INTRODUCTION

Spinal cord injuries are common neural disorders for old and young, especially in traumatology medicine. Although various approaches have been reported as possible treatments for spinal cord injury, the effective treatment has not been established in clinical practice.

Micro RNAs are 18-22 nucleotides RNAs in length and regulate gene expression at the mRNA level. They are responsible for the pathogenesis of human diseases. Several therapeutic trials to regulate the endogenous miRNAs related to various diseases have been conducted. MicroRNA-210 (miR-210) plays a crucial role in cell response to hypoxia, modulating cell survival, VEGF-driven endothelial cell migration, and the ability of endothelial cells to form capillary-like structures. VEGF would be beneficial for neuronal development, neuronal protection, and axonal growth. We focused on the association of neovascularization and neurogenesis, and hypothesized that miR-210 leads to be effective in the treatment of spinal cord injury. Here we report that transplantation of miR210 promotes regeneration following spinal cord injury.

METHODS

Mouse Spinal Cord Injury Models: we used female C57BL/6 mice, approximately 10 weeks of age. After laminectomy at the 10th thoracic spinal vertebrae with use an operating microscope. A contusive spinal cord injury was induced using an Infinite Horizon Impactor (70 kdyn; Precision Systems). One day after injury, 2.0 μl of miR-210 (100pM) per mouse was injected into the injured site of spinal cord (miR-210 group). As a negative control, the same amount of non-functional siRNA was administered in the same way (control group).

Behavioral testing: The recovery of hindlimb motor function was assessed using the Basso Mouse scale (BMS). Mice in all groups were assessed before injury and 1, 3, 5, 7, 14, 21, 28, 35 and 42 days after injury.

Electrophysiological recording: To assess functionality and recovery of descending pathways from the forebrain to the hindlimb motor neuron pool, transcranial electric motor evoked potentials (MEPs) were monitored in the hamstring muscles at 1, 7, 14, and 21 days after injury.

Real-time PCR analysis: The expressions of miR-210 in the injured spinal cord tissue was assessed by real-time PCR at 24 hours after injection.

Statistical analysis: Statistical analysis was performed using Mann-Whitney U test or repeated measures two-way ANOVA for group × time followed by Mann-Whitney U test.

RESULTS

Behavioral recovery after spinal cord injury

7 days after injury, the miR210 group could lift their trunks. On the other hand, the siRNA or miR140 groups were unable to support their body weight with their hindlimbs. The BMS score in the miR210 group was significantly higher than in the siRNA and the miR140 group at days 5 or later (Figure 1).

Figure 1: Time course of functional recovery of limbs assessed using Basso Mouse Scale (n=6/group)

Electrical recovery after spinal cord injury

The amplitude of MEP in the miR-210 group was significantly higher than that in the control group at days 7 or later.

Figure 2:

Expression of miR-210

The mean value for miR-210 expression was 1.0 ±0.1 (mean ± SD) in the control group, and 431.9 ±161.4 in the miR-210 group at 24 hours after injection (Figure 3).

Figure 3: Real time PCR analysis of miR-210 expression at 24 hours after the injection.

DISCUSSION

The findings in the current study demonstrate that administration of miR210 provides a novel therapeutic strategy for spinal cord injury.

The target genes for microRNAs are estimated to range between one and several hundred, based on target predictions using the bioinformatics approach. Therefore, many molecular networks via miR-210 might interact to promote spinal regeneration after a local injection. Several validated target genes of miR-210, which relate to spinal cord regeneration, have been reported, such as ephrin-A3 and neuronal pentraxin-1. Ephrin-A3 has a suppressive role not only in angiogenesis but also in neuronal axon guidance. Neuronal pentraxin-1 is also expressed in the brain, exclusively by neurons. It is a secreted glycoprotein that mediates hypoxic injuries in primary cortical neurons after their exposure to hypoxia.

In the current study, injected miR-210 might regulate these target genes to enhance spinal cord repair. Further work is underway to elucidate mechanisms for the enhancement of spinal cord regeneration through the regulation of these target genes. The current study showed the possibility of a new strategy for spinal cord repair regulating microRNA expressions in vivo.

REFERENCES