**Administration Of Microrna-210 Promotes Spinal Cord Regeneration**

Satoshi Ujigo, Naosuke Kamei, MD, PhD, Hadoush Hikmat, Yuki Fujioka, Shigeru Miyaki, Tomoyuki Nakasa, Nobuhiro Tanaka, Kazuyoshi Nakanishi, Toru Sunagawa, Mitsuo Ochi.

1Hiroshima University, Hiroshima, Japan, 2Hiroshima University, Hiroshima, Japan, 3Hiroshima Univ, Hiroshima city, Japan, 4Hiroshima University, Hiroshima City, Japan, 5Hiroshima University, Hiroshima City, Japan.


**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 neovascularization and neurogenesis, and hypothesized that miR-210 leads to be effective in the treatment of spinal cord injury. Here we report that administration 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, 1.0μl of miR-210 (100μM) with 1.0μl of a peptide transduction domain-double stranded RNA-binding domain as a carrier per mouse was injected into the injured site of spinal cord (miR210 group). As a negative control, the same amount of non-functional siRNA was administrated in the same way (control group).

Expression of miR-210: The expressions of miR-210 in the injured spinal cord were assessed by real-time PCR at 2, 3, 5, 7 and 14 days after injection. In situ Hybridization was also performed to confirm the distribution of the injected miR-210.

Immunohistochemistry analysis: We evaluate angiogenesis, gliosis, neurogenesis, axon growth and myelination with immunohistochemistry assay using CD31, GFAP, Nestin, 5HT and FluoroMyelin anti-body.

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.

Target gene analysis: Micro array assay was performed to narrow down the candidates of target gene with use of at day3 after injury. After that, the expression level of candidates of target gene was assessed to investigate the mechanism of regeneration.

Results: Expression of miR-210: The mean value for miR-210 expression was up-regulated compared with siRNA group from day2 to day7 after injury. The signals of miR-210 merged with CD31 positive blood vessels and GFAP positive astrocytes at the lesion site.

Immunohistochemistry: The area of CD31 positive vessels at day3, 5HT positive fiber and FluoroMyelin positive at day42 in miR210 group was greater than those of siRNA control group, respectively. The number of GFAP positive and Nestin positive cells at day 3 in miR210 group was more than those of siRNA control group. The area of GFAP negative and F4/80 positive after day7 and 14 in miR210 group was larger than those of siRNA control group (Fig 1).

Behavioral recovery after spinal cord injury: Seven days after injury, mice in the miR-210 group could lift their trunks. On the other hand, mice in the siRNA group were unable to support their body weight with their hindlimbs. The BMS score in the miR-210 group was significantly higher than in the siRNA at days 5 or later (Fig 2).

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.

Target genes of miR210: Ephrin-A3 and protein tyrosine phosphatase 1B were down-regulated after administration of miR210 in real-time PCR and Western blot assay (Fig 3). The expressions of WNT associated genes were greater or lesser in mice in the miR-210 group than those in the control in micro array assay.
Discussion:
Administration of miR-210 promoted angiogenesis and neuroprotection that led to spinal cord regeneration. Ephrin-A3 and protein tyrosine phosphatase 1B has been demonstrated to be potent angiogenesis inhibitors. Wnt pathway has been reported to be associated with neuroprotection. We suspected miR-210 promoted spinal cord repair via these genes acting.
In the current study, injected miR-210 might regulate these genes directly or indirectly to enhance spinal cord repair. Although further comprehensive studies are needed to determine their suitability as therapeutic agents, the current study showed that intradural injection of ds miR-210 into an injured spinal cord might be a novel drug target for treating SCI in humans.

Significance:
The current study showed the possibility of a new strategy for spinal cord repair regulating microRNA expressions in vivo.

Acknowledgments:

References:

Fig 1. Representative immunostaining for GFAP+ reactive astrocytes and F4/80+ cells
Fig 2. Time course of functional recovery of lower limbs

Fig 3. Evaluation of target genes of miR-210