Beneficial effects of paracrine HMGB1 action through TLR2 on ischemic oligodendrocyte death and white matter injury

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# Introduction

Ischemic stroke in white matter is a common phenomenon of human stroke and results various neurological deficits such as hemiparesis and even dementia especially subcortical ischemic vascular dementia

Therefore, treating or regenerating of ischemic white matter injury is important therapeutic strategy in human neurological diseases. However, despite its importance, recent studies reported conflicting pathophysiological features of ischemic white matter injury with demyelination and oligodendrocyte death.

Previously, we found that Toll-like receptor 2 (TLR2) expressed in OL lineage cells provide cell-autonomous protective effects. [1,2] Therefore, treating or regenerating of ischemic white matter injury is important therapeutic strategy in human neurological diseases.

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# Results

## Release of High Mobility Group Box 1 (HMGB1) during and after OGD in oligodendrocyte culture

- To show protective effect of HMGB1, oligodendrocyte conditioned media (CM) was used with or without immunodepletion of HMGB1
- HMGB1 containing CM induced OL death but not HMGB1 immunodepleted CM in wild type oligodendrocytes.
- TLR2−/− oligodendrocytes didn’t respond to HMGB1 containing CM. This result is suggestive of cell-autonomous oligodendrocyte death.
- HMGB1 silencing showed successful knock-down of HMGB1 in oligodendrocyte and bathing media after OGD.
- Application of HMGB1 knock-down oligodendrocyte bathing media showed aggravated OGD induced OL death.
- Application of glycyrrhizin, direct inhibitor of TLR2, to oligodendrocyte could aggregate OGD induced OL death with dose dependent manner in wild type OL. Exogenous recombinant HMGB1 also showed protective effect in OGD induced OL death in WT OL but not TLR2−/− OL.
- All of these data suggest paracrine protective effect of released HMGB1 in TLR2 dependent manner.

## Protective effect of recombinant HMGB1

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## Translocation of HMGB1 in Oligodendrocyte after OGD

- Translocation of HMGB1 in Oligodendrocyte during ischemic insult.
- HMGB1 was located in nucleus in normal condition. To reexamine release of HMGB1, there should be translocated HMGB1 from nucleus.
- After OGD insult, there was translocation of HMGB1 from nucleus to cytoplasm with loss of nuclear remnants.

## Protective Effect of released HMGB1 in media to oligodendrocyte

- To further confirmation of HMGB1 release from oligodendrocyte after OGD, transfection HMGB1-GFP by electroporation to primary oligodendrocyte also showed released HMGB1 from nucleus.
- This result confirmed that Oligodendrocytes release its HMGB1 to the extracellular space after ischemic insult.

## Worsening of neurobehavioral outcomes after HMGB1 inhibition in WT animal or in TLR2 (−/−) animal

- HMGB1 inhibition with co-injection of glycyrrhizin resulted in increased ET1 induced ischemic white matter lesion volume in WT animal. TLR2 (−/−) animal showed similar lesion volume with glycyrrhizin administration in ischemic white matter lesion volume and glycyrrhizin co-injection didn’t impact on ET1 induced white matter lesion volume in TLR2 (−/−) animal.
- This result showed TLR2 dependent protective effect of HMGB1 in vivo.

## Summary

- Oligodendrocyte released HMGB1 after ischemic insult.
- Released HMGB1 provided oligodendrocyte protective effect via TLR2 and HMGB1 inhibition aggravated oligodendrocyte loss and ischemic demyelination in vitro and in vivo.
- Additionally, HMGB1 also affected expression of M2 phenotype markers in microglia. HMGB1 stimulated microglia conditioned media didn’t aggravate OGD induced OL death.
- Modulation of TLR2 with endogenous ligand like HMGB1 may be a novel therapeutic strategy to treat ischemic white matter injury.