Scientific report

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„Interference with hypoxia-signalling pathways in mesenchymal stem cells prior to transplantation as a strategy to enhance myocardial recovery post infarction"

Summary:

Mesenchymal stem cells (MSC) derived from bone marrow reside *in vivo* in a low oxygen microenvironment (1-5% O_2). However, *in vitro* standard cell culture protocols are performed at atmospheric concentrations of oxygen (21% O_2), which are restrictive growth conditions with selection effects through long-term cultivation by accumulation of loss-of-function mutations that cause cells to escape from the oxygen-induced inhibition growth (Boregowda et al., Stem Cells 2012).

The goal of this project is to better understand the role of hypoxia and more specific of MIR210HG locus on the effector properties of bone marrow-derived human MSC.

The main results obtained at this stage of the project are:

1. Characterization of human bone-marrow derived MSC grown for long-term (three passages) in normoxia (21% oxygen) or hypoxia (5% oxygen). For hypoxia experiments, cells where cultivated within a controlled and sustained workstation environment using the Whitley H35 Hypoxystation. The results showed that low oxygen concentration (5% O_2) slightly increased the proliferation index and cell number, as compared with normoxia (21% O_2).
2. Assessing the metabolic phenotype of MSC as compared to that of another cell type, namely endothelial progenitor cells (EPC) isolated from human umbilical cord blood. This analysis was performed using an Agilent Seahorse XFp system. The results showed that MSC have a more energetic metabolism than EPC, both in basal and stress conditions. Under conditions that mimic hypoxia (with DMOG) both cell types become glycolytic. However, EPC lose any stress response capacity while MSC still have a metabolic activation capacity. Their coculture both under normal conditions and in the presence of DMOG, shows a metabolic response capacity, suggesting that MSC confer an advantage for EPC-based cell therapy when co-transplanted at an ischemic tissue injury.
3. Functional evaluation for the deletion of MIR210HG locus by Real Time PCR for miR-210 and lnc MIR210HG transcripts -001 and -003 expression and by functional metabolic analysis of glycolysis and mitochondrial respiration in control cells and knock-out cells. The results showed that by using CRISPR-Cas9 genome editing technology, the deletion of MIR210HG locus resulted in a decrease in the expression for the MIR210HG ncRNA transcripts with functional impact on the metabolic phenotype of the cells (affecting the mitochondrial respiration).

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