Circulating MicroRNA Expression As A Biomarker For Early Diagnosis Of Severity In Spinal Cord Injury

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Disclosures:

Introduction: Spinal cord injury (SCI) has difficulty determining the baseline severity of the injury because of unstable condition of patients including the phenomenon of spinal shock in the acute phase. This is an obstacle to the development of new treatment for spinal cord injury during the acute phase. In this study, we focused on microRNAs (miRNAs) as a candidate biomarker to predict the prognosis of patients with SCI. MiRNAs are expressed endogenously and play an important part in regulating gene expression(1).

The recent discoveries of extracellular and circulating miRNAs have been focused new biomarker regarding their potential in modulating signaling not only within cells but also between cells(2). MiRNAs are simple to measure using real-time PCR with high sensitivity. In addition, miRNAs have the advantage that these are stabilized by being contained within exosomes and microvesicles, and are unaffected of the blood-spinal cord barrier because of their small size. We evaluated the expression of miRNAs in the serum of mice after SCI, and selected candidate extracellular miRNAs for novel biomarkers of SCI.

Methods: Mouse Spinal Cord Injury Models: Female C57BL/6 mice, approximately 10 weeks of age, were used. Laminectomy was carried out at the 10th thoracic spinal vertebrae with use an operating microscope. A contusive spinal cord injury was induced using an Infinite Horizon Impactor. Severe model of mice were made at the T10 level with 70 kdyn compressing power. Mild model of mice were made with 50 kdyn compressing power. In the sham group, mice underwent a laminectomy without SCI.

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.

Collect samples: Blood samples were collected from the heart at 12h after injury. The spinal cords (the injured site and 5mm on either side) were removed during harvested for blood sample.

RNA Isolation: Total RNA of serum samples were isolated from 200 ml collected using the mirVana PARIS kit (Ambion). C. elegans spiked-in oligonucleotides (cel-miR-39) were introduced, which were used for normalization of variability in RNA isolation. Total RNA of spinal cords were extracted from spinal cords using TRIzol regent (Invitrogen).

MicroRNA microarray:
① A miRNA microarray (Invitrogen), containing probes for the complete Sanger mirBASE 9.0, was used to screen RNA from spinal cords in both the severe model and sham model harvested 12 h after injury or laminectomy, respectively.
② Toray 3D array system was used to screen RNA from serum samples in 3 groups harvested 12 h after injury or laminectomy, respectively. miRNA expression profile analysis: Based on the result of the microarray expression data, expression level of candidate for the biomarker was assessed by real-time PCR.

Statistical analysis: Holm-Bonferroni method was used for statistical analysis. Values for p of < .05 were considered statistically significant.

Results: Behavioral recovery after spinal cord injury: A significant improvement was seen in mild model group compared with sever model group at 12 hour or later after
Figure 1: Time course of functional recovery of hindlimb assessed using Basso Mouse Scale. Changes in expression of microarray analysis: Using a miRNA-based array screening, we identified 4 differentially expressed miRNAs (miR-1, -133a, -133b, -451) in the SCI group compared with the sham group. Changes in expression of real-time PCR analysis: The expression of mir-1 and mir-451 from spinal cords were significantly increased in the following order, severe group, mild group, sham group, whereas mir-133b was significantly decreased in the following order, severe group, mild group, sham group.
Figure 2; Real time PCR analysis of miR-1, -133a, -133b, -451 from spinal cords expression at 12 hour injury. In the serum samples, the expression of mir-451 in the severe group was significantly greater than that in the sham group. In the severe group, the values for mir-133b expression were significantly less than those in the sham group and the mild
groups.


discussion: In the tissue of spinal cords, the expression of mir-133b and mir-451 were significantly difference correlated with severity of injury. We considered serum level of miR-133b and mir-451 have the potential of a candidate for the biomarker and may be used to conveniently monitor neuronal damage and functional prognosis, because the expression of miR-133b and mir-451 from serum samples indicated a similar tendency with the tissue samples. The combinatorial assay of miR-133b and mir-451 might be useful for the prognosis prediction of SCI.

significance: Circulating miRNAs in the serum are potential candidates for biomarkers to predict the prognosis of patients with SCI.

acknowledgments:

2) Xi Chen et.al. Trends in Cell Biology. March 2011

figure 3; Real time PCR analysis of miR-1, -133a, -133b, -451 from the serum samples expression at 12 hour after injury.