Acta Pharmaceutica Sinica B 2014;4(6):464–469

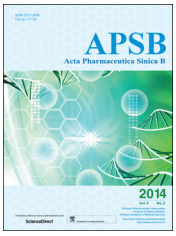
HOSTED BY

ELSEVIER

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

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



SHORT COMMUNICATION

Preferential expression of cytochrome CYP CYP2R1 but not CYP1B1 in human cord blood hematopoietic stem and progenitor cells

Shuoqi Xu[a], Zhihua Ren[b], Yanan Wang[c,*,†], Xinxin Ding[a,*,†], Yongping Jiang[a,b,*,†]

[a]*Biopharmaceutical R&D Center, Chinese Academy of Medical Sciences & Peking Union Medical College, Suzhou 215126, China*
[b]*Biopharmagen Corp., Suzhou 215126, China*
[c]*Department of Clinical Laboratory, Suzhou Municipal Affiliated Hospital of Nanjing Medical University, Suzhou 215002, China*

Received 18 August 2014; revised 29 September 2014; accepted 14 October 2014

KEY WORDS

Cytochrome P450;
CYP2R1;
CYP1B1;
CD34+;
Hematopoietic stem cells;
Gene expression

Abstract Cytochrome P450 (CYP) enzymes metabolize numerous endogenous substrates, such as retinoids, androgens, estrogens and vitamin D, that can modulate important cellular processes, including proliferation, differentiation and apoptosis. The aim of this study is to characterize the expression of CYP genes in CD34+ human cord blood hematopoietic stem and early progenitor cells (CBHSPCs) as a first step toward assessment of the potential biological functions of CYP enzymes in regulating the expansion or differentiation of these cells. CD34+ CBHSPCs were purified from umbilical cord blood *via* antibody affinity chromatography. Purity of CD34+ CBHSPCs was assessed using fluorescence-activated cell sorting. RNA was isolated from purified CD34+ CBHSPCs and total mononuclear cells (MNCs) for RNA-PCR analysis of CYP expression. Fourteen human CYPs were detected in the initial screening with qualitative RT-PCR in CD34+ CBHSPCs. Further quantitative RNA-PCR analysis of the detected CYP transcripts yielded evidence for preferential expression of CYP2R1 in CD34+ CBHSPCs relative to MNCs; and for greater expression of CYP1B1 in MNCs relative to CD34+ CBHSPCs. These findings provide the basis for further studies on possible functions of CYP2R1 and CYP1B1 in CBHSPCs'

Abbreviations: bp, base pair; Ct, threshold cycle; CBHSPCs, cord blood HSPCs; FACS, fluorescence-activated cell sorting; HSPCs, hematopoietic stem and early progenitor cells; kbp, kilobase pair; MNCs, mononuclear cells; OD, optical density; CYP, cytochrome P450; PCR, polymerase chain reaction; PE, R-phycoerythrin; RT, reverse transcription

*Corresponding authors. Tel./fax: +86 512 62831269.
E-mail addresses: wangyn1980@163.com (Yanan Wang), xding001@gmail.com (Xinxin Ding), yjiang@biopharmagen.com (Yongping Jiang).
†These authors contributed equally to this work.

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2014.10.003

proliferation and/or differentiation and their potential utility as targets for drugs designed to modulate CD34+ CBHSPC expansion or differentiation.

1. Introduction

Cytochrome P450 (CYP) enzymes play important roles in the biotransformation of numerous endogenous compounds as well as drugs and chemical carcinogens[1,2]; in humans, 57 functional or potentially functional CYP genes have been identified[3]. CYP genes show varying degrees of tissue selective expression with some expressing widely in many tissues, whereas others showing stringent tissue specificity. The collection of CYP enzymes expressed in a given tissue can have a significant impact on the metabolome and biological function of that tissue.

The aim of this study is to identify CYP genes selectively expressed in human hematopoietic stem and early progenitor cells (HSPCs). In contrast to the large amounts of data available regarding CYP expression, regulation and function in the liver and other major extrahepatic organs such as lung and intestine[4], relatively little is known about the expression and function of CYP genes in stem or progenitor cells. We speculate that the HSPCs may suppress the expression of CYP genes that support differentiation, while enhancing the expression of CYP genes that are important for the maintenance of the pluripotency. CYP enzymes may modulate these biological functions through metabolism of various endogenous substrates, such as retinoids, androgens, estrogens, vitamin D, and bile acids. Therefore, it is worthwhile to identify the CYP genes that are preferentially expressed in HSPCs, as a first step toward characterization of their possible biological functions in these important cells and their potential utility as targets for drugs that are designed to modulate HSPC expansion or differentiation.

Several studies have reported detection of CYP expression in HSPCs. CYP1B1 and CYP2E1 (mRNA and possibly also protein), but not CYP2C9 or CYP3A4, were detected in human CD34+ hematopoietic stem and progenitor cells (>96% pure) isolated from peripheral blood[5,6]. CYP1B1 mRNA was also detected in human cord blood-derived CD34+ cells, and its levels were suppressed by treatment with SR1, an agent that increased *ex vivo* expansion of these cells[7]. In a more recent study, CYP1A1, 2B6, 2E1 and 3A4 were apparently detected in cord blood-derived CD34+ cells (80–85% pure) on day 0 of differentiation and they showed time-dependent increases in expression during differentiation into neuron-like cells, although it was not clear whether the basal expression on day 0 of differentiation was in the stem cells or in the contaminating mononuclear cells[8]. However, none of these studies determined whether the detected CYPs were preferentially expressed in the CD34+ cells, and the expression of the other human CYPs in HSPCs has not been examined.

In this study, we first performed a global analysis, using qualitative RNA-PCR, of all 57 human CYP genes for their possible expression in CD34+ human cord blood HSPCs (CBHSPCs). Following sequence validation of the detected CYP transcripts in CD34+ CBHSPCs, we further compared their expression levels to those in total mononuclear cells from the same donors using quantitative RNA-PCR. Our results indicate that CYP2R1 is preferentially expressed in CD34+ CBHSPCs relative to MNCs, whereas CYP1B1 is more abundantly expressed in MNCs than in isolated CD34+ CBHSPCs.

2. Materials and methods

2.1. Materials

CD34 MicroBead kit, magnetically assisted chemical separation (MACS) unit, human CD34-PE antibody and mouse IgG2a-PE antibody were purchased from Miltenyi Biotec (Shanghai, China). Ficoll solution was purchased from Huajing Bioproduct (Shanghai, China). Trizol reagent was supplied by Invitrogen (Carlsbad, CA, USA). Deoxy-ribonucleoside triphosphate (dATP, dCTP, dGTP and dTTP) for polymerase chain reaction (PCR), Taq polymerase, $MgCl_2$ and molecular weight standards for electrophoresis (DL500) were purchased from Sangon Biotech (Shanghai, China). Ultrapure agarose was supplied by Life Technologies (Paisley, UK). Oligonucleotide primers were synthesized by BioSune (Shanghai, China). RNase-free DNase I, PrimeScript II High fidelity RT-PCR kit and agarose gel DNA extraction kit were supplied by Takara Biotechnology (Dalian, China). Fast SYBR green master mix was purchased from Applied Biosystems (Foster City, CA). Other chemical reagents were purchased from Sigma-Aldrich.

2.2. Preparation of CD34+ CBHSPCs

Anonymous human cord blood donated by pregnant mothers who gave birth to healthy babies at full term was collected at Suzhou Municipal Hospital, Suzhou, China. All studies involving human tissue samples were approved by the local Institutional Review Board. Anticoagulated cord blood was diluted with phosphate-buffered saline (PBS), and then subjected to density-gradient centrifugation as described below. Diluted blood layered on top of a 75% Ficoll solution was centrifuged at 500*g*, at 4 °C, for 30 min. MNCs in the interphase were collected and washed with PBS. Cell numbers were manually counted using a hemacytometer. CD34+ CBHSPCs were purified from the MNCs using the CD34 MicroBead kit with FcR blocking reagent and a MACS unit based on two-step magnetic bead cell sorting.

Briefly, the MNCs were washed and resuspended in PBS buffer containing 1% bovine serum albumin and 2 mmol/L edetic acid (EDTA). Cells were first incubated with monoclonal mouse anti-human CD34 antibodies labeled with MACS microbeads, in the presence of human IgG as blocking reagent, at 4 °C, for 30 min. Labeled cells were loaded onto a column installed in a magnetic field, and CD34− cells, which were not conjugated with the microbeads, were removed as unbound fraction. Trapped CD34+ cells were eluted after removing the column from the magnet according to the instructions of the CD34 MicroBead Kit.

2.3. Flow cytometric analysis

The purity and the number of freshly isolated CD34+ CBHSPCs were assessed *via* fluorescence-activated cell sorting (FACS) using human CD34-PE antibody and isotype-matched mouse IgG2a-PE. Cells were incubated with either antibody at room temperature for 15 min in the dark. After the incubation, the cells were washed once in PBS. FACS