Evaluation of the potential ability of multipotent mesenchymal stromal cells of amniotic fluid to differentiate in lung cells lineage - Partial Results

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INTRODUCTION

Pluripotent cell therapy has great potential as a breakthrough for treatment of various diseases, including lung diseases, which affect individuals of all ages. About third quarter of the amniotic fluid multipotent mesenchymal stromal cells (MMSC) has quite primitive features, which makes them a good alternative for treatment of lung diseases in preterm neonates.

OBJECTIVE

The aim of this study is isolate and characterize in vitro the MMCE amniotic fluid obtained from human term pregnancies and evaluate their capacity in differentiation into lung cell lines.

MATERIAL AND METHODS

The samples of amniotic fluid were collected during the cesarean surgery. It was cultivated in AmnioMax ® (Invitrogen) culture medium and characterized by flow cytometry. For differentiation into lung cells, we used Small Airway Epithelial Cell Growth Medium - SAGM ® (Lonza) and the control group remained in the AmnioMax ® culture medium. After treatment, the differentiation of the stem cells was evaluated by light microscopy, real time PCR and flow citometry, using surfactant protein type C (SFTPC) as a marker.

PARTIAL RESULTS

We observed three distinct morphologies in the cell cultures from amniotic fluids in the first stage of *in vitro* culture. These different cells populations disappear in the later stages and become one homogeneous population. All the cells exhibit expression of mesenchymal markers and absence of hematopoietic markers.

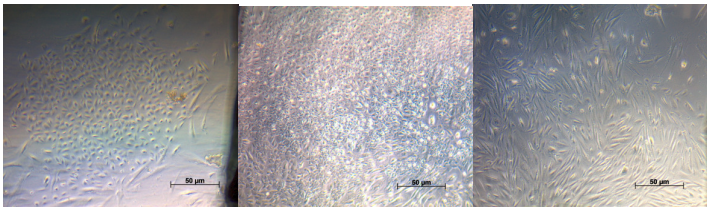

Figure 1. Culture of amniotic fluid cells presenting three different colony morphologies. Increased of 4x

When the amniotic fluid cells was induced to differentiation into lung cells they showed a shift in morphology, becoming more elongated and thin. In addition to the morphological changes, cells induced to differentiation showed the expression of the gene responsible for production of surfactant protein type C when testate by real-time PCR. This gene is expressed in type II pneumocytes, the resident population of lung tissue, a fact not observed in cells from amniotic fluid samples obtained among pregnant women in control group. For immunofluorescence methodology, the procedure lies in standardization.

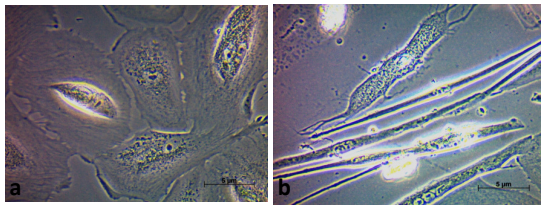

Figure 2. Morphological changes observed after treatment. a) Control group treated with the basic medium AmnioMax ® featuring larger cells with abundant cytoplasm and a large number of cell divisions; b) Group treated with SAGM ® featuring elongated cells with little material cytoplasm and nucleus condensed. Increase of 40x.

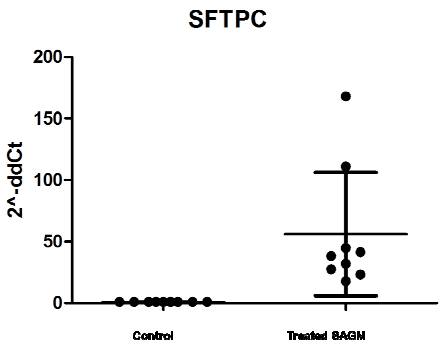

Figure 3. The graph showing the values of 2 ^-ddCt of the control group samples against the treated group for the expression of SFTPC. Analysis by RT-PCR (p <0.0001).

SUPPORT

FAPESP

INCTC

Hemocentro RP