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:

Hypoxia-responsive ncRNAs have yet undefined roles in the biology of mesenchymal stem cells (MSCs). Currently regarded as a "master hypoxamiR" with complex roles in the cellular responses to hypoxia, miR-210 was first discovered by Dr. Mircea Ivan's group (Kulshreshtha et al., Mol Cell Biol 2007) and demonstrated to be induced in response to low oxygen concentrations. As miR-210 improved angiogenesis, inhibited apoptosis, and improved cardiac function in a murine model of myocardial infarction (Hu et al., Circulation 2010), this fundamental regulator of hypoxia might have other yet unknown functions, which extends beyond cell response to hypoxia.

The goal of this project is to improve the pro-angiogenic, anti-inflammatory and immunomodulatory (effector) functions of MSC by manipulating the hypoxia-responsive mechanisms in order to consolidate their therapeutic potential for post-infarction myocardial regeneration.

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

1. Our results showed that long-term cultivation (3 weeks) of human MSCs at low O_2 concentrations (3% or hypoxia) have significantly lowered the proliferative capacity of the cells, measured by real-time monitoring of proliferation (xCELLigence), cumulative population doublings, and colony-forming unit fibroblast assay. There are conflicting reports on the effects of low O_2 levels (hypoxia) on MSCs proliferation in vitro. Some studies have shown that MSCs proliferate better when grow at low concentrations of O_2 but others have shown the harmful effect of hypoxia. A possible explanation of this response observed by us would be that the cells were derived and cultured for several passages (3-5) first at the atmospheric concentration of O_2 and thus adapted to this atmospheric microenvironment. Moreover, hypoxia reduced cell proliferation without disturbing the mitochondrial function of MSCs.
2. Adaptation of human MSCs to hypoxia (for 10 days) resulted in increased pro-angiogenic properties (measured by Proteome Profiler Human XL Cytokine Array) and in similar immunomodulatory effect on activated lymphocytes, as normoxic MSC. However, hypoxic MSCs attenuate M1 (pro-inflammatory) activation and enhance M2 (reparatory) activation of THP-1 derived macrophages at a higher level as compared with normoxic MSCs.
3. Gain-of-function and loss-of-function experiments of miR210 expression in human MSC cultivated in normoxic (atmospheric) conditions or short-term exposed to hypoxia (3% O_2) showed that the hypoxia-responsive ncRNA miR210 largely recapitulates the effects of hypoxia. Thus, activation of hypoxia-induced signalling pathways in hMSCs grounds them to increase their pro-angiogenic and anti-inflammatory potential, having a particular importance in MSC-based cell therapy.

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