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Increased expression of miRNA-146a in Alzheimer's disease transgenic mouse models

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ABSTRACT

A mouse and human brain-enriched micro-RNA-146a (miRNA-146a) is known to be important in modulating the innate immune response and inflammatory signaling in certain immunological and brain cell types. In this study we examined miRNA-146a levels in early-, moderate- and late-stage Alzheimer's disease (AD) neocortex and hippocampus, in several human primary brain and retinal cell lines, and in 5 different transgenic mouse models of AD including Tg2576, TgCRND8, PSAPP, 3xTg-AD and 5xFAD. Inducible expression of miRNA-146a was found to be significantly up-regulated in a primary co-culture of human neuronal–glial (HNG) cells stressed using interleukin1-beta (IL-1β), and this up-regulation was quenched using specific NF-κB inhibitors including curcumin. Expression of miRNA-146a correlated with senile plaque density and synaptic pathology in Tg2576 and in 5xFAD transgenic mouse models used in the study of this common neurodegenerative disorder.

Micro-RNAs (miRNAs) constitute a novel class of small, evolutionarily conserved non-coding regulatory RNAs that interact with the 3′ un-translated region (3′-UTR) of specific messenger RNA (mRNA) populations, and in doing so function in mRNA processing, inhibition, or termination of that mRNA's expression [22,11,13,14]. A number of miRNAs, such as the brain-enriched micro-RNA-146a (miRNA-146a) have been strongly implicated in regulation of innate immune, viral, and inflammatory responses in neurodegenerative disorders, including Alzheimer's disease (AD) [11,13,14,16,24,23,5,20]. In this report we have studied miRNA-146a abundance in the superior temporal lobe neocortex and hippocampal CA1 region in the early, middle and late stages of AD, in several distinct types of human brain and retinal cells in primary culture (Figs. 1 and 2), and in 5 different transgenic mouse models of AD (Tg-AD; Table 1). The expression of miRNA-146a was found to be significantly expressed in the human neocortex and the limbic system and significantly increased as the severity of AD advanced (Fig. 1A). In primary human neuronal–glial (HNG) cell co-cultures miRNA-146a was found to be induced by IL-1β, a pro-inflammatory cytokine known to be elevated in AD brain (Fig. 1B) [12]. Interestingly, the levels of miRNA-146a were found to correlate with the senile plaque density and the appearance of synaptic pathology in the brain cortex of certain Tg-AD mouse models (Fig. 2, Table 1) [19]. Transfection of HNG cells with a miRNA-146a luciferase reporter (construct A547), and the inclusion of 3 different classes of NF-κB inhibitors, indicated that in IL-1β-stressed HNG cells, as in other cell types, miRNA-146a is under transcriptional regulatory control by NF-κB. This suggests an important, potentially pathogenic, interplay between a pro-inflammatory transcription factor and an inducible miRNA under NF-κB regulatory control in specific types of human brain cells, tissues, and in amyloid over-expressing Tg-AD models (Figs. 2 and 3) (2–6,13).

Reagents used in these experiments were obtained from commercial suppliers and were used without further purification. RNAse-free plasticware and RNAse-free isolation reagents including diethyl pyrocarbonate (DEPC; Ambion, Austin TX; Invitrogen, Carlsbad CA; Sigma–Aldrich, St Louis, MO) were used as previously described [13,14,16,24,12]. The NF-κB inhibitors

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Table 1
Various neuropathological characteristics of transgenic AD (Tg-AD) mouse models derived from previous studies and unpublished data. Genotype and phenotype are further described in detail in the original 'source' references provided for these Tg-AD models.

Alzheimer transgenic model	Age of onset (senile plaques)	Tg-AD promoter/transgene	Senile plaque density	CNS-specific expression of amyloid	Synaptic pathology	Source reference
Tg2576	10	Hamster prion promoter/human APP695 cDNA with KM670/671NL	++++	++	++++	[7,9]
Tg-CRND8	3	Hamster prion promoter/APPSwe/Ind (KM670/671NL + V717F)	++	+	–	[2]
PSAPP	6	Tg2576 × PSEN1M146L	++	+		[6,10,27]
3xTg-AD	6	Thy1 promoter/APP695-Swedish/Tau isoform 4R0N(P301L mutation)/PSEN1M146L	++	++++	+	[18]
5xFAD	2	Thy1 promoter/B6SJL-Tg(APPSwFlLon,PSEN1M146L*L286V)6799Vas/J	++++	++++	++++	[17]

Symptomatic Tg-AD models exhibiting the highest senile plaque density (as seen anywhere within the CNS in aged Tg-AD models), and synaptic neuropathology (as determined by microscopic and molecular abundance measures; see text for further descriptions, unpublished observations) also displayed the highest levels of the inducible, inflammation-associated miRNA-146a species; + detected, ++ moderate abundance, ++++ extensive phenotype; see also Refs. [19,1,29,4,26,3,28,30]. The scoring system adapted from Ref. [19], also took into consideration additional Tg-AD characteristics listed at the Tg-AD website www.alzforum.org/res/com/tra/app/default.asp, in addition to consideration of deficits in the abundance of several important synaptic and cytoarchitectural support proteins, including synapsin-2, spectrin, syntenin, synaptophysin and neurofilament light chain (NF-L) protein assayed in these Tg-AD models (data not shown), and from observations made in the original source and supporting references for each Tg-AD type (rightmost column and text).

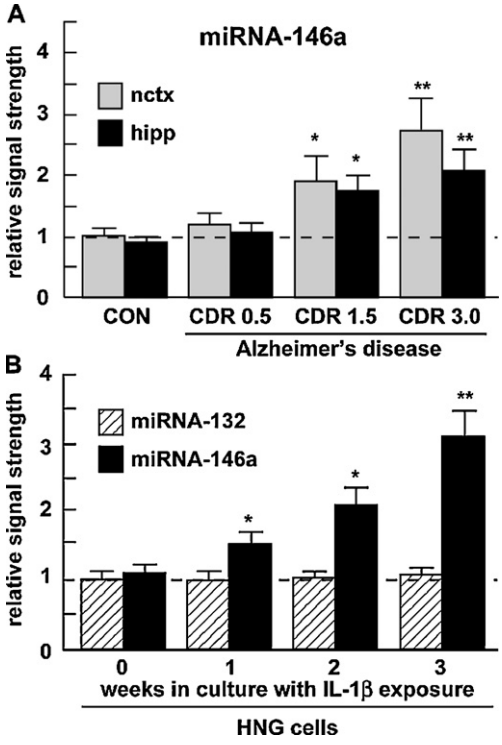

Fig. 1. Up-regulation of an inducible miRNA-146a (A) in various stages of AD and (B) in IL-1β-stressed HNG cells. (A) Abundance of miRNA-146a in superior temporal lobe neocortex (nctx) and hippocampus (hipp) compared to 5S RNA in the same sample and to age-matched controls; number of samples used CON = 6, CDR 0.5 = 6, CDR 1.5 = 6, CDR 3.0 = 6 (3 males and 3 females in each group; see text), and (B) miRNA-146a abundance in stressed HNG cells compared to miRNA-132 in the same sample and to age-matched controls [16]. For ease of comparison control miRNA-146a levels in the neocortex arbitrarily set to 1.0; dashed horizontal line. In (B) HNG cells were treated with human recombinant IL-1β (10 ng/ml cell culture medium) and were cultured for 0–3 weeks, representing inflammatory cytokine IL-1β- mediated stress for up to one half of their *in vitro* lifetime of 6 weeks. The results indicate a specific up-regulation of miRNA-146a; a related miRNA-132 showed no change in either AD or in stressed HNG cells. Hence miRNA-146a is up-regulated in HNG cells stressed with inflammatory cytokines known to be abundant in AD brain. For ease of comparison control miRNA-132 levels arbitrarily set to 1.0; dashed horizontal line; $N = 3–5$; $^{*}p < 0.05$, $^{**}p < 0.01$ (ANOVA).

curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione diferuloylmethane; purity >98.5%, Axxora, San Diego, CA), dissolved in dimethylsulfoxide as a 100 mM stock solution, was used at 5 uM ambient concentration in HNG cell medium; the metal chelator, anti-oxidant and NF-κB translocation inhibitor pyrrolidine dithiocarbamate (PTDC; P8765; Sigma, St Louis, MO) was used at 20 uM; and the polyphenolic trans-stilbene resveratrol analog CAY10512 (10009536; Cayman Chemical, Ann Arbor, MI) was used at 0.5 uM as previously described [16]. Primary HNG cells were cultured as previously described [14,16,24,15].

Stringent selection parameters were used in choosing control and AD superior temporal lobe neocortex and hippocampal CA1 tissues employed in this study [16,24]. As post-mortem interval (PMI; the time of death to brain freezing at −81 °C) is a factor that affects total RNA quality [24,15,21], all total RNA fractions were derived from tissues having a PMI of 3 h or less. CERAD/NIH criteria were used to categorize AD tissues in accordance with established guidelines [15]. AD brain tissues were obtained from patients with a clinical dementia rating (CDR; an index of cognitive impairment) ranging from 0.5 to 3.0, indicating very mild- to severe-AD [15,21,8]. Brain tissues were used in accordance with the institutional review board (IRB)/ethical guidelines at the Louisiana State University Health Sciences Center and the donor institutions [15,21]. All total RNA isolation, micro-RNA enrichment, quality control, quantitation and characterization using Northern dot blot miRNA analysis for 5S rRNA, miRNA-132, and miRNA146a, and bioinformatics analysis were performed as previously described [13,14,16,24,5,20,12,15,21]. All cell and tissue total RNA samples showed 28S/18S ratios larger than 1.45 indicating high spectral quality RNA [16,24]. Parameters involving control and AD superior temporal lobe neocortical tissues and detailed analysis of RNA spectral purity have been previously described, and most of these tissue samples have been previously subjected to wide spectrum gene expression analysis using DNA array technologies [12,15]. Samples of control and AD tissues used in these experiments exhibited no significant differences in age (69.0 ± 1.8 vs 70.3 ± 3.3 years, $p < 0.87$), PMI (mean 2.1 ± 0.7 vs 2.0 ± 0.7 h, $p < 0.96$), RNA $A_{260/280}$ indices (2.09 ± 0.2 vs 2.09 ± 0.1, $p < 0.97$), control versus AD, respectively.

Samples of age-matched control and AD tissues, cultured brain and retinal cells, and the transgenic animals Tg2576, TgCRND8, PSAPP, 3xTg-AD and 5xFAD were analyzed for 5S RNA, miRNA-132 and miRNA-146a abundance using miRNA array and/or Northern dot blot hybridization as previously described by our group [13,14,16,24,5,20,12]. Human 5S ribosomal RNA (5S rRNA), a rela-