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THE USE OF NEUROTROPHIN-3 AND SHORT HAIRPIN RNA NG2 LENTIVIRUSES TO PROMOTE AXONAL SPROUTING IN TRANSECTION SPINAL CORD INJURY *EX VIVO*

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SUMMARY

Introduction: The failure of the spinal cord to regenerate can be attributed to both a lack of trophic support for regenerating axons, and inhibitory factors that are up-regulated following injury. The inhibitory environment is mainly a result of chondroitin sulphate proteoglycans (CSPGs). Neural Glial-2(NG2), one of the main inhibitory CSPGs, is upregulated following spinal cord injury. One possible treatment strategy for spinal cord injury is the use of lentiviral vector-mediated gene therapy. This study involved testing the efficacy, biological effect and functional effect of lentiviruses expressing Neutrophin-3 (NT3) and short-hairpin RNA against NG2 (shNG2) in vitro and in an ex vivo spinal cord slice culture model of transection injury. **Methodology:** Lentivirus expressing NT3, GFP control and shNG2 knockdown lentiviral vectors were constructed and validated. Five shRNAs were designed to target mouse NG2. The titer of each lentiviral vector was determined by examination of the increase in gag sequence within the target cell genome measured using quantitative real time PCR. To determine the biological effect of Lenti NT3, the level of NT3 protein in media taken from Lenti NT3 transduced cells was measured using ELISA and the functional effect was measured using a DRG neurite outgrowth assay. Neu7 cells were used in this study to investigate the ability of Lenti shNG2 to knockdown NG2 protein in vitro. To determine whether Lenti shNG2 can decrease NG2 levels, Neu7 cells were transduced with shNG2 lentiviral vector, selected with puromycin and the NG2 RNA and protein levels were examined. To examine the effect of Lenti NT3 and combination Lenti NT3/shNG2 on transected ex vivo spinal cord slices, control and transection injured longitudinal slices were prepared and transduced with lentiviral vectors expressing a non-targeting control, GFP control, NT3 and a combination of NT3/shNG2. The cellular environment of the lesion was examined using stereological analysis. The volume fraction (Vv) of immunohistochemical staining for NG2-positive cells, □III Tubulin-positive axons and GFP-positive cells was carried out. Each spinal cord slice was divided into three regions of interest: IZ, SZ and NSZ. **Results:** Both of lentivirus expressing NT3 and shNG2 show increased neurite outgrowth and produced NT3 protein in vitro. NT3 and shNG2 lentiviruses caused a significant increase in Vv of □III Tubulin-positive axons and reduced the Vv of NG2 proteoglycans in injured group in comparison to control group. **Discussion and Conclusions:** The findings suggest that both NT3 and shNG2 lentiviruses promote axonal regeneration and reduction of NG2 expression; they therefore may prove to be a useful tool to address the possible roles of these proteins in spinal cord injury.