Acta Pharmaceutica Sinica B 2012;2(2):146–158

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

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ORIGINAL ARTICLE

Epigenetic regulation of developmental expression of *Cyp2d* genes in mouse liver

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Received 28 November 2011; revised 26 December 2011; accepted 10 January 2012

KEY WORDS

Cyp2d;
DNA methylation;
Histone methylation;
Liver development

Abstract CYP2D6 expression in liver is age-dependent. Because epigenetic mechanisms, such as DNA methylation and histone modifications, modulate age-related gene expression during development, and are highly conserved among species, the current study examined the epigenetic regulation of age-related expression of the *Cyp2d* genes in mouse liver. DNA methylation (DNAme), histone 3 lysine 4 dimethylation (H3K4me2), and histone 3 lysine 27 trimethylation (H3K27me3) was established by ChIP-on-chip tiling microarrays from mouse livers at prenatal, neonatal, and adult stages. Levels of DNAme, H3K4me2, and H3K27me3 were analyzed in a genomic region containing the *Cyp2d* clustering genes and their surrounding genes. Gradually increased expression levels of the *Cyp2d9*, *Cyp2d10*, *Cyp2d22*, and *Cyp2d26* genes from prenatal, through neonatal, to adult are associated with gradually increased levels of H3K4me2 in the nucleosomes associated with these genes. Gene expression patterns during liver development in several *Cyp2d* surrounding genes, such as *Srebf2*, *Sept3*, *Ndufa6*, *Tcf2*, *Nfam1*, and *Cyb5r3*, could be also explained by changes of DNA methylation, H3K4me2, or H3K27me3 in those genes. In conclusion, the current study demonstrates that the changes of DNA methylation and histone modifications are associated with age-related expression patterns of the *Cyp2d* genes and their surrounding genes in liver cells during development.

Abbreviations: Cyp2d, cytochrome P450 2d; DNAme, DNA methylation; H3K4me2, histone 3 lysine 4 dimethylation; H3K27me3, histone 3 lysine 27 trimethylation

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
doi:10.1016/j.apsb.2012.01.001

Production and hosting by Elsevier

1. Introduction

Cytochrome P450 2D6 (CYP2D6) is an important drug metabolizing enzyme, responsible for metabolizing 10–20% of current prescribed drugs[1]. Significant interindividual variation in CYP2D6-mediated drug metabolism exists in the general population[2,3]. The varied phenotypes can be partially explained by functional genetic polymorphisms in the *CYP2D6* gene[4–6]. In addition to the genetic factors, age-dependent developmental changes in CYP2D6 expression in human livers are other important determinants of interindividual variability in CYP2D6-mediated drug metabolism[7,8]. The developmental changes in human liver CYP2D6 expression are not dependant on genetic polymorphisms or environmental factors, but rely on mechanisms controlling ontogenic expression of CYP2D6 in liver cells during development; however, the mechanisms involved in regulating this event remains unclear.

Changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence are defined as epigenetics. It is desirable to examine whether epigenetic mechanisms are involved in controlling the age-dependent developmental changes of CYP2D6 expression in liver development. However, there are several limitations for such studies using human liver samples. First, variation in CYP2D6 expression in human livers at various ages is controlled not only by age, but also by genetic polymorphisms, environmental factors, drugs, and physiological conditions. These factors are very difficult to control when human liver samples are used in a study. Second, moral, ethical and technical limitations for studying human fetal and neonatal samples have precluded an in-depth understanding of the epigenetic mechanisms controlling CYP2D6 expression. Therefore, it would be advantageous to have a laboratory animal model that parallels the developmental patterns in humans where age, genetic background, diet and environment could be controlled.

In this respect, mouse is an ideal model. First, many epigenetic mechanisms are conserved between mice and humans, and these epigenetic mechanisms have similar roles in programming gene expression in mice and humans[9,10]. Second, organization of the mouse *Cyp2d* gene cluster is similar to the human *CYP2D* gene cluster (Fig. 1). The mouse *Cyp2d* gene cluster contains eight *Cyp2d* coding and pseudo-genes, including *Cyp2d9*, *Cyp2d10*, *Cyp2d12*, *Cyp2d13*, *Cyp2d22*, *Cyp2d26*, *Cyp2d34* and *Cyp2d40* on mouse chromosome 15. The comparable human *CYP2D* gene cluster contains one coding gene, *CYP2D6*, and two pseudo-genes, *CYP2D7P1* and *CYP2D8P1*, on human chromosome 22. Both gene clusters are in synteny with the same surrounding genes in the same order, including *Ndufa6*, *Sept3*, and *Srebf2* at the 5′-region and *Tcf20*, *Nfam1*, and *Cyb5r3* at the 3′-region. Third, the expression patterns of some mouse *Cyp2d* genes, such as *Cyp2d9*, *Cyp2d10*, *Cyp2d22* and *Cyp2d26*[11,12] are similar to human *CYP2D6* gene with gradually increased expression levels from prenatal, through neonatal, to young adults[8]. Fourth, mice have been used recently to study epigenetic regulation of membrane transporters in different tissues[13] and developmental changes of *Cyp3a* gene expression in liver[14].

DNA methylation and histone modifications are the two most extensively studied epigenetic mechanisms involving in the regulation of gene expression. DNA methylation is essential for normal development[15], and it regulates gene transcription either by directly preventing the binding of transcription factors to their DNA binding sites in gene promoters, or by indirectly interfering in the recruitment of co-repressors by methyl-CpG binding proteins[16]. Histone modifications are post-translational modifications of histone N-terminal tails at various positions, which can alter their interaction with DNA. The interactions between DNA and modified histones can influence gene transcription. More than 50 different types of histone

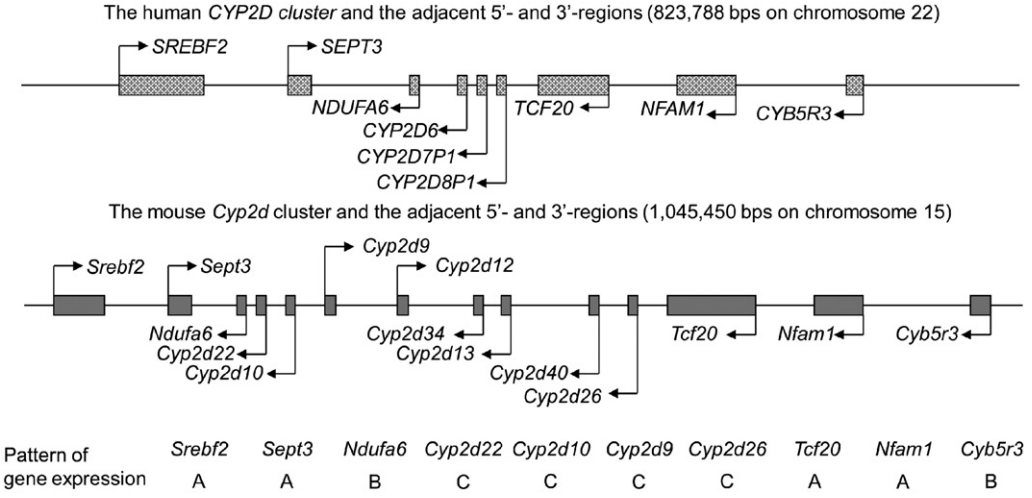

Figure 1 Comparison of genomic organization between the mouse *Cyp2d* cluster and the human *CYP2D* cluster and their adjacent 5″- and 3″-regions. Based on the UCSC Genome Browser Mouse February 2006 and human March 2006 Assemblies, two syntenic regions are located at mouse chromosome 15 from 81,964,524 to 83,009,974 and human chromosome 22 from 40,549,052 to 41,372,840. *Srebf2*, sterol regulatory element binding factor 2; *Sept3*, neuronal-specific septin-3; *Ndufa6*, NADH dehydrogenase (ubiquinone) 1 alpha; *Tcf20*, transcription factor 20; *Nfam1*, nuclear factor of activated T-cells activation molecule 1; *Cyb5r3*, NADH-cytochrome b5 reductase 3. Age-related gene expression of the *Cyp2d* genes and their surrounding genes was based on the microarray data generated by Li et al.[11], which are stored at GEO database with the accession number GSE13149 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE13149). The patterns are defined as: Pattern A for consistently low levels at any age; Pattern B for consistently high levels at any age; Pattern C for gradually increased levels from prenatal, through neonatal, to adult.