In Vitro Differentiation Of Bone Marrow – Derived Stem Cells Into Insulin Producing Cells

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ABSTRACT: Diabetes mellitus is a worldwide health problem. It constitutes a metabolic disorder that results, among many other causes, from dysfunctional pancreatic islet beta cells with inadequate release of insulin hormone responsible for the control of normal blood glucose level. As stem cells are the key to tissue regeneration and repair attention was directed towards the role of stem cells in regeneration therapy for diabetes.

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OBJECTIVES

The exploration of an in vitro induction of differentiation of human undifferentiated bone marrow derived stem cells into morphologically similar and functionally competent insulin producing cells. It also aimed at delivering an optimized work protocol as a guide for the generation of the targeted differentiated cells.

METHODS

Eleven bone marrow samples (10 ml/sample) that were obtained from consented patients admitted for bone marrow aspiration among other investigation procedures at the Main University Hospital, Alexandria Faculty of Medicine. The bone marrow derived mononuclear cells were isolated physically by a density gradient solution (Biocoll, 1.077). The isolated mononuclear were cultured for 14 days in Low glucose DMEM complete media and incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO2.

Differentiation was performed by applying the following differentiation protocol:
Stage 1: Cells were cultured in low serum high glucose DMEM (25 mmol/L) media for 14 days.
Stage 2: Exendin-4 20nm/L (Sigma-Aldrich) was added to the previous media for 14 days.

Morphological characterization of the differentiating BM-MSCs included:

1- Regular monitoring by phase contrast inverted microscope twice a week.
2- Transmission electron microscopic examination of ultrathin sections prepared from pellets of cultured cells.

Functional characterization of the differentiating cells was done by:

1- Dithizone staining of cultured cells to detect insulin containing granules.
2- Light microscopic examination of toluidine blue stained semithin sections for demonstration of metachromasia of insulin granules in differentiated cells.
3- ELISA technique before and after differentiation, and exposure of differentiated cells to glucose challenge test for assessing competence of the differentiated cells to release insulin in response to glucose.

RESULTS

Primary culture came out with fibroblast-like sheets of cells achieving an 80% confluence after 14 days as evidenced by monitoring cultures using the phase contrast inverted microscope. Differentiated cells showed tendency to aggregate in clusters of variable sizes simulating the pancreatic islets. Individually, the cells acquired a polyhedral shape with many surface

processes. The cytoplasm revealed euchromatic nuclei and was filled with granules. Dithizone staining demonstrated a positive crimson red reaction of many differentiated cells within the formed clusters. Toluidine blue stained semi-thin sections revealed purple metachromatic insulin granules. Transmission electron microscopic examination revealed ultrastructural features typical of protein synthesizing cells in contrast to lack of these features in the undifferentiated cells.

Survey of cells before and after differentiation was evaluated via ELISA test performed on the conditioned culture media. Pre-induced cultured cells didn't show any evidence of insulin synthesis. On the other hand, differentiated cells were found to produce insulin that increased more three folds after exposure to high glucose challenge.

CONCLUSION

The present study is an experimental model exploring the possibility for in vitro differentiation of human BM-MSCs into insulin producing cells. It provided an optimized protocol for induction of growth and differentiation of the BM-MSCs into the targeted cells. The morphological and functional characterization of the obtained differentiating cells proved the adequacy of the applied method to obtain morphologically similar and functionally competent insulin producing cells.

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