Title: Derivation of immune and non-immune cells from hematopoietic and non-hematopoietic progenitors. A pilot study

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Abstract

Taking in consideration the proved capability of stem/progenitor cells to differentiate to various directions depending of the respective environmental factors and conditions, the possibility about derivation of different mature types of cells from different sources in human and experimental animal models was tested. For this goal was investigated the capability of non-immune cells from precursors with both hematopoietic and non-hematopoietic origin to acquire immune characteristics in appropriate circumstances. Human mesenchymal stem cells (MSCs), isolated from bone marrow material, as well as from adipose and cartilage tissues, were *in vitro*-incubated in appropriate conditions, necessary about differentiation to chondrocyte, osteoblast and osteoclast directions, respectively. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) with subsequent label-free tandem mass spectrometry assay, combined with liquid chromatography (LC-MS/MS) were also performed. NK cells were isolated from spleen cells of mouse Balb/c and incubated in the presence of different cytokine combinations. Fibroblasts from 3T3 cell line, derived from embryos of experimental mice of the same breed, were pre-incubated in the presence of cultural fluid from different types of murine mature normal and malignant cells (both in the absence and in the presence of cells in the used suspensions).

The chondrocyte, osteoblast and osteoclast morphology was proved by Alcian Blue, Alizarin Red and TRAP+Hematoxillin staining, respectively, as well as by Hematoxillin/Eosin (H/E) staining and Giemsa dye. These morphological characteristics were proved about the three types of mature cells, derived from the human MSCs, isolated from the three sources. These features were confirmed by the data from SDS-PAGE with subsequent label free LC-MS/MS assay and were in agreement with the literature findings. When the derived from mouse spleen progenitors NK cells were pre-incubated in the presence of the cocktail IL-12/IL-15/IL-18, active production of Connexin 43 (Cx43) was proved by Western-blot assay. These results confirmed the literature data about the proved key role of this molecule in the regulation of certain immune functions, including of antibody-related immune disorders. In the sub-populations of mouse embryonic 3T3 cell line were pre-incubated with mouse malignant myeloma cells and in cultural fluid from these neo-plastic cells, was noted appearance of initial myeloid and lymphoid progenitors, respectively. When the so derived cells in the prepared mixed cultures were freezed in the presence of cryo-protector DMSO, thawed and re-incubated, appearance of availability of multi-nuclear osteoclast-like cells was observed. In pre-incubation of *de novo*-formed semi-confluent monolayers with the received osteoclast-like cells and of cultural fluid from them, differentiation to osteoblast-like and chondrocyte-like directions was assessed. These changes could be explained with probable existence of able to differentiate to different lineages sub-populations of stem-like cells in the embryonic 3T3 fibroblast line, depending of the respective environmental factors. These data were in confirmation of the literature messages about the role of DMSO and drastic temperature changes in the activation of fusion processes.

Biography:

Iskra Ventseslavova Sainova has completed her PhD at the Department of Oncovirology to the Institute of Experimental Pathology and Parasitology (IEPP) to Bulgarian Academy of Sciences (BAS) in Sofia, Bulgaria, with a title "Investigation on the replication of vaccine avipoxviral strains in different cell-culture systems". The main goal has been directed to development of maximally safe methods for application of viral strains for production of gene-engineering vaccines, but also as vectors for transfer of nucleotide sequences. She is assistant professor in the field of molecular biotechnology, molecular and cellular biology. In the last years, her work is directed to investigation on the possibility about production of immune molecules by non-immune cellular types in appropriate conditions. She has over 100 publications that have been cited over 150 times.

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