

Spinal Cord Injury: A Study Protocol for a Systematic Review and Meta-analysis of microRNA Alterations

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Protocol

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Abstract

Spinal cord injury (SCI) is a condition often resulting in life-long disability, high rehabilitation costs, lost wages, and reduced quality of life. Clinical trials have been hampered in part by a lack of poor diagnostic and prognostic markers of injury severity and neurologic recovery. Furthermore, while many therapies have shown promise in preclinical animal models, there are currently no neurorestorative treatments for SCI. The development of objective biomarkers and novel therapies for SCI represent urgent unmet clinical needs. Biological markers of SCI that objectively stratify the severity of cord damage could greatly expand the depth and scope of clinical trials and represent targets for the development of novel therapies for acute SCI. MicroRNAs (miRNAs) represent promising candidates both as informative molecules of injury severity and recovery, and as therapeutic targets. miRNAs are small, stable, regulatory RNA molecules that are often tissue-specific and evolutionarily conserved across species. miRNAs could represent powerful predictors of pathology, particularly with respect to neurologic disorders. There is great heterogeneity in the current literature describing miRNA changes after SCI with respect to animal species, SCI model, miRNA detection technology, and normalization strategies. Here, we present a protocol to perform a systematic review and meta-analysis to investigate the conserved inter- and intra-species miRNA changes that occur post-SCI and provide a comprehensive resource for the SCI community.

Background

In the last four decades, improvements in medical, surgical, and rehabilitative care have increased the quality of life and extended the life expectancy of individuals with SCI – once considered an imminently fatal condition [1]. However, while management has improved, there are no therapies for SCI that have demonstrated convincing neurologic benefit in large-scale clinical trials [2-6]. There are, therefore, urgent unmet needs for the preclinical scientific development of novel therapeutic strategies and for the subsequent clinical validation of these treatments in human trials.

miRNAs hold great promise to underlie novel treatment strategies for SCI. miRNAs are small (22 nucleotide), noncoding RNAs that regulate at least 30% of all protein coding genes [7]. Many miRNAs have been identified in the central nervous system (CNS) [8-11], with several showing CNS-specific expression [12]. Using microarray analysis, real-time PCR, and *in-situ* hybridization, Bak et al. found 44 miRNAs that were more than threefold enriched in the brain or spinal cord [8] and Liu et al. found that nearly 80% of detected miRNAs were expressed in the adult rat spinal cord [13]. miRNAs also show cell type-specific expression in the CNS, with specific miRNAs being expressed in neurons [12, 14], astrocytes [15, 16], and oligodendrocytes [17]. We have shown that miRNAs are required for the development and possibly the evolution of the corticospinal system [18], injury to which results in paralysis in SCI [19].

We and others have also shown that miRNA levels in cerebrospinal fluid (CSF) and blood serum are specifically altered in SCI in a severity-dependent fashion, both in humans [20], and in animal models [13, 21]. miRNAs are furthermore differentially altered in chronic SCI patients undergoing active exercise regimes, compared to sedentary patients [22]. miRNAs are promising biomarkers of injury severity, and by

implication – recovery and response to treatment [23-25] due to their regional abundance, specific developmental requirements, and altered levels following SCI. miRNAs represent promising therapeutic targets for CNS injuries and disease, given their intimate involvement in the development and injury of the relevant circuitry, their ability to cross barriers and membranes, and their potential for rapid transition from bench to bedside [26, 27].

To inform the next stage of miRNA biomarker and preclinical therapeutic interventions, a comprehensive and systematic understanding of the disparate existing data would allow development of unified models and testable hypotheses. There has not been a meta-analysis of miRNA changes in the setting of SCI that includes the results of recent comprehensive human trials. Here, we present a protocol outlining our approach to a meta-analysis of the current literature, in which we will provide a comprehensive review, interpretation of results, and discussion of the methodology used to document, quantify, and analyze miRNA alterations in the setting of SCI.

Methods/design

Protocol registration and standard reporting

For the preparation and development of this protocol, we followed the checklist provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA) [see Additional file 1].

Research Question

We will conduct a systematic review and meta-analysis of studies evaluating miRNA alterations following SCI. The specific research questions we aim to answer with the meta-analysis presented here are:

1. Is SCI associated with a specific pattern of miRNA expression that is conserved across species?
2. Is SCI associated with a tissue- or cell type-specific pattern of miRNA expression?
3. Which SCI-associated miRNA alterations are consistent across, and which are unique to, specific injury models?

Information sources and search strategy

Studies investigating miRNA alterations in all species of animal models of SCI and human studies will be identified from PubMed, Embase, and Scopus (including and up to 12/04/2020). Our search strategy was developed in consultation with an expert librarian and information specialist in systematic reviews.

Our PubMed search strategy is as follows: "MicroRNAs"[Mesh] or "RNA, Untranslated"[Mesh] OR "untranslated rna"/exp OR "microrna*"[tw] OR "mirna*"[tw] OR "micro rna*"[tw] OR "mir"[tw] OR "non coding rna*"[tw] OR "ncrna*"[tw] OR "non protein coding rna*"[tw] OR "noncoding rna*"[tw] OR "untranslated rna*"[tw] OR "nprna*"[tw] OR "non translated rna*"[tw] OR "non peptide coding rna*"[tw] AND "Spinal Cord Injuries"[Mesh] or "Spinal Cord Trauma*"[tw] or "Traumatic Myelopathy*"[tw] or "Spinal Cord Injur*"[tw] or

"Spinal Cord Transection*"[tw] or "Spinal Cord Laceration*"[tw] or "Post-Traumatic Myelopathy*"[tw] or "Spinal Cord Contusion*"[tw].

Our Embase search strategy is as follows: 'microrna'/exp OR 'untranslated rna'/exp OR 'microrna*':ti,ab,kw OR 'mirna*':ti,ab,kw OR 'micro rna*':ti,ab,kw OR 'mir':ti,ab,kw OR 'non-coding rna*':ti,ab,kw OR ncRNA*':ti,ab,kw OR 'non protein coding rna*':ti,ab,kw OR 'noncoding rna*':ti,ab,kw OR 'untranslated rna*':ti,ab,kw OR 'npcrna*':ti,ab,kw OR 'non translated rna*':ti,ab,kw OR 'non peptide coding rna*':ti,ab,kw AND 'spinal cord injuries'/exp OR 'spinal cord trauma*':ti,ab,kw or 'traumatic myelopathy*':ti,ab,kw OR 'spinal cord injur*':ti,ab,kw OR 'spinal cord transection*':ti,ab,kw OR 'spinal cord laceration*':ti,ab,kw OR 'post-traumatic myelopathy*':ti,ab,kw OR 'spinal cord contusion*':ti,ab,kw.

Our Scopus search strategy is as follows: (TITLE-ABS-KEY ("spinal cord trauma*" OR "traumatic myelopathy*" OR "spinal cord injur*" OR "spinal cord transection*" OR "spinal cord laceration*" OR "post-traumatic myelopathy*" OR "spinal cord contusion*")) AND (TITLE-ABS-KEY ("microrna*" OR "mirna*" OR "micro rna*" OR "mir" OR "non-coding rna*" OR ncRNA* OR "non protein coding rna*" OR "noncoding rna*" OR "untranslated rna*" OR "npcrna*" OR "non translated rna*" OR "non peptide coding rna*")).

Eligibility criteria

The inclusion criteria for this study will be as follows: 1. Studies published anytime, 2. Including all species, and sexes with SCI, 3. Relating to the alteration of miRNA after SCI, 4. Including miRNA alterations in tissues, such as spinal cord, serum/plasma, and/or CSF, and 5. Studies with a control group. The exclusion criteria for this study will be as follows: 1. Studies not relating to SCI, 2. Non-English articles, 3. Studies lacking sufficient data, 4. *In vitro*, *ex vivo*, in silico studies, 5. Non peer-reviewed articles and conference abstracts, 6. Studies focused solely on differential expression of miRNA in chronic SCI, and 7. Case reports or case series.

Study selection

All original studies will be eligible for inclusion. Two reviewers will screen the imported studies using Covidence [28], reading title and abstract, and exclude studies that do not meet the inclusion criteria. At the selection phase, two reviewers will independently include studies that meet all eligibility criteria. Any discrepancies will be resolved by consensus. The results of study selection will be presented using a PRISMA flow diagram.

Inclusion and exclusion criteria

Animals/population:

All species and sexes of animal models and studies including human patients will be included in this analysis. Any *in vitro* studies, *ex vivo* studies, *in silico* studies, and polytrauma studies that do not include *in vivo* analysis will not be included.

Intervention/exposures:

All studies including animal models of SCI (including, but not limited to: contusion, transection, traumatic, ischemic reperfusion) and human studies of SCI will be included. Any study of CNS injury not including the spinal cord will not be included.

Comparator/group

All studies with the inclusion of a comparator or control group (group without SCI) will be included. Any study without a control group will not be included.

Outcome measures

All studies including the detection of miRNAs and their alteration, in spinal cord parenchyma, blood, plasma/serum, or CSF, in response to SCI and within 7 days will be included. Any study that does not report the direction of change of miRNA in response to SCI will not be included.

Prioritized list of exclusion criteria

1. Not an original study (ie: review, meta-analysis, case report) or not peer-reviewed (ie: conference abstracts)
2. Not relating to SCI
3. *In vitro*, *ex vivo*, or *in silico* study
4. Not including a control group
5. Not reporting direction of change of miRNA post-SCI

Data extraction and data items

Data will be extracted by two reviewers. Data parameters collected will include but will not be limited to: 1. Author/year, 2. Study title, 3. Study model, 4. Study subjects (species, sex, sample size), 5. SCI model (contusion, transection, ischemic-reperfusion), 6. miRNA detection platform (RT-PCR, microarray, sequencing), 7. miRNA direction and/or magnitude of change, 8. miRNA target (predicted and/or validated), 9. Reported p-value and/or q-value of each miRNA change, and 10. Risk of bias. When values are not explicitly provided, the extraction of statistical data from graphs will be performed using the graphical data extraction application, WebPlotDigitizer (Version 4.4) [29].

Bibliographic information

Information including PubMed ID, Embase ID, authors, and year of publication will be collected.

Subject characteristics

Information including species, sex, and age will be collected. If numerical values are presented as an interval, then the mean value will be calculated and reported instead.

Injury parameters

Information including the injury model (contusion, transection, etc.), injury severity, injury location (cervical, lumbar, thoracic), and timepoint of sample collections will be collected. We will interpret and categorize injury severity as mild or severe.

Differential miRNA expression

Information including the miRNA extraction method, library generation, and detection platform will be collected. Changes in miRNA expression will be recorded as up- or down-regulated following SCI. Where fold-change, relative change, or other quantitative descriptions of changes are described, these data will be included.

Data analysis and synthesis

A narrative synthesis will be conducted to describe the studies in terms of initial results and methodological quality of primary literature. A meta-analysis will be performed for all outcome measures. If meta-analysis is not possible, data will be reported through a descriptive summary. Our primary analysis will apply a random effects meta-regression, which allows for within-study and between-study variability. The covariates will include, but will not be limited to: species, sex, injury model, injury level (cervical/thoracic/lumbar), injury severity, tissue type, timepoint, miRNA detection platform, and normalization strategy. To integrate studies where only the fold-change and p-value are available, we will additionally apply a p-value based meta-analysis using an inverse normal method, allowing us to use the data from as many studies as possible. The method will take into account of the direction of effect, the magnitude of signal, and the sample size. We will use I^2 to quantify between study heterogeneity. The advantages and disadvantages of the respective study designs (injury models, miRNA detection platforms, normalization strategies, etc) will also be discussed. Whenever a control group serves more than one experimental group, we will correct the total number of control animals in the meta-analysis by dividing the number of animals in the control group by the number of treatment groups served. Where applicable, Holm-Bonferroni correction for testing multiple subgroup analyses will be performed. If one or more subgroup analyses cannot be performed due to insufficient data, the p-value will be adjusted accordingly.

Risk of bias

The use of SYRCLE's risk of bias tool and the CAMARADES checklist for study quality will be included to assess risk of bias and study quality by two reviewers. Any disagreement will be resolved by consensus. Additionally, publication bias will be visually inspected with funnel plots.

Discussion

miRNAs represent promising molecules to address two primary goals: developing diagnostic and prognostic tools for SCI and creating novel therapeutics. The mechanisms involved in the pathogenesis of SCI include a primary mechanical injury (impact) and a secondary injury induced by multiple subsequent biological processes, including a local inflammatory response, cytotoxicity, apoptosis, and demyelination [30, 31]. In addition to local inflammation, a systemic inflammatory response, inducing organ damage, has been shown to occur following SCI [32]. Although altered gene expression significantly contributes to the pathogenesis of secondary SCI [31], the regulatory networks that control it are not well understood. One aspect of the complex nature of secondary SCI could relate to gene regulation by miRNAs [16, 33-35]. As potential biomarkers of a pathological state, miRNAs are not constrained by cell membranes and communicate in extracellular fluids as free-floating miRNA [36] or within exosomes [37], and are considered stable, with relatively long half-lives of greater than 24 hours [38] - as such, miRNA are appealing candidates for monitoring CNS pathophysiology related to SCI.

This is a critical juncture in the nascent field of miRNA biology in SCI, at which a synthesis and rigorous objective analysis of the collective data amassed over the last two decades with respect to miRNA changes after SCI will significantly inform future investigations. A systematic review and meta-analysis will be relevant to the SCI community and provide a comprehensive resource of robust miRNA changes across a spectrum of SCI paradigms with various injury models, species, tissues, and time points. This systematic review and meta-analysis will seek to serve as a resource by establishing a thorough, rigorous, and unbiased understanding of CNS miRNA and the target pathways regulated by these miRNAs for the next generation of biomarkers and therapeutic interventions.

Strengths And Limitations

Here we propose a systematic approach to search for articles in three major databases (PubMed, Embase, and Scopus), in which screening for eligible articles will be performed by two independent reviewers. This review will provide a comprehensive analysis of miRNA changes following SCI and assist researchers and clinicians alike in the pursuit of biomarkers and novel therapeutics for SCI. The main limitations of this study will be the exclusion of articles not indexed in PubMed, Embase, or Scopus, and of those articles that might not include relevant keywords or phrases used in this search.

Abbreviations

CNS: Central nervous system

CSF: Cerebrospinal fluid

miRNA: microRNA

SCI: Spinal cord injury

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated and/or analyzed in this study will be included in the final published article and its supplementary files.

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

ST, ZH, and ST initiated the study, designed the protocol, and wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1

PubMed Query	Embase Query	Scopus Query
<p>"MicroRNAs"[Mesh] or "RNA, Untranslated"[Mesh] OR "untranslated rna"/exp OR "microrna*"[tw] OR "mirna*"[tw] OR "micro rna*"[tw] OR "mir"[tw] OR "non coding rna*"[tw] OR "ncrna*"[tw] OR "non protein coding rna*"[tw] OR "noncoding rna*"[tw] OR "untranslated rna*"[tw] or "npcrna*"[tw] or "non translated rna*"[tw] or "non peptide coding rna*"[tw]</p>	<p>'microrna'/exp OR 'untranslated rna'/exp OR 'microrna*':ti,ab,kw OR 'mirna*':ti,ab,kw OR 'micro rna*':ti,ab,kw OR 'mir':ti,ab,kw OR 'non-coding rna*':ti,ab,kw OR 'ncrna*':ti,ab,kw OR 'non protein coding rna*':ti,ab,kw OR 'noncoding rna*':ti,ab,kw OR 'untranslated rna*':ti,ab,kw OR 'npcrna*':ti,ab,kw OR 'non translated rna*':ti,ab,kw OR 'non peptide coding rna*':ti,ab,kw</p>	<p>(TITLE-ABS-KEY ("spinal cord trauma*" OR "traumatic myelopath*" OR "spinal cord injur*" OR "spinal cord transection*" OR "spinal cord laceration*" OR "post-traumatic myelopath*" OR "spinal cord contusion*")) AND (TITLE-ABS-KEY ("microrna*" OR "mirna*" OR "micro rna*" OR "mir" OR "non-coding rna*" OR "ncrna*" OR "non protein coding rna*" OR "noncoding rna*" OR "untranslated rna*" OR "npcrna*" OR "non translated rna*" OR "non peptide coding rna*"))</p>
AND		
<p>"Spinal Cord Injuries" [Mesh] or "Spinal Cord Trauma*"[tw] or "Traumatic Myelopath*" [tw] or "Spinal Cord Injur*" [tw] or "Spinal Cord Transection*"[tw] or "Spinal Cord Laceration*" [tw] or "Post-Traumatic Myelopath*"[tw] or "Spinal Cord Contusion*"[tw]</p>	<p>'spinal cord injuries'/exp OR 'spinal cord trauma*':ti,ab,kw or 'traumatic myelopath*':ti,ab,kw OR 'spinal cord injur*':ti,ab,kw OR 'spinal cord transection*':ti,ab,kw OR 'spinal cord laceration*':ti,ab,kw OR 'post-traumatic myelopath*':ti,ab,kw OR 'spinal cord contusion*':ti,ab,kw</p>	

Supplementary Files

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