College of Veterinary Medicine
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Identification of miRNA Biomarkers Associated with Severity of Spinal Cord Injury

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Introduction

- Acute traumatic spinal cord injuries (SCI) are a type of neurological condition involving mixed compressive and contusive damage to neural tissue which typically require surgical intervention as a basis of treatment.
- Circulating miRNAs are small non-coding RNAs involved in post-transcriptional gene regulation that may also play a role in the physiological/pathological response to spinal cord injury through stimulation of inflammation, apoptosis, axonal regeneration, and glial scar formation.
- Currently, there are few diagnostic biomarkers available to establish therapeutic strategies for patients with SCI.

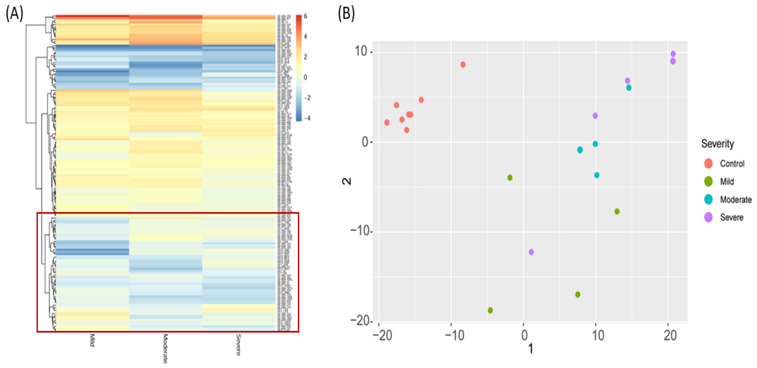


Figure 1. (A) Total sample heat map of miRNA expression demonstrating a range of miRNAs that are consistently upregulated, downregulated, or unaffected regardless of injury severity as well as a range of miRNAs that exhibit variation in expression in patients with acute SCIs *(indicated with red box)*. (B) Multi-dimensional scaling (MDS) of miRNA candidates demonstrating distinguishable expression patterns between healthy controls and patients with SCIs.

- The results of the study will determine if there is a significant up-regulation or down-regulation of specific miRNAs that occurs in patients with acute SCIs compared to healthy patients without SCIs.
- This would ideally potentiate the use of miRNA biomarkers as a diagnostic tool for distinguishing the severity of SCI which would inform the therapeutic approach and help predict the long-term clinical outcome of treatment.

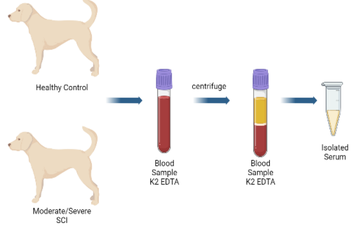
Hypothesis

The increased or decreased expression of specific miRNA candidates within circulation will reflect the severity of spinal cord injury in canine patients.

Methods

1. Plasma/Serum Collection

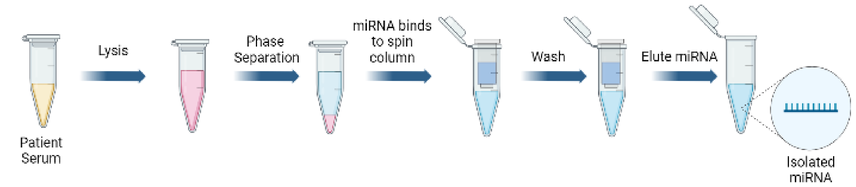
- Blood samples were collected from patients with moderate (Grade 4) SCI and severe (Grade 5) SCI as well as healthy controls.
- Samples were centrifuged to collect the serum component which was used for miRNA isolation.



Methods

2. miRNA Isolation/Purification

Purification of the total RNA, including miRNAs, from the serum samples was conducted to allow for comparison of miRNA expression levels with control miRNAs.



3. Amplification and Quantification of miRNA Candidates

cDNA was first synthesized from the miRNA isolated from patient serum samples Real-Time PCR was then performed to quantify a range of miRNA candidates that were expected to be uniquely enriched in either severe or moderate SCIs (Table 1).

Table 1. Target miRNA candidates selected for use as qRT-PCR primers

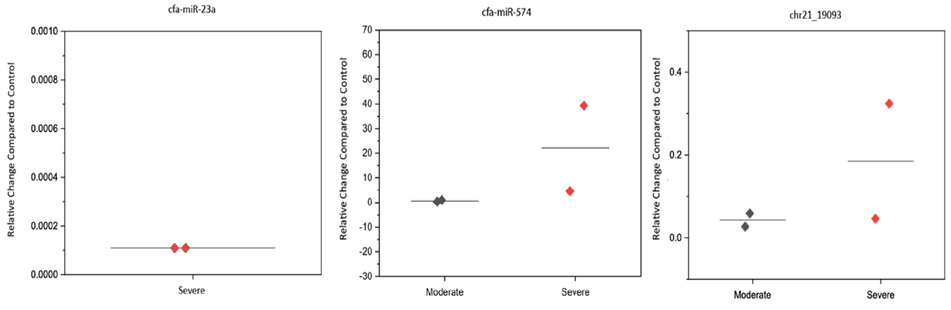
Primer	Sequence	Expected Result
cfa-miR-23a	cgcagatcacattgcca	up-regulated in severe only
cfa-miR-23a	tccagttttttttttttttttaaatccct	
cfa-miR-574	acgctcatgcacacac	up-regulated in severe only
cfa-miR-574	gtccagttttttttttttttgtgg	
chr21_19093	cagatcgaggctagagtca	up-regulated in severe only
chr21_19093	tccagttttttttttttttaagcgt	
cfa-miR-195	cgcagtagcagcacaga	up-regulated in moderate only
cfa-miR-195	tccagttttttttttttttgcca	
cfa-let-7e	gcagtgaggtaggaggttg	up-regulated in moderate only
cfa-let-7e	ggtccagttttttttttttttaactatac	

Results

Delta-Delta Ct Analysis of qRT-PCR Results

The relative fold gene expression of each of the miRNA candidates within the patient samples was determined using the delta-delta Ct method. Two samples were derived from moderate SCI patients and two samples were derived from severe SCI patients. A total of three healthy controls were used for comparison.

A. miRNA candidates expected to be enriched in severe SCI



B. miRNA candidates expected to be enriched in moderate SCI

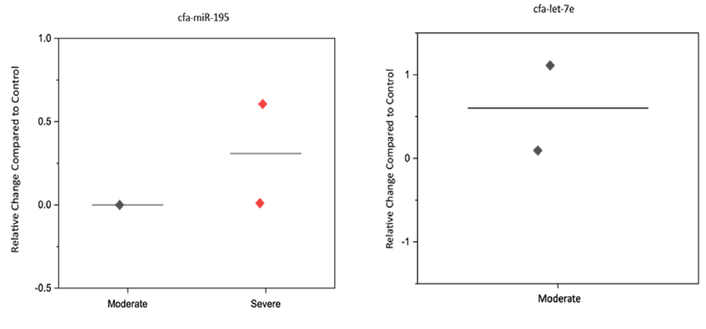


Figure 2. Selected miRNA candidates that were expected to be enriched in severe SCI (A) and miRNA candidates that were expected to be enriched in moderate SCI (B) were quantified in terms of relative gene fold expression. These values were then compared across patients with moderate SCI and severe SCI to a healthy control.

Summary and Conclusions

- Protocols for the collection of blood/serum and for the isolation of miRNA were optimized to enhance the quality of the sample and improve amplification potential.
- The miRNA candidates, cfa-miR-574 and chr21_19093, were enriched only in patients with severe SCI which supports our hypothesis. This suggests that there may be distinguishable expression patterns of miRNA candidates between moderate and severe cases that will allow for an accurate diagnosis of injury severity.

Future Directions

- Continued profiling of miRNA samples from SCI patients will be needed to validate the observed trends and determine their potential as diagnostic biomarkers capable of predicting long-term clinical outcomes.
- Understanding the physiological/pathological mechanism underlying the expression of specific miRNAs in response to SCI may also allow for them to be used as focused therapeutic targets.

Acknowledgments

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