

## Special Section on Epigenetic Regulation—Commentary

# Epigenetic Regulation of ADME-Related Genes: Focus on Drug Metabolism and Transport

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### ABSTRACT

Epigenetic regulation of gene expression refers to heritable factors that are functionally relevant genomic modifications but that do not involve changes in DNA sequence. Examples of such modifications include DNA methylation, histone modifications, noncoding RNAs, and chromatin architecture. Epigenetic modifications are crucial for packaging and interpreting the genome, and they have fundamental functions in regulating gene expression and activity under the influence of physiologic and environmental factors. Recently, epigenetics has become one of the fastest-growing areas of science and has now become a central issue in biologic studies of development and disease pathogenesis. The interest in epigenetics is also true for studies of drug metabolism and transport. In

this issue of *Drug Metabolism and Disposition*, a series of articles is presented to demonstrate the role of epigenetic factors in regulating the expression of genes involved in drug absorption, distribution, metabolism, and excretion in organ development, tissue-specific gene expression, sexual dimorphism, and in the adaptive response to xenobiotic exposure, both therapeutic and toxic. The articles also demonstrate that, in addition to genetic polymorphisms, epigenetics may also contribute to wide interindividual variations in drug metabolism and transport. Identification of functionally relevant epigenetic biomarkers in human specimens has the potential to improve prediction of drug responses based on patient's epigenetic profiles.

### Introduction

Epigenetics refers to genomic modifications that do not involve changes in DNA sequence but are heritable and have functional influence on gene expression and cellular phenotypes (Bird, 2007). Various types of genomic modifications have been defined as epigenetic modifications, including DNA methylation, histone modifications, noncoding RNAs, nucleosome positioning, and chromatin remodeling. DNA methylation is a biochemical process involving the addition of a methyl group to cytosine or adenine DNA nucleotides. Depending on occurrence at different genomic locations, DNA methylation contributes to the formation of chromatin structure in cells and is involved in the regulation of cell differentiation and organ development. Uncontrolled DNA methylation may lead to the development of various types of human diseases, such as cancers (Baylin and Jones, 2011). Histone proteins are subject to posttranslational enzymatic modifications, such as acetylation, methylation, phosphorylation, and ubiquitination. Modified histones may alter their interactions with associated DNA to further change activity of gene expression. A noncoding RNA is a functional RNA molecule that is not translated into a protein. A microRNA is a small noncoding RNA molecule that ranges in size from 17 to 25 nucleotides and functions in the transcriptional and posttranscriptional regulation of gene expression (Wang et al., 2013).

These epigenetic modifications intertwine with each other to regulate gene expression in the processes of cellular differentiation (Reik, 2007) and organ development, and in response to environmental stimuli.

Currently, there is considerable interest in the role of epigenetic factors in the regulation of absorption, distribution, metabolism, and excretion (ADME)-related genes as indicated by a session dedicated to the topic at the recent American Society for Pharmacology and Experimental Therapeutics Annual Meeting at Experimental Biology 2013 in Boston, Massachusetts. A meeting report by Ingelman-Sundberg et al (2013). is included in this special issue to summarize current progress in studies of epigenetic mechanisms, such as DNA methylation, histone modifications, and noncoding RNAs, in the regulation of drug metabolism and transport.

Ingelman-Sundberg and colleagues have assembled a comprehensive listing of ADME genes subject to epigenetic regulation (Kacevska et al., 2011), and this compendium was recently updated to include ADME genes associated with drug response (Kacevska et al., 2012a). The articles included in this special issue provide additional evidence to demonstrate epigenetic mechanisms involved in regulating ADME genes, with a particular focus on drug metabolizing enzyme and transporter genes in organ development, tissue-specific regulation of gene expression, sexual dimorphism, drug induction, and interindividual phenotypic variability.

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**ABBREVIATIONS:** ADME, absorption, distribution, metabolism, and excretion; C/EBP $\beta$ , CCAAT/enhancer binding protein  $\beta$ ; HNF1, hepatocyte nuclear factor 1, OAT, organic anion transporters; P450, cytochrome P450; T-DMRs, tissue-dependent differentially methylated regions; XREM, xenobiotic responsive module.

### Epigenetic Regulation of ADME Genes during Development

Among the earliest investigations of epigenetic regulation of ADME genes were studies of the developmental trajectory of rat CYP2E1 expression conducted approximately 25 years ago (Umeno et al., 1988). Although not expressed in fetal hepatocytes, activation of CYP2E1 occurred within hours after birth. Studies using the cytosine methylation-dependent restriction endonucleases, *HhaI* and *HpaII*, associated the rapid postnatal increase in mRNA expression with DNA demethylation at the 5'-region of the gene (Umeno et al., 1988). In the mid-1990s, similar studies of CYP2E1 ontogeny in humans implicated progressive demethylation of specific CpG residues in exon 1 and intron 1 with postnatal increases in CYP2E1 mRNA expression throughout the neonatal period (Vieira et al., 1996). Low levels of CYP2E1 mRNA in human fetal, neonatal, and adult kidney and lung tissue were also associated with extensive methylation of the same region of the gene (Vieira et al., 1998). A similar phenomenon has been reported for postnatal activation of *Cyp1a2* in mice (Jin et al., 2004).

A more complex picture has begun to emerge with respect to regulation of the CYP3A developmental trajectory. The ontogeny of the CYP3A locus in humans is largely characterized by a "switch" from predominantly CYP3A7 expression in fetal hepatocytes to CYP3A4 expression in adult liver, whereas CYP3A5 expression is relatively stable throughout development, at least in those individuals with genotypes compatible with functional activity (Schuetz et al., 1994; Lacroix et al., 1997; Hines, 2008). Investigation of the mechanisms underlying this developmental pattern of expression has tended to focus on *trans*-acting factors, such as the age/developmental stage-specific change in the ratio of alternative transcripts of CCAAT/enhancer binding protein  $\beta$  (C/EBP $\beta$ ), known as the LAP/LIP ratio, which function as transcriptional activators and repressors (Descombes and Schibler, 1991), and the nuclear receptors pregnane X receptor and constitutive androstane receptor (Vyhldal et al., 2006). More recently, epigenetic regulatory mechanisms involving specific patterns of histone methylation have been demonstrated for an analogous switch from *Cyp3a16* in neonatal mouse liver to *Cyp3a11* in adult mouse liver (Li et al., 2009). DNA hypermethylation within the murine *Cyp3a* locus was not observed at any developmental stage; however, data recently have been presented indicating that silencing of CYP3A4 in human fetal liver is associated with DNA hypermethylation of individual CpG sites in close proximity to key regulatory elements, such as CLEM4, HNF4 $\alpha$ , XREM, and C/EBP sites within the CYP3A4 regulatory region (Kacevska et al., 2012b). In addition, microRNA mmu-miR-298, which is decreased in wild-type mouse livers after puberty but increased in CYP3A4-transgenic mouse livers after puberty, has been shown to suppress CYP3A4 gene expression through direct and indirect targeting (Pan et al., 2009). In this issue, Xie et al. (2013) implicate a role for microRNA in cytochrome P450 (P450) ontogeny in their investigation of sexually dimorphic expression of *Cyp2b9*. For example, when the ability to generate microRNAs was compromised by knocking out *Dicer1*, the age-dependent decline in *Cyp2b9* expression was abolished, providing indirect evidence for microRNAs and regulation of *Cyp2b9* ontogeny.

### Epigenetic Regulation of Tissue-Specific Expression of ADME Genes

DNA methylation and histone modifications represent important mechanisms for tissue-specific gene expression; it has even been suggested that it may be possible to identify specific cells/tissues based on their unique DNA methylation profiles (Shiota, 2004). In general, hypomethylation of a gene promoter region combined with expression of tissue-specific regulatory factors is permissible for

driving cell- or tissue-specific gene expression. For example, experiments involving demethylation of cytosine residues and overexpression of C/EBP $\delta$ , a lung-enriched form of C/EBP, implicate epigenetic modulation of C/EBP binding sites as a mechanism underlying the expression of human CYP2A13 in the olfactory mucosa of transgenic mice and in human lung cancer cells (Ling et al., 2007). A series of studies investigating kidney- and liver-specific expression of multiple transporters reveals a pattern of tissue-dependent differentially methylated regions (T-DMRs) that influence the interaction of hepatocyte nuclear factor 1 (HNF1). HNF1 is an integral transactivating factor for organic anion transporters (OATs) in liver and kidney, with its cognate binding site in the promoter region of targeted transporters. In the case of amino acid transporters and OATs, hypomethylation of Hnf1 $\alpha$  binding sites leads to transporter expression in kidney whereas hypermethylation silences gene expression in the liver. In contrast, Hnf1 $\alpha$  binding sites are hypermethylated in cerebrum and are not available to drive transporter expression in that tissue (Kikuchi et al., 2010). The opposite scenario (hypomethylated T-DMRs in liver and hypermethylated in kidney) is operative for liver-specific expression Oatp transporters (Imai et al., 2013). In this issue, Oda et al. (2013) provide evidence that decreased expression of UGT1A1 in kidney is a function of decreased binding of HNF1 $\alpha$  due to both DNA hypermethylation and histone hypoacetylation, implicating multiple levels of epigenetic regulation in tissue-specific expression of UGT1A1.

### Epigenetic Regulation of Sexual Dimorphism

Sexual dimorphism refers to phenotypic differences between males and females of the same species. Studied extensively, sex differences in the expression of P450s and other drug-metabolizing enzymes in liver have been attributed to the differences in the temporal pattern of growth hormone release by the pituitary (pulsatile in males and continuous in females) that are more pronounced in rodents than in humans (Waxman and Holloway, 2009). Although the results of early studies of male-specific CYP2C11 expression in liver implied a role for epigenetic regulation (Ström et al., 1994), Yokomori et al. provided more definitive evidence for epigenetic regulation by demonstrating that the *Cyp2a4* promoter was differentially demethylated in female CD-1 mice, and the *Cyp2d9* promoter was preferentially demethylated in male mice (Yokomori et al., 1995). Furthermore, binding of GABP was sensitive to the methylation status of the *Cyp2d9* promoter, confirming the association between promoter methylation status, transcription factor recruitment, and sex-specific expression (Yokomori et al., 1995). In this special issue, Xie et al. report that several microRNAs are associated with the hepatic expression of *Cyp2b9* in female C57Bl/6J mice, and that the expression of these microRNAs is lower in female mice than in male mice. Furthermore, *Cyp2b9* expression is increased in male *Dicer1* knockout mice, providing evidence that regulation by microRNA can be added to the list of mechanisms underlying sexually dimorphic P450 expression.

### Contribution of Epigenetics to Interindividual Variability in Drug Disposition and Response

Interindividual variability in drug biotransformation has been subject to extensive investigation at multiple levels, including genetic and nongenetic factors as well as multiple in vitro and in vivo approaches (Zanger and Schwab, 2013). In addition to variations in the regulatory and coding regions of P450 and other genes themselves, genetic variation in transcription factors, such as pregnane X receptor and constitutive androstane receptor (Chai et al., 2013), and in the electron donor cytochrome P450 oxidoreductase (Pandey and Flück, 2013) also contributes to interindividual variability in ADME gene expression

and function. There is also growing interest in studying epigenetic factors, which may be additional causes of large variations in drug disposition and response. As an example of how epigenetic regulation of ADME genes may influence drug response, this issue's report by Pan et al. (2013) provides data linking hsa-miR-1291-directed down-regulation on *ABCC1* to an increase in the intracellular accumulation of doxorubicin, which was accompanied by an increase in chemosensitivity to the effects of the drug. From a more clinical perspective, the current hope/hype is that changes in epigenetic modifications and other factors may account for some of the interindividual variability in epigenetic markers, either as a consequence of genetic variation, environmental exposures (Choudhuri et al., 2010), or other factors. In doing so, these factors may also account for some of the "missing heritability" observed in large population studies (Mill and Heijmans, 2013). Epigenetic mechanisms therefore may be used along with genetic, environmental, and other factors to guide drug and dose selection toward an improved treatment outcome.

Although a compelling argument can be made for aberrant DNA methylation in the pathogenesis of various cancers (Baylin and Jones, 2011), a causal role for epigenetic dysregulation of cellular processes leading to complex diseases is less well established. A quick review of the list of studies supporting a role for epigenetic factors in the regulation of ADME genes (Kacevska et al., 2011) reveals that the majority were conducted in tumor cell lines. To begin exploring the role of epigenetic mechanisms in vivo, Rieger et al. (2013) describe the variability in expression of 56 microRNAs selected on the basis of their potential relevance for regulation of ADME genes in a panel of 92 (40 male and 52 female) human liver samples. They identified a set of six microRNAs that demonstrated considerable (1000- to 30,000-fold) variability in the samples, and another set of four microRNAs that varied <10-fold. Furthermore, significant correlations between microRNA expression and age as well as disease processes (e.g., inflammation and cholestatic liver disease) were observed; no significant correlation between expression and sex were observed. However, attempts to validate previously reported associations (Yokoi and Nakajima, 2013) between various microRNAs and CYP mRNA, protein, and activity levels with this dataset met with varying degrees of success. This finding serves as a reminder that the results from in vitro studies conducted using optimized cell-based model systems may differ from "population-based" studies; the latter involves more confounding factors, such as disease processes, exposure to concurrent medications, lifestyle factors (e.g., smoking, alcohol use), and other environmental exposures. In other words, study designs best suited for mechanistic insights come at a cost in terms of generalizability to populations of human patients. On the other hand, studies designed to characterize population variability may result in hypothesis-generating associations, whereas they are limited with respect to apparent or casual associations. Both types of studies nevertheless provide valuable information that must be interpreted in the proper context.

### Epigenetic Regulation of ADME Genes in the Adaptive Response to Xenobiotic Exposure

In this issue, Ramamoorthy et al. (2013) pursue sources of interindividual variability in ADME gene expression further by characterizing the induction of microRNA expression by rifampin in a set of seven primary human hepatocyte cultures. In contrast to the targeted approaches used by Rieger et al., this group adopted a global profiling strategy and identified 33 microRNAs that were upregulated by rifampin and a set of 35 microRNAs that were downregulated by rifampin. As expected, they observed negative correlations between

various microRNAs and expression of some P450s (including orphan P450s), reflecting suppression of target gene expression by microRNA. Nevertheless, some positive correlations were also noted, indicating that changes in the expression of microRNAs in response to perturbing factors, such as enzyme-inducing agents, might have indirect effects on gene expression by affecting multiple components of the gene regulatory machinery and thereby influence downstream phenotypes. The studies by Rieger et al. (2013) and Ramamoorthy et al. (2013) provide a glimpse of the complexity of microRNAome in a highly dynamic system among which the diseases (e.g., inflammation and cholestasis) and other perturbing factors (e.g., concurrent medications) have the potential to alter the microRNA repertoire and might further influence ADME processes. The potential complexity of the regulatory process is further illustrated in the paper by Shukla et al. exploring the role of microRNAs in insulin-mediated down-regulation of CYP2E1 (2013). In primary rat hepatocytes, insulin treatment increased the expression of miR-132 and miR-122, which target the 3' untranslated region of CYP2E1, and mechanistic studies implicated the phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin pathway in the induction of target microRNAs by insulin.

To fully understand the impact of epigenetic regulation (especially microRNAs) on disease pathogenesis and on drug disposition and response, more comprehensive in vivo studies are required. Prior to integration of epigenetic factors in clinical research, the stability of microRNAs in biologic specimens obtained in the course of pharmacokinetic studies and clinical trials needs to be determined. In a *Short Communication* in this issue, Benson and Skaar (2013) demonstrate, using Mir-16 and Mir-223 as examples, that microRNAs are stable in whole blood at room temperature for up to at least 12 hours, supporting the use of circulating microRNAs as potential biomarkers in diagnosis and prognosis. However, additional investigation is required to establish whether the stability patterns reported by Benson and Skaar are generalizable to other plasma microRNAs, or whether stability is microRNA-dependent.

### Conclusion

As research in epigenetic regulation of ADME genes progresses, there are many opportunities and challenges in understanding the epigenetic mechanisms in drug metabolism and disposition as well as clinical significance. More comprehensive profiling technologies and strategies will lead to identification of DNA methylation profiles and patterns of small noncoding RNAs that are associated with phenotypes of interest and generate testable hypotheses and new mechanistic insights. A better understanding of the mechanisms by which the epigenetic machinery regulates gene expression has implications for developing therapeutic interventions to reverse aberrant gene expression through inhibition of DNA methylation or gene silencing via therapeutic small interfering RNA molecules. Prior to clinical application, preclinical model systems will be needed to inform the optimal design of these novel therapeutic compounds with respect to their ADME properties and interactions with the drug target. Ultimately, the promise of epigenetic research is its potential to identify additional sources of interindividual variability in drug disposition and response that can be applied to more accurately predict the consequences of xenobiotic exposure. Multiple opportunities exist in this exciting new field.

### Authorship Contributions

Wrote or contributed to the writing of the manuscript: Zhong, Leeder.

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