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MicroRNAs from biology to future pharmacotherapy: regulation of cytochrome P450s and nuclear receptors

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Abstract

MicroRNAs (miRNAs) are a family of short, non-coding RNA whose final product is a 22-nucleotide functional RNA molecule. They regulate the expression of target genes by binding to complementary regions of transcripts to repress their translation or promote mRNA degradation. Since miRNAs regulate every aspect of cellular function, their dysregulation is associated with a variety of diseases including cancer, diabetes, and cardiovascular diseases. Therefore, miRNAs are now considered new therapeutic targets. However, the roles of miRNAs in the metabolism of xenobiotics and endobiotics have only recently been revealed. This review describes the current knowledge on the regulation of cytochrome P450s and nuclear receptors by miRNAs, the physiological and clinical significance. The miRNA expression is readily altered by chemicals, carcinogens, drugs, hormones, stress, or diseases, and the dysregulation of specific miRNAs might lead to changes in the drug metabolism potency or pharmacokinetics as well as pathophysiological changes. In the field of pharmacogenomics, the evaluation of miRNA-related polymorphisms would provide useful information for personalized medicine. Utilizing miRNAs opens a new era in the fields of drug metabolism and pharmacokinetics as well as toxicology.

Keywords: microRNA, P450, CYP, transcription factors, post-transcriptional regulation.

Contents

1. Introduction

MicroRNAs (miRNAs) are short (~22-nucleotide), endogenous non-coding RNAs that lead to gene silencing through translational repression or mRNA degradation (Bartel, 2004). They are now recognized as critically important regulators of gene expression. So far, more than 1000 human miRNAs have been identified (miRBase, release 16, http://www.mirbase.org/). Computational prediction estimates that 60% of human mRNAs could be targets of miRNAs (Friedman et al., 2009). The miRNAs have roles in fine-tuning the expression of their target genes forming intricate networks. Research on miRNA is growing exponentially, and it is now clear that miRNAs can potentially regulate every aspect of cellular processes such as differentiation, proliferation and apoptosis as well as a large range of physiological processes such as development, immune response, metabolism, tumor formation, and disease development (Kloosterman and Plasterk, 2006). Since 30% of miRNA are expressed in a tissue- or cell-specific manner, the expression patterns are associated with the physiological functions of miRNAs. Dysregulation of the miRNA function may lead to certain diseases.

In the fields of drug metabolism and pharmacokinetics (DMPK), the roles of miRNAs have only recently started to become clear. The purpose of this review is to summarize recent findings concerning the roles of miRNA in the regulation of cytochrome P450s (P450, CYP) and nuclear receptors and their clinical significance with consideration of their potential application for pharmacotherapy.

2. Biogenesis of miRNAs and gene regulation by miRNAs

Genes encoding miRNA are located in intergenic or intragenic (intronic or exonic) regions with both sense and antisense orientations (Lagos-Quintana et al., 2001). In human, the distribution of miRNA is as follows: 52% intergenic miRNAs, 43% intronic miRNAs, and 5% exonic miRNAs (Hinske et al., 2010). The miRNAs are first transcribed as long primary transcripts, generating a stem-loop containing primary miRNA (pri-miRNA). The pri-miRNA is processed to a 70- to 100-nucleotide hairpin-shaped precursor miRNA (pre-miRNA) by a

microprocessor complex including Drosha and DiGeorge syndrome critical region 8 protein (DGCR8) in the nucleus. The pre-miRNA is exported into the cytoplasm by Exportin 5, processed to miRNA duplex of about 22 nucleotides by Dicer or Ago2, and then unwound into the single strand form of mature miRNAs. The functional guide strand is loaded onto the RNA-induced silencing complex (RISC), composed of Dicer, TAR RNA binding protein (TRBP) and Argonaute protein Ago2, and guides the complex to its mRNA targets with imperfect pairing causing translational repression or mRNA degradation (Krol et al., 2010). Target sites of animal and human mRNAs are usually at the 3'-untranslated region (UTR), although there are examples of target sites in the coding region as well (Duursma et al., 2008). The passenger strand, named miRNA*, is usually degraded, although it is sometimes functional. Gene silencing by miRNAs include multiple mechanisms and the details can be found in other review articles (Chekulaeva and Filipowicz, 2009; Fabian et al., 2010).

3. Prediction/identification of miRNA(s) for a given target gene

Identification of miRNA(s) for a target gene is a challenging task because miRNAs bind to their target by partial complementarity over a short sequence. The nucleotides 2–8 at the 5'-end of the miRNA, called the seed sequence, are critical and sometimes sufficient to repress the target translation (Lewis et al., 2005). To predict the miRNAs for the target genes, several computational programs such as MicroCosm Targets (John et al., 2004), TargetScan (Lewis et al., 2003; 2005), Pictar (Krek et al., 2005), and microrna.org (Betel et al., 2008) are available. In most cases, many kinds of miRNAs are predicted. The predicted miRNAs vary significantly depending on the different algorithms used in the programs, because these algorithms place variable weight on complementarity to the miRNA seed sequence, evolutionary conservation of the microRNA recognition element (MRE) of the target gene, free energy of the miRNA-mRNA duplex, and accessibility of the target site. The false positive rate of the predicted candidate targets of a given miRNA is thought to be 30-50% (Alexiou et al., 2009; Watanabe et al., 2007). Confirming experimentally the validity of all of them is a time-, money, and energy-consuming process. We consider the following points to

choose miRNAs that are to be experimentally verified: 1) convergence of algorithms and 2) conservation of the target site sequence among species, although these points are neither a necessity nor a guarantee of function, 3) accessibility to target: it seems to be a critical feature of miRNA target recognition, and 4) whether the miRNA is substantially expressed in the tissue where the target mRNA is expressed.

To validate specific miRNA:mRNA interaction, several lines of experiments can be used. The most commonly employed technology is the use of luciferase assays using reporter constructs containing the MRE of the target mRNA downstream of the reporter gene. The constructs are co-transfected into the cells with precursor miRNA (or the expression plasmid of miRNA) or antisense oligonucleotide (AsO) for miRNA to overexpress or inhibit miRNA. Evidence can be established whether the reporter activity is significantly decreased or increased. The overexpression or inhibition of miRNA is an effective method to determine potential miRNA:mRNA interactions. In the overexpression experiments, a possible concern is that the decrease of target expression might be an artifact of overexpression. From this point, experiments to inhibit endogenous miRNA are valuable. Furthermore, an inverse relationship between the protein and miRNA levels is expected, for example, cancer versus normal tissues, or among individuals or cell lines.

In addition to the above direct methods, it is useful to determine the change of mRNA or protein expression by microarray or proteome analysis after the overexpression or inhibition of miRNA to determine the targets of a given miRNA comprehensively (Lim et al., 2005; Baek et al., 2008). It should be noted that these include secondary targets, the expression of which may change owing to the expression changes of the primary targets. We should also pay attention to the fact that different proteins have different turnover rates. This means that the level of protein with high turnover will change rapidly, whereas stable proteins will be affected later. In addition, if one determines only the changes in mRNA levels, some targets whose expression repression is caused by translational repression could be missed. Thus, for comprehensive analysis, both mRNA and protein analysis should be considered. Another method is immunoprecipitation of RISC and sequence analysis of mRNAs in the

immunoprecipitant (Beitzinger et al., 2007). In such a experiment, we don't know which mRNAs are targets of which miRNA. To overcome this problem, the overexpression or inhibition of miRNA before the immunoprecipitation and comparison with control by microarray analysis will be useful (Karginov et al., 2007; Chi et al., 2009).

4. miRNAs that regulate P450s and nuclear receptors

P450s are important enzymes that catalyze the metabolism of xenobiotics including drugs, environmental chemicals, and carcinogens as well as endobiotics such as steroids, bile acids, and fatty acids. Most P450s are transcriptionally regulated by nuclear receptors. The understanding of the mechanisms of the transcriptional regulation of P450s has progressed greatly, but post-transcriptional regulation largely remains to be clarified. Recently, some P450s and nuclear receptors have been found to be post-transcriptionally regulated by miRNAs (Table 1, Fig. 1). We introduce the examples that have been confirmed as targets of miRNA by direct experimental methods as described above, and the clinical significance.

4.1 Relationship to cancer

miRNA studies have achieved much progress in the field of cancer. There are many publications concerning miRNAs related to cancer. The miRNA expression in most types of cancer cells is quite different from that in normal cells. Certain miRNAs have been found to serve as oncogenes or tumor suppressor genes, being associated with cancer initiation and/or progression. P450s are also one of the factors associated with the initiation or progression of cancer. In cancer tissues, some P450 expressions are modulated, and that would lead to an imbalance in the metabolism of exo/endobiotics. Some P450s related to cancer have been found to be regulated by miRNA as follows.

4.1.1 CYP1B1

CYP1B1 catalyzes the metabolic activation of a variety of procarcinogens and promutagens, including polycyclic aromatic hydrocarbons and aryl amines (Shimada et at.,

1996). In addition, CYP1B1 metabolizes 17β-estradiol to form a catechol metabolite that cause DNA damage (Han and Liehr, 1994). It has been demonstrated that the expression level of CYP1B1 protein is higher in various types of cancer compared with normal tissue (Murray et al., 1997), whereas there is no difference in the CYP1B1 mRNA levels between cancerous and normal tissues (Cheung et al., 1999) implying post-transcriptional regulation. This background prompted us to investigate the possibility that human CYP1B1 might be regulated by miRNA, and we found that it is negatively regulated by miR-27b via translational repression (Tsuchiya et al., 2006). Interestingly, the expression level of miR-27b was lower in breast cancer tissues than in adjacent normal tissues. A significant inverse association was observed in the expression levels of miR-27b and CYP1B1 protein between breast cancer and normal tissues. The decreased expression of miR-27b would be one of the causes of the high expression of CYP1B1 protein in cancer tissues. The study by Tsuchiya et al (2006) is the first to reveal the regulation of P450 by miRNA.

4.1.2 CYP2A3

Kalscheuer et al (2008) have reported that chronic administration of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) to F344 rats reduced the expression of several miRNAs including miR-126* in lung. They found that CYP2A3, which catalyzes the metabolic activation of NNK, is regulated by miR-126*. Since the reduced miR-126* expression was accompanied by increased CYP2A3 expression (at both mRNA and protein levels) in the NNK-treated rats, they concluded that the expression changes would reinforce NNK genotoxicity.

4.1.3 CYP24 and vitamin D receptor (VDR)

1,25-Dihydroxyvitamin D_3 (calcitriol) has now received much interest for its anti-tumor activity (Deeb et al., 2007), although it is well known as a regulator of calcium and bone homeostasis. Most of the biological effects of calcitriol are elicited by the binding to VDR (Carlberg and Polly, 1998). Calcitriol is then inactivated by CYP24, a key metabolic enzyme,

the expression of which is regulated by VDR (Chen and DeLuca, 1995). It has been reported that CYP24 (Deeb et al., 2007) and VDR (Friedrich et al., 2002; Khadzkou et al., 2006) are overexpressed in various cancers. We found that both human CYP24 (Komagata et al., 2009) and VDR (Mohri et al., 2009) are post-transcriptionally regulated by miR-125b. It was demonstrated that the miR-125b expression is decreased in breast cancer tissues, being a causal factor of the overexpression of CYP24 and VDR. Since CYP24 is a target of VDR, the miR-125b would directly and indirectly regulate CYP24. The increase of VDR in cancer tissues would augment the anti-tumor effects of calcitriol, whereas the increase of CYP24 would attenuate the anti-tumor effects. When we investigated the effects of miR-125b on the anti-proliferative effects of calcitriol using human breast cancer-derived MCF-7 cells, the overexpression of miR-125b restored the cell growth suppressed by calcitriol, indicating that miR-125b had a great impact on the VDR function in this cell system.

4.1.4 Estrogen receptor α (ERα)

Three fourths of diagnosed breast cancers express ERα, which is a target of estrogen, exerting proliferative effects. ERα is an important marker for the prognosis and is predictive of the response to endocrine therapy in breast cancer patients. ERα regulates the expression of human CYP1B1, which catalyzes the metabolism of estradiol to a carcinogenic metabolite, 4-hydroxyestradiol (Tsuchiya et al., 2004). It has been demonstrated that human ERα is regulated by miR-206, whereas the activation of ERα results in the decreased expression of miR-206, showing mutually inhibitory regulation (Adams et al., 2007). After this report, it was reported that miR-221, miR-222 (Zhao et al., 2008), and miR-22 (Xiong et al., 2010) also regulate human ERα expression. The authors suggested the possibility that these miRNAs may be potential targets for anti-estrogen therapy.

4.2 Relationship to metabolism of xenobiotics/endobiotics

4.2.1 CYP2E1

CYP2E1 catalyzes the metabolism of a variety of low molecular-weight xenobiotics including drugs, organic solvent, and procarcinogens. CYP2E1 is inducible by some compounds through post-transcriptional or post-translational mechanisms. In a panel of human liver samples, no positive correlation between the CYP2E1 activity and CYP2E1 mRNA levels was observed (Sumida et al., 1999), indicating the post-transcriptional regulation for the constitutive expression. As for the molecular mechanism, we found that human CYP2E1 is regulated by miR-378 (Mohri et al., 2010). The significance of miR-378 in the regulation of CYP2E1 was supported by the finding of an inverse correlation between the miR-378 level and CYP2E1 protein level in a panel of human liver samples. This study could provide a new insight into the unsolved mechanism of the post-transcriptional regulation of CYP2E1.

4.2.2 CYP3A4 and pregnane X receptor (PXR)

CYP3A4 is the most abundant P450 in human liver, and it is responsible for the metabolism of more than one half of prescription drugs and endogenous compounds such as steroids. There are large interindividual differences (~50 fold) in the CYP3A4 expression level in human livers. CYP3A4 is inducible by a wide variety of exogenous and endogenous compounds through the activation of PXR, possibly being responsible for the variability in the expression levels. We studied whether CYP3A4 and PXR may be regulated by miRNA focusing on miR-148a among the various predicted miRNAs, because miR-148a was commonly predicted and is substantially expressed in liver. Another reason was that miRNA tends to regulate genes in the same pathway to exert its biological function (*i.e.*, miR-148a repression of both PXR and CYP3A4 would result in both direct and indirect mechanisms to regulate CYP3A4).

Our data demonstrated that the miR-148a regulates PXR but not CYP3A4 (Takagi et al., 2008). Interestingly, the miR-148-dependent regulation of PXR affected the induction of endogenous CYP3A4 in human colon carcinoma-derived LS180 cells. Correlation analysis using human liver samples showed that the PXR protein level was not positively correlated

with PXR mRNA supporting the post-transcriptional regulation. In contrast, the CYP3A4 protein level showed a significantly positive correlation with the CYP3A4 mRNA level indicating that the transcriptional regulation mainly contributes to the expression. Although we didn't examine CYP3A4 further, Pan et al (2009a) subsequently reported that CYP3A4 protein in LS180 and human pancreas cancer-derived PANC1 cells was decreased by the overexpression of miR-27b, accompanied by a decrease of the CYP3A4 mRNA level. A limitation of their study may be that the conclusion was drawn from only an overexpression study. Again, to determine the impact of miRNAs on their targets, an inhibition study of endogenous miRNA as well as correlation analysis between the miRNA and target mRNA levels would be necessary. All proteins are produced by mRNA, which means that transcriptional regulation certainly participates. The balance between the transcriptional and post-transcriptional regulation is critical. If miRNA cannot overcome the strong transcriptional regulation of a target gene, the involvement of miRNA may be invisible. We think that in CYP3A4 this might be the case.

In our study, since the translational efficiency of PXR was inversely correlated with the miR-148a levels, it is suggested miR-148a would actually be involved in the regulation of PXR in human livers. In addition, the positive correlation between the PXR protein level and CYP3A4 at both the mRNA and protein levels suggests that the miR-148a-dependent PXR regulation would have great impact on the basal expression of CYP3A4 in human livers.

PXR regulates a variety of drug-metabolizing enzymes and transporters. We found that the induction of CYP2B6 (2-fold) and multi-drug resistance 1 (MDR1)/P-glycoprotein (5-fold) mRNA by rifampicin in LS180 cells was also attenuated by the overexpression of miR-148a. Thus, the miR-148a-dependent regulation of PXR appeared to affect target genes in common.

4.2.3 Hepatocyte nuclear factor 4α (HNF4α) and CYP7A

HNF4α is a master regulator of drug-metabolizing enzymes, drug transporters, and genes involved in the synthesis/metabolism of bile acids, fatty acids, cholesterol, glucose, and urea (Gonzalez, 2008). HNF4α regulates gene expression not only via direct binding to the gene's

regulatory sequences but also through the regulation of other transcriptional factors such as PXR and constitutive androstane receptor (CAR). HNF4α forms large transcriptional regulatory networks in the liver. Therefore, it is believed that the change of HNF4α expression has a great impact upon the function of the liver. We found that human HNF4α is regulated by miR-24 and miR-34a (Takagi et al., 2010). Interestingly, miR-34a recognizes MRE in the 3'-UTR and causes translational repression, whereas miR-24 recognizes MRE in the coding region and causes mRNA degradation. The down-regulation of HNF4α by these miRNAs resulted in the decrease of various target genes such as CYP7A1, CYP8B1 (bile acid-synthesizing enzymes), CYP27A1 (a cholesterol-metabolizing enzyme), and phosphoenolpyruvate carboxykinase (PEPCK, a gluconeogenetic enzyme). We also could provide a novel feedback loop for bile acid synthesis as follows: bile acids activate protein kinase C (PKC)/mitogen-activated protein kinase (MAPK) and reactive oxygen species (ROS) pathways. The PKC/MAPK and ROS pathways increase miR-24 and miR-34a expression, respectively. These miRNAs down-regulate the HNF4α expression resulting in the expression of bile acid-synthesizing enzymes. Thus, the involvement of miRNAs in the fine-tuning of bile acid synthesis was demonstrated.

4.3 Others

Several research groups (Karbiener et al., 2009; Lin et al., 2009; Jennewein et al., 2010; Kim et al., 2010) have reported that miR-27a and miR-27b repress the expression of peroxisome proliferator-activated receptor γ (PPARγ) suggesting an association with the change in adipocyte differentiation and anti-inflammatory effects. In addition, a recent study reported that adipogenesis-regulated miR-130 represses PPARγ, thereby controlling adipocyte gene expression programs (Lee et al., 2010). Interestingly, an inverse correlation was observed in which PPARγ mRNA was high in obese women but low in lean women, whereas miR-130 was low in obese women but high in lean women. Since it was reported that PPARγ agonists such as rosiglitazone and pioglitazone increased the expression of CYP26 (retinoic acid-metabolic enzyme) in HepG2 cells (Tay et al., 2010), it would be interesting and worth

investigating whether the miRNA-dependent regulation of PPARγ affects retinoid metabolism.

Lin et al (2009) have reported, using mouse embryonic fibroblast-derived 3T3-L1 preadipocytes, that overexpression of miR-27a or miR-27b decreased the expression of C/EBPα, which is involved in the regulation of some P450s (Akiyama and Gonzalez, 2003). Pan et al (2009a) have reported, using LS180 and PANC1 cells, that overexpression of miR-27b decreased the expression of VDR. Ji et al (2009) have reported, using rat hepatic stellate cells, that the inhibition of miR-27a and miR-27b increased the expression of retinoid X receptor α (RXRα), a heterodimer partner of various nuclear receptors such as PXR, VDR, CAR, PPAR, farnesoid X receptor, and liver X receptor. They described that the sequences of MRE on the RXRα mRNA are highly conserved, implying that human RXRα may also be regulated by miR-27. Taken together, miR-27 seems to regulate a wide variety of key transcriptional factors that are involved in the regulation of various drug metabolizing enzymes. It would be interesting to investigate the impact of miR-27 on the metabolism of xenobiotics/endobiotics in human livers, in addition to our finding that CYP1B1 is a direct target of miR-27b.

Vreugdenhil et al (2009) have reported that rat and human glucocorticoid receptors (GR) are regulated by miR-18 and miR-124a. Uchida et al (2009) also reported that rat GR is regulated by miR-18 and that the sequences of MRE are well conserved among rat, mouse, and human. Although the expression of miR-124a is restricted to brain, miR-18 is widely expressed throughout the body. Since GR is involved in the regulation of CYP2B6, CYP2C9, CYP3A4, PXR, and CAR (Rezen et al., 2011), additional studies are needed to determine whether the miRNA-dependent regulation of GR might affect drug metabolism.

Collectively, it has been revealed that various nuclear receptors are regulated by miRNAs. The regulation of nuclear receptors by miRNA results in changes in the expression of a variety of target genes, constructing complex regulatory networks. Since this knowledge has only recently been taken into consideration in drug metabolism, further studies are warranted to clarify the clinical significance of miRNAs in the control of pharmacokinetics for better

understanding of inter- and intraindividual differences in drug responses and adverse reactions.

5. Modulation of miRNA expression

5.1 Modulation of miRNA expression by chemicals, drugs, and hormones

Similar to coding RNA, miRNAs are controlled by transcription factors (Krol et al., 2010). There are several reports regarding the changes of miRNA expression through ligand-activated nuclear receptors. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a potent environmental toxicant, is a ligand of aryl hydrocarbon receptor (AhR), which forms a heterodimer with AhR nuclear translocator (ARNT) and binds to xenobiotic response elements on the promoter of many kinds of drug-metabolizing enzymes such as CYP1A, alcohol dehydrogenase (ALDH), and glutathione *S*-transferase (GST). It is well known that TCDD causes vast changes in mRNA expression, but the administration of TCDD to mice and rats caused few changes in miRNA (45 and 17 miRNAs, respectively) levels in liver (Moffat et al., 2007). Mouse and rat hepatoma cells in culture also exhibited few changes in miRNAs in response to TCDD (Moffat et al., 2007). A recent study has reported that the oral administration of benzo(a)pyrene, another ligand of AhR, to mice caused no changes in miRNA, in spite of widespread changes (> 400 genes) in mRNA expression (Yauk et al., 2010).

It has been demonstrated that the administration of Wy-14,643, a specific PPARα agonist, to mice for 2 weeks caused changes (>1.5-fold) in the expression of 27 miRNAs (12 miRNAs were decreased and 15 miRNAs were increased) (Shah et al., 2007), but greater changes in 707 mRNAs (671 mRNAs were decreased and 36 mRNAs were increased, >4-fold). When rat primary thymocytes were treated with dexamethasone, a synthetic glucocorticoid that binds to GR, the expression of 56 out of 350 miRNAs was altered (44 miRNAs were decreased and 12 miRNAs were increased) (Smith et al., 2010). Testosterone, which regulates gene expression through androgen receptor (AR), affects the expression of 6 miRNAs in female mouse liver

(Delić et al., 2010). Treatment of estradiol, a ligand of ERα, caused changes in the expression of a subset of miRNAs in mice, rats, and human breast cancer-derived MCF-7 cells (Klinge, 2009). Taken together, the expression of miRNAs actually changes in response to chemicals and steroid hormones, but the changes are likely smaller than those of mRNA expression.

Various stressors also affect miRNA expression. Arsenite, which is known to activate nuclear factor-erythroid 2-related factor 2 (Nrf2) (Aono et al., 2003), affects miRNA expression in human lymphoblastoid TH-6 cells (Marsit et al., 2006). Cigarette smoking causes the down-regulation of many miRNAs in the lungs of both mice and rats (Izzotti et al., 2011) as well as human airway epithelial cells (Schembri et al., 2009). The administration of the hepatotoxicants acetaminophen or carbon tetrachloride to rats caused changes in the expression of some miRNAs (there were only 13 commonly changed miRNAs in the two groups) in liver (Fukushima et al., 2007). As described above, the chronic administration of NNK, a tobacco-specific carcinogen, to rats reduced the expression of several miRNAs in lung (Kalscheuer et al., 2008). There is accumulating evidence that these up-regulated or down-regulated specific miRNAs could alter the expression of target mRNAs and lead to some phenotypic changes (Shah et al., 2007; Kalscheuer et al., 2008; Maillot et al., 2009; Ribas et al., 2009).

Interestingly, it has been demonstrated that enoxacin, an antibiotic, affects the processing of miRNAs by facilitating the interaction between TRBP and RNA (Shan et al., 2008). Yamagata et al (2009) reported that the ligand-activated ERα inhibits the Drosha processing of pre-miRNA. Smith et al (2010) have reported that the expression of miRNA-processing enzymes such as Dicer, Drosha, and DGCR8 were significantly reduced at the mRNA and protein levels during glucocorticoid-induced apoptosis. Thus, it is likely that chemicals and steroids modulate the expression of miRNAs not only transcriptionally but also post-transcriptionally.

5.2 Modulation of miRNA expression in disease

A growing number of reports have shown that aberrant miRNA expression is a common feature of human diseases such as cancer, Alzheimer's disease, cardiovascular disease, schizophrenia. Since miRNAs regulate cellular functions, it is not surprising that miRNAs are implicated in a wide variety of diseases. We can refer to a database for miRNA-diseases associations, the Human MiRNA & Disease Database (HMDD) (http://202.38.126.151/hmdd/mirna/md/) (Lu et al., 2008). Because of the growing evidence that a variety of diseases are associated with the dysregulation of miRNAs, miRNAs are now considered as a new tool for diagnosis and therapy, as described below in detail (Calin and Croce, 2006). Our great concern is that such dysregulation of specific miRNAs in diseases or miRNA manipulation in therapy may lead to changes in drug responses in patients, although it has never been noticed so far.

6. Potential therapeutic applications of miRNAs

Since miRNAs are differently expressed in diseases and they play critical roles in various biological pathways, miRNAs are expected to be potential targets of therapeutics. The modulation of the levels of miRNAs can be adapted from existing gene therapy and antisense technology. For miRNAs whose expression is reduced in diseases, re-introduction of miRNA into the proper tissue could provide a therapeutic benefit by restoring the regulation of target gene(s). An RNA-interference-based method using chemically modified antagomirs or locked nucleic acid (LNA)-modified oligonucleotides is already being developed. The therapeutic potency has been demonstrated in vivo using experimental animals such as mouse and monkey (Krützfeldt et al., 2005; Lanford et al., 2010). Furthermore, there are several ongoing clinical trials (Seto et al., 2010; Wahid et al., 2010). The most advanced miRNA-based therapeutics is targeted to the liver-specific miR-122, which is involved in hepatitis C replication and cholesterol metabolism. The company reported that there have been no apparent adverse reactions so far. In addition, some pioneering pharmaceutical companies have initiated studies on creating therapeutic candidates with miRNA mimics or miRNA inhibitors for cancer, cardiovascular diseases, neurological disorders, and viral infections. It is

possible that the miRNA-based therapy could be used to regulate drug sensitivity. For example, the expression changes of miRNA in cancer cells are associated with resistance to anti-cancer drugs (Saker et al., 2010), and there is accumulating evidence that many drug transporters such as MDR1/P-glycoprotein, breast cancer resistance protein (BCRP/ABCG2), and multi-drug resistance-associated protein 1 (MRP1) are regulated by miRNAs (Zhu et al., 2008; Pan et al., 2009b, Liang et al., 2010). It is also possible that miRNAs could be utilized to selectively modulate drug metabolizing enzymes, drug transporters, and nuclear receptors toward optimal drug responses in pharmacotherapy. Targeting miRNAs for therapy could be an emerging field, although there are many hurdles to be overcome: stability, appropriate in vivo delivery systems, and selectivity. An individual miRNA could regulate several genes and pathways simultaneously suggesting that miRNA modulation could be powerful. However, attention must be paid to the possibility that miRNA manipulation may cause unanticipated effects, because the targets of the each miRNA have not been thoroughly clarified.

7. miRNA-related polymorphisms

Single nucleotide polymorphisms (SNPs) are the most common human genetic variants. The SNPs may affect either the expression or activities of various enzymes, and therefore may be associated with differences in the physiological or pharmacological outcomes. Polymorphisms can be present not only in the mRNA but also in mature miRNA sequences. In addition, polymorphisms in pri-miRNA and pre-miRNAs affect the expression level of mature miRNAs (Iwai et al., 2005; Duan et al., 2007) and might lead to modification of the expression level of target genes. A polymorphism in an mRNA target site would be target-specific, whereas a polymorphism in an miRNA may affect the expressions of multiple genes and have serious consequences. A polymorphism in the genes encoding miRNA-processing enzymes may have a large impact (Horikawa et al., 2008).

Thousands of miRNA-related polymorphisms have been identified and are catalogued in databases such as Patrocles (http://www.patrocles.org/Patrocles.htm) and PolymiRTS (http://compbio.uthsc.edu/miRSNP/). Many miRNA-related polymorphisms have been shown

to be associated with diseases (Sethupathy and Collins, 2008), because a gain-of function or loss-of-function of miRNA polymorphisms would result in changes in the expression of target mRNAs that are related to diseases: e.g., *SLITRK1* (Slit and Trk-like 1) gene/miR-189/Tourette's syndrome, *AGTR1* (angiotensin receptor 1) gene/miR-155/hypertension, and *FGF20* (fibroblast growth factor 20) gene/miR-433/Parkinson disease.

Pharmacogenetics research has matured considerably during the past few decades. The importance and implications of genetic polymorphisms in genes encoding drug metabolizing enzymes are largely recognized. Representative polymorphisms that can be used clinically are these of UDP-glucuronosyltransferase/irinotecan and thiopurine methyltransferase/thiopurines. In contrast to the accumulating evidence of polymorphisms on coding genes that affect drug responses and adverse effects, there are few examples regarding miRNA-related polymorphisms that can affect drug responses. A SNP in the binding site of miR-24 in the 3'-UTR of human dihydrofolate reductase gene leads to the overexpression of dihydrofolate reductase and methotrexate resistance (Mishra et al., 2007). Interestingly, it has been reported that SNPs in the pri-miR26a-1 or pri-miR-100 genes were significantly associated with the tumor response or time to progression in patients treated with 5-fluorouracil and CPT-11 (Boni et al., 2011), although the molecular mechanism by which these polymorphisms act is not yet understood.

Actually, the SNPs exist on the 3'-UTR of many genes encoding P450s and other drug metabolizing enzymes. So far, most of them might be overlooked, because they are unlikely to affect the enzyme property or expression levels. Further studies are warranted to investigate whether the SNP may lose or gain the recognition site of miRNA, causing differences in the clinical outcome. Pharmacogenomics research on miRNAs should provide clues to understanding the cause of interindividual variability in drug responses and adverse reactions.

8. Conclusions

We now recognize the critical roles of miRNAs in numerous physiological processes and their involvement in human diseases. There is increasing interest in understanding the contribution of miRNAs to pharmacological and toxicological outcomes. How is miRNA expression changed under physiological conditions (diet, alcohol, smoking, environmental chemicals, stress, supplement, medication, or diseases)? Do the differences in the miRNA expression affect the drug response or susceptibility to xenobiotic toxicity? How do the miRNA-related polymorphisms affect pharmacokinetics and pharmacodynamics? Clarifying such issues will provide us better understanding of the intra- and interindividual variability in drug responses. miRNAs have clearly opened a new field in DMPK and toxicology.

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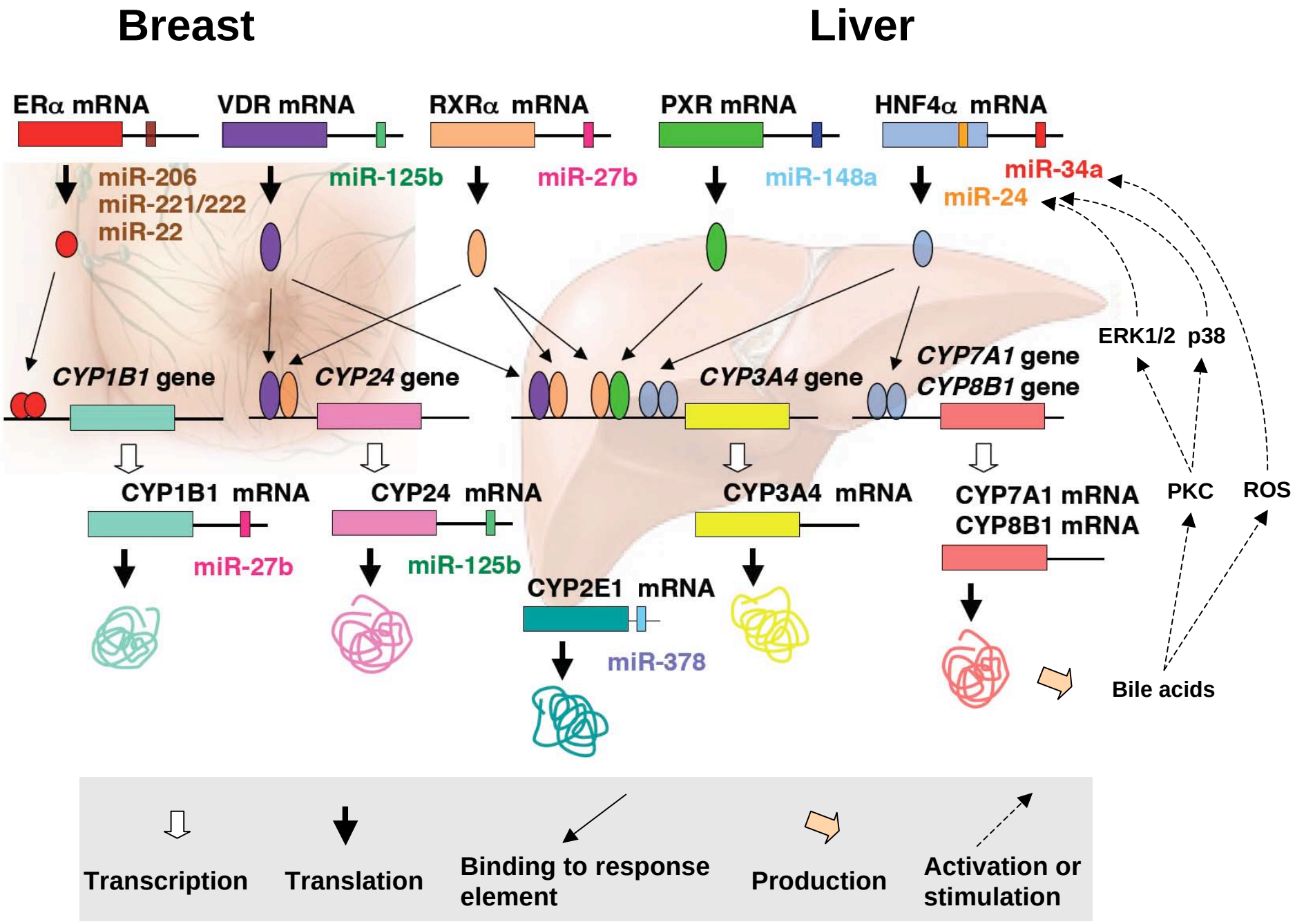
Table 1. Cytochrome P450s and nuclear receptors that are regulated by miRNAs.

Target	miRNA	Reference
CYP1B1	miR-27b	Tsuchiya et al., 2006
CYP2A3 (rat)	miR-126*	Kalscheuer et al., 2008
CYP2E1	miR-378	Mohri et al., 2010
CYP3A4	miR-27b	Pan et al., 2009a
CYP24A1	miR-125b	Komagata et al., 2009
PXR	miR-148a	Takagi et al., 2008
VDR	miR-125b	Mohri et al., 2009
PPARγ	miR-27a	Kim et al., 2010, Lin et al., 2009
	miR-27b	Karbiener et al., 2009, Jennewein et al., 2010
	miR-130	Lee et al., 2010
RXRα (rat)	miR-27	Ji et al., 2009
HNF4α	miR-24a, miR-34	Takagi et al., 2010
ERα	miR-206	Adams et al., 2007
	miR-221/222	Zhao et al., 2008
	miR-22	Xiong et al., 2010
GR	miR-18, miR-124a	Vreugdenhil et al., 2009

If not specified, the targets mean human mRNAs.

Figure legends

Fig. 1. Cytochrome P450s and nuclear receptors that are regulated by miRNAs. The miRNAs regulate targets forming an intricate network with transcriptional factors and signal transduction pathways.


Breast
Liver
ERα mRNA
VDR mRNA
RXRα mRNA
PXR mRNA
HNF4α mRNA
miR-206
miR-221/222
miR-22
miR-125b
miR-27b
miR-148a
miR-34a
miR-24
ERK1/2
p38
CYP1B1 gene
CYP24 gene
CYP3A4 gene
CYP7A1 gene
CYP8B1 gene
CYP1B1 mRNA
CYP24 mRNA
CYP3A4 mRNA
CYP7A1 mRNA
CYP8B1 mRNA
PKC
ROS
miR-27b
miR-125b
CYP2E1 mRNA
miR-378
Bile acids
Transcription
Translation
Binding to response element
Production
Activation or stimulation