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Regular Article

Developmental regulation of CYP3A4 and CYP3A7 in Chinese Han population

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ABSTRACT

CYP3A4 and CYP3A7 are generally served as the major adult and fetal liver forms, respectively, and exhibited a developmental switch during liver maturation. The objective of this study was to explore the potential mechanisms associated with the developmental switch of CYP3A4 and CYP3A7 in the Chinese Han population. We analyzed CYP3A4/7, nuclear receptors, and epigenetic modifications in human liver samples. We found that the expression levels of CYP3A4 mRNA in adults were significantly higher than the levels in fetus. In contrast, CYP3A7 mRNA expression reached a maximal level at an estimated gestational age of 25 weeks and then substantially decreased during the first year after birth. We also found that the expression level of hepatocyte nuclear factor 4 alpha (HNF4A) was most associated with CYP3A4 expression in adult liver; whereas the expression level of glucocorticoid receptor (GR) was intensively correlated with CYP3A7 expression in fetal liver. Furthermore, we illustrated the dynamic changes of H3K4me2 and H3K27me3 in the developmental switch of CYP3A7 and CYP3A4. In summary, our data suggested that HNF4A and GR, and epigenetic changes of H3K4me2 and H3K27me3 are associated with the ontogenic expressions of CYP3A4/3A7 in the livers of the Chinese Han population.

1. Introduction

The human cytochromes P450 3A (CYP3A), the most ample human drug metabolizing enzymes, contribute to the metabolism of more than 50% of all marketed drugs [1]. The activities of these enzymes are known to show a significant variability not only among different individuals but also within the same individual at different time-points. The expression patterns of CYP3A forms are subjected to developmental influence [2]. A shift in the expression from CYP3A7 to CYP3A4 was first observed in 1997 [3]. In most individuals, the shift of expression from CYP3A7 to CYP3A4 occurs within the first month after birth. CYP3A7 activity is high before birth, with a rapid decrease after birth, while CYP3A4 activity is low at birth with a sharp increase in the neonatal period in order to reach adult levels at one year of age. Therefore, CYP3A7 predominates in fetuses, whereas CYP3A4 accounts for the main CYP3A enzyme in adults [4–6].

A variety of factors are involved in the developmental regulation of CYP3A4 and CYP3A7. The interindividual variability of CYP3A4/7 expression levels could be partly attributed to genetic polymorphisms. CYP3A7*1C, a polymorphic variant of the CYP3A7 promoter, causes a CYP3A7 expression surge in some adults. Due to the mutation of CYP3A7*1C allele, CYP3A7 promoter was replaced by the proximal ER6 motif of CYP3A4 containing the element of pregnane X receptor (PXR) and constitutive androstane receptor (CAR) [7]. In addition, the changes in expression of transcription factors during development, in particular, hepatocyte nuclear factors 4A (HNF4A), CCAAT/enhancer-binding protein A, B(C/EBPA, C/EBPB), and PXR can play a vital role on CYP3A4 and CYP3A7 development expression [8,9]. Furthermore, a hepatocyte nuclear

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factor (HNF) 3γ-containing complex from prenatal liver regulated CYP3A7 basal expression by interacting with CYP3A7 (−242/−219) [10,11].

DNA methylation and histone modifications are two vital reversible epigenetic modifications [12]. DNA methylation affects gene transcription during development and may play a major role in the developmental switch of CYP3A7 and CYP3A4. The data denoted that dynamic DNA methylation elements in CYP3A4 promoter regions (mainly −1547, −1521 and −1452) were likely correlated with the interindividual difference of CYP3A4 and may have potentially contributed to the hepatic developmental shift in its expression [13]. This developmental switch involved in changes in histone modification patterns also exhibited in mice in which Cyp3a16 was expressed in neonatal age while Cyp3a11 was expressed in adults. High expression levels in Cyp3a16 in the neonatal liver and Cyp3a11 in the adult liver were correlated with an increase of demethylated lysine 4 of histone H3 (H3K4me2), and a decrease of trimethylation of lysine 27 in the same histone (H3K27me3) around Cyp3a16 and Cyp3a11 [14]. Less is known about the mechanisms controlling the developmental switch during liver maturation. On the basis of the hypothesis that histone modification patterns may play a major role in the developmental switch of CYP3A7 and CYP3A4, we performed this study.

The objective of this study was to explore the potential mechanisms that are associated with the developmental switch of CYP3A4 and CYP3A7 in the Chinese Han population. We analyzed CYP3A4/7, nuclear receptors, and epigenetic modifications in human liver samples from an estimated gestational age (EGA) of 13 weeks (wks) to a postnatal age (PNA) of 72 years (yr). Our data suggested that nuclear receptors HNF4A and GR, and epigenetic changes of H3K4me2 and H3K27me3 are associated with the ontogenic expressions of CYP3A4/3A7 in the livers of the Chinese Han population.

2. Materials and methods

2.1. Human liver specimens

This study was approved by the Medical Ethical Committee of First Affiliated Hospital of Zhengzhou University, and written consent was obtained from each patient. The investigation was carried out with a developmental tissue bank with 53 fetal samples and 97 postnatal samples. The detailed information is shown in Supplemental Tables 1–5. Human fetal tissues were obtained from legally aborted fetuses of gestational age determined by ultrasonic measurement of the diameter of the skull and ranged from 13 to 38 weeks. All the donors were screened serologically for hepatitis B and C and human immunodeficiency virus (HIV) 1 and 2. The postnatal liver tissue specimens were promptly removed at the time of surgery. A part of each tissue sample was reserved for histopathological examination and the remainder was rapidly frozen in liquid nitrogen and stored at −80 °C.

2.2. Isolation of RNA and RT-qPCR analysis

Total RNA was extracted from human fetal, postnatal liver samples with tripure reagent (Roche, Germany) according to the manufacturer's instructions. The concentration and purity of total RNA were confirmed by a 260:280 nm absorbance ratio greater than 1.9–2.1, and agarose gel electrophoresis with ethidium bromide staining was used to confirm the integrity of 18S and 28S ribosomal RNA bands.

RNAs were reverse-transcribed into cDNA using a Primer Script RT reagent kit with gDNA Eraser (TAKARA, Japan) according to the manufacturer's instructions. Real-time quantitative PCR for expression levels of CYP3A4, CYP3A7, and nuclear receptors was performed by the fluorescent dye SYBR Green methodology using the SYBR Premix Ex TaqTM (TAKARA, Japan) and the ABI 7500 fast (Applied Biosystems). Primer pairs for each transcript is shown in Supplemental Table 6 starting with conditions consisting of 95 °C for 30 s followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s. The increase in fluorescence emission (Rn) was measured during the course of PCR amplification, and the difference (ΔRn) between the fluorescence emission of the product and the baseline was calculated by the Sequence Detection System software (Applied Biosystems) and plotted versus the cycle number. Furthermore, the amplification curves were read with the ABI Prism 7500 SDS software using the comparative cycle threshold method. The dissociation curve was performed after the PCR to verify the specificity of the amplification. The relative quantification of the steady state mRNA levels was calculated after normalization of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Relative quantification analysis was performed using the comparative CT ($2^{-\Delta\Delta CT}$) method.

2.3. Preparation protein samples and western blot analysis

One milliliter of liver homogenate was prepared in RIPA (Beyotime Biotechnology, Shang hai,China) with protease inhibitor cocktail (Roche, Germany)and PMSF(Sigma, USA) at 4 °C and dissociated on ice for 30 min. The samples were vortexed and centrifuged at 12,000 g for 10 min at 4 °C, and the supernatant was removed, homogenized and concentrations were determined using a BCA protein assay kit (Beyotime Biotechnology, Shanghai, China) then boiled for 5 min at 100 °C. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membrane. The membranes were blocked for 1.5 h in 5% nonfat dry milk in TBS. CYP3A4/3A7, PXR, HNF4A and GR were detected with primary antibodies of CYP3A4 rabbit polyclonal antibodies with a dilution of 1:20,000 (ab124921, Abcam, Cambridge, MA), CYP3A7 rabbit polyclonal antibodies with a dilution of 1:5000 (ab151701, Abcam, Cambridge, MA), PXR mouse polyclonal antibodies with a dilution of 1:1000 (ab 41930, Abcam, Cambridge, MA), HNF4A mouse polyclonal antibodies with a dilution of 1:1000 (ab 41898, Abcam, Cambridge, MA), GR rabbit polyclonal antibodies with a dilution of 1:50,000 (ab109022, Abcam, Cambridge, MA) respectively and detected with a chemiluminescent agent (Millipore, USA) after incubating with the corresponding secondary antibodies with a dilution 1:5000 of coupled to horseradish peroxidase (HRP) (SA00001-1, SA00001-2, proteintech, Chicago, USA). Samples were normalized to GAPDH, which was detected with Mouse monoclonal antibody with a dilution of 1:10,000 (60004-1-Ig, proteintech, Chicago, USA). Reaction intensity was determined by computer assisted densitometry (Proteinsimple, California, USA).

2.4. Histone modification

ChIP was performed using a Chromatin Immunoprecipitation Assay Kit (Millipore, Billerica, MA). In brief, frozen liver tissues homogenate were fixed with 1% formaldehyde in phosphate buffered saline for 15 min at room temperature and quenched with 0.125 M glycine for another 5 min and were lysed in the SDS lysis buffer on ice for 10 min. Then DNA was sonicated to shear to lengths between 200 and 1000 base pairs (checked by agarose gel electrophoresis/ethidium bromide staining). Methylated histone H3 proteins at lysine 4 and 27 were immunoprecipitated with monoclonal antibodies (Millipore 17–677) for H3K4me2 and with polyclonal antibodies (Millipore 17–622) for H3K27me3 (Millipore, Billerica, MA) and followed by adding 60 μl of Protein A Agarose/Salmon Sperm for one hour at 4 °C with rotation to collect the