In vivo transduction of the injured rat peripheral nerve by lentiviral vectors as a putative strategy to promote nerve regeneration

Tannemaat MR*/**, *Hendriks WT***, Eggers R**, Cramer SJ***, Hoeben RC***, Malessy MJA*, Boer GJ**, Verhaagen J**
*Department of Neurosurgery, Leiden University Medical Center, Leiden, **Department of neuroregeneration, Netherlands Institute of Brain Research, Amsterdam, ***Department of Molecular Cell Biology, Leiden University Medical Center, Leiden

Exogenously applied neurotrophic factors have the potential of enhancing regeneration of the peripheral nerve after transection. To exert a beneficial effect they must be applied locally, preferably distal to the lesion site and, most likely, for a prolonged period of time.
These conditions can be met through viral vector-mediated gene transfer, i.e., transduction of neural cells to produce neurotrophic factors. Therefore, we investigated whether it is possible to transduce Schwann cells in vivo by direct injection of lentiviral (LV) vector in several peripheral nerve lesion/repair rat models. LV vectors integrate their gene into the target cell DNA and were shown to give non-toxic long-term transgene expression in Schwann cell cultures.
LV-GFP, a vector coding for a green fluorescent protein as a marker for transduction, was injected in 1) intact sciatic nerve, 2) distal to a crush or 3) transection/resuture lesion of the sciatic nerve, and 4) in the avulsed/re-inserted ventral root of the spinal cord. Transgene expression was evaluated at 1, 2 and 4 weeks using fluorescence microscopy.
In all models, large numbers of GFP-fluorescent Schwann cells could be identified at 1 week, but expression of GFP clearly diminished at later time points, with expression being virtually absent at 4 weeks. At 1 week GFP-positive macrophages were found in some animals.
We hypothesized that direct in vivo injection leads to invasion of macrophages to the injection/lesion site and that either through apoptosis of transduced Schwann cells, or through direct transduction of macrophages, the foreign protein GFP is presented as an antigen, leading to an immune reaction against transduced cells.
To test this hypothesis, we obtained an LV vector coding for GFP with the Gly-Ala repeat domain of the Ebstein Barr nuclear antigen 1. This fusion protein is still fluorescent, but the Gly-Ala repeat prevents cytotoxic T-lymphocyte-epitope generation, creating a “stealth” gene product. The results of this ongoing experiment will be presented during the presentation.
In the future we plan to investigate the direct transduction of the injured nerve as well as the application of ex-vivo transduced Schwann cell implants that can bridge large defects of transected peripheral nerves, both using LV vectors for the overexpression of glial cell derived neurotrophic factor (GDNF) and nerve growth factor (NGF).

William Hendriks, Department of Neuroregeneration, Netherlands Institute of Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, e-mail w.hendriks@nih.knaw.nl

Speaker Session 19