TRANSFECT HUMAN CCR7 GENE IN MESENCHYMAE STEM CELL IMPROVES CELL MIGRATION AND ANTI-APOPTOSIS ABILITY IN VITRO

Huang Xin1, Kong Huijia2, Wang Lihan3, Hu Xinyang3, Cao Jiang1, Wang Jianan3

1. Clinical Research Center at Second Affiliated Hospital of Zhejiang University, Zhejiang, China; 2. School of Medicine at Zhejiang University, Zhejiang, China; 3. Key Laboratory of Cardiovascular Research

Objectives Bone marrow mesenchymal stem cells (BMSCs) are ideal source for cell therapy in treating ischemic cardiomyopathy (ICM). Hypoxia preconditioning can enhance the migrating ability as well as reduce the apoptosis of BMSCs. This study explored the impact of a hypoxia related gene product, CCR7 chemokine receptor, in BMSC in vitro migration and anti-apoptosis ability.

Methods Human bone marrow mononuclear cells (BMNCs) were isolated from human bone marrow using Ficoll 400 lymphocytes separation medium. BMSCs are separated by adhering to flask bottom and cultured in vitro. BMSCs were characterised by detecting cell surface antigen using flow cytometry. Human CCR7 sequence was amplified from human genome, and constructed into pCCL-CMV-IRES-GFP plasmid backbone. BMSCs were transfected with the pCCL-CMV-CCR7-IRES-GFP plasmid with the presence of lipofectamine 2000 (Invitrogen) to generate BMSC/CCR7+ cells. BMSC/CCR7+ were incubated under 0% O2, glucose and serum deprived (OGSD) condition for 36 h, BMSCs treatment with lipofectamine 2000 were cultured under the same condition as control group. In vitro BMSCs was determined using transwell assay. BMSCs were stained with DAPI dye, and the anti-apoptosis ability of BMSCs was assessed using fluorescence microscope.

Results Human CCR7 gene could be transduced into BMSCs by lipofectamine 2000 reagent. BMSCs/CCR7+ gained a higher survival rate, versus control BMSC cells. BMSCs/CCR7+ were more able to migrate through the transwell membrane.

Conclusions Transduce exogenous human CCR7 gene could enhance BMSCs in vitro migration and anti-apoptosis ability.