



P233- Effects of A549-Condition Medium on Mouse Embryonic Stem Cells Differentiation into Alveolar Epithelial Type II Cells

Mohammad Reza Mokhber Dezfouli^{1,2}, Sirous Sadeghian Chaleshtori^{1,2*},
Hossein Baharvand^{3,4}, Yaser Tahamtani³, Shokufe Yadollahi⁵

¹ Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

² Department of Stem Cells and Tissue engineering, Institute of Biomedical Research, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

³ Department of Stem Cells and Developmental Biology at the Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

⁴ Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

⁵ Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Corresponding Author Email: s.sadeghian@ut.ac.ir

Abstract

Objective: A549 cells are adenocarcinomic human alveolar basal epithelial cells. These cells are responsible for the diffusion of some substances, such as water and electrolytes, across the alveoli of lungs. A549 cell line are widely used as an in vitro model for a alveolar epithelial type II (AETII) cell model for drug metabolism and as a transfection host. The main functions of AETII cells, including the synthesis and secretion of surfactants (a mixture of proteins and lipids which reduces surface tension and preventing collapse of the alveolus), hyperplasia in reaction to alveolar epithelial injury and act as progenitor cells for AETI cells (which are essential for gas exchange), thus being able to renew the alveolar epithelium. The aim of the study was to evaluate the effects of the A549-Condition Medium (A549-C.M) on the AETII cells differentiation from mouse embryonic stem cells (mESCs). **Material and Methods:** Royan 20 cells were induced to differentiate using adherent culture method and without the formation of embryoid body (EB). After endoderm formation on day 6, cells was treated with DMEM/F12 supplemented with A549-C.M (filtered and added at 50:50 v/v to serum-free medium as the working solution) for 9 days. In end on 15 day, the produced cells were analyzed by quantitative PCR, immunocytochemistry and flowcytometry for markers of embryo, definitive endoderm and alveolar type II cells. **Results:** Differentiated cells displayed increased SP-A, SP-B, SP-C and SP-D (surfactant proteins, AETII cells specific markers) expression consistent with AETII cells production. We founded ~ 11.09% of ESCs displayed immunoreactivity to SP-C (unique feature of AETII cells which is commonly used to identify these cells from other lung parenchymal cells) after exposure to A549-C.M. There was minimal production of Foxa2 a marker of definitive endoderm and Oct4, a marker of pluripotency. **Conclusion:** We have shown A549-C.M stimulate the generation of AETII cells from mESCs. The application of A549-C.M will facilitate, a more robust and efficient generation of mESCs-derived AETII cells for future basic research and potential therapeutic application.

Keywords: A549-C.M, AETII Cells, mESCs, Differentiation