BIORCHESTRA

Targeting microRNA-485-3p blocks Alzheimer's disease progression

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BACKGROUND

Alzheimer's disease (AD) is a form of dementia characterized by progressive memory decline and cognitive dysfunction, which affects more than 44 million people worldwide. Currently, there is no effective therapy for AD despite its increasing global incidence; thus, effective treatment strategies for AD are urgently needed. While several drugs that decrease amyloid beta (Aβ) production or increase Aβ clearance in the brain have been identified, treatment with these drugs is poorly correlated with improvements in AD severity and cognitive dysfunction.

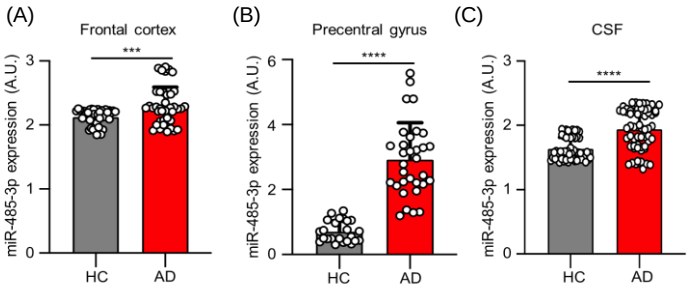
METHODS

Expression of miR-485-3p was analysed by real-time PCR in the human frontal cortex (8 healthy controls (HC), 7 AD patients), precentral gyrus (6 HC, 8 AD), cerebrospinal fluid (CSF) (6 HC, 7 AD), plasma exosomes (10 HC, 17 mild cognitive impairment (MCI), 12 AD). Aβ 1–42 plaque immunofluorescence and tau pathology were imaged in primary cultured mouse neurons after lentivirus-derived miR-485-3p transduction. MiR-485-3p antisense oligonucleotide (ASO, 1.5 μg) or control oligonucleotide formulated with the in vivo jetPEI reagent was injected into 8-month-old 5XFAD mice (n = 5-7) by ICV injection once weekly for two weeks. Behavioral tests were performed at 8 months and their brain pathology was examined after 8-week-washout at 10 months.

RESULTS

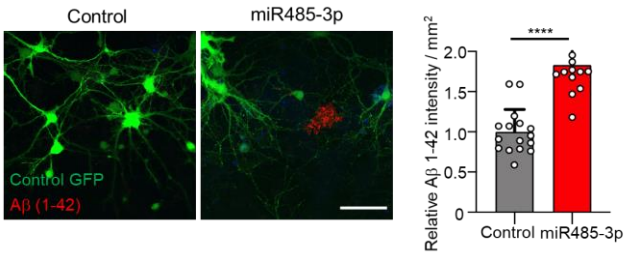
1. miR-485-3p as a potential AD biomarker

1) Overexpression of miR-485-3p in human brain and CSF



(**A**) Expression of miR-485-3p in the human frontal cortex (healthy control [HC] *n* = 8; AD patients *n* = 7) (**B**) precentral gyrus (healthy control [HC] *n* = 6; AD patients *n* = 8) (**C**), and cerebrospinal fluid (CSF) (healthy control [HC] *n* = 6; AD patients *n* = 7).

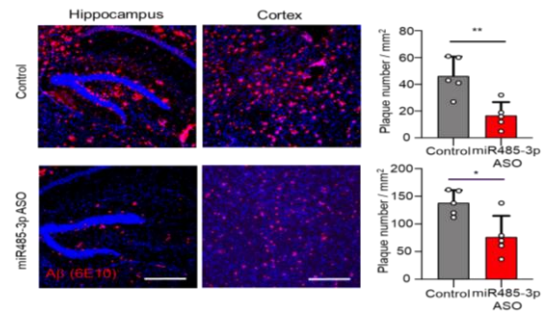
2) miR-485-3p induces AD pathology



Aβ 1–42 plaque immunofluorescence images of primary mouse neurons after lentivirus-derived miR-485-3p transduction. Scale bars, 20 μm.

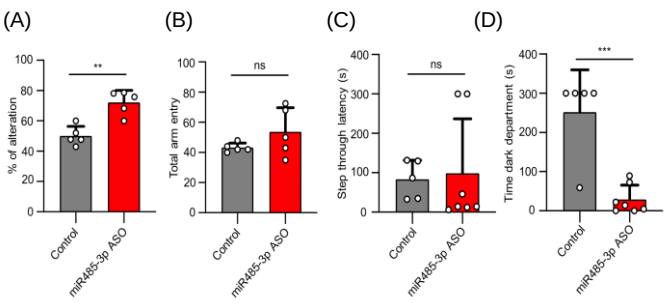
2. miR-485-3p ASO as a potential therapeutic candidate for AD

1) miR-485-3p ASO reduces Aβ plaque



Representative images of immunohistochemical staining with Aβ (6E10) in the hippocampus and cortex region after miR-485-3p antisense oligonucleotide injection. Scale bars, 300 μm.

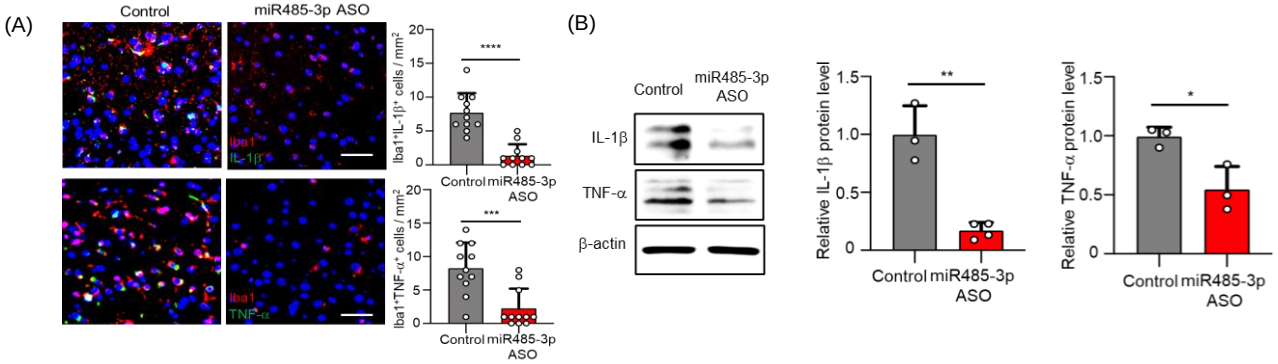
2) miR-485-3p ASO rescues cognitive impairment



Behaviour tests in control- (*n* = 5–7) or miR-485-3p ASO- (*n* = 5–7) injected 8-month-old 5XFAD mice. (**A, B**) Y-maze and (**C, D**) passive avoidance test in control- or miR-485-3p ASO-injected 8-month-old 5XFAD mice.

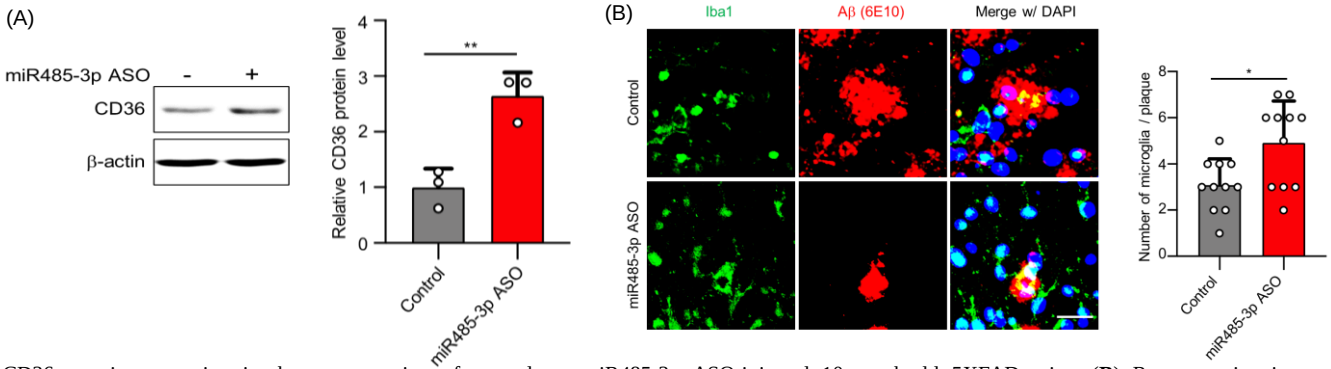
RESULTS

3) miR-485-3p ASO reduces neuroinflammation



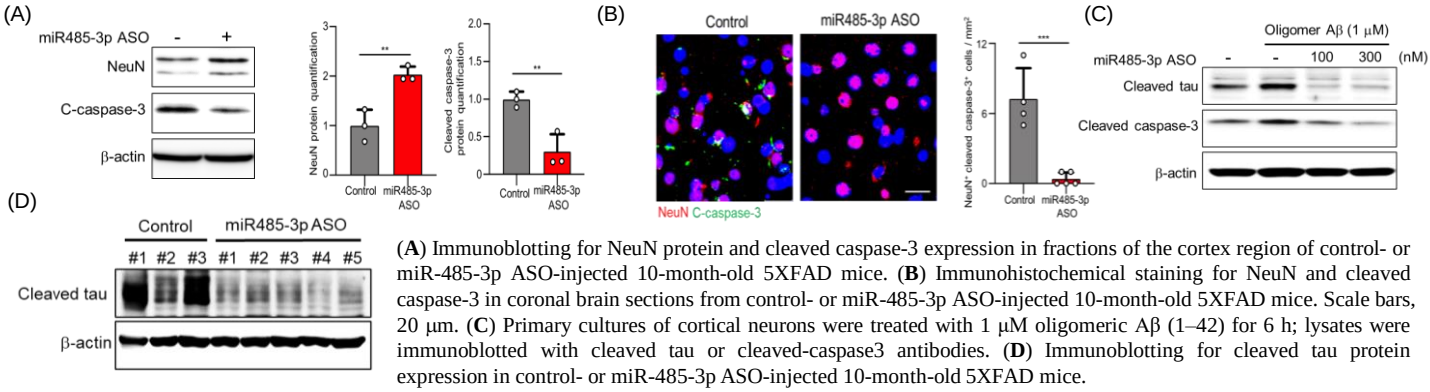
(**A**) Representative images of immunohistochemical staining for Iba1 and IL-1β or TNF-α in the cortex region of control- or miR-485-3p ASO-injected 10-month-old 5XFAD mice. Scale bars, 20 μm. (**B**) The protein levels of IL-1β and TNF-α in microglia in miR-485-3p ASO-injected 5XFAD mice.

4) miR485-3p ASO enhances phagocytosis of Aβ by regulation of CD36 in vitro and in vivo.



(**A**) CD36 protein expression in the cortex region of control- or miR485-3p ASO-injected 10-month-old 5XFAD mice. (**B**) Representative images of immunohistochemical staining for Iba1 and Aβ (6E10) in the cortex region of control- or miR-485-3p antisense oligonucleotide (ASO)-injected 10-month-old 5XFAD mice.

5) miR-485-3p ASO reduces apoptosis and truncated tau



(**A**) Immunoblotting for NeuN protein and cleaved caspase-3 expression in fractions of the cortex region of control- or miR-485-3p ASO-injected 10-month-old 5XFAD mice. (**B**) Immunohistochemical staining for NeuN and cleaved caspase-3 in coronal brain sections from control- or miR-485-3p ASO-injected 10-month-old 5XFAD mice. Scale bars, 20 μm. (**C**) Primary cultures of cortical neurons were treated with 1 μM oligomeric Aβ (1–42) for 6 h; lysates were immunoblotted with cleaved tau or cleaved-caspase3 antibodies. (**D**) Immunoblotting for cleaved tau protein expression in control- or miR-485-3p ASO-injected 10-month-old 5XFAD mice.

CONCLUSION

We found that the miR-485-3p is overexpressed in brain tissues and CSF of AD patients, and the therapeutic ASO reduces Aβ plaques, tau pathology, neuroinflammation (cytokines, IL-1β and TNF-α), and eventually relieved cognitive impairment in a transgenic mouse model of AD. Mechanistically, the ASO enhanced Aβ clearance via CD36-mediated phagocytosis of Aβ *in vitro* and *in vivo*. We found that the ASO reduces apoptosis, which effectively decreases truncated tau levels. Collectively, our findings suggest that miR-485-3p is a useful biomarker as well as a causative factor of the inflammatory pathophysiology in AD. Furthermore, the ASO represents a therapeutic candidate for AD pathology and cognitive decline, establishing a new paradigm in the AD field.

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