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Effect of BMSCs-LV5-GAP on retinitis pigmentosa

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Purpose: To observe the therapeutic effects of growth associated protein-43 (GAP-43) gene modified rat bone marrow mesenchymal stem cells (BMSCs) on experimental retinal degeneration.

Methods: SD rat BMSCs were isolated and cultivated by adherence method then identified cell surface markers by flow cytometry. Construct lentiviral-mediated GAP-43 overexpression virus (LV5-GAP) after infection BMSCs get GAP-43 gene-modified BMSCs (BMSCs-LV5-GAP-43). A total of 67 male RCS rats were divided into 3 groups at postnatal 21 (P21) randomly. A cell suspension of 5x104 BMSCs modified with GAP-43 in 2 μl PBS was injected into the sub-retinal space of BMSCs+GAP-43 group rats, BMSCs group animals received 5x104 BMSCs in 2 μl PBS and NC group rats received 2 μl PBS. The expression of GAP-43, GS and RHO was analyzed by immunofluorescence, western blot. The thickness of ONL was assessed by the method of 'HE' staining and photoreceptor apoptosis was assessed by the method of TUNEL detection. The expression of Caspase-8 and Caspase-9 was analyzed by western blot.

Results: The BMSCs were cultured and passaged stably *in vitro*, flow cytometry analysis showed that CD90 and CD44 expression was positive, CD11b and CD45 expression was negative; GAP-43 gene sequence went through digestion, transformation, packagement and transfection to get lentiviral vector LV5-GAP; after infection BMSCs, Western Blot and immunofluorescence results showed that the expression of GAP-43 in BMSCs-LV5-GAP-43 was increased ($P<0.05$) after transplanted the cells into the sub-retinal spaces, western blot revealed the expression of GAP-43 was significantly increased during 14 days after sub-retinal transplantation in BMSCs+GAP-43 group; 30 days following sub-retinal injection, Western blot revealed the expression of Rho in BMSCs+GAP-43 group was significantly up-regulated ($P<0.05$), but the GS in BMSCs group was up-regulated compared with BMSCs+GAP-43 group ($P<0.05$). Histological analysis revealed that the thickness of outer nuclear layer (ONL) in BMSCs+GAP-43 group compared with the other two groups was significantly increased ($P<0.05$). After 30 days following treated, TUNEL positive cells in BMSCs+GAP-43 group compared with the other 2 groups were decreased ($P<0.05$). Western blot revealed the expression of Caspase-8 and Caspase-9 in BMSCs+GAP-43 group was significantly up-regulated ($P<0. 05$).

Conclusions: The rat BMSCs were successfully cultured and we constructed GAP-43 gene overexpression of lentiviral vectors successfully. Lentiviral-mediated GAP-43 can efficiently infect BMSCs. BMSCs-LV5-GAP-43 were transplanted into the sub-retinal space of RCS rat cells, they were gradually disappeared in the degeneration retina environment but the expression of GAP-43 gradually increased. GAP-43 can reduce Muller proliferation after transplantated BMSCs-LV5-GAP-43 into the sub-retinal space and prevent "glial blocked" and BMSCs-LV5-GAP-43 can protect photoreceptor cells maintain the integrity of photoreceptor cells. The TUNEL method results showed that the apoptotic cells were mainly located in the ONL of the retina, BMSCs-LV5-GAP-43 has the anti-apoptotic effect to photoreceptor cells and it is through blocking exogenous and endogenous apoptotic pathway mediated by Caspase-9 and Caspase-8 to inhibit neuronal apoptosis and maintain the integrity of ONL. The results suggest that GAP-43 gene modified BMSCs have the therapeutic effect on the early stage of retinal degeneration which might make RP retina preserved.

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