DIFFERENTIATION OF MESENCHYMAL STEM CELLS INTO HEPATOCYTES

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Introduction. Acute and chronic liver diseases are common in Kazakhstan and other countries. These diseases are known to cause significant disability and death. In many cases, liver transplantation is the last resort for patients with end stage liver disease, but it is an extremely expensive procedure and is associated with many risks. The most important among them is an immune rejection. Autologous cell transplantation is a potential therapeutic approach for liver regeneration and could become an alternative to organ transplantation. In this regard, mesenchymal stem cells (MSCs) are a very attractive source for differentiation into hepatocytes. These cells can be isolated from bone marrow and adipose tissue of the patient and exponentially expanded *in vitro*. Transplantation of hepatocytes differentiated from MSCs could become a new promising approach in treatment of the patients with chronic liver conditions.

Materials and methods. MSCs were isolated from bone marrow of 2-month-old male rats. For the differentiation of MSCs into hepatocytes Snykers technique was applied. MSCs were seeded into type I collagen-coated culture flasks containing basal medium and supplemented with 2% fetal bovine serum. At 100% cell confluence, medium was replaced with differentiating medium (basal medium + 100 ng/ml FGF-4, 10 ng/ml HGF, 20 ng/ml EGF, 1 x ITS and 20 |ig/L dexamethasone). The degree of differentiation was assessed at days 4 and 14 of cultivation in differentiating medium by examining the morphology of the cells and the expression of hepatocyte-specific proteins. MSCs cultured in basal medium served as control.

Results and discussion. Microscopic examination of cells in phase contrast mode demonstrated that after two weeks of exposure by differentiating agents, MSCs changed their morphology from fibroblast-like cells to polygonal cells. Results of Western blot analysis showed an expression of an early hepatocyte cell marker (C/EBP a) on the day 4 and 14. At the same time, a late marker of hepatocytes (cytokeratin 18) was not expressed on day 4, but on day 14. Additionally, we observed increased production of glycogen and urea, and accumulation of an indocyan green dye on day 14, which also indicates hepatogenic differentiation.

Conclusions. We report that exposure of MSCs isolated from bone marrow to dexamethasone, insulin, transferrin, selenite, hepatocyte growth factor, and fibroblast growth factor for 14 days leads to differentiation of these cells into hepatocyte-like cells.