Ruprecht-Karls-Universität Heidelberg Medizinische Fakultät Mannheim Dissertations-Kurzfassung

Influence of ABCB5+ Mesenchymal Stem Cells on Hypoxic Damage in vitro and in vivo

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Hypoxia is a common pathological process that commonly occurs in many diseases such as peripheral artery disease (PAD), cerebral ischemia, myocardial infarction, different kinds of trauma and tumour growth. The angiogenic potential of endothelial Cells (ECs) is essential to maintain the homeostasis of tissues and organs after exposition to hypoxic injury. Stem cell therapy is a promising therapeutic approach for peripheral artery disease (PAD), with the mode of action still remaining to be elucidated.
The aim of the project was to evaluate the influence of ABCB5+ mesenchymal stem cells (MSCs) on the Ca2+ homeostasis of Human Umbilical Vein Endothelial Cells (HUVECs) under hypoxia in vitro and in an in vivo mouse model. The Ca2+ homeostasis within the endoplasmic reticulum (ER) and the cytosol may play a key role.
In this study, we applied Cobalt (II) chloride (CoCl2) and Deferoxamine (DFO) to establish mimic hypoxia HUVECs model. The HUVECs angiogenic function under mimic hypoxic and ABCB5+ MSCs therapeutic situations was evaluated by cell viability assay, migration assay, and tube formation assay. The dynamic changes of Cytosolic (Cyto) Ca2+, ER Ca2+ and Reactive Oxygen Species (ROS) under different situations were detected. For the in vivo experiments, the operation of Double ligature of the right femoral artery (DFLA) was performed to establish the hindlimb ischemia ApoE-/- mice model. They were divided into control and ABCB5+ MSC therapy groups according to the treatment they got after the DFLA. To evaluate the ABCB5+ MSCs therapeutic effect, we record the functional recovery within 7 days after the operation. The histological analysis of Gastrocnemius (Gm), aorta and plasma were performed. Besides, Western blot (WB) was performed to check the in vitro and in vivo expression level of Sarco-/endoplasmic reticulum atpase 2a (SERCA2a), Phospholamban (PLN) and Phospho-phospholamban (pPLN).
In the in vitro results, the cell viability, migration rate and tube formation of HUVECs were all significantly impaired by mimic hypoxia. But ABCB5+ MSCs therapy can improve angiogenic ability. Under the mimic hypoxia, the ER Ca2+ homeostasis was disturbed as the ER Ca2+ significantly decreased that then induced the Cyto Ca2+ overloading. ABCB5+ MSCs therapy can reverse these trend by increased the expression of SERCA2a and phosphorylation of PLN, which was proved by WB. In the in vitro results, the ABCB5+ MSCs group showed better functional recovery than the control group. The microvascular density results indicated that ABCB5+ MSCs therapy significantly enhanced angiogenesis after the DFLA. The in vivo WB results showed that SERCA2a expression of the ABCB5+ MSCs group was higher than the control group but not significant. However, the ratio of pPLN in the ABCB5+ MSCs group was significantly higher than the control group.
The mimic hypoxia decreases angiogenic function by reducing ER Ca2+ restoring ability, while the ABCB5+ MSCs therapy has the ability to improve the ECs angiogenic function through enhancing the ER Ca2+ restoring ability and eliminating ROS accumulation. While the detailed mechanism needs further research. These may provide new insight into the future development of MSCs therapy in the clinic.