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Journal

Drug Metabolism and Disposition, 41(10)

Authors

Ingelman-Sundberg, Magnus
Zhong, Xiao-Bo
Peng, Lai
et al.

Publication Date

2013-10-01

DOI

10.1124/dmd.113.053157

Peer reviewed

Special Section on Epigenetic Regulation of Drug Metabolizing Enzymes and Transporters—Symposium Report

Potential Role of Epigenetic Mechanisms in the Regulation of Drug Metabolism and Transport

Magnus Ingelman-Sundberg, Xiao-Bo Zhong, Oliver Hankinson, Sudheer Beedanagari, Ai-Ming Yu, Lai Peng, and Yoichi Osawa

Department of Physiology and Pharmacology, Section of Pharmacogenetics, Karolinska Institutet, Stockholm, Sweden (M.I.-S.); Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut (X.-B.Z., L.P.); Interdepartmental Molecular Toxicology Program and the Department of Pathology and Laboratory Medicine, University of California, Los Angeles, California (O.H., S.B.); Department of Biochemistry and Molecular Medicine, University of California at Davis School of Medicine, Sacramento, California (A.-M.Y.); and Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan (Y.O.)

ABSTRACT

This is a report of a symposium on the potential role of epigenetic mechanisms in the control of drug disposition sponsored by the American Society for Pharmacology and Experimental Therapeutics and held at the Experimental Biology 2013 meeting in Boston, MA, April 21, 2013. Epigenetics is a rapidly evolving area, and recent studies have revealed that expression of drug-metabolizing enzymes and transporters is regulated by epigenetic factors, including histone modification, DNA methylation, and noncoding RNAs. The symposium speakers provided an overview of genetic and epigenetic mechanisms underlying variable drug metabolism and drug response, as well as the implications for personalized medicine. Considerable insight into the epigenetic mechanisms in differential regulation of the dioxin-inducible drug and carcinogen-metabolizing enzymes CYP1A1 and 1B1 was provided. The role of noncoding microRNAs in the control of drug metabolism and disposition through targeting of cytochrome P450 (P450) enzymes and ATP-binding cassette membrane transporters was discussed. In addition, potential effects of xenobiotics on chromatin interactions and epigenomics, as well as the possible role of long noncoding RNAs in regulation of P450s during liver maturation were presented.

Introduction

Drug absorption, distribution, metabolism, and excretion (ADME) are critical processes that must be understood for the development of safe drugs. These processes are mediated by drug-metabolizing enzymes and transporters that are expressed in various tissues, including the small intestine, liver, and kidney. These processes can limit or enhance the systemic and target organ exposure to xenobiotics. In particular, drug-metabolizing enzymes such as cytochrome P450 (P450) isoforms govern the metabolic elimination of drugs, and membrane transporters such as ATP-binding cassette (ABC) transporters can affect drug absorption, distribution, and excretion processes. Thus the interplay of drug-metabolizing enzymes and transporters may determine the pharmacokinetic properties of a drug such as bioavailability, volume of distribution, and half-life, and understanding the regulation of drug-metabolizing enzymes and transporters is necessary for the prediction of consequent pharmacological and toxicological effects.

There are considerable variations in drug metabolism and transport in humans (Ingelman-Sundberg et al., 2007; Yu, 2009; Giacomini et al., 2010; Yokoi and Nakajima, 2013) that can alter drug efficacy or cause adverse drug reactions. The latter is a leading cause of morbidity and mortality during pharmacotherapy. Indeed, the expression of ADME genes is tightly controlled by a variety of molecular regulatory mechanisms, among them transcription factors and cellular processes such as membrane trafficking and subcellular organization (Correia and Liao, 2007; Klaassen and Aleksunes, 2010). Genetic polymorphisms of ADME genes are also recognized for their clinical significance. Furthermore, concurrent use of drugs may lead to drug-drug interactions (DDIs) through the inhibition of enzyme or transport functions, or the alteration of ADME gene expression. Nevertheless, the interindividual differences in expression of the ADME genes are

ABBREVIATIONS: 3′UTR, 3′-untranslated region; 5-AzadC, 5-aza-2′-deoxycytidine; 5hmC, 5-hydroxymethylcytosine; ABC, ATP-binding cassette; ADME, absorption, distribution, metabolism, and excretion; AHR, aryl hydrocarbon receptor; DDI, drug-drug interactions; dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; lncRNAs, long noncoding RNAs; miRNA, microRNA; ncRNA, noncoding RNA; P450, cytochrome P450; PCAF, p300/CBP-associated factor; Pol II, polymerase II; PXR, pregnane X receptor; TBP, TATA-binding protein; XRE, xenobiotic responsive element.

tremendously large and this variation cannot be solely explained by these factors. Additional mechanisms such as the more recently identified epigenetic factors warrant investigation in relation to ADME processes. The symposium was organized to highlight and stimulate studies on such epigenetic factors, including DNA methylation, histone modification, and noncoding RNA (ncRNA)–mediated post-transcriptional regulation (Gomez and Ingelman-Sundberg, 2009b; Nakajima and Yokoi, 2011; Yu and Pan, 2012; Yokoi and Nakajima, 2013), studies which are expected to enhance mechanistic understanding of variable pharmacokinetics and multidrug resistance, and provide novel insights into individualized medication.

A symposium sponsored by the American Society for Pharmacology and Experimental Therapeutics was held at the Experimental Biology Annual Meeting in Boston, MA, April 21, 2013. Presentations were given by M.I-S., X.-B.Z., O.H., and A.-M. Y. to overview and exemplify the potential role of epigenetic regulatory mechanisms in cellular drug metabolism and transport, consequent effects on pharmacokinetics and pharmacodynamics, and implications for improved pharmacotherapy. In addition, one abstract relating to long noncoding RNAs (lncRNAs) was presented by L.P. at the symposium. This article summarizes the presentations.

Overview of Genetic and Epigenetic Mechanisms Underlying Variable Drug Metabolism and Drug Response (M.I.-S.)

Interindividual differences in expression of genes responsible for drug metabolism, transport, and response are caused by genetic, epigenetic, physiologic, and environmental factors. Much information has evolved regarding the role of genetic polymorphism in such variability. The number of genetic biomarkers for prediction of drug dosage and choice are increasing. Recent genome-wide association studies reveal that polymorphisms among the drug-metabolizing enzymes affect endogenous functions such as blood pressure, suicide risk, and bilirubin levels, as well as exogenous factors like coffee intake, cigarette consumption, and drug efficacy (Sim et al., 2013). Such polymorphisms and others, including variations in the human leukocyte antigen (HLA) system, as well as the levels of expression of different tyrosine kinases and other signal transduction polymorphisms, provide together an arsenal of pharmacogenomic biomarkers that can help optimize drug treatment, particularly in the areas of oncology, cardiovascular disease, infection, and psychiatry.

The epigenetic causes for variation in drug metabolism and response have been much less studied. A novel method for target enrichment–based analyses of 174 ADME genes in fetal and adult livers was presented (Ivanov et al., 2013). Genomic DNA from fetal and adult livers were sheared and subjected to Agilent Sure select target enrichment for the ADME genes, followed by next generation sequencing using protocols with or without bisulfite treatment (Kacevska et al., 2012). In total 0.5 million cytosine-phosphate diester-guanine (CpG) islands were analyzed. In general the variability in DNA methylation of the ADME genes between liver samples was conserved, but at some DNA regions variable methylation was seen. The extent of total methylation variation between different ADME genes was very different and occurred primarily in the open reading frames. An in depth analysis of the *CYP3A4* gene revealed that specific elements in the 5′ upstream region were highly methylated in fetal livers, where no expression of the gene occurs, but severely decreased in adult livers, which suggested the importance of the higher *CYP3A4* gene expression in this organ. Some DNA elements in the *CYP3A4* gene were much conserved with respect to methylation in the adult livers, whereas others were very variable. The methylation of

specific CpG sites correlated with the expression of the *CYP3A4* gene as analyzed at the transcriptomic level (Kacevska et al., 2012a).

We found that besides methylation of cytosine, 5-hydroxymethylation is indeed an important modification of cytosine in liver (Ivanov et al., submitted manuscript). Thus, we found using mass spectrometry that up to 1% 5-hydroxymethylcytosine (5hmC) of the total cytosine content in adult liver consists of 5hmC, whereas in fetal livers it is below 0.125%. Genome-wide mapping of the distribution of 5hmC in human adult liver samples showed that 5hmC occupancy was overrepresented in genes involved in active catabolic and metabolic processes, whereas 5hmC elements that were found in genes from fetal livers were not present in the adult state. Overall we found a localization of 5hmC in enhancers and in CpG islands and in the active genes, and that the amount of 5hmC was indeed high. The data emphasize the importance of using methods that permit the discrimination between 5mC and 5hmC when investigating the liver epigenome, as methods based on bisulfite sequencing will cause erroneous conclusions.

It was emphasized that altered gene methylation is important for creating drug resistance in tumors during anticancer treatment and that the progress of tumor removal and treatment can be monitored by quantification of tumor-specific methylation of circulating DNA. This indicates the possibility of following tissue-specific epigenetic alterations in circulating blood. It was concluded that much more research is needed before the mechanisms behind and the role of gene methylation for drug metabolism (Fig. 1), transport, and action can be understood.

Role of Epigenetic Mechanisms in Differential Regulation of the Dioxin-Inducible Human *CYP1A1* and *CYP1B1* Genes (O.H. and S.B.)

The human *CYP1A1* and *CYP1B1* genes are highly inducible by agonists of the aryl hydrocarbon receptor (AHR), including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin) and certain polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene. Induction of these genes involves the following steps: activation of the AHR by agonists such as dioxin, subsequent translocation of AHR to the nucleus, its dimerization with the aryl hydrocarbon receptor nuclear translocator, the binding of the AHR/aryl hydrocarbon receptor nuclear translocator dimer to xenobiotic response elements (XREs) located in the upstream enhancer regions of the above genes, the recruitment of coactivator proteins to the regulatory regions of the genes, the binding of general transcription factors to the promoter, followed by recruitment of the TATA-binding protein (TBP) and RNA polymerase II to the promoter, and then activation of gene transcription. CYP1A1 and CYP1B1 are particularly effective at metabolically activating polycyclic aromatic hydrocarbons, carcinogenic components prevalent in cigarette smoke, smog, and cooked foods. It is well established that methylation of cytosine residues of genomic DNA (DNA methylation) of the regulatory region(s) of a gene can lead to silencing or diminished expression of the gene. Of particular interest, the promoter of the *CYP1B1* gene exhibits DNA methylation in a portion of colorectal (Habano et al., 2009) and gastric (Kang et al., 2008) cancers. Studies conducted in our laboratory demonstrated the mechanisms through which DNA methylation causes *CYP1B1* silencing and also shed light on the mechanisms of gene regulation in mammalian cells in general (Beedanagari et al., 2010a).

Our studies emanated from our initial observations that CYP1A1 mRNA is highly induced by dioxin in both the human breast cancer cell line MCF-7 and the human hepatoma cell line HepG2, but whereas CYP1B1 mRNA is induced in MCF-7, it is not induced in
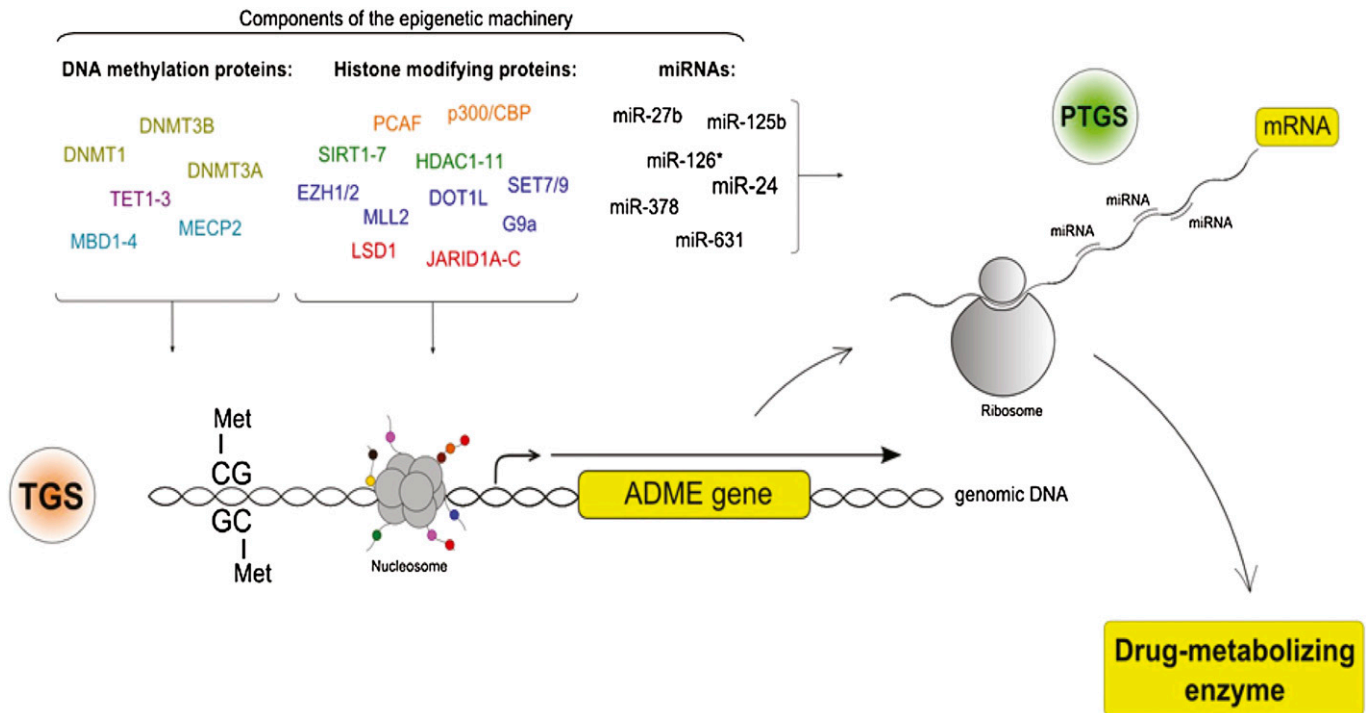
Fig. 1. Overview of the epigenetic control of ADME genes. Epigenetic machinery consists of DNA methylation proteins [e.g., DNA methyltransferases DNMT1, DNMT3A, and DNMT3B; Tet methylcytosine dioxygenase (TET1-3); methyl-CpG-binding domain proteins (MBD1-4 and MECP2)], histone-modifying enzymes [e.g., histone deacetylase HDAC1-11; p300/CBP and PCAF; sirtuin (SIRT1-7); enhancer of zeste homolog (EZH1/2); DOT1-like histone H3 methyltransferase (DOT1L); myeloid/lymphoid or mixed-lineage leukemia (MLL2)], and miRNAs (e.g., miR-27b, -125b, -126*, -24, -378, and -631), which contribute to transcriptional and/or posttranscriptional gene silencing (TGS and PTGS). Adapted from Pharmacogenomics, 2012, 13(12):1373-1385, with permission of Future Medicine Ltd. (Kacevska et al., 2012b).

HepG2 cells. The *CYP1B1* promoter abutting the transcriptional start site, and the gene's upstream enhancer region (which contains XREs to which the liganded AHR can bind) have a high density of CpG dinucleotides, representing CpG "islands." Bisulphite sequencing revealed that 22 of the 24 CpG sites within 280 bp encompassing the promoter region of *CYP1B1* (–260 to +20) are fully methylated in HepG2 cells, whereas only one of the 24 CpG sites was methylated in MCF-7 cells. The CpG sites in a 360-bp sequence (–560 to –920 bp) encompassing the enhancer of *CYP1B1* were either only partially methylated (0–60%) or not methylated in HepG2 cells, while these CpG sites in MCF-7 cells were not methylated. There are two XREs in the *CYP1B1* enhancer (Fig. 2). The XRE sequence contains a CpG site, methylation of which is known to interfere with AHR binding. DNA methylation analysis of these XREs located in the *CYP1B1* enhancer region revealed that they are only partially methylated (30%) in HepG2 cells, but not methylated in MCF-7 cells. Treatment of HepG2 cells with the DNA methyltransferase inhibitor 5-aza-2′deoxycytidine (5-AzadC) reactivated dioxin-induced CYP1B1 mRNA expression to a level comparable to that in MCF-7 cells. Furthermore, 5-AzadC treatment led to significant demethylation of the CpG sites in the *CYP1B1* promoter and enhancer regions in HepG2 cells. Collectively these data indicate that DNA methylation directly inhibits dioxin inducibility of the *CYP1B1* gene in HepG2 cells.

We investigated the consequences of DNA methylation of the *CYP1B1* gene. Using the chromatin immunoprecipitation assay, we demonstrated that dioxin treatment fails to lead to the recruitment of RNA polymerase II (Pol II) and the TBP to the promoter of the *CYP1B1* gene in HepG2 cells. However, these recruitments were restored after 5-AzadC treatment. These observations are consistent with the observation that CYP1B1 mRNA inducibility is reactivated in

this cell line by 5-AzadC. Importantly, dioxin treatment led to recruitment of AHR to the enhancer region of the *CYP1B1* gene in HepG2 cells. It is known that the transcriptional coactivator p300 is required for maximal dioxin induction of CYP1B1 (Taylor et al., 2009). We demonstrated that dioxin treatment led to recruitment of p300 and the coactivator p300/CBP-associated factor (PCAF) to the enhancer of *CYP1B1* in HepG2 cells. Thus loss of dioxin inducibility of CYP1B1 in HepG2 cells due to DNA methylation is ascribable to events in the induction pathway subsequent to AHR, p300, and PCAF recruitment to the enhancer.

Chromatin modifications play an important role in the epigenetic regulation of the transcription of genes. Acetylations of histone H3 at lysines 9 and 14, acetylation of histone H4, and trimethylation of histone H3 at lysine 4 are generally associated with actively transcribed genes. We found that the above histone modifications were markedly increased at the *CYP1B1* promoter of MCF-7 cells after dioxin treatment, and that these increases were dependent upon p300 (which is capable of directly catalyzing the above acetylations). However, in dioxin-treated HepG2 cells, Pol II recruitment did not increase despite enhanced coactivator recruitment, implying a dissociation between this event and chromatin modification under these conditions. This is most likely due to DNA methylation at the promoter.

These studies therefore provided a detailed description of the mechanism whereby DNA methylation inhibits dioxin induction of the *CYP1B1* gene, many aspects of which may be applicable to other genes whose expression is modified by DNA methylation. The following of our observations are of particular interest: 1) Recruitment of AHR to the enhancer of the *CYP1B1* gene does not necessarily equate with dioxin inducibility, consistent with the authors' other studies (Beedanagari et al., 2010b) and others (Yang et al., 2008). 2)
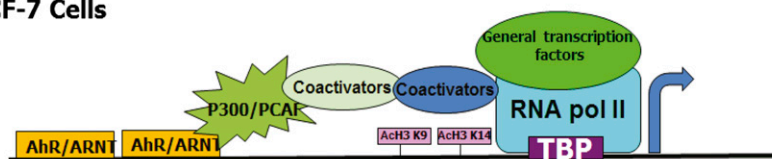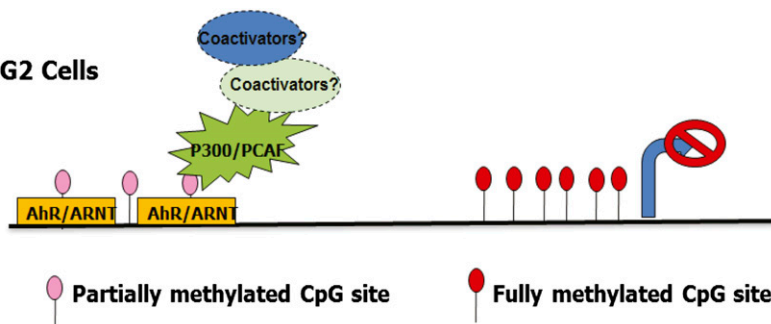
Fig. 2. CYP1B1 silencing model in HepG2 cells. The presence of a fully methylated CpG site at the *CYP1B1* promoter inhibits dioxin-induced binding of TBP, which provides a molecular explanation for the silencing of CYP1B1 in HepG2 cells.

The binding of TBP to gene promoters generally seeds the association of other general transcription factors. Our observation that DNA methylation at the *CYP1B1* promoter inhibits dioxin-induced binding of TBP suggests that this may represent a critical step in the process whereby DNA methylation at the promoter inhibits gene expression; and this may be the case for other genes subject to silencing by methylation. 3) Our observations indicate that p300 recruitment to the enhancer is not sufficient for the generation of histone modifications at the promoter, but that communication with other protein(s) at the promoter is probably required. 4) Transcriptional coactivators are generally incorporated in large multiprotein complexes straddling both the enhancer and promoter of responsive genes. We observed much greater levels of p300 and PCAF (as well as AHR) at the *CYP1B1* enhancer than at the *CYP1B1* promoter in MCF-7 cells treated with dioxin. This observation is consistent with the notion that these proteins are in closer proximity to AHR at the *CYP1B1* enhancer than to TBP and the general transcription factors located at the promoter. It is therefore of considerable interest that p300 and PCAF are recruited efficiently at the *CYP1B1* enhancer after dioxin treatment in HepG2 cells, despite the fact that TBP and Pol II are not recruited to the corresponding promoter. These observations strongly imply that p300 and PCAF can be recruited to the enhancer even when they are not incorporated into a multiprotein complex spanning the enhancer and the promoter (Fig. 2). The lack of a requirement for coactivators to be tethered at both the promoter and enhancer can probably be generalized to other coactivators and other genes, and this represents an important area for future research.

Chromatin Interactions, Epigenomics, and Transcriptional Outcomes in Response to Xenobiotics (X.-B.Z.)

DDI is a significant clinical concern especially for people at ages above 50 because ~78% of people over 50 currently using drugs actually take more than one drug a day, and 4% of them are at risk for either a reduced therapeutic efficacy or adverse drug reactions (Qato et al., 2008). One major cause of DDI is that some drugs have the ability to alter the expression of drug-metabolizing enzymes or transporters responsible for the ADME of coadministrated drugs. It has been known that at the molecular levels ADME genes are induced through the activation of transcriptional factors such as nuclear receptor pregnane X receptor (PXR) and constitutive androstane receptor by the drugs (Urquhart et al., 2007). Upon activation by the

drugs, the nuclear receptor binds to its response elements at multiple genomic locations to regulate the transcription of ADME genes. However, many questions remain to be answered for a thorough understanding of the mechanisms underlying the induction of ADME genes by drugs. For example, many nuclear receptor binding sites are located far away from the promoters of their target genes. How are those binding sites involved in the transcription regulation of their target genes? Are long-range chromatin interactions involved in bringing these binding sites close to their target promoters for possible interactions, and thus facilitating the transcriptional regulation? Are the regulatory elements acting alone or being coordinated in clusters in response to xenobiotics? What are the epigenomic features associated with ADME genes and regulatory elements?

Dr. Zhong from the University of Connecticut and his collaborators, Drs. Yijun Ruan and Guoliang Li from The Jackson Laboratory (Farmington, CT), intend to improve the understanding of molecular mechanisms of drug-induced change in ADME gene expression. They have selected rifampicin as a model drug in a planned study. Based on the literature and their preliminary data, they formed a central hypothesis that rifampicin activates the transcription factor PXR, which binds to its DNA response elements at multiple genomic locations and participates in long-range chromatin interactions in a three-dimensional conformation, thus helping to bring relevant genomic elements such as the enhancers and promoters into close spatial proximity for an efficient and coordinated transcription regulation. The changes in ADME gene expression further alter therapeutic efficacy of drugs coadministered with rifampicin. They will use human primary hepatocytes and established human liver cell lines (e.g., HepaRG for loss-of-function and HepG2 for gain-of-function) as in vitro models, and *PXR*-humanized mice as an in vivo model to test their hypothesis. They plan to define PXR-mediated chromatin interaction architecture, epigenomic features, and transcriptional outcomes in response to rifampicin in human liver cells. They will characterize PXR-specific transcriptional regulatory programs in *PXR*-humanized mice. Then they will analyze PXR-mediated changes in chromatin interactions, transcription, and histone modifications by computational and network approaches. The data are expected to provide a comprehensive repertoire of regulatory elements such as promoters and enhancers, and specific interaction networks of the regulatory functions in responding to rifampicin. Through the integrated analyses of multiplex datasets generated, they hope to understand how PXR activated by rifampicin participates in maintaining higher-order

chromatin structure and transcriptional outcomes, which would be helpful for the prediction of related interactions and development of proper strategies to prevent unwanted drug effects.

Noncoding MicroRNAs in the Control of Drug Metabolism and Transport (A.-M.Y)

In recent years there has been increasing interest in identifying ncRNAs and understanding their functions in regulation of cellular processes. A major portion (>96%) of the human genome comprises noncoding DNAs (Matera et al., 2007; Kung et al., 2013), which include ribosomal RNAs, transfer RNAs, small nucleolar RNAs, microRNAs (miRNAs), small interfering RNAs, P-element–induced wimpy testis–interacting RNAs, and lncRNAs. Among them the miRNAs, a class of small (18–25 nt in length) ncRNAs in the control of post-transcriptional regulation of target genes, represent one of the groups of ncRNAs most intensively studied toward the elucidation of their roles, along with protein-coding target genes, in human diseases, as well as development of therapeutic strategies (Trang et al., 2008; Kasinski and Slack, 2011). MicroRNAs usually reduce the expression of target genes through the inhibition of translation or acceleration of mRNA degradation after imperfectly complementary Watson-Crick base pairings with miRNA response element within the 3′-untranslated regions (3′UTRs) of mRNA targets. Over 2000 miRNAs have been identified in humans, and they are predicted to govern post-transcriptional regulation of thousands of protein coding genes in control of essentially all life processes.

There is also increasing evidence supporting miRNA-controlled post-transcriptional regulation of ADME genes (Gomez and Ingelman-Sundberg, 2009a; Yu, 2009; Nakajima and Yokoi, 2011; Yu and Pan, 2012; Yokoi and Nakajima, 2013), which were highlighted at the symposium. More and more studies have demonstrated that miRNAs are able to target the 3′UTR of ADME genes and modulate the expression of P450 enzymes and ABC transporters (Table 1). Following the discovery of developmental and sexual *CYP3A4* transgene expression in *CYP3A4*-transgenic mouse models (Yu et al., 2005), we demonstrated the involvement of miR-27b and murine miR-298 (mmu-miR-298) in regulation of CYP3A4 through targeting of its 3′UTR as well as the vitamin D receptor transcription factor (Pan et al., 2009a). Other studies also revealed the importance of miR-27b in regulation of P450 enzyme CYP1B1 (Tsuchiya et al., 2006) and nuclear receptors retinoid X receptor alpha and peroxisome proliferator-activated receptor alpha

(Ji et al., 2009; Kida et al., 2011). These findings indicate that miRNAs may modulate drug metabolism through "direct" and "indirect" regulation of drug-metabolizing enzymes. Meanwhile, multiple miRNAs were shown to regulate the same transporters, such as ABCG2 (Pan et al., 2009b), thus potentially controlling cellular drug disposition and multidrug resistance. Our further studies demonstrated a higher efficiency for miR-519c and miR-328 in the modulation of ABCG2 expression in MCF-7 cells, and an mRNA degradation mechanism for miR-519c–controlled regulation (Li et al., 2011). Our most recent study identified the contribution of miR-1291, a small nucleolar RNA–derived miRNA, toward the regulation of ABCC1 and subsequent modulation of doxorubicin disposition and cytotoxicity (Pan et al., 2013). Delineation of the role of ncRNAs in the control of ADME genes will inevitably improve mechanistic understanding of variable pharmacokinetics and drug response as well as multidrug resistance, and offer new clues to rational drug therapy.

Long Noncoding RNAs and Transcriptional and Post-Transcriptional Regulation of Cytochromes P450 in Mouse Liver during Maturation (L.P.)

LncRNAs are nonprotein-coding RNA transcripts longer than 200 nucleotides (Kapranov et al., 2007). Their genomic DNAs are either present in the intergenic regions and thus called long intergenic noncoding RNAs, or partially overlapped with protein-coding genes that can be transcribed from sense or antisense strands. LncRNAs may be alternatively spliced like protein-coding genes, whereas they have fewer exons (2.9 exons on average). Comparative analyses have found that lncRNAs are evolutionarily conserved, especially at the promoter regions. The expression levels of lncRNAs are usually lower than protein-coding RNAs, and lncRNAs are expressed in tissue- and development-specific manners. LncRNAs have been demonstrated to regulate various biologic processes, including the cell cycle, pluripotency, and cell differentiation and development. Recent studies have also revealed that some lncRNAs are functional in the regulation of gene expression during organ maturation (Guttman et al., 2011; Pauli et al., 2011). The expression levels of P450 drug-metabolizing enzymes critical for the biotransformation of xeno-/endo-biotics have been shown to be altered during postnatal liver maturation, whereas the molecular mechanisms are not yet clear. Therefore, a systematic analysis of lncRNA-expression profiles during liver maturation was performed

TABLE 1

Some P450 drug-metabolizing enzymes and ABC transporters shown to be targeted by noncoding miRNAs

		MicroRNA	Reference
Enzymes	CYP1B1	miR-27b	Tsuchiya et al., 2006
	CYP2E1	miR-378	Mohri et al., 2010
	CYP3A4	miR-27b, mmu-miR-298	Pan et al., 2009a
Transporters	ABCB1	miR-451	Kovalchuk et al., 2008
		miR-27a	Zhu et al., 2008
	ABCG2	miR-520h	Liao et al., 2008; Wang et al., 2010; Li et al., 2011
		miR-519c	To et al., 2008; To et al., 2009; Li et al., 2011
		miR-328	Pan et al., 2009b; Li et al., 2011
	ABCC1	miR-134	Guo et al., 2010
		miR-326	Liang et al., 2010
		miR-199a, miR-199b, miR-296	Borel et al., 2012
		miR-1291	Pan et al., 2013
	ABCC2	miR-379	Haenisch et al., 2011
	ABCC3	miR-9-3p	Jeon et al., 2011
	ABCC4	miR-125a, miR-125b	Borel et al., 2012
	ABCC5	miR-101, miR-125a, Let-7a	Borel et al., 2012
		miR-128	Zhu et al., 2011
	ABCC6	miR-9-3p	Jeon et al., 2011

to determine whether lncRNAs are involved in the regulation of P450 ontogeny.

Male C57BL/6 mouse livers were collected at 12 different ages from prenatal, neonatal, adolescent to adult. Poly-T selected RNAs were sequenced and the expression levels of both protein-coding and -noncoding genes were determined. Approximately 15,000 genes, including ~2000 lncRNAs, were expressed in liver during maturation. About 70% of these lncRNAs were significantly changed across age groups. Three major ontogenic expression patterns were identified for lncRNAs, and they fell into neonatal-, adolescent-, and adult-enriched groups. The same patterns were also found for P450 genes. LncRNAs with potential roles in regulation of P450s ontogeny were initially screened. In addition, we recognized one lncRNA that displayed an inverse expression pattern with Cyp4b1 during liver maturation. With a gene symbol *Gm12839*, this lncRNA is located approximately 40 kb downstream of *Cyp4b1* gene, and it was annotated as a pseudogene of *Cyp4b1*. This lncRNA exhibits more than 90% sequence identity as the 3′ end of Cyp4b1 RNA. As the 3′UTR usually contains regulatory elements for the ncRNAs, it would be interesting to determine whether Gm12839 RNA downregulates the expression of Cyp4b1. This is the first attempt to examine the correlation of lncRNAs and P450 ontogeny in liver and should facilitate future study on the possible role of lncRNAs in the regulation of P450 expression during liver maturation.

Summary

In summary, this symposium has given a glimpse of some of the ongoing studies defining the important role of epigenetics in regulation of drug-metabolizing enzymes and drug transporters. It is clear from the presentations and the questions raised during discussion that further studies on the role of epigenetics in drug metabolism and disposition are forthcoming and highly warranted.

Authorship Contributions

Performed data analysis: Ingelman-Sundberg, Zhong, Hankinson, Beedanagari, Yu, Peng, Osawa.

Wrote or contributed to the writing of the manuscript: Ingelman-Sundberg, Zhong, Hankinson, Beedanagari, Yu, Peng, Osawa.

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Address correspondence to: Dr. Yoichi Osawa, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109. E-mail: osawa@umich.edu