USE NT-3 AND BDNF LENTIVIRUS VECTOR TRANSFECT ADIPOSE DERIVED STEM CELLS AND DETECT ITS EXPRESSION

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Background: Spinal cord injury (SCI) always leads to everlasting damage. So how to treat it becomes a challenge to all the people. Tissue engineering is a new method to cure SCI which including three factors: seed cell, scaffold, growth factor the most important is the seed cell which takes effect through secrete growth factors. So how to promote the seed cell secrete growth factor stably is extremely important. We construct two lentivirus carriers which carry the NT-3 and BDNF gene independently, transfect the adipose-derived stem cell (ADSCs) to detect whether it could secrete gene stably. At last we want to obtain the seed cell which could stably express the growth factor.
Method: According to the literature we construct the NT-3 and BDNF lentivirus vector, use Lv-GFP to transfect the third generation of ADSCs according to the MOI of 0, 10, 20, 40, 50, 100 to determine the best MOI value. Divide the experiment into four groups: BDNF group, NT-3 group, BDNF+NT-3 group and control group (No transfection). Use RT-PCR and Westbolt to detect the gene expression. Apply the SPSS12.0 to analysis result.
Result: BDNF and NT-3 lentivirus vector concentration reach to 3×10^7 TU/mL and 2×10^7 TU/mL; according to the cell which express green fluorescent and cell viability, we find the best MOI value is 50. RT-PCR result show that the BDNF group, NT-3 group, BDNF+NT-3 group are separately express its own gene, the control group is also could express BDNF and NT-3,but very weak compare to the other group ($P<0.05$), the Westblot result is consistent with the RT-PCR.
Conclusion: We construct the BDNF and NT-3 lentivirus vector, the concentration of which satisfy to the experiment, after transfect to the ADSCs, the cell could express the gene highly compare to the control group which laying a solid foundation for the later experiment which use tissue engineering to cure the spinal cord injury.