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

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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 $\text{MOI}=1 \times 10^6$, 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 ($\text{PDGF-B}/\text{GAPDH}=15.6 \pm 1.1\%$), after 6 days reached a peak ($63.7 \pm 2.7\%$), after 14 days ($46.6 \pm 1.1\%$), after 21 days ($34.5 \pm 1.2\%$), after 28 days ($34.4 \pm 1.9\%$). 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 $65.7 \pm 2.6\%$ and $39.6 \pm 1.8\%$ ($p < 0.01$), 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.