Original Article

Ineffectiveness of Methylation in Rgulation of VHL, ECAD, and RUNX3 Genes in Erythroid Cells Differentiated by Erythropoietin

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Abstract

Background: Vast variety of intermediate factors including cell cycle regulators, growth factors, transcription factors, and signaling pathways are involved in hematopoietic stem cell (HSC) commitment and differentiation into distinct lineages. VHL, Ecad, and RUNX3 are among these. Epigenetics is currently introduced as a potential mechanism to control the gene regulation. The aim of this study is to reveal the correlation between the expression level and methylation pattern of mentioned genes after in vitro differentiation of cord blood HSCs into erythroid lineage mediated by erythropoietin.

Materials and Methods: After isolation and expansion, the CD34+ cord blood stem cells were divided into two parts. The first part was used to extract the DNA and RNA and the second to differentiate into erythroid lineage. Methylation specific PCR (MSP) and Real-time PCR were used to determine the methylation status and expression levels of the genes, respectively.

Results: Although the significant upregulation observed for VHL and Ecad genes and a down-regulation for RUNX3 gene after differentiation, no remarkable changes were seen in methylation pattern compared with cord blood HSCs by MSP technique.

Conclusion: It is appearing that methylation pattern in promoter region has not an effective role in expression of VHL, Ecad, and RUNX3. Moreover, considering the inability of MSP method to detect subtle differences in methylation level a more sensitive method is needed to distinguish the methylation levels of these genes before and after erythroid differentiation.

Keywords: Methylation, gene expression, erythropoietin, differentiation

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Introduction

Currently abundance of knowledge on fundamental principles of cell biology and evolutionary mechanisms of tissues especially about gene expression has been gained[1]. Differentiation of a primary stem cell into distinct cell lineages is regulated by highly complicated pathways such as cytokines, transcription factors, cell cycle regulators, proliferation, apoptosis, and a variety of signaling