Targeting Fibrotic Scar Formation with MicroRNA-133b: A Novel Therapeutic Strategy for Traumatic Brain Injury

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Introduction

Traumatic brain injury (TBI) triggers lesion scar formation that prevents neuronal re-growth and reconnection with target cells, resulting in loss of function. Inhibiting fibrous scar formation could enhance functional recovery. MicroRNAs (miRs) are small noncoding RNAs that regulate gene expression by binding target mRNAs and initiating translational repression or mRNA degradation. Our prior work in a spinal cord injury mouse model showed that miR133b administration (IV, IN) specifically targets fibrotic scarring with improved motor outcomes (1-3). Targeting fibrotic scar formation through inhibition of extracellular matrix components could be an effective way of promoting healing and functional recovery following an TBI.

Methods

Cortical Contusion Injury (CCI): Under isoflurane, the C57/BL6 mouse's head is fixed in a stereotaxic frame; the galea is incised/reflected and a 3.5 mm craniotomy made over right sensorimotor cortex via stereotax-mounted drill, opened with a microrongeur. Body temperature is held at 37°C. Injury is delivered with a Leica Impact One Stereotaxic Impactor (5.25 m/sec, 3.5-mm tip, 0.1 sec dwell, 1.5 mm depth, perpendicular) (Fig. 1). Galea/skin are sutured closed. Shams receive incision only, no craniotomy/impact.

Intravenous (IV) tail vein injections of either a) miR-133b (mmu-miR-133b-5p mimic; *Dharmacon, Cat No. C-311305-00*) complexed with mouse Argonaute 2 (Ago2; *Sino Biological Inc., Cat No. 50683-M07B*) or b) miR-Negative Control (mmu-miR-Negative Control, NC-miR, *Ambion Cat No. 4464058*) complexed with Ago2. The miR and Ago2 were dissolved in saline solution and administered in a total injection volume of 20µL/mouse. Final doses were 10-20µmol/L (miR-133b), 20µmol/L (NC-miR) and 0.1–0.2µmol/L (Ago2).

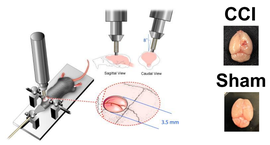

Fig. 1: CCI experimental setup. https://www.sciencedirect.com/science/article/pii/S0165027006004559

miRNA extraction and expression: miR was extracted from brain tissue using mirVana miRNA Isolation Kit (Invitrogen) and copied using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosys.). Gene expression was assessed by qPCR using TaqMan Universal Master Mix II (Life Tech.), TaqMan assay for miR133b, and Taq Man Control miRNA assay for U6 snRNA (Applied Biosys.). U6 snRNA was used as an internal control for normalization of polymerase chain reaction (PCR) products.

Behavioral studies: Effectiveness of miR-133b/Ago2 treatment in the CCI model were determined at 8 weeks post-injury. Tests included Open field behavior, grip strength, horizontal beam walking, grid walking, and novel object recognition.

Results

Endogenous miR-133b Levels Decrease after CCI

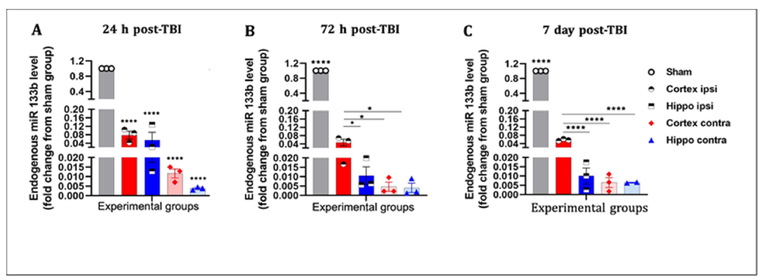

Fig. 2: Endogenous brain miR-133b levels following cortical contusion injury (CCI)

miR-133b (IV) Reduces Astrocyte Activation After CCI

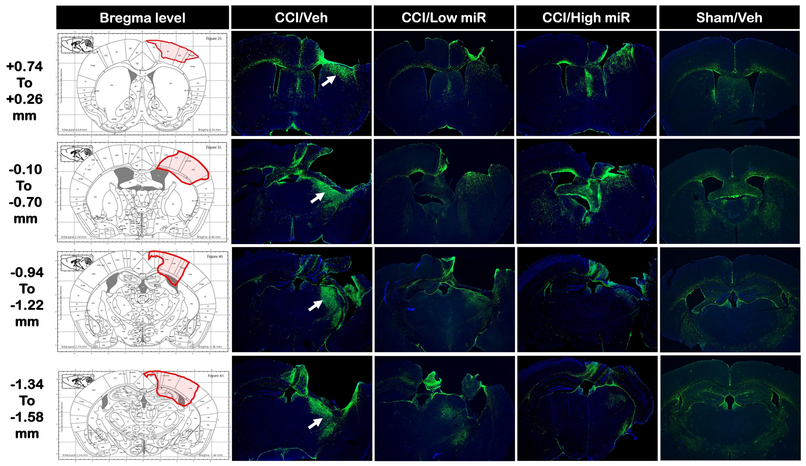

Fig. 3: Representative images of the brain sections immunolabeled for glial fibrillary astrocytic protein (GFAP), a marker of reactive astrocytes (astrogliosis) 2 weeks post-CCI.

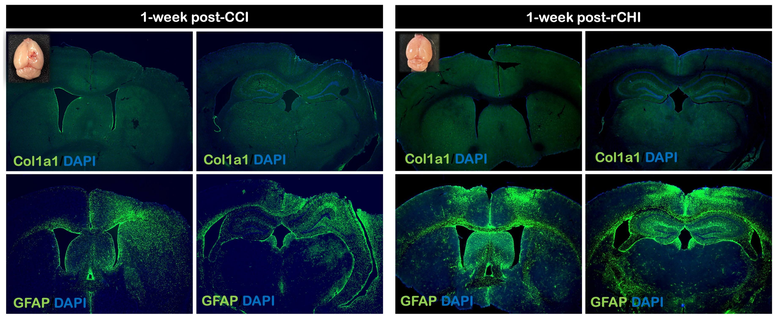

Fig. 4: Absence of Cerebral Collagen 1 (Col1a1) immunostaining 1-week following CCI (left panel) and in a separate set of pilot studies using a repeat closed head injury model rCHI* (right panel), with marked GFAP expression in both injury models.

**concussive impacts on days 0, 2, 4, 6, and 8 (3.0 m/s velocity, 3.5-mm tip, 0.2-s dwell, 1.0-mm depth)*

Improved CCI-related motor deficits after miR-133b (IV)

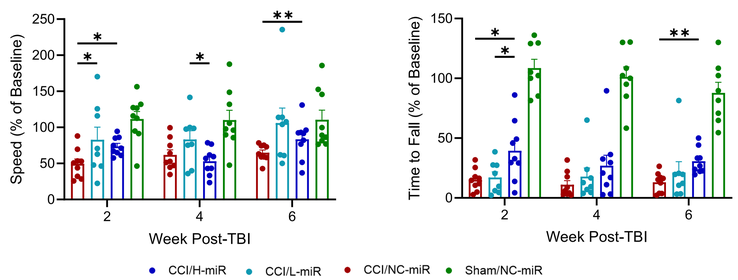

Fig. 5: Round Beam Crossing 2-Way ANOVA with repeated measure:
Group effect, P = 0.01;
Time effect, P = 0.0004;
*, Fisher's LSD test (uncorrected)

Fig. 6: Grip strength. 2-Way ANOVA with repeated measure:
Group effect, P < 0.0001;
Time effect, P = 0.13;
*, Fisher's LSD test (uncorrected)
(NC-miR = scrambled RNA)

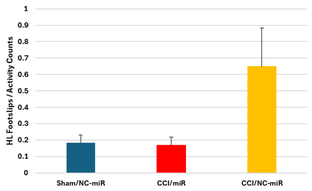

Fig. 7: Hindlimb footslips during gridwalk. Sham/NC-miR (n=7), CCI/miR (n=10), CCI/negative control-miR (n=4). Footslips were normalized by locomotor activity counts over 5 min. (NC-miR = scrambled RNA)

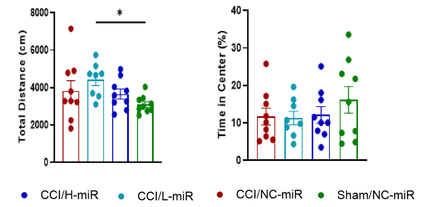

Fig 8. Open Field: Lesioned compared to nonlesioned mice (CCI/NC-miR vs. Sham/NC-miR) showed significant but mild hyper-activity, but no effect of miR-133b over the 20min recording period. One-Way ANOVA, P = 0.09; *, Fisher's LSD test (uncorrected) One-Way ANOVA, P = 0.52

miR-133b (IV) improves working memory after CCI

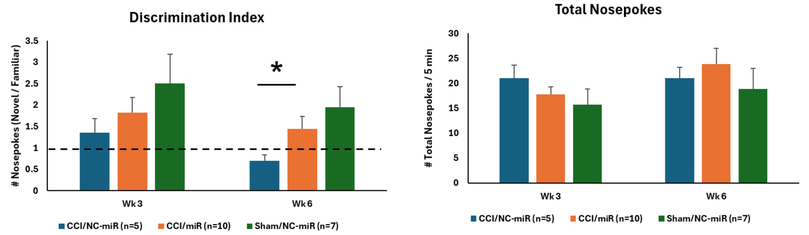

Fig 9. Novel object recognition (NOR) test: Depicted are group averages (+/- SEM) of the discrimination index, the ratio of between nosepokes to the novel object compared to the familiar object over 5 minutes. * P< 0.05 CCI/NC-miR vs. CCI/miR. (NC-miR = scrambled RNA)

Conclusions

This is the first study applying miR therapy targeting recovery from TBI. miR133b administration improved behavioral deficits following CCI, with evidence of an attenuation in lesion-associated astrogliosis. Surprising, and different from our results in spinal cord injury, was the lack of miR-133b effect in the brain on col1a, a molecular marker of fibrosis. Future work will examine changes in target gene expression in astrocytes, microglia, macrophages, and perivascular fibroblasts by single-cell RNA sequencing analysis. We will include screening for inflammatory, apoptotic, as well as neuroplastic markers, as we suspect that miR133b may have alternate mechanisms in injury recovery outside of effects on tissue fibrosis.

Selected References

1. Danilov, C.A., et al. (2020) Intravenous delivery of microRNA-133b along with Argonaute-2 enhances spinal cord recovery following cervical contusion in mice. Spine J, 20, 1138-1151.
2. Danilov, C.A., et al. (2023) Intranasal Delivery of miR133b in a NEO100-Based Formulation Induces a Healing Response in Spinal Cord-Injured Mice. Cells, 12.
3. Yu, J.Y.H. et al. (2024) MicroRNA-133b Dysregulation in a Mouse Model of Cervical Contusion Injury. Int J Mol Sci, 25.

Funding source: DoD CDMRP TBI & Psychological Health Res. program FY23 #HT9425-23-TBIPHRP-IIRA. The views expressed in this presentation are those of the author and do not reflect the official policy or position of the Department of War or the U.S. Government.

MHSRS 2026 Military Health System Research Symposium