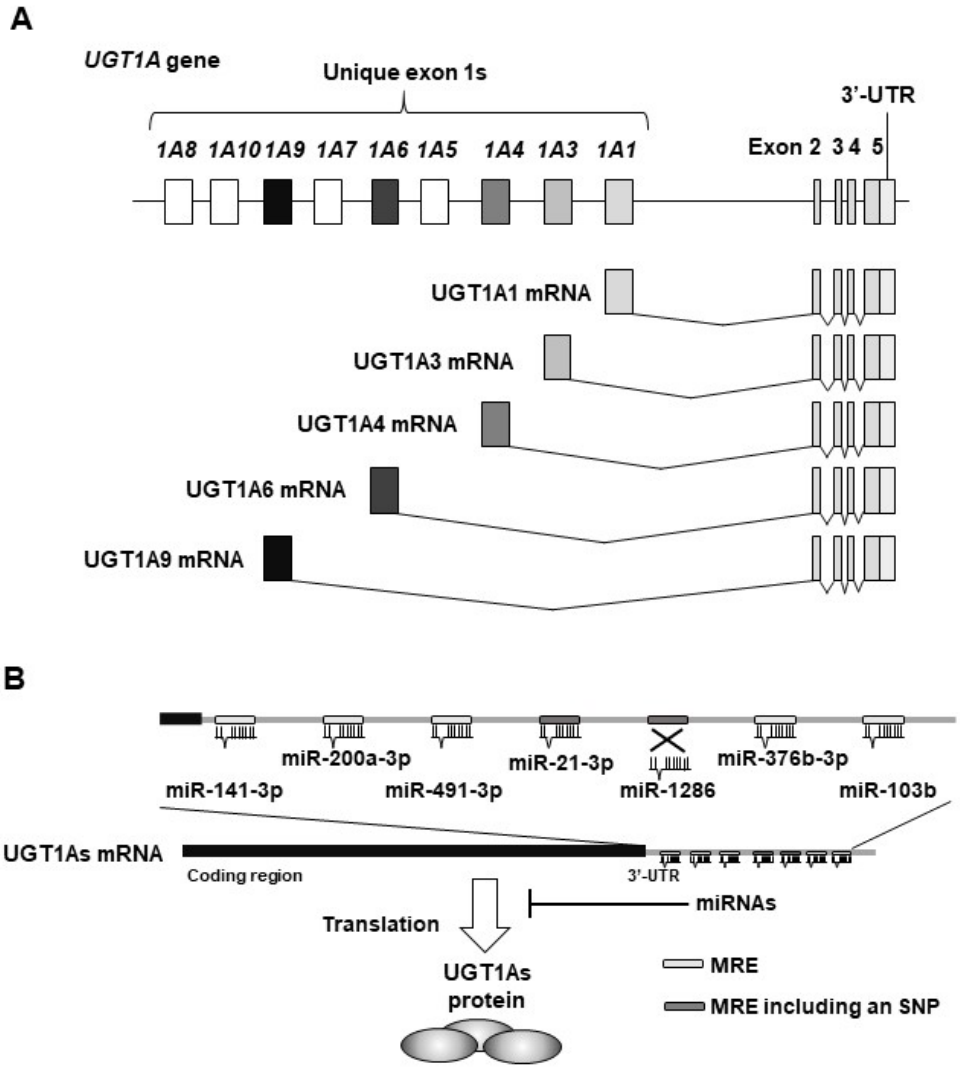
A
UGT1A gene
Unique exon 1s
3'-UTR
1A8 1A10 1A9 1A7 1A6 1A5 1A4 1A3 1A1
Exon 2 3 4 5
UGT1A1 mRNA
UGT1A3 mRNA
UGT1A4 mRNA
UGT1A6 mRNA
UGT1A9 mRNA
B
miR-200a-3p
miR-21-3p
miR-376b-3p
miR-141-3p
miR-491-3p
miR-1286
miR-103b
UGT1As mRNA
Coding region
3'-UTR
Translation
miRNAs
UGT1As protein
MRE
MRE including an SNP

Figure 5

Table 1. CYPs and UGTs that are regulated by miRNAs.

CYP isoform	miRNA	Reference
CYP1A1	miR-892a	19
CYP1A2	miR-132-5p	21
CYP1B1	miR-27b	15
	miR-187-5p	30
	miR-200c	28
CYP2A6	miR-126-5p	43
CYP2B6	miR-25-3p	46
	miR-1275	47
CYP2C8	miR-103	49
	miR-107	49
CYP2C9	miR-103	49
	miR-107	49
	miR-128-3p	50
	miR-130b	52
CYP2C19	miR-23a-3p	51
	miR-29a-3p	51
	miR-103	49
	miR-107	49
CYP2D6	miR-370-3p	56
CYP2E1	miR-214-3p	67
	miR-378	61
	miR-552	66
	miR-570	62
CYP3A4	miR-1	72
	miR-27a	71
	miR-27b	70
	miR-206	74
	miR-532-3p	72
	miR-577	72
	miR-627	72

UGT isoform	miRNA	Reference
UGT1A	miR-21-3p	84
	miR-103b	84
	miR-141-3p	84
	miR-200a-3p	84
	miR-376b-3p	84
	miR-491-3p	83
	miR-1286	84
UGT2B4	miR-135a-5p	88
	miR-216b-5p	87
	miR-410-3p	88
UGT2B7	miR-142-3p	89
	miR-216-5p	87
	miR-513c-3p	89
	miR-1293	89
	miR-3664-3p	88,89
	miR-4317	89
	miR-4483	89
UGT2B10	miR-216b-5p	87
UGT2B15	miR-103b	89
	miR-376b-3p	89
	miR-376c	90, 91
	miR-455-5p	89
	miR-548as-3p	89
	miR-605	89
	miR-624-3p	89
	miR-770-5p	89
	miR-3675-3p	89
	miR-3924	89
	miR-4292	89
	miR-4712-5p	89

	miR-628-3p	73		miR-6500-5p	89
	miR-641	73			

CYP, cytochrome P450; UDP-glucuronosyltransferase