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TDP-43 Phase Separation Does Not Likely Regulate LPS-Induced Neuroinflammation

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TDP-43 Phase Separation Does Not Likely Regulate LPS-Induced Neuroinflammation

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Abstract

- Immunohistochemistry (IHC) was performed to assess whether Transactive response Deoxyribonucleic acid binding Protein 43 (TDP-43) liquid-liquid phase separation (LLPS) regulates lipopolysaccaride (LPS)-induced neuroinflammation. Quantification and intensity results of glia cells and cytokines indicate that TDP-43 LLPS does not likely regulate LPS-induced neuroinflammation.

Background

- TDP-43 is encoded by the TARDP gene and is a key player in pathogenesis of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD), Alzheimer's Disease (AD), and Inclusion Body Myositis. Neuroinflammation is a pathological feature of neurodegenerative diseases and is characterized by increases in glia cells and cytokines. TDP-43 proteinopathy has been found to increase the number of microglia and astrocytes, and intensity of interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α). Recently, research has been performed in Dr. Xinglong Wang's laboratory investigating the *in vivo* physiological function TDP-43 LLPS in mice. LLPS of proteins underlies the formation of membrane-less organelles and is suspected to possibly play a role in disease. TDP-43 LLPS regulation of neuroinflammation remains elusive and warrants further investigation. LLPS function can be removed via deletion of the conserved region (CR) of TDP-43. To investigate the relationship between TDP-43 LLPS and neuroinflammation, IHC was performed to assess the quantity of microglia and astrocytes and intensity of IL-1beta and TNF-alpha in brain tissue of wild type (WT) and transgenic (TDP-43ΔCR+/-) mice with and without LPS treatment.

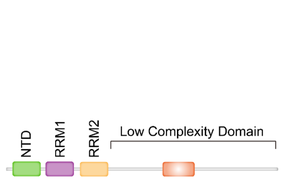

Fig. 1 Domains of TDP-43. CR located in the low complexity domain in red.

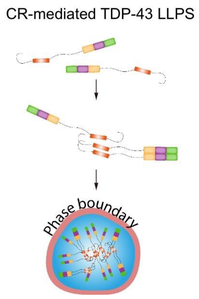

Fig. 2 Schematic of TDP-43 LLPS via the CR.

Results

1. No significant difference in number of astrocytes between WT and TDP-43ΔCR+/- with and without LPS treatment.

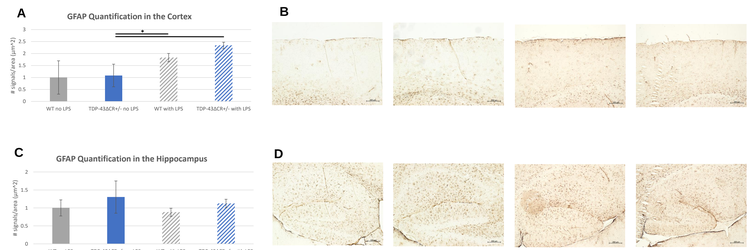

Fig. 3 (A) Bar graph comparing GFAP quantification in the cortex for WT and TDP-43ΔCR+/- with and without LPS treatment, (B) cortex images of WT no LPS, TDP-43ΔCR+/- no LPS, WT with LPS, TDP-43ΔCR+/- with LPS, (C) Bar graph comparing GFAP quantification in the hippocampus for WT and TDP-43ΔCR+/- with and without LPS treatment, (D) hippocampus images of WT no LPS, TDP-43ΔCR+/- no LPS, WT with LPS, TDP-43ΔCR+/- with LPS. * indicates $p < 0.05$

2. No significant difference in number of microglia between WT and TDP-43ΔCR+/- with or without LPS treatment.

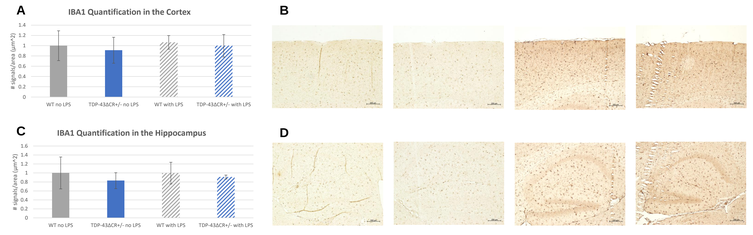

Fig. 4 (A) Bar graph comparing IBA1 quantification in the cortex for WT and TDP-43ΔCR+/- with and without LPS treatment, (B) cortex images of WT no LPS, TDP-43ΔCR+/- no LPS, WT with LPS, TDP-43ΔCR+/- with LPS, (C) Bar graph comparing IBA1 quantification in the hippocampus for WT and TDP-43ΔCR+/- with and without LPS treatment, (D) hippocampus images of WT no LPS, TDP-43ΔCR+/- no LPS, WT with LPS, TDP-43ΔCR+/- with LPS.

3. No significant difference in intensity of IL-1β between WT and TDP-43ΔCR+/- with or without LPS treatment.

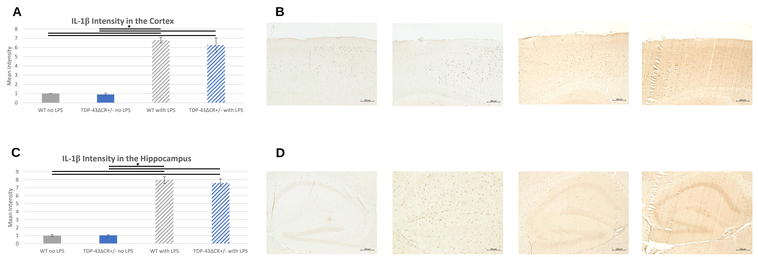

Fig. 5 (A) Bar graph comparing IL-1β quantification in the cortex for WT and TDP-43ΔCR+/- with and without LPS treatment, (B) cortex images of WT no LPS, TDP-43ΔCR+/- no LPS, WT with LPS, TDP-43ΔCR+/- with LPS, (C) Bar graph comparing IL-1β quantification in the hippocampus for WT and TDP-43ΔCR+/- with and without LPS treatment, (D) hippocampus images of WT no LPS, TDP-43ΔCR+/- no LPS, WT with LPS, TDP-43ΔCR+/- with LPS. * indicates $p < 0.05$

4. No significant difference in intensity of TNF-α between WT and TDP-43ΔCR+/- with or without LPS treatment.

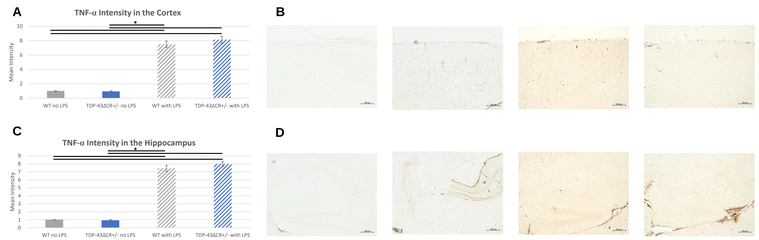

Fig. 6 (A) Bar graph comparing TNF-α quantification in the cortex for WT and TDP-43ΔCR+/- with and without LPS treatment, (B) cortex images of WT no LPS, TDP-43ΔCR+/- no LPS, WT with LPS, TDP-43ΔCR+/- with LPS, (C) Bar graph comparing TNF-α quantification in the hippocampus for WT and TDP-43ΔCR+/- with and without LPS treatment, (D) hippocampus images of WT no LPS, TDP-43ΔCR+/- no LPS, WT with LPS, TDP-43ΔCR+/- with LPS. * indicates $p < 0.05$

Methods

- Peroxidase Anti-Peroxidase IHC
  - Sectioning via microtome
  - Incubation
  - Deparaffinization/rehydration
  - Antigen retrieval via pressure cooker
  - Application of primary and secondary antibody and PAP complex
  - Development
  - Dehydration
  - Imaging via Zeiss Axio Imager.A2

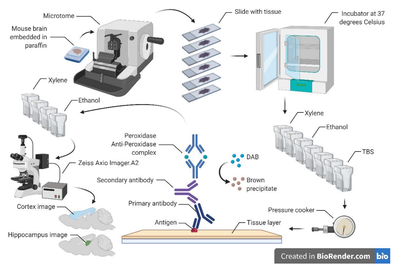

Fig. 7 Schematic of IHC.

Scan QR code for detailed IHC protocol.

Conclusion

- TDP-43 LLPS did not show a significant difference in regulation of glia cells or cytokines in LPS-induced neuroinflammation. Since three mice were used for each group, this study could be replicated again in the future with more mice for each group to generate better results. Future studies may also investigate TDP-43 phase LLPS in different areas locally or globally.

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