Lentiviral Vector Treatment of the Inhibitory Spinal Cord Injury Environment in vitro and in vivo

E. Donnelly¹, N. Madigan¹,², G. Rooney², P. Strappe³, A. Windebank², T. O’Brien¹, S. McMahon⁴
¹REMEDI, Galway, Ireland, ²Mayo Clinic, Rochester MN, USA, ³Centre for Brain Research, Sydney, Australia, ⁴Dept of Anatomy, Galway, Ireland

Introduction

Following spinal cord injury (SCI), a highly inhibitory environment for axonal regeneration develops. One of the main sources of this inhibition is the glial scar which is formed after injury by reactive astrocytes. The inhibitory environment is mainly due to chondroitin sulphate proteoglycans (CSPGs). NG2, is one of the main inhibitory CSPGs. Studies have shown that NG2 is unregulated following SCI as early as 2 days. Neurotrophins are a family of proteins which play a major role in the development, survival and plasticity of neurons. Neurotrophin-3 (NT-3) is produced during embryonic development and is essential in the survival of sensory neurons. NT-3 has been seen to guild axons through the site of injury to the correct target and result in the formation of functional synapses. Due to its ability to transduce non dividing cells, Lentiviral vectors are a suitable vector system for a gene therapy approach to SCI.

Methods

Lentiviral vectors containing a shRNA to NG2 and a vector containing the gene for NT-3 were produced using a widely accepted transient transfection method. Sprague Dawley female rats were anesthetized and a laminectomy was preformed at T9. A contusion injury at 200kd was administered using an Infinite Horizon impactor. Lentiviral vector expressing NT-3, shRNA to NG2 or a combination of both vectors were directly injected into contused rat spinal cord one week post injury, 2 x 1ul injections were administered, rostral and caudal to the site of injury. Control animals received injections of PBSA or LVGFP. Animals were sacrificed six weeks post injection and examined by histology for changes in scar size and immunohistochemistry for changes in expression of NG2 and NT-3. Motor and sensory function was assessed using the Basso, Beattie and Bresnahan (BBB) locomotor scale, Von Frey filaments and Hargreaves apparatus.

Results

Sirius red staining show a reduction in scar size in the animals that received the combination of shNG2 and NT-3 treatments (Figure 1). These animals also showed a significant functional recovery, as assessed by the BBB locomotor scale (Figure 2). Also a reduction in the size of the glial scar was seen with the combination of shRNA for NG2 and NT-3. Preliminary results has shown the present of NT-3 around the injection site.

Discussion

The combination of NG2 knockdown and over expression of NT-3 can increase the locomotor improvement of contused rats. And the same treatment appears to reduce the size of the scar. This suggests that there is a link between the size of the scar and regeneration capacity. Though the total removal of the scar would not be desirable, as it has been shown that the scar is involved in the primary protection of surviving axons after injury. In vitro studies in this lab have shown that both NG2 knockdown and NT-3 overexpression, individually, can increase neurite growth in an inhibitory environment. This study shows the potential for gene therapy as a therapeutic for SCI.