GENERATION OF HEPATOCYTE-LIKE CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Introduction: The demand for donor livers in transplantation medicine continues to increase every year with the difficulties in finding compatible human hepatocytes. Therefore, generation of patient specific human hepatocytes from pluripotent stem cells (PSCs) possess the possibility for cell-based therapeutics, bioengineering and modeling of fetal liver development. In this regard, the aim of this study was to generate of human hepatocyte-like cells from human PSCs under xeno-free and feeder-independent conditions.

Methods: Homogeneous and karyotypically normal PSCs were maintained in xeno-free and feeder-independent conditions. Pluripotent characteristics of PSCs were confirmed with alkaline phosphatase staining, immunocytochemistry and RT-PCR analysis. For hepatocyte differentiation, PSCs were cultured in RPMI-1640 medium, containing B27, activin A, FGF-2, BMP-4, HGF for 15 days and in hepatocyte growth medium with oncostatin M for last 5 days. After differentiation, hepatocyte-like cells were characterized using morphological and immunofluorescence analysis.

Results: Morphological analysis of developed hepatocyte-like cells demonstrated formation of cuboidal shape and accumulation of glycogen and lipid in cytoplasm. Loss of pluripotency was determined by negative expression of transcription factor Oct-4 on the 15-th day. In PSC-derived hepatocyte-like cells the expression of hepatic transcription factor HNF4a was detected on the 10-th day, alpha-fetoprotein (AFP) on the 15-th day and albumin (ALB) on the 20-th day of culturing. Both morphological and immunofluorescence analysis indicated acquisition of fetal hepatocyte-like cell characteristics under xeno-free and feeder-independent conditions.

Conclusion: Thus, we demonstrate that human PSCs retain full potential for fetal liver development and describe a procedure that facilitates the efficient generation of highly differentiated human hepatocyte–like cells.