Co-Targeting MG53 and CitH3 Attenuates Cerebral Ischemia-Reperfusion Injury

Kelvin Chen[1]; Kyung Eun Lee, PhD[1]; Ki Ho Park, PhD[1]; Jianjie Ma, PhD[1]

[1]Division of Surgical Sciences, Department of Surgery, University of Virginia School of Medicine, Charlottesville, VA, USA

ABSTRACT

Cerebral ischemia-reperfusion (I/R) injury is a major contributor to neuronal death and sensorimotor deficits, in part through the induction of neuroinflammation driven by microglia/macrophage polarization and excessive neutrophil extracellular trap (NET) formation. MG53, a TRIM family protein involved in cell membrane repair, and citrullinated histone H3 (CitH3), a central mediator of NETosis, represent potential therapeutic targets in this context. Here, we investigate the efficacy of recombinant human MG53 (rhMG53) in combination with a humanized anti-CitH3 monoclonal antibody (hCitH3-mAb) in treating I/R-induced neuroinflammation in a mouse model of transient middle cerebral artery occlusion. Following acute treatment, our data demonstrate that relative to either monotherapy or vehicle, combined rhMG53 and hCitH3-mAb treatment significantly reduced infarct size in mouse brain tissue 24 hours post-I/R. Behavioral outcomes assessed by neurological scoring confirmed improved sensorimotor function in the combination therapy group. Biochemical analyses further reveal upregulated protein and/or mRNA expression levels of anti-inflammatory (M2) cytokine and pro-survival signaling, whereas pro-inflammatory (M1) and cell death markers were downregulated. Together, these findings suggest that co-targeting both MG53-mediated cell membrane repair and CitH3-dependent NET formation confers synergistic neuroprotection against acute cerebral I/R injury, supporting its application in the treatment of neuroinflammatory diseases.

INTRODUCTION

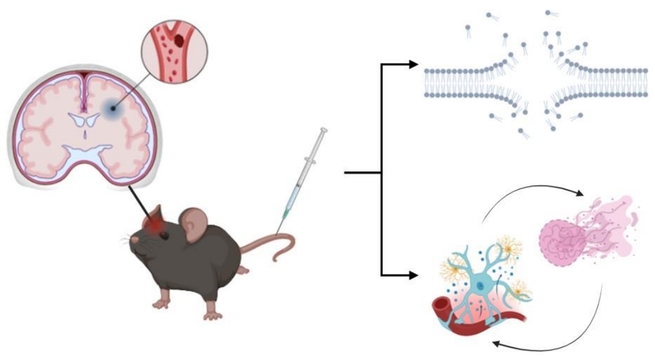

Figure 1. Cerebral I/R injury occurs when blood flow is restored to a localized area after a period of ischemia.[1] Prior studies using animal/in vitro models have shown rhMG53 promoting tissue regeneration in multi-organ injuries,[2] while hCitH3-mAb modulates the immune response in sepsis.[3]

Hypothesis: rhMG53 and hCitH3-mAb improve cerebral I/R injury outcomes by respectively targeting MG53-mediated cell membrane repair and excessive CitH3-mediated NETosis to preserve cellular integrity and reduce pro-inflammation.

METHODS

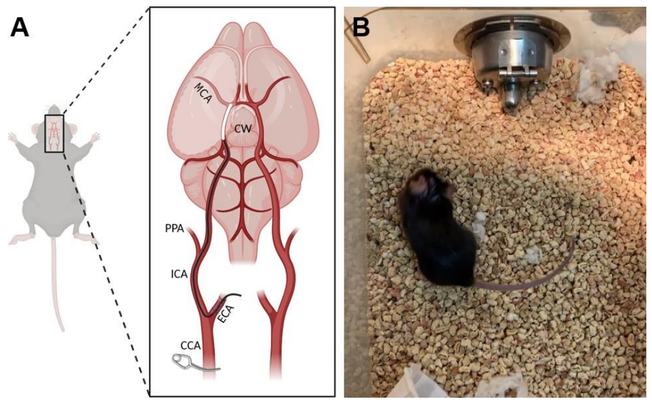

Figure 2. (A) Schematic representation of endovascular occlusion of the middle cerebral artery by a silicone monofilament inserted via an arteriotomy at the external carotid artery in C57BL/6J mice. (B) Mice were scored based on the Zea Longa neurological criteria 24 hours post-I/R.

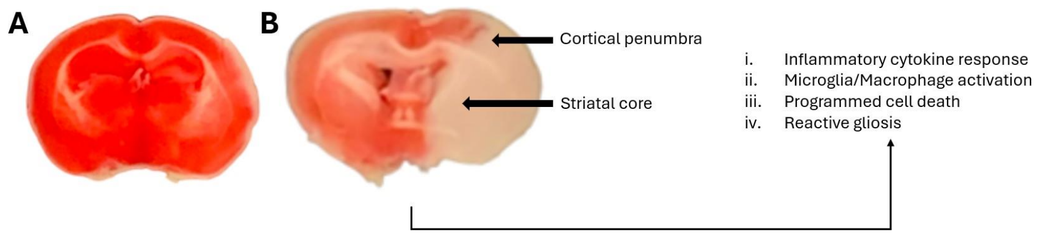

Figure 3. Representative TTC-stained coronal sections from a sham- (A) and I/R- (B) operated mouse brain. The pale infarcted region in the striatum corresponds to tissue with irreversible metabolic failure, while peri-infarct tissue in the cerebral cortex is metabolically compromised but potentially salvageable. Postoperative treatments were administered intravenously, after which cryopreserved brain tissues were lysed for further biochemical analysis post-I/R.

RESULTS & DISCUSSION

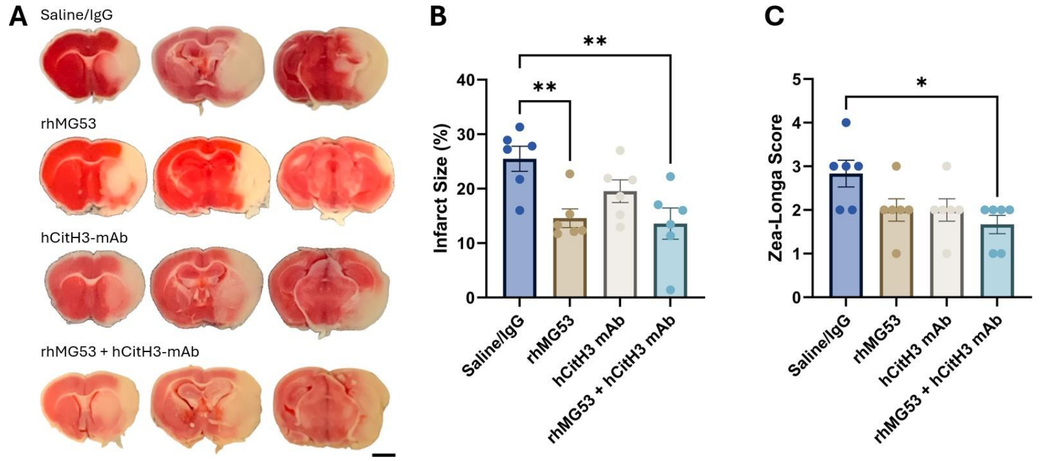

Figure 4. (A) TTC-stained tissue indicated a reduced cerebral infarction following intravenous delivery of 2 μg/kg of rhMG53 and/or hCitH3-mAb (Scale bar: 2 mm). (B) Total infarct size was estimated by image analysis; data analyzed by one-way ANOVA with Dunnett's test. (C) An improvement in sensorimotor phenotypes in the combined treatment group was observed 24 hours post-I/R; data analyzed by the Kruskal-Wallis test with Dunn's corrections.

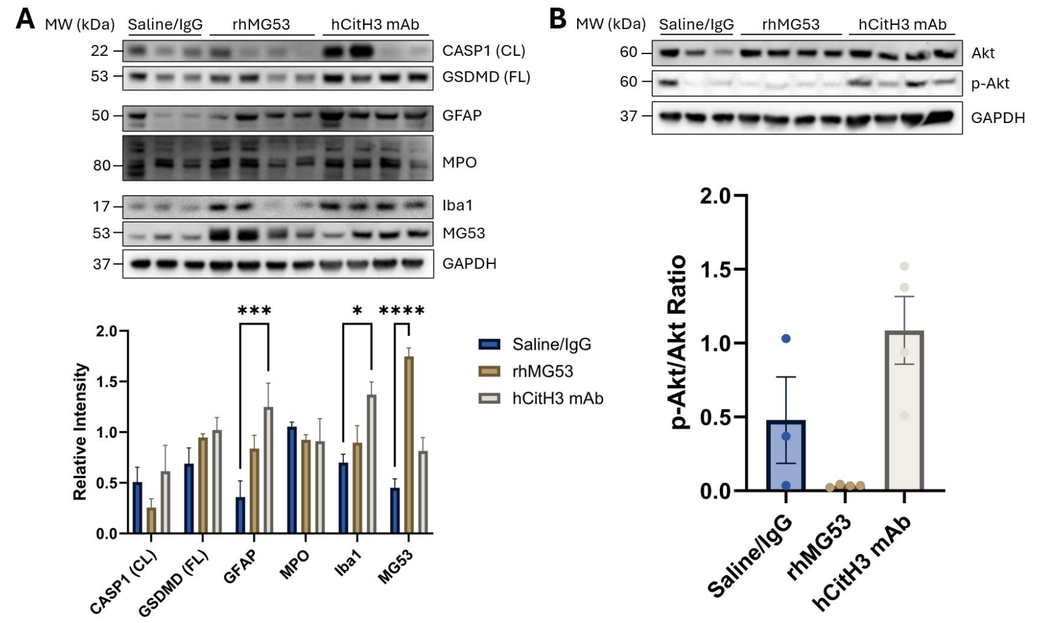

Figure 5. Western blot analysis of relative intensity of proteins associated with pyroptosis (CASP1, GSDMD), immune cell infiltration (MPO, Iba1), and astrogliosis (GFAP) across all treatment groups; data analyzed by two-way ANOVA with Šidák corrections. (B) Protein expression patterns of Akt and p-Akt showed upregulated Akt activation linked to cell survival; data analyzed by one-way ANOVA with Dunnett's test. All data were normalized to GAPDH.

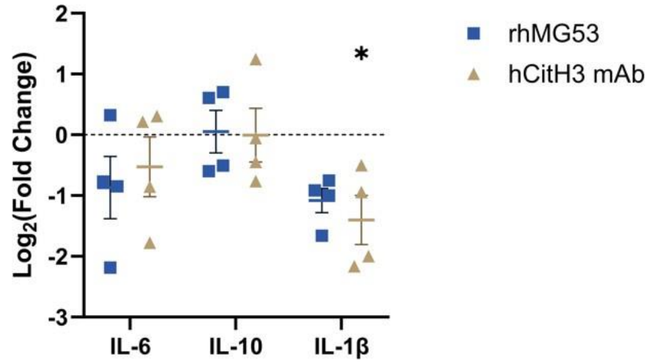

Figure 6. Relative fold change determined by real-time quantitative reverse transcription PCR analysis of interleukin signaling genes, showing the upregulation of anti-inflammatory (M2) IL-10 expression in tandem with the downregulation of pro-inflammatory (M1) IL-6 and IL-1β expression following rhMG53 and hCitH3-mAb administration. Data analyzed by two-way ANOVA with Šidák corrections. All data were normalized with β-actin expression and given as relative to control.

CONCLUSION

- Acute rhMG53 and hCitH3-mAb treatments administered post-I/R yielded a reduction in infarct size and neurological deficits.
- A trend toward downregulated pro-inflammatory cytokine signaling and pyroptotic cell death and upregulated cell survival pathways was found.
- Co-targeting MG53 and CitH3 protects the brain against neuroinflammation.

Limitations: Variability in lesion size due to differences in either cerebrovascular anatomy or surgical technique is inherent to the model used herein, thereby making outcome reproducibility difficult, as the data are not normally distributed.

Future Directions: Further investigations include delineating the localization of NETs within infarcted tissue, 2) transcriptomic and proteomic changes in M1/M2 markers, and 3) effects of a chronic treatment regimen on long-term recovery.

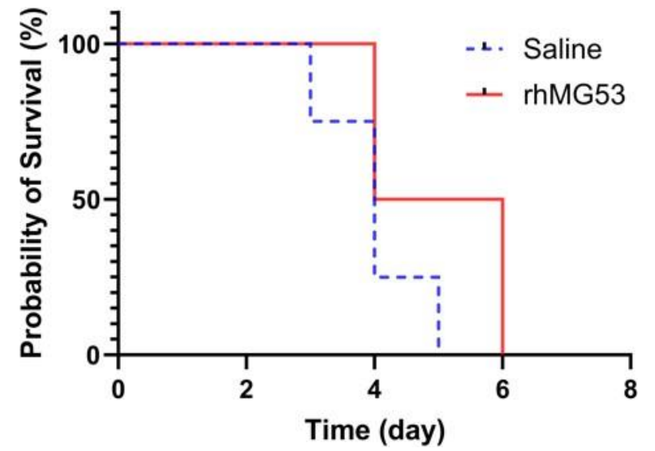

Figure 7. Kaplan-Meier analysis of animal survival rate after I/R with treatment, with the end-point event defined as the loss of ≥30% in total body weight. The product limit estimate of the cumulative survival was assessed with the log-rank test to evaluate for significant differences in survival.

ACKNOWLEDGEMENTS

This work was supported by both the Harrison Undergraduate Research Award and the Ingrassia Family Research Award. KC thank members of the Ma Laboratory, particularly KEL, KHP, and JM for their mentorship and feedback on this work.

REFERENCES

1. Li et al. (2022). *Neuroscience*, 507, 112-124.
2. Whitson et al. (2021). *Curr. Opin. Pharmacol.*, 59, 26-32.
3. Ouyang et al. (2025). *Nat. Commun.*, 16(1), 7435.