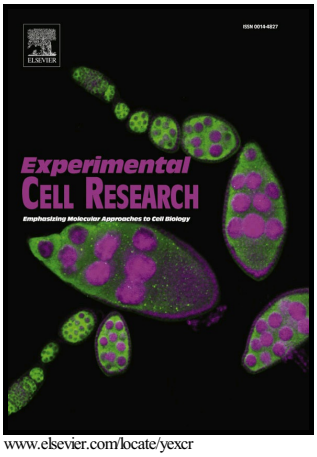

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Validation of Reference Genes for Real-Time PCR of Cord Blood Mononuclear Cells, Differentiating Endothelial Progenitor Cells, and Mature Endothelial Cells

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Abstract

In the last ten years, endothelial progenitor cells (EPCs) have gained interest as an attractive cell population in regenerative medicine for vascular applications. This population is defined as the precursor of endothelial mature cells (ECs) through a process of differentiation. To our knowledge, no single marker can be used to discriminate them from mature ECs. To effectively study their differentiation kinetics, gene expression must be assessed. Quantitative real-time PCR (RT-qPCR) is widely used to analyze gene expression. To minimize the impact of variances from RT-qPCR, a rigorous selection of reference genes must be performed prior to any experiments due to variations in experimental conditions. In this study, CD34+ mononuclear cells were extracted from human cord blood and differentiated into EPCs after seeding for a maximum period of 21 days. To choose the best combinations of reference genes, we compared the results of EPCs, CD34+ mononuclear cells, and mature endothelial cells to ensure that the differentiation kinetics did not affect the expression of our selected reference genes. The expression levels of seven genes, namely, YWHAZ, GAPDH, HPRT1, RPLP0, UBC, B2M, and TBP were thus compared. The algorithms geNorm, NormFinder, BestKeeper, and the Comparative ΔCt method were employed to assess the expression of each candidate gene. Overall results reveal that the expression stability of reference genes may differ depending on the statistical program used. YWHAZ, GAPDH, and UBC composed the optimal set of reference genes for the gene expression studies performed by RT-qPCR in our experimental