

GW23-e2158

# THE STUDY OF BONE MARROW MESENCHYMAL STEM CELLS GENE MODIFIED BY RECOMBINANT ADENO-ASSOCIATED VIRUS-9 COMBINED WITH PDGF-B IN VITRO

doi:10.1136/heartjnl-2012-302920a.205

Ma Xiang, Yao Yong-zhao, Zhao Ai-chao, Yuan Qing-hua, Lui Fen, Cheng Bang-dang, Ma Yi-tong. *First Affiliated Hospital of Xinjiang Medical University*

**Objectives** To explore the feasibility, stability and safety of using recombinant adeno-associated virus-9 which contained platelet-derived growth factor-B (rAAV9-PDGF-B) transduction to bone marrow mesenchymal stem cells in vitro.

**Methods** Bone marrow mesenchymal stem cells (MSCs) were transduced with rAAV9-eGFP at MOI=1×10<sup>6</sup>, and GFP expression was detected by converted fluorescence microscope. Experimental group: MSCs were transduced with rAAV9-PDGF-B, control group: MSCs were untreated. Collected stem cells from different times after transduction, respectively at 3 days, 6 days, 14 days, 21 days, 28 days. Proteins were extracted from the cells for Western blot analyse and observed the expression of PDGF-B, the transfection efficiency of after transduction 6 days and 21 days were measured by cell immunofluorescence. Alamar Blue assays were used to evaluate the safety of rAAV9-PDGF-B transduction to cardiomyocytes.

**Results** After transduction (rAAV9-eGFP) 48 h, only individual cells own GFP, however the expression of GFP was gradually enhanced, 5–6 days reached a peak, and then GFP expression was began to fade. Cells of control group did not found the expression of PDGF-B, cells of experimental group were expressed PDGF-B after transduction 3 days (PDGF-B/GAPDH=15.6±1.1%), after 6 days reached a peak (63.7±2.7%), after 14 days (46.6±1.1%), after 21 days (34.5±1.2%), after 28 days (34.4±1.9%). By Statistical Analysis, the expression of PDGF-B in control group compared to experimental group p<0.01). Compared to each times in experimental group, pDGF-B level in 3 days was lower than other times, the level of 6 days was the highest. Moreover, there was no statistical significant difference between the level of 21 days and 28 days (p=1.0). Cell immunofluorescence showed that the transfection efficiency of after transduction 6 days and 21 days, was 65.7±2.6% and 39.6±1.8% p<0.01), the transfection efficiency of 6 days was higher than 21 days. Alamar Blue assays, which evaluated the toxicity of rAAV9-PDGF-B transduction to MSCs, showed that the reduction ratios of cardiomyocytes at different times were all close to 1.0, there was no statistical significant difference in each times.

**Conclusions** rAAV9-PDGF-B could effectively transduce MSCs cultured in vitro and persistently express PDGF-B gene at least 28 days. Furthermore, rAAV9-PDGF-B was no significant toxic effects on MSCs.