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Evaluation of Gene Expression after in Vitro Differentiation of Human Bone Marrow-Derived Stem Cells to Insulin-Producing Cells

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Abstract : Many protocols were publicized for differentiation of human mesenchymal stem cells (MSCS) into insulin-producing cells (IPCs) in order to excrete insulin hormone ingoing to treat diabetes disease. Our aim is to evaluate relative gene expression for each independent protocol. Human bone marrow cells were derived from three volunteers that suffer diabetes disease. After expansion of mesenchymal stem cells, differentiation of these cells was done by three different protocols (the one-step protocol was used conophylline protein, the two steps protocol was depending on trichostatin-A, and the three-step protocol was started by beta-mercaptoethanol). Evaluation of gene expression was carried out by real-time PCR: Pancreatic endocrine genes, transcription factors, glucose transporter, precursor markers, pancreatic enzymes, proteolytic cleavage, extracellular matrix and cell surface protein. Quantitation of insulin secretion was detected by immunofluorescence technique in 24-well plate. Most of the genes studied were up-regulated in the in vitro differentiated cells, and also insulin production was observed in the three independent protocols. There were some slight increases in expression of endocrine mRNA of two-step protocol and its insulin production. So, the two-step protocol was showed a more efficient in expressing of pancreatic endocrine genes and its insulin production than the other two protocols.

Keywords : mesenchymal stem cells, insulin producing cells, conophylline protein, trichostatin-A, beta-mercaptoethanol, gene expression, immunofluorescence technique
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