

# Microglial Mir-320 Restoration As A Novel Therapeutic Strategy for Alzheimer's Disease

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Targeting microglial miR-320 restores AD-linked cognitive functions, inflammation and mitochondrial dysfunction

INTRODUCTION

Alzheimer's disease (AD), the leading cause of dementia worldwide, involves complex mechanisms beyond amyloid and tau pathology. MicroRNAs (miRNAs) are critical post-transcriptional regulators implicated in AD-related processes such as amyloid precursor protein (APP) processing, tau phosphorylation, neuroinflammation, and synaptic dysfunction. Among these, miR-320 is consistently downregulated in AD brains and targets key tau pathway genes, including MAPT, yet its functional role remains poorly understood. Microglia, the brain's resident immune cells, are central to the neuroinflammatory landscape of AD, where their dysregulated activation contributes to chronic inflammation, impaired clearance of pathological proteins, and neuronal damage. Given that microglia are highly sensitive to miRNA-mediated regulation, targeting them offers a strategic opportunity to modulate disease progression. We hypothesize that restoring miR-320 levels specifically in microglia could recalibrate their activation state, suppress neuroinflammatory cascades, and enhance neuronal and mitochondrial resilience.

METHODS

**1. Behavioural assays**

**A** Spatial memory: Y maze

**B** Associative memory: Fear conditioning

**C** Motor function: Rota Rod

**2. Mitochondrial functions**

**A** Mitochondria isolation

**B** Calcium retention capacity assay

**C** Mitochondrial respirometry analysis

**3. Molecular functions**

**A** RNA isolation

**B** qRT-PCR

**C** Protein isolation

**D** Western blotting

**4. Immunohistochemistry**

**A** IHC

**B** Western blotting

RESULTS

### miR-320 expression is downregulated under AD conditions

**A** miR-320 expression (Healthy controls vs AD patients)

**B** miR-320 expression (6NJ vs App<sup>SAKI/KI</sup>)

**C** miR-320 expression (HMC3-WT vs HMC3-APPsw/F/L)

**D** miR-320 expression (SH-SY5Y-WT vs SH-SY5Y-APPsw/F/L)

### miR-320 expression rescues cognitive functions in App<sup>SAKI/KI</sup> mice

**A** miR-320 expression (Microglia)

**B** Y Maze % Alternations

**C** Total arm #

**D** Contextual recall Freezing (%)

**E** Cued recall Freezing (%)

**F** Latency to fall (sec)

RESULTS

### miR-320 reduces APP-processing BACE1 and PSEN1 protein levels

**A** Western blot analysis (APP, ADAM10, BACE1, APOE, PSEN1, NCT, sAPPα, sAPPβ, C99, TUBULIN)

**B** BACE1 Normalized to tubulin

**C** PSEN1 Normalized to tubulin

### miR-320 expression decreases inflammatory status in brain

**A** Western blot analysis (IBA1, GFAP, TUBULIN)

**B** Pro-inflammatory mRNAs (TNF-α, IL-1β, IL-6, IL-1R)

### miR-320 improves mitochondrial functions in App<sup>SAKI/KI</sup> mice brain

**A** Respirometry analysis (O2 flux, mass, Time [min])

**B** Mito-CRC traces (Fura-FF, Time [sec])

**C** Mito-CRC traces (Fura-FF, Time [sec])

**D** Mito-CRC traces (Fura-FF, Time [sec])

RESULTS

### App<sup>SAKI/KI</sup> mice model shows no difference in the neuronal density and lipid peroxidation levels

**A** Immunohistochemistry images (4-HNE, Nissl, NeuN)

CONCLUSION

Improved Spatial and Associative memory response

Decreased Neuroinflammatory markers

Inhibit APP processing enzymes - PSEN1 and BACE1

Improved mitochondrial functions

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