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The involvement of miRNAs in *CYP450* gene expression: A brief review of the literature

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ABSTRACT: Drug biotransformation is a critical process in metabolic elimination of drugs. It occurs mainly in the liver as well as in other tissues such as kidney and small intestine and consists of three stages: Phase I, Phase II and Phase III. Drugs are converted to a more polar and hydrophilic metabolites during Phase I and Phase II, which are excreted by a variety of membrane transporters such as P-glycoprotein and Multidrug Resistance Protein 1 in Phase III. Cytochrome P450 enzyme isoforms achieve 80% of Phase I metabolism. It is well described that there is inter-individual drug response variability such as adverse drug reactions or reduced drug efficacy and that genetic variation within the sequence of biotransformation-related genes affects these different therapeutic outcomes observed between individuals. However, genetic factors cannot completely explain inter-individual differences. Recent studies have shown that epigenetic factors such as histone modification, DNA methylation and non-coding miRNAs heavily influence drug biotransformation through post-transcriptional regulation of metabolism gene expression. It is important to understand the causes of alterations in drug metabolism since varied biotransformation may lead to adverse drug effects or a loss of efficacy. Therefore, in this review, the effects of miRNAs in *CYP450* gene expression will be discussed briefly in the light of recent studies.

KEYWORDS: miRNA; CYP450; Gene Expression; Epigenetics; Inter-individual difference.

1. INTRODUCTION

Humans are continually exposed to various chemicals such as food additives, cosmetics, environmental pollutants and drugs, most of which are not essential for normal physiological processes in the body. These foreing compunds are handled in the body with several processes such as absorption, distribution, metabolism and elimination [1]. In order to reach the systemic circulation, drugs and other chemical compounds should first cross the cell membrane either through simple diffusion or membrane transporters such as organic anion transporters (OAT) [2]. Following absorption (entering the bloodstream) and distribution via circulatory and lymphatic vessels drugs are chemically modified by Phase I enzymes such as Cytochrome P450 (CYP) monooxygenase enzymes, flavin-containing monooxygenases (FMOs), alcohol dehydrogenases, and esterases and Phase II conjugation enzymes such as glutathione S-transferases and sulfotransferases. These enzyme-catalysed steps (biotransformation) convert hydrophobic drugs to more polar and hydrophilic metabolites and facilitate the excretion of them. In Phase I, xenobiotics are converted to their metabolites with polar groups, which enables conjugation reactions catalysed by Phase II enzymes. Hydophilic conjugates with the anionic groups produced in Phase I and/or II are excreted by Phase III transporter proteins belonging to two clusters: ATP-Binding Cassette Proteins (P-glycoprotein and Multidrug Resistance Protein 1) and Solute Carrier Transporters. Finally, biotransformed drugs are eliminated through the feces, urine and bile. Although all these pathways of pharmacokinetics act to detoxify xenobiotics and protect cells and tissues from harmful concentrations (detoxification), in some cases, more harmful metabolites with varying degrees of toxicity may be produced (bioactivation). Thus, biotransformation reactions influence the biological effects of drugs [1].

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It is now clear that altered expressions of genes encoded metabolizing enzymes of Phase I and II as well as transporter proteins of Phase III by a variety of factors such as genetic variations have an impact on drug sensitivity, efficacy and toxicity and cause the inter-individual drug response variability such as adverse drug reactions or reduced drug efficacy. Furthermore, pharmacological responses to a specific medication can be seen in only 25–60% of patients [3]. On the other hand, genetic factors cannot completely explain inter-individual differences. In recent years, regulation of genes via microRNAs (miRNAs) has emerged as a critical mechanism. miRNAs have crucial roles in modulating expression levels of the genes involved in absorption, distribution, metabolism and excretion of drugs via post-transcriptional gene silencing. Epigenetic factors have ability to alter the clinical efficacy, pharmacological impacts and toxicity of the drugs. In order to prevent potential adverse drug events and to achieve the desired drug efficacy, the underlying mechanisms of the inter-patient variability in pharmacokinetics and pharmacodynamics of drugs should be well understood. Therefore, in this review, we aimed to briefly focus on the effects of miRNAs on CYP gene expressions.

2. MICRORNAS

A portion of genetic material of an organism is firstly transcribed to messenger RNA (mRNA) and then translated into protein, an important functional molecule. miRNAs are not translated into proteins however; they have crucial roles in regulatory steps of cellular mechanisms. It is estimated that approximately 2/3 of human mRNA is regulated by miRNAs and the ratio of the genes regulated by miRNAs are above 60% meaning that miRNAs are involved in the translation of 60 proteins in every 100 proteins [4,5].

The first small RNAwas discovered in 1993 by Lee and colleagues studying on developmental biology of *Caenorhabditis elegans* [6]. There are various small RNAs with different characteristic and can be divided into 3 classes due to their mechanisms of biogenesis: miRNAs, Piwi-interacting RNAs and endogenous small interfering RNAs [7]. miRNAs, single stranded small RNAs with 19-25 nucleotides in length, are a part of the epigenetics mechanism and can regulate the gene expression without altering the genetic code [8-10].

miRNAs of human genome are localized on chromosomes either as single or as clusters and miRNA genes have been reported to be through the genome [11-13]. miRNAs are classified according to location as intronic (intron-derived), exonic (exon-derived), intergenic and intragenic. While approximately 70% of mammalian miRNA genes are located within the introns of noncoding or protein-coding transcription units, the rest are encoded by exons of noncoding transcripts [11]. Chromosomal locations of miRNAs may affect their gene expressions and functions.

miRNAs are specific to different species and determined in several tissues [14]. miRNAs can regulate basic cellular processes through diverse mechanisms [15]. A single miRNA can bind approximately to 200 target genes possessing different functions and regulate hundreds of functions in a cell [16,17]. It has been considered that miRNAs may endorse novel functions in gene expression due to diverse cellular localizations and binding regions [18].

According to recent investigations, miRNAs have been detected in extracellular fluids including saliva, tears, breast milk, colostrum, plasma, semen, amniotic fluid, serum, bronchial lavage, pleural fluid, cerebrospinal fluid, peritoneal fluid and shown to be extremely stable [19,20]. Circulating miRNAs in peripheral blood have also been reported [21]. Their extracellular stability and accessibility make them potential candidates for biomarker discovery [22]. Several studies concerning the miRNAs as clinical biomarker have been done [21-23]. Vachev et al. stated that expression of miRNA *let-7d-3p* and *let-7b-3p* decreased in peripheral bloods of children with autism. Their study supported that miRNAs which can be found in body fluids such as whole blood may be a crucial clinical biomarker [24]. Additionally, it was reported that 8 miRNAs (*miR-21*, *miR141*, *miR-203*, *miR-200a*, *miR-200b*, *miR-214, miR-200c*and*miR205*) present in peripheral blood were determined as biomarkers for ovarium cancer [25]. Eventough biogenesis and functions of miRNAs have been well detailed, the dynamic interaction between miRNAs and mRNAs is needed to be further investigated. miRNAs can be detected by using in-situ hybridization, microarray, quantitative real time PCR (qRT-PCR) and RNA sequencing methods and therefore it has gain more attention amongst other noncoding RNAs [18].

3. BIOGENESIS OF MIRNAS

miRNAs were shown to be processed through two subsequent steps occurring in the nucleus and cytoplasm, respectively [26]. RNA polymerase II/III enzymes transcribed miRNAs. RNA polymerase II synthesizes double stranded RNA molecule using an exogenous or endogenous RNA as a model. This double-stranded RNA folds within intronic regions and forms primary miRNA (pri-miRNA). pri-miRNAs are large stem-loop structures containing mature mRNA sequence and similar to mRNAs they carry 7-methyl guanylate (m7G) caps and poly (A) tails at 5′ and 3′ ends, respectively. pri-miRNAs are cleaved by a multiproteincomplex (also known as "the microprocessor"). This complex consists of a "RNase III endonuclease Drosha" and the double-stranded RNA-binding protein "DiGeorge syndrome critical region gene 8 (*DGCR8*)" to form approximately 60-70 nucleotide long precursor miRNAs (pre-miRNAs). Pre-miRNAs migrate to cytoplasm by means of a nucleocytoplasmic transporter complex (Pre-miRNA/Exportin-5/Ran-GTP). Hairpin arm of the pre-miRNA is cleaved by a RNase III enzyme (Dicer) in the cytoplasm and double-stranded microRNA duplex (miRNA:miRNA*, mature strand:passenger strand*), 21-24 nucleotides in length, is generated. The mature strand of miRNA duplex is subsequently loaded into Argonaute (Ago) through "RNA induced silencing complex (RISC)" which is essential for mature miRNA to bind its target messenger RNA. While the mature strand of miRNA duplex assembles into RISC, passenger strand and the rest of precursor structure are destroyed. RISC activated and guided by mature miRNA ultimately binds its target mRNA. This binding relies on base pairing between 3′ untranslated region (3′UTR) of target mRNA and the seed region referring to 2-8 nucleotides at the 5′ end of miRNA. The ratio of complementarity between mRNA and miRNA determines the degree of degradation. When the base-pairing occurs in a complete complementary manner, mRNA is degraded, however, miRNAs may bind target mRNA partially leading translational inhibition [27-29]. The biogenesis of miRNAs and their effects on CYP450 genes were summarized in "Figure".

4. THE EFFECT OF MIRNAS ON CYP EXPRESSION

Cytochrome P450 enzymes are responsible for the biotransformation of xenobiotics via hepatic oxidative (Phase I) pathways [30]. They are hem proteins and are responsible for metabolizing steroids, fatty acids, prostaglandins, drugs, chemicals, carcinogens and xenobiotics [31-33]. Since the wavelength of light absorbed after binding to carbon monoxide shows a peak at 450 nm, these enzymes have been named as Cytochrome P450 [34]. They are found in the highest concentration in hepatocytes [31] and are also located in many other tissues (excluding skeletal muscles and mature erythrocytes), including the central nervous system (CNS). Most CYPs are localized in the endoplasmic reticulum and some mainly in the mitochondria [32]. Among individuals, tissues such as liver, brain and plasma contain widely varied levels of CYP forms. The CYP superfamily is divide into 18 families and 57 subfamilies in humans [35]. Therefore, this enzyme superfamily is capable of catalyzing many diverse reactions such as epoxidations, ester cleavages, dehydrations and N- and S- dealkylations [36]. CYP families, CYPs 1–3, play a role in the Phase I metabolism of xenobiotic compounds and drugs. In contrast, other CYP families such as CYP4, CYP11, CYP17, CYP19 and CYP21 play a role in the biotransformation of endogenous components including fatty acids, fat-soluble vitamins, steroids and bile acids.There are 6 CYP subfamilies (*CYP3A4, CYP1A2*, *CYP2E1, CYP2C9*, *CYP2D6* and *CYP2C19*) that are particularly important in drug metabolism, which catalyze the oxidative reactions of 90% of drug metabolism in humans [37].

Alterations in expressions of *CYP450*s due to a variety of factors and/or induction and inhibition of these enzymes by drugs are an important source of inter-individual drug response variability such as an adverse drug reaction or reduced drug efficacy [30,38]. As adverse drug reaction is one of the leading causes of morbidity and mortality [39], therapies would be specific not only to individual but also to population by the help of revealing genetic profiles of every population. As not all variants have been determined yet and only specific variants are known, individualised therapy applications are limited in clinical use [40].

In general, it is known that genes of drug metabolising enzymes are tightly regulated at transcriptional, post-transcriptional and post-translational levels [39]. At transcriptional level, when the genes encoding these enzymes are absent or inactive then drugs cannot be metabolised. Partial synthesis of an enzyme leads to decreased metabolic rate of a drug. When genetic polymorphisms can cause alterations in enzyme function, substrate specificity may be modified or synthesis of enzyme can increase and drug can be metabolised fast [40,41].

Genetic variations influencing CYP expressions are single nucleotide polymorphisms, pseudogenes and copy number variations. Transcriptional regulators such as transcription factors and nuclear receptors as well as xenobiotic sensor receptors including aryl hydrocarbon receptor and pregnane-X receptor can affect the CYP expression [36]. However, the inter-individual variability in drug responsiveness or expression of drug-

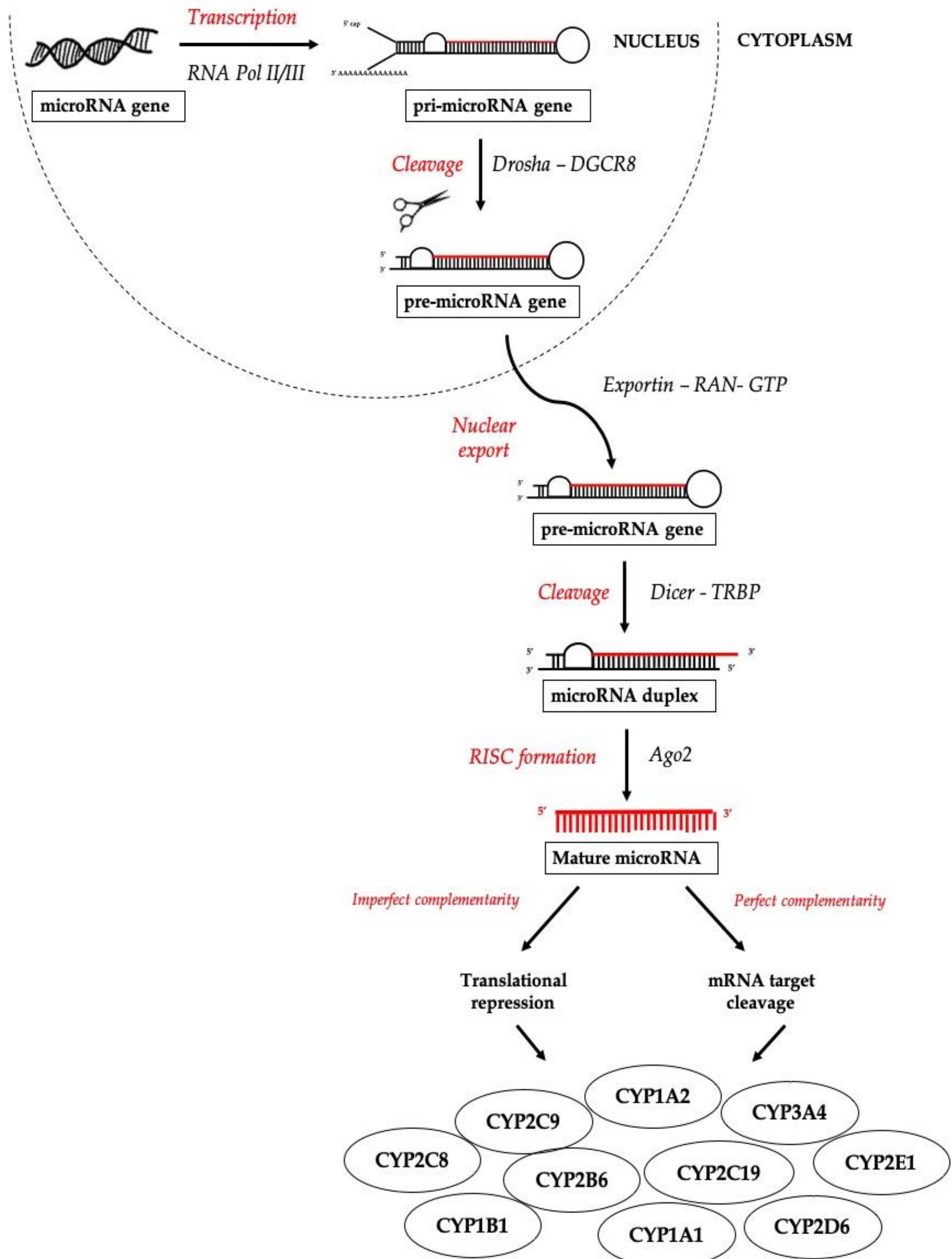

Figure. Biogenesis of miRNAs and posttranscriptional silencing mechanism on CYP450 genes. RNA pol II/III: RNA polymerase II and III, pri-microRNA: primary microRNA, Drosha: RNase III enzyme, DGCR8: DiGeorge critical syndrome region 8, pre-microRNA: precursor microRNA, RAN: Ras-related nuclear protein; GTP: guanosine triphosphate, Dicer: RNase III enzyme, TRBP: transactivation response element RNA-binding protein, Ago2: Argonaute protein-2, RISC: RNA induced silencing complex.

metabolising enzymes cannot be explained fully by the involvement of these genetic factors in gene expression [3]. Therefore, recently, epigenetic mechanisms underlying the inter-individual variability in the expression of drug-metabolising enzymes is a hot research topic in pharmacogenetics and pharmacoepigenetics.

Since epigenetic regulation of genes encoding drug metabolising enzymes may also affect the drug response of a patient, attention is drawn to the epigenetic mechanisms including DNA methylation, histone modifications and noncoding RNA (ncRNA)-mediated post-transcriptional regulation [33,39]. DNA methylation regulated by a family of DNA methyltransferases occurs at CpG sites in any context of the genome and causes a stable silencing of gene expression [42]. Habano and coworkers (2015) examined the DNA methylation profiles of hepatoma cells and normal liver tissues and found that significant inverse correlations between DNA methylation and mRNA expressions of *CYP1A2*, *CYP2C19* and *CYP2D6* genes, which indicated that some genes encoding drug metabolizing enzymes are downregulated due to DNA methylation and have different mRNA expression levels among individuals. The other epigenetic mechanism histone modifications are closely associated with inappropriate gene expression. For example, Yang and coworkers showed that CYP2E1 transcription was increased due to histon modification at the promoter region [43].

In this review, we aimed to focus on the involvement of miRNAs in *CYP450* gene expression. Thus, studies examining the effect of miRNAs on CYP expression will be given in detail in the next part of the paper. miRNAs are epigenetic regulators altering the expression of target gene post-transcriptionally and mediates the regulation of biotransformation enzymes comprising Phase I and II enzymes [33,44]. There have been studies demonstrating that miRNAs can regulate drug metabolism and affect toxicity [38]. Currently, more than 38.000 miRNAs have been determined and among them around 2812 miRNAs belong to human [45]. miRNAs have been shown to target 60% of all genes [46]. miRNAs bind to complementary region of target transcript and regulates gene regulation either by mRNA degradation or translational inhibition [44]. miRNAs may contribute drug response by possessing a key role in regulating expressions of drug metabolising genes such as *CYP450* family [30,38,44,47,48]. According to a recent analysis done by microrna.org database, it was estimated that miRNAs may regulated the expression of at least 56 CYP enzymes [30].

Although the impact of miRNAs on drug metabolising enzymes is still not understood clearly, many studies have stated that various miRNA may contribute to regulate expression of some CYP genes (Table). In a recent study, inter-individual variation and expression of seven miRNAs in human liver samples were investigated by qRT-PCR method. It has been suggested that *miR-34a* and*miR-27b* may be directly or indirectly potential major regulators of CYPs and contribute to variations in drug metabolism mediated by CYP enzymes [49]. Kugler et al. (2020) reported that strong negative correlations between liver tissue miRNA levels (*miR-155-5p*, *miR-452-5p* and *miR6807-5p*) and CYP phenotypes and *miR-155* may contribute to the downregulation of drug metabolism enzymes in case of inflammation [50]. Gill et al. (2017) reported that miRNA regulation of *CYP2E1, CYP3A4* and *CYP1A2* expressions in acetaminophen toxicity. The in-vitro experiments demostrated that the translational repression of CYPs was associated with the upregulation of *miR-378a-5p* and *miR-122-5p* [51].

CYP3A4 involves in metabolising around 50% of clinically used drugs such as benzodiazepines and antivirals [39,52]. A number of endogenous molecules such as steroid hormones, cholesterol, vitamin D and bile acids are also metabolised by CYP3A4 [53]. It is predominantly found in human liver, followed by gut, colon prostate, small intestine and brain, and catalyses wide range of substrates among all the CYPs [52]. The levels of *mmu-miR-298, miR-27a*, *miR-27b-3p* and *miR-27b* was found to be associated with *CYP3A4* expression in several previous studies [30,44,54,55]. Pan et al. (2009) suggested that miRNAs could regulate *CYP3A4* gene expression by directly targeting the 3'-UTR of *CYP3A4* at the post-transcriptional level. They showed that *mmu-miR-298* and *miR-27b* down-regulates the CYP3A4 protein expression over 30% in PANC1 and LS-180 cells, which was associated with significantly decreased *CYP3A4* mRNA levels [54]. On the other hand, Ekström et al. (2015) suggested an inhibitory effect of *miR-27b* at a translational level, but not affecting mRNA levels. There are also studies demonstrating that *hsa-miR-627, hsa-miR-532-3p, hsa-miR-1* and *hsa-miR-577* repress the *CYP3A4* mRNA translation using in vitro, in vivo and in silico methods [52,55]. Huang et al. (2019) reported that 3′-UTR of *CYP3A4* was directly targeted by *miR-150-5p* and *miR-200a-3p,* which contributed to the depletion of the expression and function of *CYP3A4* and, thus, to the development of free fatty acid-induced steatosis that is associated with [56]. Vuppalanchi et al. (2013) suggested that *miR-32* and

Table. CYP450 enzymes targeted by miRNAs.

Enzymes	Organism	Sample population	Biological sample	Method and total numbers of miRNAs	miRNAs significantly changed	Reference
CYP2C8 *CYP2C19*	Human	31	Liver	Real-Time PCR, 2 miRNA	*miR-103/107* [↑]	[66]
CYP2C19 *CYP3A4*	Human	50 [25 K, 25 E]	Liver	Real-Time PCR, 22 miRNA	*miR-34a* [↑] *miR-148a* [↓]	[75]
CYP2C19	Human	25	Human liver cell line	Real-Time PCR, 2 miRNA	*miR-29a-3p* [↓]	[67]
CYP1A1	Human		Human breast cancer cell line [MCF-7]	Real-Time PCR, 1 miRNA	*miR-892a* [↑]	[72]
		92	Liver	Real-Time PCR, 56 miRNA	*miR-132*[↑] *miR-142-3p* [↑] *miR-21*[↑]	[64]
CYP1B1	Human		Cancerous tissue	Real-Time PCR, 1 miRNA	*miR-27b* [↓]	[77]
CYP2B6	Human		Human embryonic kidney cell culture	Real-Time PCR, 2 miRNA	*miR-25-3p* [↑]	[70]
CYP2C9	Human		Hepatocellular carcinoma cell culture	Real-Time PCR, 2 miRNA	*miR-128-3p* [↑]	[76]
CYP2D6	Human		Human liver tissue	Real-Time PCR-1 miRNA	*miR-370-3p* [↑]	[68]
CYP3A4	Human	293	Embryonic kidney cell culture	Real-Time PCR-1 miRNA	*miR-27b* [↑]	[54]
CYP2E1	Human		Human hepatoma and hepatocyte-like cells	Real-Time PCR-4 miRNA	*miR-214-3p* [↑]	[78]

[↑] : Upregulation: suppresses the expression of target gene.
[↓] : Downregulation: increases the expression of target gene.

miR-155 could be two potential miRNAs which may play role in the pathogenesis of cirrhosis associated with decreased hepatic *CYP3A4* activity [57]. The indirectly inhibition of CYP3A4 expression at both the miRNA

and protein levels by miR-148 that regulates Pregnane X receptor was shown by Takagi et al. (2008) [58]. At the transcriptional level, it has been suggested that miRNAs may influence the expression of CYP genes by targeting nuclear receptors serving as transcriptional regulators of CYP genes [36]. In addition, there have been studies showing the downregulation of CYP3A4 by suppression of retinoid X receptor α and Pregnane X receptor by miR-34a [59] and miR-30c-1-3p [60], respectively. All these previous studies indicated that CYP3A4 expression was regulated by multiple miRNAs including *miR-27b*, *hsa-miR-627* and *miR-155*.

Human *CYP2E1*, predominantly expressed in the liver, catalyses the biotransformation of toxicologically important chemicals such as ethanol and benzene as well as therapeutic drugs including acetaminophen [30,39]. The substrates of *CYP2E1* can induce its expression and cause *CYP2E1*-mediated toxicity. The epigenetic regulation of *CYP2E1* gene expression by miRNAs have also been investigated by current studies. miRNAs can regulate *CYP2E1* expression by diverse molecular mechanisms with different efficiencies of inhibition at transcriptional and post-transcriptional levels. For example, cytoplasmic miR-522 binds to miRNA recognition elements (MREs) in the 3′-UTR of the *CYP2E1* mRNA transcript and inhibits *CYP2E1* translation. *Hsa-miR-214–3p* suppresses constitutive *CYP2E1* expression by targeting the coding region of *CYP2E1* mRNA in HepaRG cells [30]. Mohri and coworkers reported the involvement of *miR-378*in the regulation of *CYP2E1*post-transcriptionally. *CYP2E1* protein levels and enzyme activity significantly decreased by the overexpression of *miR-378* in the cells expressing *CYP2E1*with3′-UTR, but not in the cells expressing *CYP2E1* without 3′-UTR. This study showed that the 3′-UTR is important for the *miR-378*-dependent repression [61]. Moreover, Nakano et al. (2015) demonstrated that single nucleotide polymorphisms in 3'-UTR of CYP450 affected the binding of miRNA and *miR-570* regulates human *CYP2E1* in a genotype-dependent manner [62]. CYP2E1 is also expressed in the kidney tubular cells. Matthews and coworkers (2020) demonstrated that liver-derived miR-122 could be transferred from liver to kidney in order to regulate kidney CYP2E1 expression and they found that the transfer of this miRNA could modulate the nephrotoxic tubular cell injury. They speculated that miR-122 could be a therapeutic agent to treat renal injury [63]. Rieger et al. (2013) also reported a negative correlation between the expressions of let-7g, miR-200c and miR-10a and the protein level and activity of CYP2E1 [64].

CYP2C19 and *CYP2D6* are the enzymes responsible for metabolising more than 30% of all prescribed drugs. However, the genes encoding those important enzymes are found to be highly polymorphic leading to variations in drug response, toxicity and pharmacokinetics between individuals and ethnic groups [65]. *CYP2C19* is a monooxygenase metabolising many therapeutic agents used clinically such as diazepam, imipramine, proton pump inhibitors [44]. Decreased level of *CYP2C19* protein has been reported following overexpression of *miR-103* and *miR-107* in human primary hepatocytes [66]. In addition, negative correlation between *CYP2C19* mRNA level and *miR-34a*, *miR-130b*, *miR-185* has been reported in human liver [64]. Moreover, both in silico analysis and in vitro tests on HepaRG cells and human liver tissue samples showed the suppressing effect of *hsa-miR-29a-3p* on *CYP2C19* expression by binding directly to the homologous cognate targets in the *CYP2C19* transcript [67]. The modulation of *CYP2D6* gene expression by several miRNAs was also demonstrated. Zeng et al. (2017) demonstrated an inverse correlation between the expressions of CYP2D6 and miRNA hsa-miR-370-3p in human liver tissue samples. miRNA hsa-miR-370-3p supresses the expression of CYP2D6 by binding to the coding region of the CYP2D6 transcript. hsa-miR-370-3p decreases the expression and activity of CY2D6 enzyme through the degradation of CYP2D6 mRNA [68]. Recently, Guo and coworkers (2020) reported that overexpression of miR-544 activates CYP2D6 by inhibiting the activity of the YY1/PRC2 complex and by promoting the expression of the ten-eleven translocation 2 (TET2) gene [69].

CYP2B6 is a critical enzyme, expressed mainly in liver and brain, in metabolising drugs used widely. The changes in the expression levels of corresponding gene have been correlated with decreased drug efficacy and side effects. It was reported that *miR-25-3p* suppressed expression and thus, drug induction activity of *CYP2B6* in human liver cells [70]. Furthermore, it was stated that *miR-370-3p* repressed the expression and induction of *CYP2D6* in liver samples via mRNA degradation [68].

CYP1A1 enzyme is the member of CYP450 family concerning the biotransformation of carcinogenic agents such as benzo(a)pyrene [44]. It also contributes to biotransformation of the antiestrogen tamoxifen, some flavonoids or benzo(a)pyrene derivatives [71]. Choi et al. (2012) showed the inhibition effect ofthe overexpression of *miR-892a* on the expression of the *CYP1A1* protein through a perfect match between *miR-892a* anda sequence in the 3'-UTR of *CYP1A1*[72]. Furthermore, overexpression of *miR-21, miR-142-3p* and*miR-132* resulted in decreased level of *CYP1A1* expression in human liver tissue [44,64]. Wang and coworkers (2009) found a significant correlation between *CYP1A1* expression and *miRNA-18b* and *miRNA-20b*[73]. Lim et al. (2005) reported that *miR-124*, a brain specific miRNA, downregulates *CYP1B1* directly [74].

CYP2C8 detoxifies more than 60 clinical drugs mainly in liver and it has been indicated that expression level of *CYP2C8* can be modified by miRNAs including*miR-103*, *miR-539, miR-223, miR-142-3p*, *miR-27a*, *miR-21* and *miR-107* [64,66]. Zhang et al. (2012) found that the expression of *CYP2C8* protein was inversely regulated by *miR-107* and*miR-103*post-transcriptionally though MRE within the 3′-UTR of *CYP2C8* in human liver cells[66]. Moreover, Rieger et al. (2013) showed that *CYP2C8* mRNA level exhibited reverse correlation with *miR-21*, *miR-223, miR-27a*, *miR-142-3p* and *miR-539* at transcriptional level in human liver tissue [64].

CYP2C9 is able to metabolise approximately 20% of therapeutic drugs and miRNAs including*miR-16*, *miR-17*, *miR-28-3p*, *miR-29a* and *miR-128-3p* have been demonstrated to be associated with *CYP2C9* expression [64]. Additionally, it has been reported that *miR-148a* involves in increasing the expression levels of enzymes metabolising diazepam such as *CYP2C19* and *CYP3A4*, whereas *miR-34a* supresses them [75]. Yu et al. (2015) applied integrative approaches such as in silico, in vivo, and in vitro analyses in order to examine the effect of *hsa-miR-128-3p*on the regulation of *CYP2C9* expression and demonstrated an interaction between this miRNA and the *CYP2C9* transcript. It was also revealed that the expression of *CYP2C9* is inversely correlated with the expression of *hsa-miR-128-3p* in hepatocellular carcinoma tumour tissues [76]. Rieger et al. (2013) found that *miR-130b* directly regulatesthe *CYP2C9* 3'-UTR with reporter gene assays [64].

CYP1B1, an extrahepatic cytochrome P450 enzyme, is mainly expressed in breast, uterus and ovary, and is responsible for metabolising of many structurally different substrates [77]. It plays also important role in bioactivation of a numerous of procarcinogens and promutagens such as aryl amines and polycyclic aromatic hydrocarbons. It was stated that its expression is supressed by *miR-27b*post-transcriptionally via direct action on the *miR-27b* MRE site near the poly(A) tail [39,77].

5. CONCLUSION

There are growing evidences concerning the involvement of miRNAs in drug-metabolizing enzymes-*CYP450* gene expression. However, more prospective clinical investigations are needed to be done in order to determine the causal relationship between miRNAs and drug response. miRNA expressions may also be changed by drugs, which can cause various diseases. If it is determined that particular drugs may modulate the levels of miRNAs regulating the expressions of drug metabolising genes, it would be possible to reveal a novel miRNA-dependent mechanism in drug-drug interactions.

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