Expression of microRNA-223 following spinal cord injury in mice.

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ABSTRACT
INTRODUCTION:
Spinal cord injury (SCI) provokes an inflammatory response and the recruitment of neutrophils and macrophages to the site of injury, following the primary mechanical insult. In inflammatory response, leukocytes play important role in SCI and greatly participate in the recovery. The recent discovery of microRNAs suggests a novel regulatory control over gene expression during plant and animal development. MicroRNAs are short noncoding RNAs that suppress the translation of target genes by binding to their microRNAs, and play a central role in gene regulation in health and disease. In the current study, microRNA-223 (miR-223) has been reported to relate to the inflammatory response, and take part in the differentiation adjustment of neutrophils.

We reported the time course of miR-223 expression after spinal cord injury in mice, and the high expression at 12 hours and 3 days after SCI. The purpose of this study was to reveal the distribution of miR-223 in injured spinal cord. We analyzed the existence of miR-223, neutrophil, and macrophage using real time PCR, immunohistochemistry, and in situ hybridization.

METHODS:
Mice of the C57BL/6 (wild-type), 8-12 weeks old, were subjected to moderate SCI. Control group was performed only laminectomy at T11. Spinal cord contusions were based on the methods of Faulkner, forceps with a spacer so that at maximal closure a 0.5 mm space remained, were used to compress the cord laterally from both sides for 10 seconds. And we analyzed following examination. 1) Motor activity in hind limb was assessed after 12 hours with the Basso, Beattie, and Bresnahan (BBB) open field locomotor test. 2) Spinal cord was divided to pieces that the segment was 2 mm in length, and taken up to 6 mm rostral and caudal to the lesion center. Each segment was examined the expression of miR-223 at 12h, 3 days after SCI using real time PCR. 3) The miRNA expression was also confirmed by in situ hybridization. The double staining of in situ hybridization and the immunohistochemistry at 12 hours and 3 days after SCI was examined and we compared the distribution of miR-223, Ly-6G, and CD68 positive cells.

RESULTS SECTION:
1) BBB score was 11/21 points on the average at 12 hours after SCI. 2) PCR revealed significant high expression within the range of the head caudal 2mm compared with another level at 12 hours after SCI (The SCI group: 0.53 on the average, control group: 0.17 on the average). There was no significant difference within the range of the head caudal 2–6mm at 12 hours after SCI (The SCI group: 0.18 on the average, control group: 0.16 on the average).

3) In situ hybridization demonstrated the presence of the miR-223 positive cell in the gray matter of spinal cord. The signal accumulated at the epicenter (the head caudal 2mm). Immunohistochemistry revealed the accumulation of the Ly-6G positive cell in the epicenter at 12 hours and 3 days after SCI, and appearance was not admitted excluding the lesion center. The appearance of the CD68 positive cell which was the marker of the macrophage was confirmed at 3 days after SCI.

DISCUSSION:
Our results indicate the expression pattern of miR-223, and Ly-6G positive cells in a mouse model of SCI. The signal of these makers accumulated within the range of the head caudal 2mm around epicenter after SCI. MiR-223 was reported that it took part in the differentiation adjustment of the neutrophils, therefore, this study suggested that neutrophils were accumulated rapidly in lesion center after SCI in a mouse model and were also regulated by miR-223.