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MicroRNA regulation of Alzheimer's Amyloid precursor protein expression

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ABSTRACT

Gene dosage effects of *Amyloid precursor protein* (*APP*) can cause familial AD. Recent evidence suggest that microRNA (miRNA) pathways, implicated in gene transcriptional control, could be involved in the development of sporadic Alzheimer's disease (AD). We therefore investigated whether miRNAs could participate in the regulation of *APP* gene expression. We show that miRNAs belonging to the miR-20a family (that is, miR-20a, miR-17-5p and miR-106b) could regulate APP expression *in vitro* and at the endogenous level in neuronal cell lines. A tight correlation between these miRNAs and APP was found during brain development and in differentiating neurons. We thus identify miRNAs as novel endogenous regulators of APP expression, suggesting that variations in miRNA expression could contribute to changes in APP expression in the brain during development and disease. This possibility is further corroborated by the observation that a statistically significant decrease in miR-106b expression was found in sporadic AD patients.

Introduction

The Aβ peptides accumulating in the brain of AD patients result from proteolytic cleavage of APP by β- and γ-secretases (De Strooper et al., 1998; Vassar et al., 1999; Haass, 2004). Increased expression of APP is associated with the pathogenesis of Alzheimer's disease. Indeed, gene duplications of the *app* locus on chromosome 21 is believed to cause AD pathology (Podlisny et al., 1987; Rovelet-Lecrux et al., 2006). Similarly in Down's syndrome (trisomy 21) it is believed that the associated extra copy of the *app* gene is the cause of the large incidence of AD in these patients (Podlisny et al., 1987). Moreover, polymorphisms in the APP promotor that increase transcription have been associated with AD (Theuns et al., 2006). Thus, understanding the mechanisms that regulate APP expression is relevant for our understanding of the pathogenesis of AD.

The small non-coding microRNAs (miRNAs) control gene expression networks at the posttranscriptional level (Ambros, 2004; Bartel, 2004) *via* imperfect complementary binding to the 3′ untranslated region (3′UTR) of target mRNAs leading to their translational repression and sometimes degradation. Abundantly expressed in the central nervous system, several miRNAs show a high degree of temporal and spatial specificity (Barad et al., 2004; Miska et al., 2004; Sempere et al., 2004; Smirnova et al., 2005) and are thought to play a role in neuronal cell specification, differentiation and synaptic plasticity (Smirnova et al., 2005; Kosik, 2006; Schratt et al., 2006).

The possibility that miRNA pathways could contribute to neurodegenerative disorders in human is gaining popularity (Hebert and De Strooper, 2007). Indeed, potential roles for miRNA dysfunction in the development of sporadic Parkinson's disease have been proposed (Kim et al., 2007; Wang et al., 2008a). In addition, we (Hebert et al., 2008) and others (Wang et al., 2008b) have shown that miRNAs might contribute to increased BACE1/β-secretase expression in sporadic AD. Here, we extended this investigation and asked whether miRNAs could be involved in the gene expression regulation of APP.

Materials and methods

Patient information

The non-dementia ($n=11$) and AD dementia ($n=19$) patients were from the Geriatric Department of E. Roux Hospital at Limeil-Brevannes and the Lille CH&U Hospital, France (ADERMA network). Clinical, neuropathologic, biochemical and genetic data for these patients were presented elsewhere (Hebert et al., 2008). Blocks from the anterior temporal cortex or cerebellum were dissected from each case and snap frozen in liquid nitrogen.

Abbreviations: ORF, Open reading frame; qRT-PCR, Quantitative RT-PCR; 3′UTR, 3′ untranslated region.

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Cell lines

HeLa, Neuro2A and SK-N-SH cells were cultured DMEM/F12 medium supplemented with 10% FCS as described (Hebert et al., 2006). Primary cultures of cortical neurons were prepared from embryonic day 17 C57/BL6 mice as described (Camacho et al., 2004). Mouse ES cells were differentiated into pure (~90–95%) glutamatergic neurons as described (Bibel et al., 2004). Specific technical details are available on demand.

Antibodies

Polyclonal APP B63 (previously named B10) (Hebert et al., 2006), monoclonal E2F1 (#KH95, Santa Cruz Biotechnology, Inc.), monoclonal N-RAS (#F155, Santa Cruz Biotechnology, Inc.) and monoclonal β-Actin (Sigma-Aldrich) were used.

Protein extraction and Western blot analysis

Cells were rinsed with cold PBS and lysed in buffer: 1% Triton X-100, 50 mM HEPES pH 7.6, 150 mM NaCl, 1 mM EDTA and complete protease inhibitors (Roche). Protein from brain (human and mouse) and neurons was extracted using the miRVana PARIS kit (Ambion). Immunoblot analysis was performed as described (Hebert et al., 2006).

RNA extraction and quantitative RT-PCR

2–6 total brains from wild-type BL/6 mice or 2–3 sister cultures from neurons (primary or ES cell-derived) were pooled per time point. Total RNA was extracted using the miRVana PARIS kit (Ambion) according to the manufacturer's instructions. RT-PCR as well as quantitative PCR procedures was carried out as described (Hebert et al., 2006). Primer sequences to quantify mRNA are: human APP forward 5′ AAAAC-GAAGTTGAGCCTGTTGAT 3′, reverse 5′ GAACCTGGTCGAGTGGTCAGT 3′; human β-Actin forward 5′ CACCCTGAAGTACCCCATGG 3′, reverse 5′ TGCCAGATTTTCTCCATGTCG 3′. For miRNA quantifications, probe-specific Taqman miRNA assays (Applied Biosystems) were used according to the manufacturer's instructions. Relative expression was calculated by using the comparative CT method.

Northern blotting

A pre-made 'mouse brain aging blot' (See-gene) was used which contained ~20 μg total RNA per lane isolated from mouse brain at

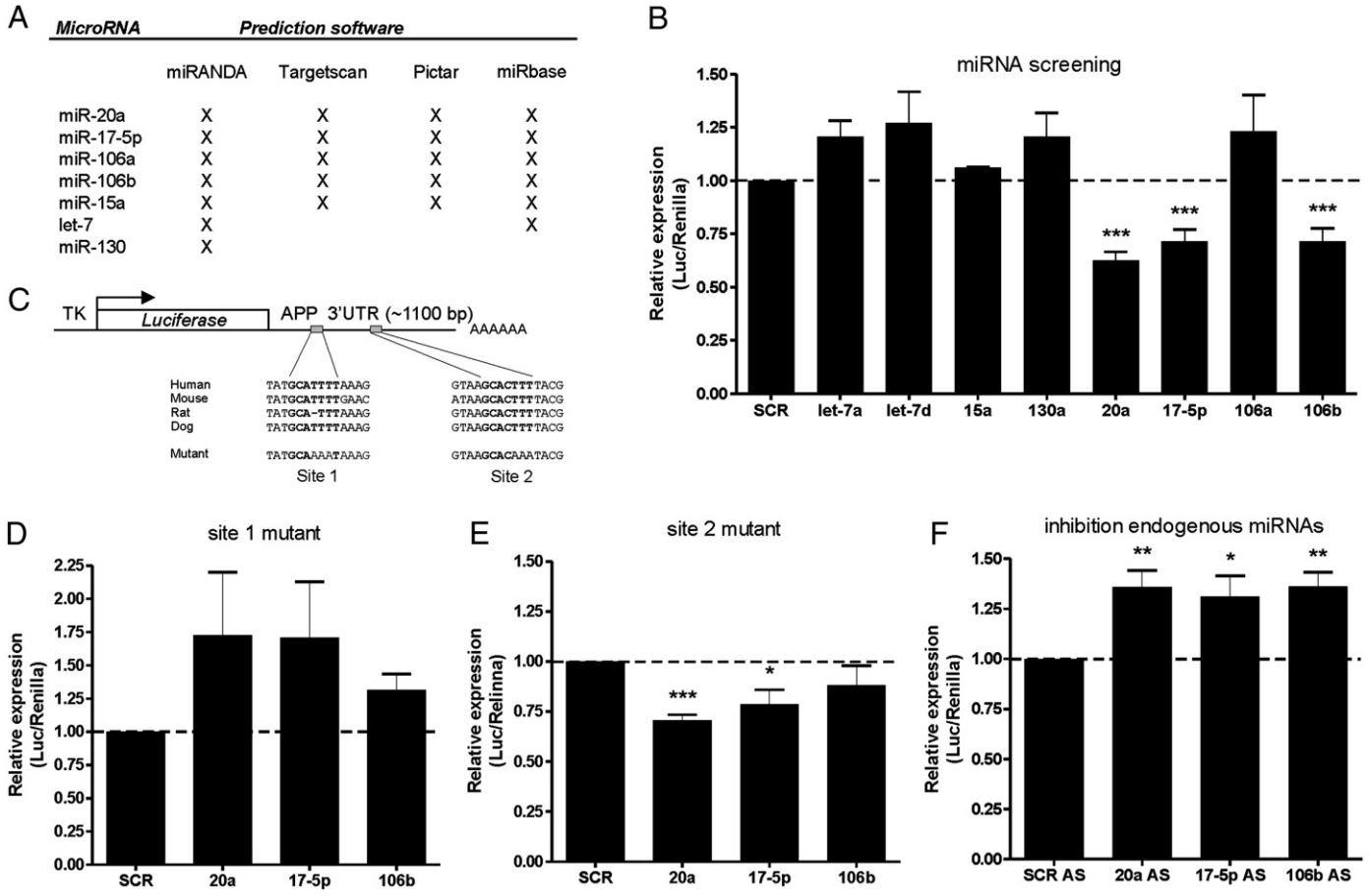

Fig. 1. Validation of candidate miRNAs. (A) List of candidate miRNAs used in this study identified by various algorithms: miRanda (microrna.org), Targetscan (targetscan.org), Pictar (pictar.bio.nyu.edu) and miRbase (microrna.sanger.ac.uk). "X" indicates positive hit in the algorithm. (B) APP 3′UTR wildtype luciferase and *Renilla* luciferase constructs were co-transfected into HeLa cells with the indicated pre-miRNA oligonucleotides (scrambled sequence, let-7a, let-7d, miR-15a, miR-130a, miR-106a, miR-106b, miR-17-5p and miR-20a) at a final concentration of 75 nM. (C) Schematic representation (not to scale) of the APP 3′UTR luciferase construct used in this study. TK, thymidine kinase promotor. The sequence as well as the "top score" putative binding sites for miR-20a, miR-17-5p, miR-106a and miR-106b is shown. The miRNA seed sequences are in bold. In the APP 3′UTR mutant constructs (site 1 or site 2), the binding sites for miRs are mutated as indicated. (D) (E) APP 3′UTR mutant constructs were co-transfected with the indicated pre-miRs at a final concentration of 75 nM. (F) APP 3′UTR wildtype luciferase construct was co-transfected into HeLa cells with the indicated anti-(complementary) miRNA oligonucleotides at a final concentration of 75 nM. In all experiments, normalized sensor luciferase activity is shown as a fold difference of the scrambled oligonucleotide control. Error bars represent standard deviations derived from three or more independent experiments performed in duplicate. Statistical significance between control (scrambled miR-treated) and candidate pre-miR-treated HeLa cells was determined by a Student's paired *t* test (*$p<0.05$, **$p<0.01$, ***$p<0.001$).