

Project's title: Activity and function of auxiliary glucose metabolic fluxes in response to low oxygen (physioxia) during long-term AD-MSC culture

Stem Cells (SC)-based therapy is one of the most popular and fast developing branches of modern regenerative medicine still being in the experiment phase. Numerous ongoing clinical trials confirm the efficacy and safety of somatic and foetal types of SC transplantation. However, ongoing studies indicate the need for multiple/cyclic cells administration in order to achieve and maintain therapeutic effect.

Therefore, it is a necessary to increase the cell expansion in laboratory conditions. Despite many years of experience and constantly broaden knowledge in the stem cell cultures field, the optimized and standardized procedures for long-term expansion, cryopreservation and graft preparation have not been yet still established and accepted. Cell senescence during long-time culture *in vitro* still becomes a problem which leads to slowdown cell proliferation, decrease the immunomodulatory and protective properties and cells genetic stability. What is the most dangerous all this imperfections may result in increasing danger of mutagenesis and sensitivity of the cells to cancer transformation.

Published results, as well as our previous and ongoing experiments indicate that MSC cultures in hypoxic/physioxic (5%O₂) environment is the key and easy available factor for maintaining the cells in the safe, therapeutically required state of high proliferation, differentiation and regenerative factors secretion. At the same time we have found not only increased cell growth timing but also enhancement of the phenotypic and genetic cells stability which lower a risk of oncogenic transformation and carcinogenesis.

Based on these information, in the proposed project we will elucidate the key biochemical and molecular mechanisms underlying the above beneficial effects of the low oxygen on MSC culture. The research will include an extended and detailed comparative analysis of the fundamental function and molecular parameters supposedly engaged in the differences in stability and safety of AD-MSC cultures growing under physiological (5%) and atmospheric (21%) oxygen conditions.

In addition, an innovative aspect of the proposed research will be focused on key molecular mechanism that binds metabolic pathways regulating proliferation, development and differentiation of cells with biological improvement and safety of MSC culture in lowered oxygen tension. It seems that important control points for the above processes may include two reactions controlling the metabolic switches from the main glycolytic pathway to auxiliary shunts (**competing with glycolysis**) dominating in undifferentiated and extensive proliferating cells. Both of these regulatory branching points governed either by **G6PDH** (glucose-6-phosphate-dehydrogenase) or **PFK-2** (phospho-fructo-kinase2) enzymes, control the exploit intermediate glycolysis products and modify and/or redirect glucose flux towards the so-called alternative transformations, including these leading to synthesis of metabolites determining such functions as growth, anti-radical protection and the degree of cell differentiation.

Project results will provide us with information of the basic mechanism and character/nature of connections between the examined pathways of glucose metabolism and the development, proliferation and differentiation of hAD-MSC in hypoxic/physioxic surrounding. These knowledge will help to understand and perhaps control the mechanisms leading to therapeutically desired and effective hAD-MSC phenotype changes such as high proliferation rate and maintaining of genetic stability during long-term culture period. This will allow develop better, optimized culture protocols that improve the quality, safety and efficiency of the cells needed for transplantation.