PROTEOMIC AND METABOLOMIC ANALYSIS OF THE PROCESSES OF INDUCTION OF PLURIPOTENCY IN MYOBLASTS AND DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS TOWARDS CARDIAC CELLS

SUMMARY

Studies based on a mouse model of post-infarction heart allow us to understand the mechanisms of processes occurring during myocardial infarction (MI), assess its severity, and allow preclinical testing of new technologies for regeneration of damaged tissue. However, the novel approach involving the use of autologous induced pluripotent stem cells (iPS) obtained from patients could contribute to the development of methods for personalized regenerative medicine and significantly improve the quality of life of post-MI patients. Eventually, it may improve the function of damaged myocardium. Although several different ways of obtaining iPS cells are available, intensive *in vitro* and *in vivo* studies are still needed to elucidate the changes that occur during the dedifferentiation and subsequent differentiation of iPS cells, which is necessary to be sure that the proposed therapy is efficient and above all safe.

The aim of this study was to describe at the proteomic and metabolomic level the changes occurring during induction of pluripotency in human myoblast cells, as well as during subsequent differentiation of the obtained iPS cells into cardiac-like cells. We also evaluated the changes arising in the proteome and metabolome of the heart after administration of the obtained cardiac-like cells to mice. In the latter case myocardial infarction had been previously induced by ligation of the left artery. The results obtained using mass spectrometry methods were analyzed using bioinformatic functional analyses to identify metabolic processes and signaling pathways that change during the dedifferentiation and differentiation processes. The study was complemented by karyotype analyses, immunofluorescence staining, RT-PCR analyses, and morphological evaluation of cells by light microscopy.

The performed analyses allowed characterizing the proteome and metabolome of the examined populations of myoblasts, iPS cells and cardiac-like cells at different stages of dedifferentiation and differentiation processes. Two different protocols to derive iPS cells were also compared. The use of STEMCCA vector for myoblast dedifferentiation resulted in activation of xenobiotic metabolism and lipid oxidation process and inhibition of ephrin receptor signaling, in contrast to the SENDAI virus-based approach. Comparison between iPS

cells obtained using the SENDAI viral vector and the myoblasts from which they were derived revealed that during the dedifferentiation process, the accumulation of many proteins intensively increases. This may be due to the high potential of iPS cells to differentiate into a variety of other cell types, in contrast to functionally stable myoblasts. During the differentiation of cells, activation of processes characteristic for rapidly proliferating cells including: splicing, EIF2 receptor signaling, and homologous recombination, as well as inhibition of muscle cell-specific functions was observed. In contrast, during the differentiation of iPS cells into cardiac-like cells, an upregulation of proteins involved in oxidative phosphorylation and processes related to muscle formation was observed, which could confirm the following specialization of the obtained cells. The results of metabolomic profiling showed that the greatest changes in the accumulation of low molecular compounds were induced by induction of the mesodermal pathway during the process of iPS cells differentiation, and the elevated level of these compounds was observed until the end of the differentiation process.

Administration of the obtained cardiac-like cells to post-infarction mouse hearts resulted in downregulation of lactic acid, with a concomitant upregulation of proteins involved in the ubiquitin-proteasome system. This may suggest a return to aerobic energy acquisition characteristic of healthy myocardium and initiation of regeneration processes.

Comparative proteomic and metabolomic analyses based on mass spectrometric techniques, supported by bioinformatics platforms, provide a very good tool to describe the changes occurring during iPS cells dedifferentiation and differentiation processes. Complemented by studies at other "omic" levels and functional analyses of the studied cells *in vitro* and *in vivo*, they can contribute to a better understanding of the mechanisms occurring during these processes and thus accelerate the introduction of iPS-based techniques into subsequent stages of clinical trials.