FEBS Letters 589 (2015) 726–729

journal homepage: www.FEBSLetters.org

MicroRNA-138 promotes tau phosphorylation by targeting retinoic acid receptor alpha

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ARTICLE INFO

Article history:
Received 9 January 2015
Revised 30 January 2015
Accepted 3 February 2015
Available online 11 February 2015

Edited by Jesus Avila

Keywords:
miR-138
Alzheimer's disease
Retinoic acid receptor alpha
Tau
Phosphorylation
Glycogen synthase kinase-3β

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative dementia characterized by Aβ deposition and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau. Emerging evidence shows that microRNAs (miRNAs) contribute to the pathogenesis of AD. Herein, we investigated the role of miR-138, a brain enriched miRNA, which is increased in AD patients. We found that miR-138 is increased in AD models, including N2a/APP and HEK293/tau cell lines. Overexpression of miR-138 activates glycogen synthase kinase-3β (GSK-3β), and increases tau phosphorylation in HEK293/tau cells. Furthermore, we confirm that retinoic acid receptor alpha (RARA) is a direct target of miR-138, and supplement of RARA substantially suppresses GSK-3β activity, and reduces tau phosphorylation induced by miR-138. In conclusion, our data suggest that miR-138 promotes tau phosphorylation by targeting the RARA/GSK-3β pathway.

1. Introduction

Alzheimer's disease (AD) is common dementia among elderly people, which is characterized histologically by the appearance of extracellular senile plaque (SP) formed by amyloid β (Aβ) and intracellular neurofibrillary tangles (NFTs) resulting from hyperphosphorylated tau protein [1]. Accumulating evidence shows that microRNAs (miRNAs) contribute to the pathogenesis of AD [2–4].miRNAs are short non-coding RNAs whose sequences are highly conserved [5,6]. miRNAs are essential regulators of genes involved in a variety of biological processes. Many brain specific and brain enriched miRNAs have been identified, and some of them are associated with normal brain development and neuronal differentiation [7,8]. Recent studies indicated that some miRNAs regulated Aβ production and tau phosphorylation, control synaptic plasticity, or participate in the formation of memory [9–11]. The expression patterns of miRNAs are altered in AD patients and AD mouse models [12,13]. miR-138, a brain enriched miRNA increased in AD patients [14], has been demonstrated to negatively regulate synaptic plasticity. However, its role in the pathogenesis of AD remains unclear.

In this study, we examined the expression of miR-138 in AD model cell lines, and investigated the effects of miR-138 on tau phosphorylation and the underling mechanisms. We found that miR-138 was increased in AD cell models, and overexpression of miR-138 increased tau phosphorylation. Furthermore, we identified retinoic acid receptor alpha (RARA) was a direct target of miR-138, and miR-138 induced tau hyperphosphorylation by targeting RARA.

2. Materials and methods

2.1. Cell culture

N2a/APP cells which stably expressed human APP695, and HEK293/tau cells which stably expressed human tau were maintained in DMEM, supplemented with 10% FBS, 0.2 g/L G418 (Invitrogen, Carlsbad, CA, USA) at 37 °C with 5% CO_2. Transfection was performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). N2a/APP and HEK293/tau cells were kindly gifted by Prof. Jianzhi Wang (Department of Pathophysiology, Tongji Medical College, Huazhong University of Science and Technology. Key Laboratory of Neurological Diseases of Education Ministry of China).

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http://dx.doi.org/10.1016/j.febslet.2015.02.001
0014-5793/

2.2. Plasmids

The overexpression plasmid of miR-138 was purchased from GeneCopoeia (Rockville, MD, USA). Overexpression construct of RARA was generated by amplifying the CDS of RARA, and the PCR fragment was subcloned into pcDNA4 (Invitrogen, Carlsbad, CA, USA). 3′-UTR of RARA containing the binding sites of miR-138: TCACCACATCTTCATCACCAGCA (Position 314-336 of RARA 3′-UTR) was synthesized by Tsingke (Beijing, China), and inserted into psi-CHECK2 (Promega, Madison, WI, USA) digested by XhoI and NotI. Mutation was performed using a fast mutation kit from Agilent (Brighton, MA USA).

2.3. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA). MiRNAs were extracted using miRNA extraction and cDNA synthesis kits from Tiangen (Beijing, China). The qRT-PCR was performed on ABI Stepone Plus (Invitrogen, Carlsbad, CA, USA) using SYBR Green mix (Takara, Tokyo, Japan). RARA primers: sense 5′-CTGCCCGGCTTCACCAC-3′, antisense 5′-CGAAGCCAGC GTTGTGC-3′. Primers for miR-138 and U6 were obtained from GeneCopoeia (Rockville, MD, USA). The expression was determine using $2^{-\Delta\Delta CT}$ method and normalized with β-actin or U6.

2.4. Luciferase activity assay

HEK293 cells were co-transfected with the miR-138 or the control vector and wild-type (WT) or the mutant (Mut) of RARA 3′-UTR. Cells were collected for luciferase activities 48 h after transfection using luciferase activity reporter assay kit (Promega, Madison, WI, USA).

2.5. Western blotting

Cells were homogenated with RIPA buffer (Beyotime, Shanghai, China) on ice. Proteins were separated by 10% SDS–PAGE and transferred to NC membranes, incubated with primary antibodies overnight at 4 °C. Then the membranes were further incubated with secondary antibodies (LI-COR, Lincoln, NE, USA) for 1 h at 37 °C and detected under the Odyssey Imaging System (LI-COR, Lincoln, NE, USA).

2.6. Statistical analysis

Data were expressed as means ± S.D., analyzed using SPSS 12.0 (SPSS, Chicago, IL, USA) by Student's *t* test or one-way ANOVA. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Expression of miR-138 was increased in AD models

The expression of miR-138 in several AD cell models was detected. The expression of miR-138 was significantly increased in both N2a/APP (Fig. 1A) and HEK293/tau (Fig. 1B) cell lines. These data indicate that miR-138 was elevated in AD models.

3.2. Overexpression of miR-138 promoted tau phosphorylation

One of the histological hallmark of AD is NFT, formed by hyperphosphorylated tau protein. We then investigated the effects of miR-138 on tau phosphorylation. HEK293/tau cells were transfected with miR-138 overexpression plasmid or the scramble control, and the phosphorylation level of tau at several sites were detected.

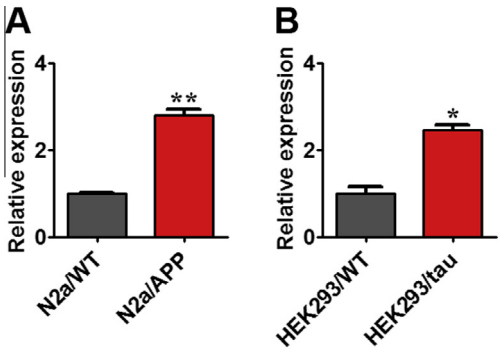

Fig. 1. Expression of miR-138 was increased in AD models. (A) Expression of miR-138 in N2a/WT and N2a/APP cells were detected by qPCR. (B) Expression of miR-138 in HEK293/WT and HEK293/tau cells were detected by qPCR. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with wild type cell line.

We found that miR-138 increased the phosphorylation of tau at Ser396 and Thr231 sites (Fig. 2A). The activities of kinase and phosphatase which functioned at these sites were examined, and we revealed that the activity of Glycogen synthase kinase-3β (GSK-3β) was activated, indicated by decreased phosphorylation of Ser9 of GSK-3β, while the activity of phosphatase, protein phosphate 2A (PP2A) was not affected, indicated by phosphorylation at Tyr307 of PP2A, which is inversely correlated with PP2A activity (Fig. 2B). These data suggest that miR-138 increased tau phosphorylation, and activated the activity of GSK-3β.

3.3. RARA was a direct target of miR-138

miRNAs function mainly by targeting their downstream targets. TargetScan 6.2 (http://www.targetscan.org/) and miRanda (http://www.microrna.org/) were used to search the potential target of miR-138, and RARA contained potential binding sites of miR-138 (Fig. 3A). Luciferase activity assay showed that miR-138 significantly inhibited luciferase activity of the wild type (WT) but not mutant (Mut) 3′-UTR of RARA (Fig. 3B). Forced overexpression of miR-138 significantly suppressed RARA protein levels (Fig. 3C). These data suggest that RARA was a target of miR-138.

3.4. miR-138 promoted tau phosphorylation by targeting RARA

We further studied whether miR-138 increased tau phosphorylation by targeting RARA. HEK293/tau cells were co-transfected with miR-138 and RARA or the control vector, the results showed that supplement of RARA by pcDNA4-RARA significantly attenuated the phosphorylation of tau (Fig. 4A) and GSK-3β activation (Fig. 4B) induced by miR-138. These data indicate that miR-138 increased tau phosphorylation partially via targeting RARA.

4. Discussion

In the present study, we found that miR-138 was upregulated in AD cell models. Forced overexpression of miR-138 induced GSK-3β activation and tau phosphorylation. Furthermore, miR-138 bound directly to the 3′-UTR of RARA mRNA and reduced RARA expression. Overexpression of RARA remarkably attenuated GSK-3β activation and tau phosphorylation induced by miR-138.

Expression profiling of miRNAs has been studied in the brain, cerebrospinal fluid (CSF), and blood of AD patients and transgenic models [14–16]. miR-138 has been known to regulate a variety of essential biological processes, including dendritic spine morphogenesis, thermotolerance acquisition, and cardiac patterning modulating during embryonic stage [17–19]. Siegel et al. reported

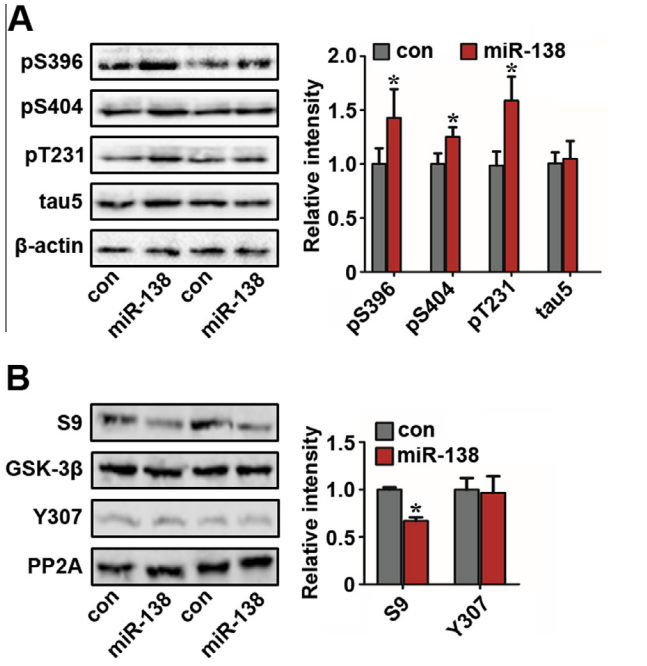

Fig. 2. Overexpression of miR-138 promoted tau phosphorylation. HEK293/tau cells were transfected with miR-138 or the control vector. 48 h later, cells were harvested for Western blotting. (A) Phosphorylation level at several sites of tau was detected. (B) The phosphorylation of GSK-3β and PP2A was detected. These antibodies, pS396, pS404, pT231 were used to detect the phosphorylation level at Ser396, Ser404, and Thr231 sites of tau. Tau5 detected the total level of tau. S9 and Y307 were used to detect the phosphorylation level at Ser9 and Tyr307 sites of GSK-3β and PP2A, respectively. $^{*}P < 0.05$ compared with control vector.

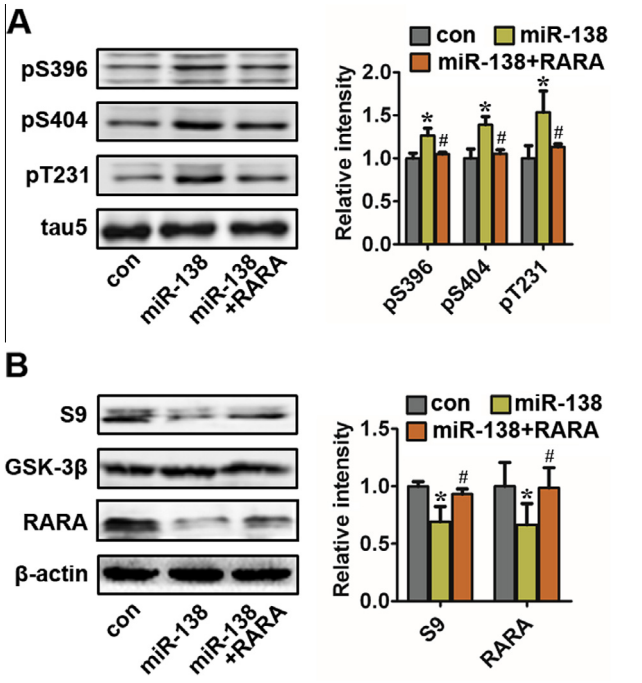

Fig. 4. miR-138 promoted tau phosphorylation by targeting RARA. HEK293/tau cells were co-transfected with miR-138 or the control vector and pcDNA4-RARA. 48 h later, cells were harvested for Western blotting. (A) Phosphorylation level at several sites of tau was detected. (B) The phosphorylation of GSK-3β and PP2A was detected. $^{*}P < 0.05$ compared with control vector. $^{\#}P < 0.05$ compared with miR-138 group.

that miR-138 negatively regulated dendritic spine morphogenesis by targeting acyl protein thioesterase 1 (APT1), an enzyme which regulated the palmitoylation status of proteins at the synapse [17]. Furthermore, Liu et al. found that miR-138 acted as a novel suppressor of axon regeneration via decreasing the expression of SIRT1, an NAD-dependent histone deacetylase [20]. Dysfunction of synaptic plasticity was found in AD patients, and miR-138 may also contribute to this dysfunction [21]. Schröder et al. performed genome-wide association studies (GWAS) in episodic and working memory phenotypes, and they found that miR-138 might be a potential regulator of episodic memory performance [22]. In our study, for the first time, we investigated the effects of

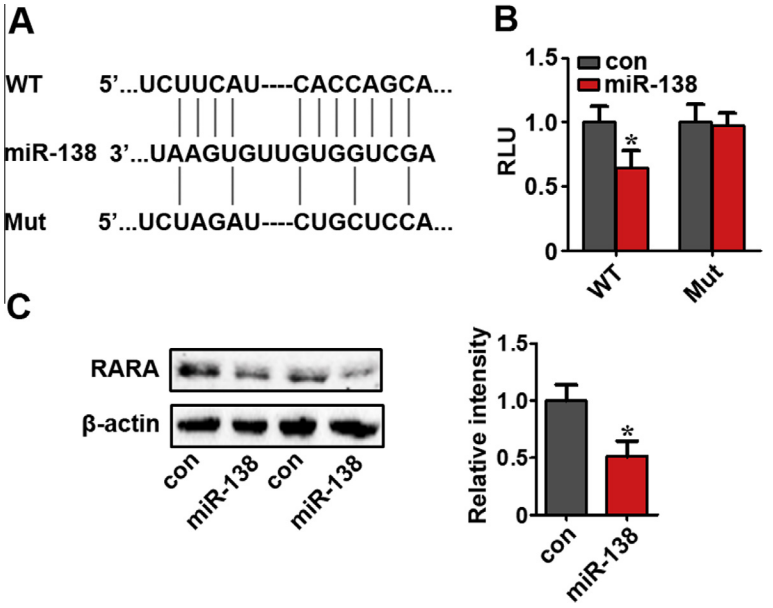

Fig. 3. RARA was a direct target of miR-138. (A) RARA 3′-UTR contained potential binding sites of miR-138. (B) HEK293 cells were co-transfected with miR-138 or the control vector and Wild type (WT) or mutant (Mut) RARA 3′-UTR. 48 h later, cells were harvested and the dual luciferase activity were determined. RLU, relative luciferase unit. (C) HEK293 cells were transfected with miR-138 or the control vector. 48 h later, cells were harvested and the protein level of RARA was detected by Western blotting. $^{*}P < 0.05$ compared with control vector.

miR-138 on tau phosphorylation. We showed that miR-138 could induce GSK-3β activation and tau phosphorylation at several sites, while miR-138 did not affect the activity of PP2A, an essential phosphatase in the regulation of tau phosphorylation. Our data suggest that miR-138 may contribute to tau hyperphosphorylation in AD.

The retinoic acid (RA) and RARA signaling pathway is essential for normal brain maintenance, and previous studies revealed that deficit in this pathway was associated with AD [23]. RARA was found to increase the expression of a disintegrin and metalloprotease 10 (ADAM10), suppress the activity of γ-secretase, leading to a reduction of Aβ production [24,25]. Moreover, RARA could also reduce tau phosphorylation by inhibiting GSK-3β activity [26]. In this work, we found that miR-138 overexpression increased the phosphorylation level of tau at Thr231, Ser396, and Ser404. The kinase GSK-3β and phosphatase PP2A are two essential regulators of tau phosphorylation at these sites [1]. We showed that GSK-3β activity was increased after miR-138 overexpression, without effect on PP2A. Moreover, we confirmed that RARA was a target of miR-138, and supplement of RARA substantially attenuated these effects of miR-138. Our work expanded the role of RARA in AD pathogenesis.

In conclusion, our data suggest that miR-138 may induce tau hyperphosphorylation by targeting RARA/GSK-3β pathway.

5. Conflict of interest

No conflict of interest.

Acknowledgment

This work was partially supported by the National Natural Science Foundation of China (NSFC) Grants 31371368 (to Y. Zhang).

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