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REVIEW

Epigenetic regulation of drug metabolism and transport



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Abstract The drug metabolism is a biochemical process on modification of pharmaceutical substances through specialized enzymatic systems. Changes in the expression of drug-metabolizing enzyme genes can affect drug metabolism. Recently, epigenetic regulation of drug-metabolizing enzyme genes has emerged as an important mechanism. Epigenetic regulation refers to heritable factors of genomic modifications that do not involve changes in DNA sequence. Examples of such modifications include DNA methylation, histone modifications, and non-coding RNAs. This review examines the widespread effect of epigenetic regulations on genes involved in drug metabolism, and also suggests a network perspective of epigenetic regulation. The epigenetic mechanisms have important clinical implications and may provide insights into effective drug development and improve safety of drug therapy.

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Abbreviations: CAR, constitutive androstane receptor; DNMTs, DNA methyltransferases; H3K4me1, histone 3 lysine 4 monomethylation; H3K4me2, histone 3 lysine 4 dimethylation; H3K4me3, histone 3 lysine 4 trimethylation; H3K9me2, histone 3 lysine 9 dimethylation; H3K9me3, histone 3 lysine 9 trimethylation; H3K27me3, histone 3 lysine 27 trimethylation; H3K36me3, histone 3 lysine 36 trimethylation; HATs, histone acetyltransferases; HDAC, histone deacetylases; lncRNAs, long non-coding RNAs; miRNAs, microRNAs; ncRNAs, non-coding RNAs; P450s, cytochrome P450s; SULTs, sulfotransferases; TSS, transcription start sites; UGTs, UDP-glucuronosyltransferases; UTR, untranslated region

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1. Introduction

Drug metabolism is the biotransformation process of pharmaceutical substances or xenobiotics, usually conducted by specialized enzymatic systems. Drug metabolism is divided into three phases. Phase-I contains the modification reactions of oxidation, reduction, and hydrolysis. Typical examples of phase-I enzymes include cytochrome P450s (P450s), flavin monooxygenases (FMOs), alcohol dehydrogenases (ADHs), aldehyde dehydrogenases (ALDHs), cytochrome P450 oxidoreductase (POR), aldo-keto reductase (AKR), quinone oxidoreductase (NQO), dihydropyrimidine dehydrogenase (DPYD), carboxylesterase (CES), paraoxonase (PON), and epoxide hydrolase (EPHX). Phase-II consists of conjugation reactions usually catalyzed by transferase enzymes, such as UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), glutathione S-transferases (GSTs), N-acetyltransferases (NATs), thiopurine methyltransferase (TPMT), catechol-O-methyl transferase (COMT). Phase-III is the uptake and excretion process of drugs and their metabolites through transporters. In a coordinated fashion to transport endogenous and exogenous substances into and out of cells, transporters can be divided into two groups: uptake and efflux transporters. Major drug transporters include solute carrier transporters (SLCs) and ATP-binding cassette transporters (ABCs). The function of enzymes and transporters involved in the three phases determines the duration and intensity of a drug's effect.

Factors influencing the expression and function of drug-metabolizing enzymes and transporters are critical to the metabolic process and therapeutic outcome of a drug. It is well known that genetic variations of enzymes and transporters can lead to altered drug response. Many genetic polymorphisms in genes encoding drug-metabolizing enzymes and transporters have been identified^{1,2}, which can be translated into clinical differences. However, the large interindividual variations in drug response can only be partly explained by genetic factors. For most cases, only 10%–30% of phenotype variations can be explained by genetic polymorphisms in genes encoding drug-metabolizing enzymes and transporters³. The genetic information can be differentially expressed in one individual over time and space, and even monozygotic twins do not always show the same phenotype, so epigenetic factors are now considered as an important part of the molecular control for gene expression⁴. In this review, the general concept of epigenetics will be briefly introduced, and its regulation on genes encoding proteins involved in drug metabolism and transport will be overviewed. We will also examine the epigenetic mechanisms as a regulatory network and discuss the implications of epigenetic research on pharmacotherapy.

2. Property of epigenetics

Epigenetics studies heritable changes in gene expression that are not caused by alterations in DNA sequences. Epigenetic regulation leads to relatively stable changes that are potentially affected by many other factors, such as age, diet, life style, disease and environment. Several mechanisms of epigenetic regulation have been extensively studied, including DNA methylation, histone modifications, and non-coding RNAs, each of which can change gene expression without altering the underlying DNA sequences.

2.1. DNA methylation

DNA methylation usually refers to the addition of a methyl group to the cytosine pyrimidine ring at the 5 position in a CpG

dinucleotide context. Status of DNA methylation on a CpG site is maintained by DNA methyltransferases (DNMTs) and demethylases and can be inherited through cell divisions. DNA methylation is necessary for proper gene regulation, chromosomal stability, and parental imprinting⁵. It plays an important role in long-term silencing of transcription and in heterochromatin formation. One way DNA methylation can silence transcription is that it directly alters chromatin structure and prohibits the binding of transcription factors or co-activators to their targeted sites containing this modification, and therefore decreases the gene expression. DNA methylation may also recruit methyl binding proteins (MBPs) that interact with co-repressors and lead to an inactive chromatin status^{6,7}.

2.2. Histone modifications

Histone proteins in the nucleosome, surrounded by 146 bp DNA, play a dominant role in the regulation of gene expression. Several covalent modifications on the N-termini of histone proteins have been discovered, many of which contain distinct regulatory functions and are transmissible through cell divisions⁸. Histone modifications can be divided into those that correlate with activation or repression of gene expression. Histone H3 acetylation is an important epigenetic modification regarding the ability for genes to be transcribed. Acetylation has the most potential to unfold chromatin, because it neutralizes the basic charge of the lysine residual and loosens the interaction between histone and DNA. Acetylation is generally associated with activation of transcription. Several histone acetyltransferases (HATs) have been identified as transcription co-activators. In contrast, histone deacetylation is generally associated with repression of transcription. Histone deacetylases (HDACs) have been identified as transcriptional co-repressors⁹.

Methylation of histone by methyltransferases can lead to different effects on gene transcription, depending on which residual is methylated and the level of methylation (mono-, di-, tri-methylation) occurs¹⁰. By analyzing the chromatin landmark and transcription initiation at most promoters in human ES cells, three classes of genes with various histone methylations have been identified¹¹. The majority of actively transcribed genes have H3K4me2/3-modified nucleosomes around the transcription start sites (TSS), and H3K36me3 along the gene-coding regions after TSSs. Certain genes do not have H3K4me2/3-modified nucleosomes around their TSS; some of them are enriched with H3K27me3 along the genes. A ChIP-on-chip study demonstrates that H3K27me3 forms broad local enrichments over silent genes and intergenic regions on gene-rich regions on mouse chromosomes¹². Vast data have confirmed that H3K4me3 and H3K4me2 are related to initiation of gene transcription, H3K36me3 is correlated to elongation of gene transcription, whereas H3K27me3 is associated with suppression of gene transcription^{10,13–18}. Although both H3K4me3 and H3K4me2 are associated with activation of transcription initiation, H3K4me3 is enriched more frequently around TSSs of genes, whereas H3K4me2 is found in a broader range of promoters, enhancers, and long-range regulatory elements. H3K4me2 sites also have a higher degree than H3K4me3 to associate with tissue-specific gene regulation¹³. Interestingly, the co-occurrence between histone acetylation of H3K9/H3K14 and methylation of H3K4 is very high for actively transcribed genes^{13,19}. One of the reasons may be that the complex of general transcription factors is selectively

anchored to nucleosomes with methylated H3K4, and binding of general transcription factors to methylated H3K4 is enhanced by coincident H3K9 and H3K14 acetylation²⁰. Methylation and demethylation of H3K4, H3K36, and H3K27 serve as a “multi-stable-switch” model of epigenetic memory to control gene expression in cellular differentiation and development²¹. With a widespread in chromatin, H3K9me2 is frequently associated with DNA methylation and is both a repressive mark in euchromatin and a hallmark of heterochromatin^{22–24}. H3K9me2 associated genes are enriched in many tightly-controlled signaling and cell-type specific pathways²⁵. Approximately 46% of mouse genome is covered by H3K9me2 in adult liver, and large H3K9me2-modified DNA regions are associated with tissue-specific gene silencing in differentiated tissues²⁶. There is little overlap between H3K9me2 and H3K27me3 in mouse liver²⁶, indicating a unique and essential role of H3K9me2 in epigenetic control of tissue-specific gene expression. In liver, H3K9me2 mediates gene repression by small heterodimer partner (SHP)^{27,28}, an orphan nuclear receptor that inhibits the transcriptional activities of many nuclear receptors^{29,30}. H3K4me2 and H3K9me2 inhibit each other *in vitro*³¹, and increase and decrease of H3K4me2 and H3K9me2 correlate with up- and down-regulation of genes by ethanol in hepatocytes³².

2.3. Non-coding RNAs (ncRNAs)

ncRNAs are functional RNA molecules that are not translated into proteins. More and more researches have focused on the regulatory function of ncRNAs. The best characterized ncRNAs are a group of small ncRNAs, the microRNAs (miRNAs), which regulate the expression of protein-coding genes. Matured miRNAs contain approximately 22 nucleotides, and they can base-pair with complementary sequences usually located in the 3' untranslated region (UTR) of the targeted mRNA molecules. This interaction either promotes destabilization and degradation of the mRNAs, or leads to decreased translational efficiency to achieve the gene silencing effect of miRNAs³³. Recent studies have also identified the role of miRNAs in cellular memory and epigenetics³⁴. Long non-coding RNAs (lncRNAs) are generally defined as non-protein coding transcripts with longer than 200 nucleotides. lncRNAs have been discovered to perform regulatory functions in various biological processes, including cell cycle, pluripotency, cell differentiation and development, due to its special property to interact with both DNAs and proteins^{35,36}. Although conceptually not epigenetic marks, ncRNAs are an integrated part of epigenetic regulation whereby relevant ncRNAs contribute to the final transcriptional outcome of protein-coding genes without genetic alterations³⁷.

3. Epigenetic regulation of genes involved in drug metabolism and transport

Several full lists of drug metabolism and transport genes under epigenetic regulation have been summarized^{37–39}. This review is not attempting to make a comprehensive coverage on this topic, but rather a personal selection of epigenetic interplay with drug metabolism that is exciting and promising for the future.

DNA methylation is perhaps the best known epigenetic process, and up to date approximately 90% of identified epigenetic regulation in drug-metabolizing genes involve DNA methylation. One of the major reasons is that aberrant DNA methylation is a hallmark of cancer. The disease of cancer has been intensively

studied, and changes of drug-metabolizing gene expression associated with altered DNA methylation in various tumor cells have been well identified. Examples of drug-metabolizing genes regulated by cancer-related DNA methylations occur in all three phases of drug metabolism, including phase-I enzymes, such as CYP1A1⁴⁰ and CYP7B1⁴¹, phase-II enzymes, such as SULT1A1⁴² and NAT1⁴³, and phase-III transporters, such as ABCC6⁴⁴ and SLC22A3⁴⁵. Some genes display reduced expression and are hypermethylated at promoter regions in different tumor cells, like *ALDH1A2*⁴⁶ and *CYP24*⁴⁷. On the other hand, several transporters showed increased expression that correlates promoter hypomethylation in certain tumor cells, like *SLC19A1*⁴⁸ and *ABCG2*⁴⁹, leading to drug resistance phenotype of cancers. Besides promoter regions, cancer-related changes in enhancer methylation have been shown to alter the induction of drug-metabolizing genes. For example, enhancer hypermethylation suppresses CYP1A1 dioxin responsiveness in prostate cancer⁵⁰.

In addition to cancer, many other situations can lead to altered DNA methylations that regulate drug-metabolizing gene expression. Parkinson's disease may be associated with epigenetic modifications, and decreased DNA methylation in the gene region was linked to increased expression of CYP2E1 in the brains of Parkinson's disease patients⁵¹. Differences in life style may affect epigenetics. For instance, tobacco smoking was associated with hypomethylation of *CYP1A1* and may be involved in the induction of CYP1A1 in the lung⁵². Differences in diet may also regulate drug-metabolizing genes through DNA methylation. Prenatal famine has been linked to timing- and sex-specific alteration of DNA methylations in *ABCA1* gene⁵³. Moreover, DNA methylation is implicated in tissue specific gene expression. For example, the promoter CpG methylation status of *CYP1A2* was strongly associated with *CYP1A2* mRNA expression in different human cell lines and tissues¹². The CpG-rich region near the *UGT1A1* promoter was hypermethylated in the kidney, and *UGT1A1* expression was defective in human kidney⁵⁴. The variable level of *CYP2E1* RNA in full-term placenta was associated with a DNA methylation pattern distinct from other tissues⁵⁵.

Although DNA methylation is the most common epigenetic mechanism of mammalian genome regulation, it is only one component of a broad epigenetic program that includes other modifications of the chromatin. Histone acetylation is an epigenetic modification that shows close crosstalk with DNA methylation. The methyl-CpG domain binding protein MeCP2 and MBD2 were found to be associated with HDAC⁵, and increased histone acetylation by HDAC inhibitor induced global DNA demethylation in human cancer cells⁵⁶. Therefore, DNA methylation controlled drug-metabolizing gene expressions are commonly coupled with changes in histone acetylation⁵⁷. For example, both histone hyperacetylation and DNA hypomethylation contributed to the tissue specific expression of *UGT1A1* in the liver and intestine⁵⁴; inhibitor treatment to decrease histone deacetylation and DNA methylation induced *CYP1* family RNA expression in MCF-7 and HeLa cells⁵⁸; the suppression of CYP27B1 by the ligand-activated vitamin D receptor was mediated through dual epigenetic modifications—DNA methylation and recruitment of histone deacetylase⁵⁹. With the help of histone acetylation modulating molecules, typically histone deacetylase inhibitors, histone acetylation is also demonstrated to be involved in regulation of drug-metabolizing genes in all three phases, examples of which include phase-I CYP3A4⁶⁰, phase-II UGT2B7⁶¹, and phase-III SLC22A2⁶².

Histone methylation is another important epigenetic mechanism in regulation of drug-metabolizing genes. In some cases, histone

methylations work in combination with other epigenetic modifications. The repression of *CYP24A1* gene expression in human prostate cancer cells was mediated by increased promoter DNA methylation and repressive histone mark H3K9me₂, together with decreased histone H3K9ac and H3K4me₂ modification⁶³. In other cases, histone modifications can work independently to silence gene transcription in the absence of DNA methylation. The knockdown of retinoic acid receptors in embryo carcinoma cell lines led to *CYP26A1* repression through increased level of repressive histone mark H3K9 methylation and hypoacetylation of histone H4 without changes in DNA methylation⁶⁴.

Histone modifications play a role in drug-metabolizing gene regulation in response to nuclear receptor ligand treatment. The induction of *CYP1A1* through aryl hydrocarbon receptor activation involved increased active histone mark of H3K4me₃ at the gene promoter and increased histone acetylation at both the promoter and enhancer region⁶⁵. Histone methylation may also function in developmental regulation of drug-metabolizing gene expression. The increases of *CYP3A16* expression in neonatal mouse livers and *CYP3A11* in adult livers were associated with increase of active histone mark H3K4me₂, while the suppression of *CYP3A16* expression in adult livers coincided with the decrease of H3K4me₂ and increase of inactive mark H3K27me₃ around *Cyp3a16*⁶⁶. Neonatal activation of the nuclear receptor CAR by a CAR-specific ligand (1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, TCPOBOP) can result in alteration of H3K4me₃ and H3K9me₃ levels around the CAR targeted drug-metabolizing genes, *Cyp2b10* and *Cyp2c37*, which further results in an induced expression level of these P450s throughout the life of exposed mice⁶⁷.

Different from the above epigenetic mechanisms, miRNAs affect drug-metabolizing gene expression at the post-transcriptional stage rather than transcriptional regulation. Similar to DNA methylations, alterations of miRNAs are common in cancer. And many of them were initially studied in the regulation of key cancer-related pathways, such as cell cycle control and the DNA damage response⁶⁸. The first study that demonstrated miRNAs in regulation of essential genes in physiology and drug metabolism was on miR-27b and *CYP1B1* in cancerous tissues, which identified a significant inverse association between the expression levels of miR-27b and *CYP1B1* protein⁶⁹. Following this, many other miRNAs were also found in regulating drug-metabolizing genes. The *CYP2E1* mRNA and protein levels showed no positive correlation in human liver samples, suggesting post-transcriptional regulation. The miR-378 was reported to potentially recognize the 3'-UTR of *CYP2E1* mRNA by *in silico* analysis, and its level was inversely correlated with the *CYP2E1* protein level and translational efficiency of *CYP2E1*, which provided insight into the unsolved post-transcriptional regulatory mechanism⁷⁰. Another special property of miRNA differing from other *cis*-regulating epigenetic marks is that it can perform the regulatory function in *trans* and therefore interact with multiple targets. The human miR-27b not only directly targeted the 3'-UTR of *CYP3A4* to down-regulate *CYP3A4* level through post-transcriptional mechanism, but also targeted the 3'-UTR of vitamin D receptor, which indirectly control *CYP3A4* expression through transcriptional regulation⁷¹. In addition to the above phase-I genes, miRNAs were also identified in regulation of phase-II enzymes and transporters. For example, the *UGT1A* gene family was regulated by miR-491-3p, which binds to the shared *UGT1A* 3'-UTR common to all *UGT1A* enzymes. And altered miRNA levels showed an inverse correlation with *UGT1A* mRNA level and enzyme activity both *in vitro* and *in vivo*⁷². Transporter

ABCG2 protein expression could be affected by multiple miRNAs, including miR-328, -519c, and -520h, probably through accelerated mRNA degradation mechanism⁷³.

Evidences of epigenetic regulation of expression involving lncRNAs have been discovered in many genes. Some well-known cases include the lncRNA *Xist* mediated X-chromosome inactivation and *Air* mediated *Igf2r* gene imprinting⁷⁴. However, findings about drug-metabolizing gene regulation are quite limited. Transporters *Slc22a2* and *Slc22a3* are located in the *Igf2r* gene cluster, and thus are also silenced by *Air* during imprinting. One example of lncRNA regulating drug-metabolizing gene in human is related to the short-chain dehydrogenase/reductase family member 4 (*DHRS4*) gene cluster⁷⁵. The lncRNA AS1DHRS4, a natural antisense transcript of the *DHRS4* gene, could either recruit DNA methyltransferases to induce DNA methylation or interact with H3K9 and H3K27 methyltransferases to maintain epigenetic silencing in the *DHRS4* gene cluster. Most chromatin modifying enzymes do not have the capacity to bind DNA, and lncRNAs usually bridge the gap and precisely target proteins to DNA.

4. Epigenetic network

Epigenetic mechanism has been universally accepted as a critical factor in gene regulation, and its general role can be applied to almost every genes in the genome, including the field of drug metabolism, as reflected by the numerous examples summarized above. However, most of the current studies have only identified the involvement of certain epigenetic elements in gene regulation, revealing the association of altered epigenetic modifications with changes of gene expression, yet a bigger scenario of how these epigenetic modifications occur is not well understood. The universal role of epigenetic mechanism involved in gene regulation strongly indicates that epigenetics is an executor rather than an initiator in control of gene expression. Epigenetic regulations are not proactive, but responsive. They commonly happen in response to changes of environment, diet, physiological or pathological conditions. The inheritance of epigenetics simply memorizes what has been set up by other cellular mechanisms or pathways. Moreover, the known examples also suggest that there are interactions between different epigenetic modifications, either mutually reinforced or inhibitory. Thus, understanding the cellular regulatory network that depicted the epigenetic modifications would be more rewarding in the study of gene regulation. We have recognized the association of different epigenetic modifications with gene expression, but the triggers for the differential marking of epigenetic modifications throughout the genome is largely unclear. Epigenetic modifications are like the "code" that control gene expression levels, yet from a network view, there must be "pens" that write down the "code" and "writers" that hold the "pens". For instance, Pozzi et al.⁶⁴ demonstrated that retinoic acid (RA) induced epigenetic activation of *CYP26A1* required the activity of *RARβ2*, which was further activated by functional *RARα* in response to RA. Future researches investigating the coordinated network involving chains of signaling upstream of the epigenetic codes are essential to unveil the mystery of gene regulation.

Examining the epigenetic regulation as a network has another important implication. Various epigenetic enzymes have been known to modify the epigenetic signature of genes, such as DNMT, HAT, and HDAC. A number of drugs targeting these enzymes are under development for treatment of certain diseases,

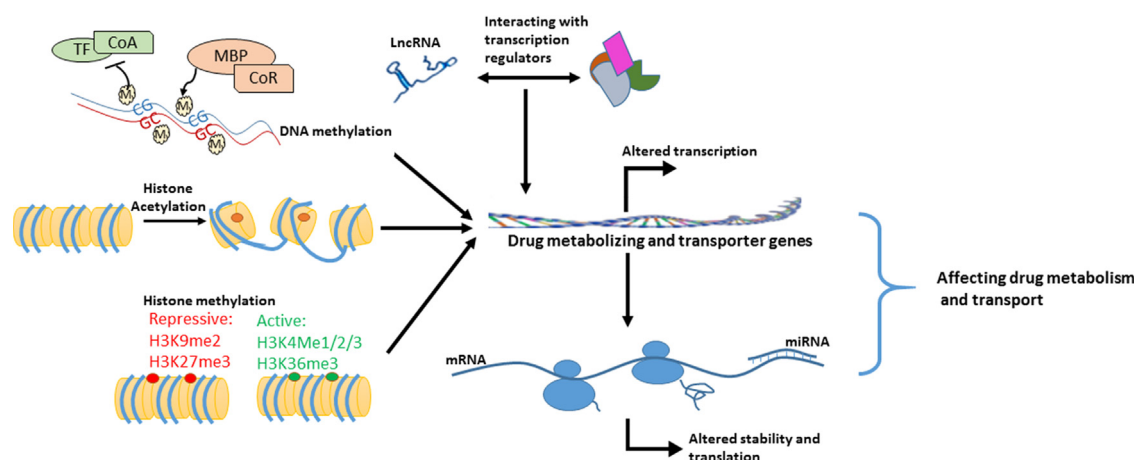


Figure 1 Schematic illustration of epigenetic regulation on drug metabolism and transport. DNA methylation at CpG sites may alter chromatin structure and prohibit the binding of transcription factors (TF) or co-activators (CoA) and therefore decrease the gene expression. DNA methylation may also recruit methyl binding proteins (MBP) that interact with co-repressors (CoR) and lead to decreased gene expression. Histone acetylation has the potential to unfold chromatin and may activate gene expression. Histone methylations can be divided into gene-activating and gene-repressive histone marks. LncRNAs may interact with epigenetic and basic transcription machinery to alter gene transcription. microRNAs can decrease the mRNA stability or inhibit translation through binding with mRNAs. All of these epigenetic factors targeting drug-metabolizing enzyme genes and transporter genes may lead to altered drug metabolism and transport.

because epigenetic changes are involved in disease progression. But most of the epigenetic drugs have strong toxic effects due to lack of specificity. The modifying enzymes modulate epigenetic signatures all over the genome, yet most disease-related epigenetic changes only occur locally. So improved understanding of the epigenetic regulatory network may suggest new drug targets with better specificity and provide novel insight into drug discovery and development. Furthermore, altered drug-metabolizing gene expressions are often linked to drug efficacy and safety issues. The study of epigenetic regulatory pathways in drug-metabolizing enzymes and transporters may facilitate the understanding of drug-drug interactions and multi-drug resistance in clinical pharmacotherapy.

5. Clinical significance of epigenetic mechanisms in regulation of drug metabolism

It has been clearly demonstrated that epigenetic mechanisms contribute to the differential expression of drug-metabolizing genes in various situations. One important thing to infer from this is that we should be cautious about the epigenetic property of drugs, which may affect drug-metabolizing gene expression and drug disposition. Certain compounds with epigenetic modulating capacities are helpful in research, such as DNMT inhibitor 5-aza-2'-deoxycytidine to decrease DNA methylation and HDAC inhibitor trichostatin A to increase histone acetylation⁷⁶. Treatment experiments with these compounds have aided in the identification and validation of epigenetic regulation in a large number of drug-metabolizing genes. Yet some clinically used drugs may also have the property to alter epigenetic signatures of drug-metabolizing genes, and lead to unexpected changes in drug metabolism and toxicity. For example, the anticonvulsant and mood-stabilizing drug valproic acid has been discovered as histone deacetylase inhibitor with potent antitumor activity⁷⁷. So potential changes in the expression of drug-metabolizing genes under epigenetic regulation may also need to be taken into consideration when this kind of epigenetic drug is administered.

6. Conclusions

In summary, epigenetic regulation of drug-metabolizing genes and transporters is a critical factor in control of drug metabolism and transport as schematically illustrated in Fig. 1. Epigenetic mechanism is an important source of interindividual variability in drug metabolism and transport. Combining the current understanding with further studies about the network of epigenetic regulations in drug metabolism may help to improve the safety and effectiveness of drug therapy.

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