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MicroRNA-130b targets *Fmr1* and regulates embryonic neural progenitor cell proliferation and differentiation

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ABSTRACT

Fragile X syndrome, one of the most common forms of inherited mental retardation, is caused by expansion of the CGG repeat in the 5′-untranslated region of the X-linked *Fmr1* gene, which results in transcriptional silencing and loss of expression of its encoded protein FMRP. The loss of FMRP increases proliferation and alters fate specification in adult neural progenitor cells (aNPCs). However, little is known about *Fmr1* mRNA regulation at the transcriptional and post-transcriptional levels. In the present study, we report that miR-130b regulated *Fmr1* expression by directly targeting its 3′-untranslated region (3′ UTR). Up-regulation of miR-130b in mouse embryonic neural progenitor cells (eNPCs) decreased *Fmr1* expression, markedly increased eNPC proliferation and altered the differentiation tendency of eNPCs, suggesting that antagonizing miR-130b may be a new therapeutic entry point for treating Fragile X syndrome.

1. Introduction

Fragile X syndrome, the most common form of inherited mental retardation, is almost always caused by expansion of the CGG repeat in the 5′-untranslated region of the X-linked Fragile X Mental Retardation 1 gene (*Fmr1*), which results in transcriptional silencing and loss of expression of its encoded Fragile X Mental Retardation Protein (FMRP) [1,2]. The CGG triplet region is highly polymorphic in the population. Normal alleles have between 6 and 54 copies and are stably transmitted to offspring. A pre-mutation allele arises when repeats expand to between 55 and 200 copies. Pre-mutation alleles are rather unstable and can evolve into a full mutation during maternal transmission. A full mutation has over 200 repeats and leads to hypermethylation of the CGG region, transcriptional silencing and abolished production of FMRP [3].

FMRP is an evolutionarily conserved RNA-binding protein that is particularly abundant in the brain due to its high expression in neurons [4–6]. Several studies have shown that FMRP plays a critical role in regulating mRNA translation, transport and stability [7–9]. In neurons, FMRP may modulate mRNA expression by controlling recognition, export, translational efficiency and stability of target mRNAs [9,10]. FMRP silencing abnormally regulates brain mRNA translation and disrupts the composition of the appropriate protein milieu, which mediates defects in neuronal development and synaptogenesis [8,11,12]. Deletion of FMRP from adult neural progenitor cells (aNPCs) leads to reduced hippocampal neurogenesis both in vitro and in vivo and markedly impairs hippocampus-dependent learning in mice [13]. Loss of FMRP increases proliferation and alters fate specification in adult neural progenitor cells. The altered function of adult neural progenitor cells (aNSCs) is partially dependent on CDK4 and GSK3β signaling, which are both known FMRP targets of translational repression [14].

MicroRNAs (miRNAs) are a class of short (~22 nucleotide), single-stranded non-coding RNAs that usually bind their target mRNAs through imperfect base pairing in the 3′-untranslated regions (3′ UTRs) and impact protein expression by translational repression, mRNA degradation or the promotion of mRNA decay [15,16]. Cumulating evidence allocates miRNA function to integral parts of regulatory cellular processes, including cell proliferation and differentiation, regulation of metabolic activities and tumorigenesis [17–19]. To date, many miRNAs that are specifically or richly expressed in the mammalian brain have been identified. For example, miR-9, a brain-specific miRNA, regulates neural progenitor cells (NSCs) proliferation and differentiation by binding to the 3′ UTR of TLX mRNA [20]. MiR-137 modulates the proliferation and differentiation of adult NSCs by targeting EZH2, a histone H3 lysine 27 methyltransferase [21]. Additionally, miR-137 regulates neuronal maturation by inhibiting dendrite formation by binding Mind bomb 1 (Mibl) [22]. Mibl is an ubiquitin ligase that

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is known to be important for neurodevelopment. Although some miRNA functions have been revealed, the precise roles of many miRNAs in the proliferation, differentiation and migration of embryonic neural progenitor cells (eNPCs) remain largely unknown. In this study, we show that miR-130b regulates FMRP translation and therefore modulates mouse eNPCs proliferation and fate determination. These data suggest that the functional interaction between miRNA and *Fmr1* plays an important modulatory role in eNPCs fate determination.

2. Materials and methods

2.1. Prediction of microRNAs that target Fmr1 mRNA

The miRNAs targeting *Fmr1* mRNA were predicted using three different prediction programs: PicTar (http://pictar.mdc-berlin.de/cgi-bin/new_PicTar_mouse.cgi), TargetScan Mouse (http://www.targetscan.org/mmu_61) and miRDB (http://mirdb.org/miRDB). Only the miRNAs predicted by all three algorithms were considered to be putative regulators of *Fmr1* and were selected for further experimental identification.

2.2. Cell culture

Human HEK-293T cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 4500 mg/l glucose, 4 mM L-glutamine, and 10% heat-inactivated fetal bovine serum (Gibco) without sodium pyruvate at 37 °C in a 5% CO_2 atmosphere.

Primary embryonic NPCs were isolated from E12.5 fetal mice. Pregnant mice were treated, anesthetized and euthanized in accordance with institutional guidelines. The fetal mice were quickly removed out of the uterus and put into the cold PBS. After being washed with cold PBS for 3 times, the brain tissues were chopped and enzymatically digested by using Papain at 37 °C for 20 min. Dissociated cells were cultured in DMEM-F12 (1:1) medium (Gibco, 11320033) supplemented with 10% B27 (Gibco, 0080085-SA), 20 ng/ml epidermal growth factor (EGF, R&D, 2028-EG-200)) and 10 ng/ml basic fibroblast growth factor (bFGF, R&D, 3139-FB-025).

2.3. Construction of plasmid-mFmr1-3′ UTR and plasmid-mFmr1-3′ UTR containing the miR-130b target site mutant

Mouse genomic DNA was isolated and purified from eNPCs with QIAamp® DNA Mini Kit according to the manufacturer's protocol. The 3′-untranslated region (UTR) sequence of *Fmr1* was PCR amplified directly from the genomic DNA using the following primers: 5′-GATACTCGAGGGCTGCGCACGGGTAAAGA-3′ (forward) and 5′-CATGGCGGCCGCTGTAATATGAATCAACTCCAACTC-3′ (reverse). The primers were designed incorporating *Xho* I and *Not* I restriction sites and 4 bp of extra random sequence to aid in restriction digestion. *Xho* I and *Not* I-digested PCR products were cloned into a *Xho* I- and *Not* I-digested psiCHECK-2 dual luciferase reporter vector (Promega, C8021). The recombinant plasmids (psiCHECK2-*mFmr1*-3′ UTR) were confirmed by sequencing. The miR-130b target site mutant plasmid was constructed by ShangHai RayGene Biotechnology Co., Ltd.

2.4. Construction of a lentiviral vector expressing sh-miR-130b

U6-miR130b or U6-control expression constructs were derived from a previously generated U6-shRNA lentiviral construct (Lenti-137) by using a PCR-shagging approach as described previously [23]. A lenti-130b reverse primer (5′-TATCGATAAAAAAA-CAGTGCAATGATGAAAGGGCATTCTCTTGAAATGCCCTTTCATCATTGC ACTGAAACAAGGCTTTTCTCCAAGGGA-3′) and a lenti-control reverse primer (5′-TATCGATAAAAAAAAATTCTCCGAACGTGTCACGTT CTCTTGAAACGTGACACGTTCGGAGAATTAAACAAGGCTTTTCTCCAA GGGA-3′) were used in combination with a common forward primer complementary to the 5′ end of the U6 promoter (5′-AAAGTTAACTAGTGGATCCGACGCCGCCATCTC-3′) to amplify the entire U6 promoter and shRNA in a single PCR product. Amplification was initiated by denaturing at 94 °C for 5 min, followed by 30 cycles of 30 s each at 94 °C, 30 s at 60 °C and 40 s at 72 °C, with a final extension step of 5 min at 72 °C. The PCR products were cloned into the TOPO TA vector, and the recombinant constructs were then verified by sequencing. U6-miR130b or U6-control expression constructs were removed from the TOPO vector for transfer to a lentiviral vector by *Hpa* I and *Cla* I restriction digestion. The lentiviral vectors were also verified by sequencing.

2.5. Virus production

The lentiviral vector expressing sh-miR-130b and the packing vectors pMDL, pRev, and pVSV-G were co-transfected into human embryonic kidney (HEK) 293T cells, and culture medium was collected at 48, 72 and 96 h. The medium was filtered through 0.22-μm pore nitrocellulose filters and then centrifuged in a Beckman ultracentrifuge (Beckman, Avanti J-301) at 55,000*g* for 2 h at 16 °C. The precipitate was resuspended in NPC complete medium. The viral supernatant was directly infected into cells or stored at −80 °C.

2.6. Luciferase reporter assay of candidate miR-130b target Fmr1

Luciferase reporter experiments were performed in the HEK293T cell line. Cells were plated 1 day before transfection in a 24-well plate at a density of 2×10^5 cells/well. Cells were co-transfected with 1 μg of psiCHECK2-3′ UTR plasmid, 1 μg of sh-miR-130b TOPO (sh-miR-130b cloned into a TOPO vector) or TOPO-control using the calcium phosphate method. At 48 h after transfection, the cell extract was obtained, and *Firefly* and *Renilla* luciferase activities were measured with the dual-luciferase reporter system (Promega, E1980) according to the manufacturer's instructions. *R*-Luc activity was normalized to *F*-Luc activity to account for variation in transfection efficiencies. Luciferase experiments were repeated three times.

2.7. Quantitative RT-PCR

Total RNAs were isolated from the viral over-expressing NPCs using RNAiso (TakaRa, D9108A). RNA samples were reverse transcribed into cDNA with oligo $(dT)_{20}$ and SuperScript III (Invitrogen, 18080051). Real-time PCR was performed with gene-specific primers and Power SYBR Green PCR Master Mix (Applied Biosystems, 4367660) using the 7500 Standard Real-Time PCR System (Applied Biosystems). GAPDH was used as an endogenous control for all samples. Primers for qPCR were as follows. m*GAPDH*: 5′-CCTC GTCCCGTAGACAAAATG -3′ (forward) and 5′-TCTCCACTTTGCC ACTGCAA-3′ (reverse); m*FMR1*: 5′-AGGTGCCAGAAGATTTACGACA-3′ (forward) and 5′-CTCGCTTTGAGGTGACTTCATT-3′ (reverse). All real-time PCR reactions were performed in triplicate, and RQs were calculated using the ΔΔCt method with calibration to control samples.

Mature miR-130b expression was also detected by qPCR reaction. The Bulge-Loop™ miRNA qRT-PCR Primer and the U6 negative control were purchased from Guangzhou RiboBio Co. Ltd. Reverse transcription and qPCR reactions were performed according to the manufacturer's instructions.

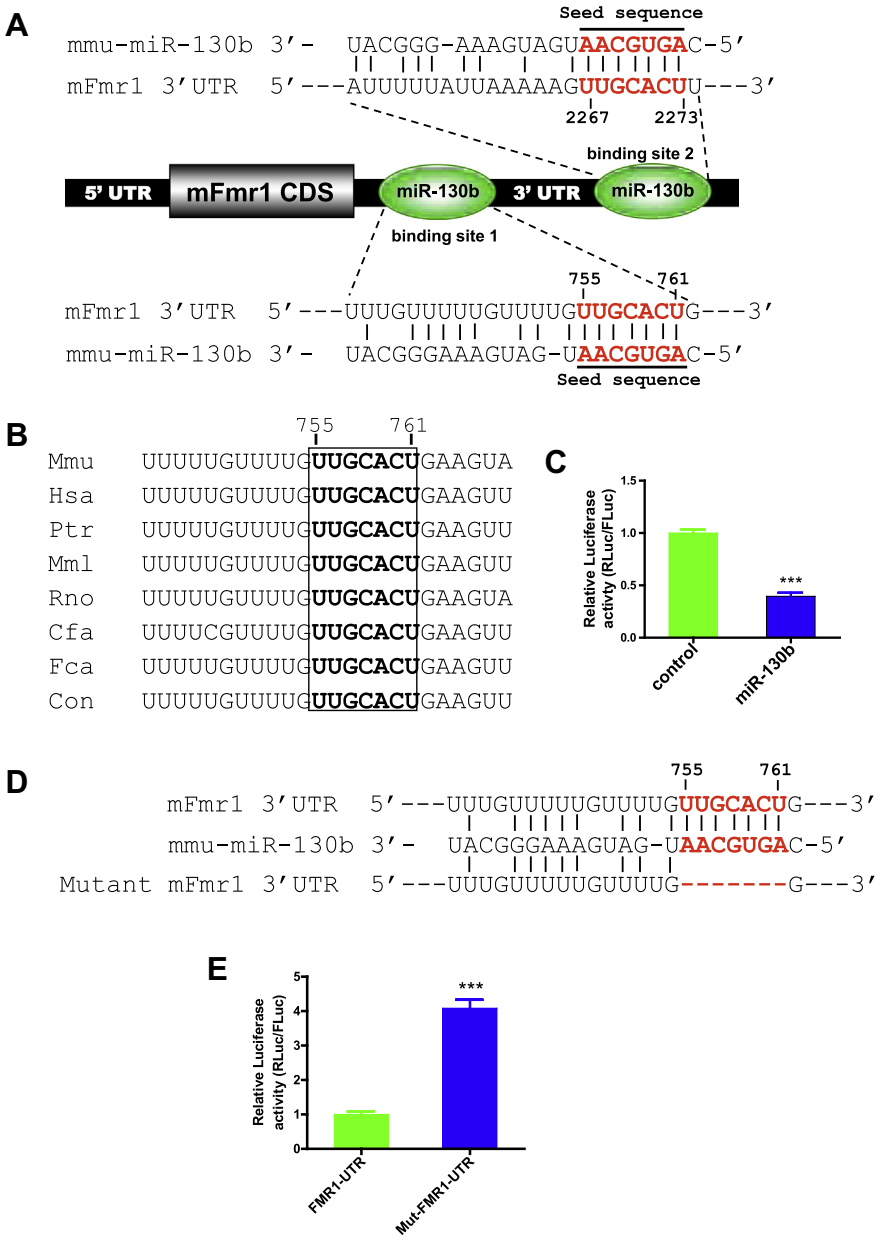

Fig. 1. Identification of target sites in the 3′ UTR of mouse *Fmr1* (*mFmr1*). (A) Schematic diagram of the *mFmr1* message including mouse miR-130b (mmu-miR-130b) binding sites in the 3′ UTR. (B) miR-130b binding site 1 in the 3′ UTR of *Fmr1* is highly conserved in mammals. (C) Schematic diagram showing the predicted seed region where miR-130b is expected to bind the *mFmr1* 3′ UTR (above) and the mutated version (below) lacking the binding site for miR-130b. (D) and (E) HEK-293T cells were transiently co-transfected with the luciferase reporter vector containing wild-type *mFmr1* 3′ UTR (D) or mutant (E) in the presence of mmu-miR-130b together or shRNA-control. Luciferase activity was evaluated 24 h after transfection as described in Section 2. These data are a representative of at least three independent experiments.

2.8. Protein isolation and Western blotting

Cells were washed twice in ice-cold phosphate-buffered saline and lysed in RIPA lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40, 0.1% SDS) in the presence of protease inhibitors. Protein concentration was determined using the BCA protein assay (TianGen, PA115) with bovine serum albumin as the standard, and equal amounts of protein were analyzed by SDS–polyacrylamide gel electrophoresis. The proteins were then electroblotted onto a polyvinylidene fluoride (PVDF) membrane (Millipore, IPVH00010). For immunoblot experiments, membranes were blocked in TBS-T containing non-fat 5% dried milk for 1 h and then incubated at 4 °C over night with anti-FMRP primary antibody (Abcam, ab69815). Anti-GAPDH (Sigma–Aldrich, G8795) was used as an internal loading control. Detection was performed with peroxidase-conjugated secondary antibodies using the enhanced chemiluminescence system (Thermo, 34094).

2.9. Proliferation and differentiation analyses of cultured eNPCs

To study cell proliferation, we plated eNPCs on 24-well plates containing glass coverslips coated with poly-L-ornithine and laminin at a density of 50,000 cells/well in proliferation medium [DMEM-F12 (1:1) medium (Gibco, 11320033) supplemented with 10% B27 (Gibco, 0080085-SA), 20 ng/ml epidermal growth factor (EGF, R&D, 2028-EG-200), and 10 ng/ml basic fibroblast growth factor (bFGF, R&D, 3139-FB-025)]. At 20 h post-plating, 5 μM 5-bromo-2′-deoxyuridine (BrdU, Sigma–Aldrich) was added to the culture medium for 2 h. NPCs were fixed with 4% paraformaldehyde for 30 min at room temperature and then washed with

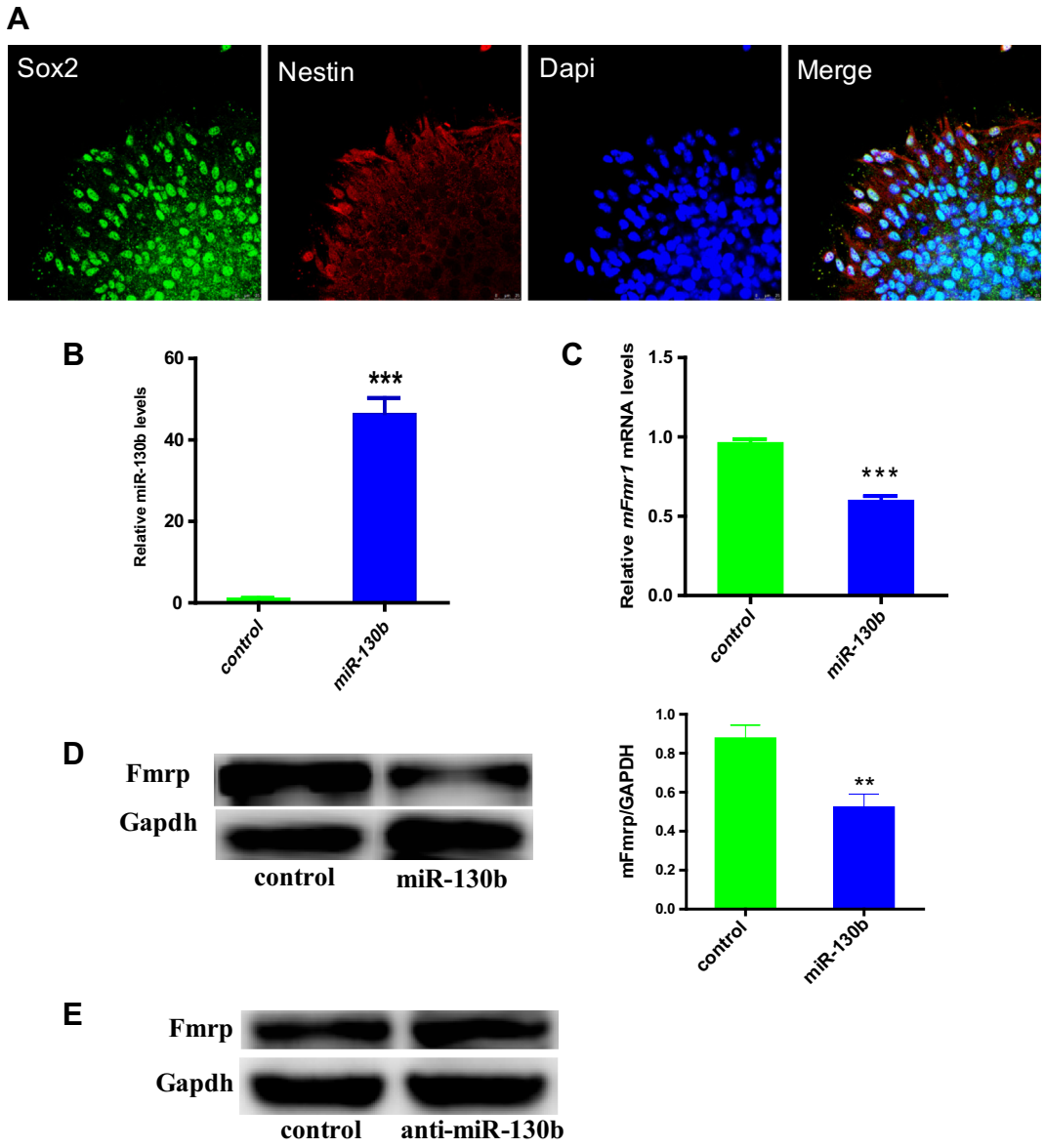

Fig. 2. MiR-130b over-expression suppresses FMRP expression. (A) eNPCs cultured under proliferating conditions expressed the neural progenitor markers Nestin (cytoplasmic, red) and Sox2 (nuclear, green; DAPI in blue). (B) miR-130b was over-expressed in eNPCs and confirmed by qRT-PCR (***$P < 0.05$). (C) *Fmr1* mRNA level was decreased (***$P < 0.05$) when miR-130b was over-expressed in eNPCs. (D) Western blot shows that over-expression of miR-130b significantly reduced *Fmr1* expression in protein levels. (E) Western blot shows that *Fmr1* expression was increased in protein levels when endogenous miR-130b was down-regulated by anti-miR-130b in mouse eNPCs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PBS, followed by immunohistochemical analysis. To detect BrdU incorporation, fixed cells were pretreated with 1 M HCl for 30 min at 37 °C and then washed with borate buffer, pH 8.5, for 30 min. For the differentiation assay at 24 h post-plating, cells were changed into differentiation medium for 4 days, followed by fixation with 4% paraformaldehyde for 30 min and then washing with PBS.

For immunocytochemistry staining, cells were preblocked using 0.1 M TBS containing 5% normal donkey serum and 0.3% Triton X-100 for 1 h, followed by overnight incubation with primary antibodies: anti-Brdu (Abcam, ab6326), anti-GFP (Abcam, ab1218), mouse neuron-specific type β-III tubulin (Promega, G712A), or rabbit glial fibrillary acidic protein (DAKO, Z-0334). After being washed with TBS, the cells were incubated with secondary antibodies that included Alexa Fluor 594 donkey anti-rat IgG (H+L) (Invitrogen, A21209) and Alexa Fluor 488 donkey anti-mouse IgG (H+L) (Invitrogen, A21202), followed by counterstaining with the fluorescent nuclear dye 4′,6-dimidino-2′-phenylindole dihydrochloride (DAPI, Sigma–Aldrich, B2261). After the cells were mounted, the numbers of Tuj1-, GFAP- or BrdU- positive cells were examined with a conventional fluorescence microscope.

2.10. Statistical analysis

Statistical analysis was performed using ANOVA and Student's *t*-test, unless specified with the aid of SPSS v.17. All data are shown as the means with standard error of mean (mean ± SEM). Probabilities of $P < 0.05$ were considered significant.

3. Results

3.1. The 3′ UTR of the Fmr1 mRNA is a target of miR-130b

According to the PicTar [24], TargetScan [25], and MiRtarget2 [26] algorithms for microRNA target prediction, several miRNAs putatively targeted the 3′ UTR of *Fmr1* mRNA. We focused our

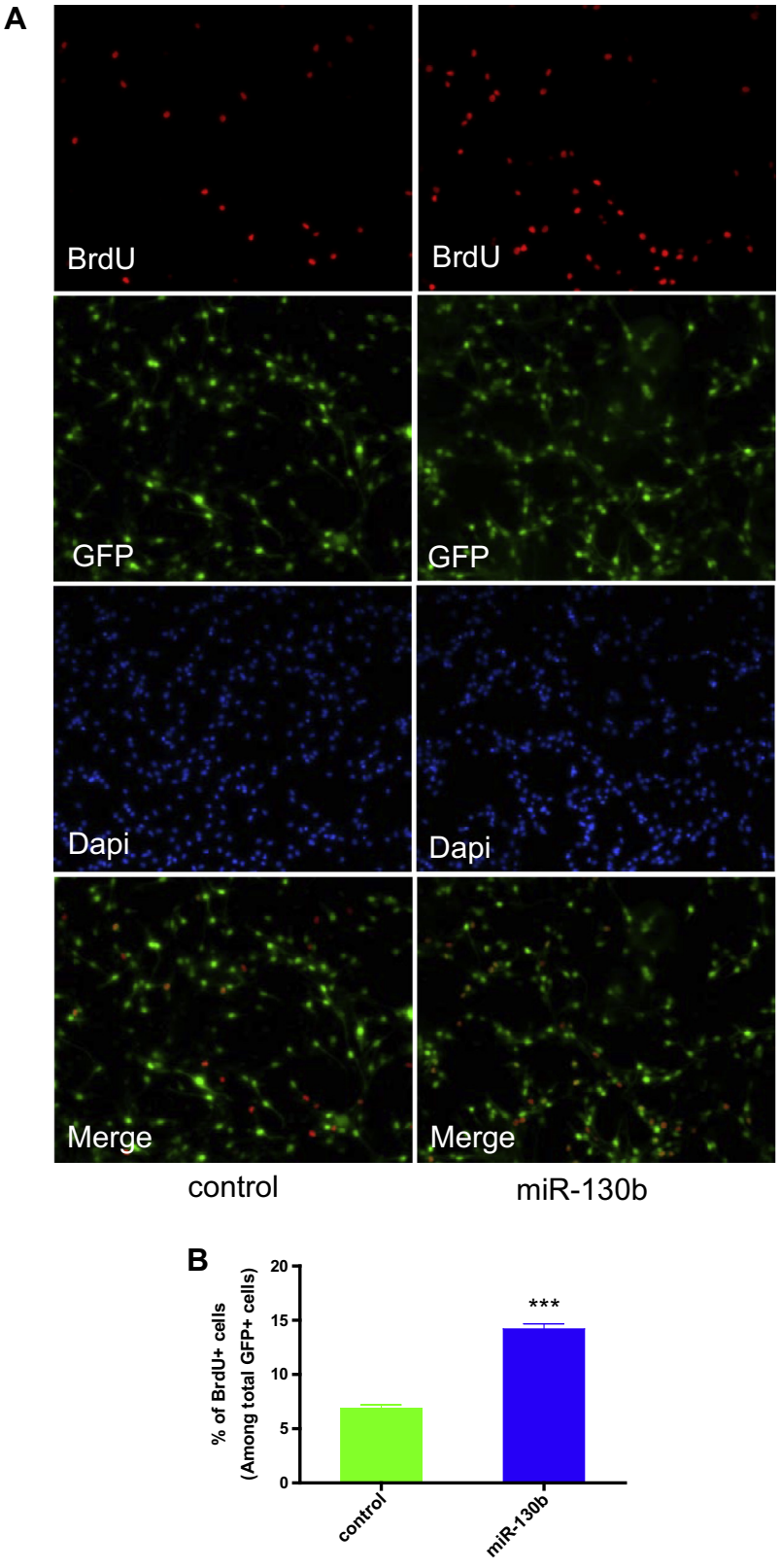

Fig. 3. Down-regulation of FMRP by miR-130b leads to increased eNPCs proliferation. (A) Immunocytochemistry shows an increased number of BrdU-positive cells in miR-130b-over-expressing cells. (B) Quantitative analysis shows that a higher percentage of miR-130b eNPCs incorporated BrdU.

attention on miR-130b, which is known to be expressed in the brain and is highly conserved between human and mouse [27]. Comparing the results obtained from different searches, we found that the 3′ UTR of the *Fmr1* gene comprises two miR-130b binding sites (Fig. 1A), and these sites are highly conserved in mouse, rat, and human (Fig. 1B).

The most direct approach for experimentally validating miRNA targets is to clone the predicted miRNA-binding sequence

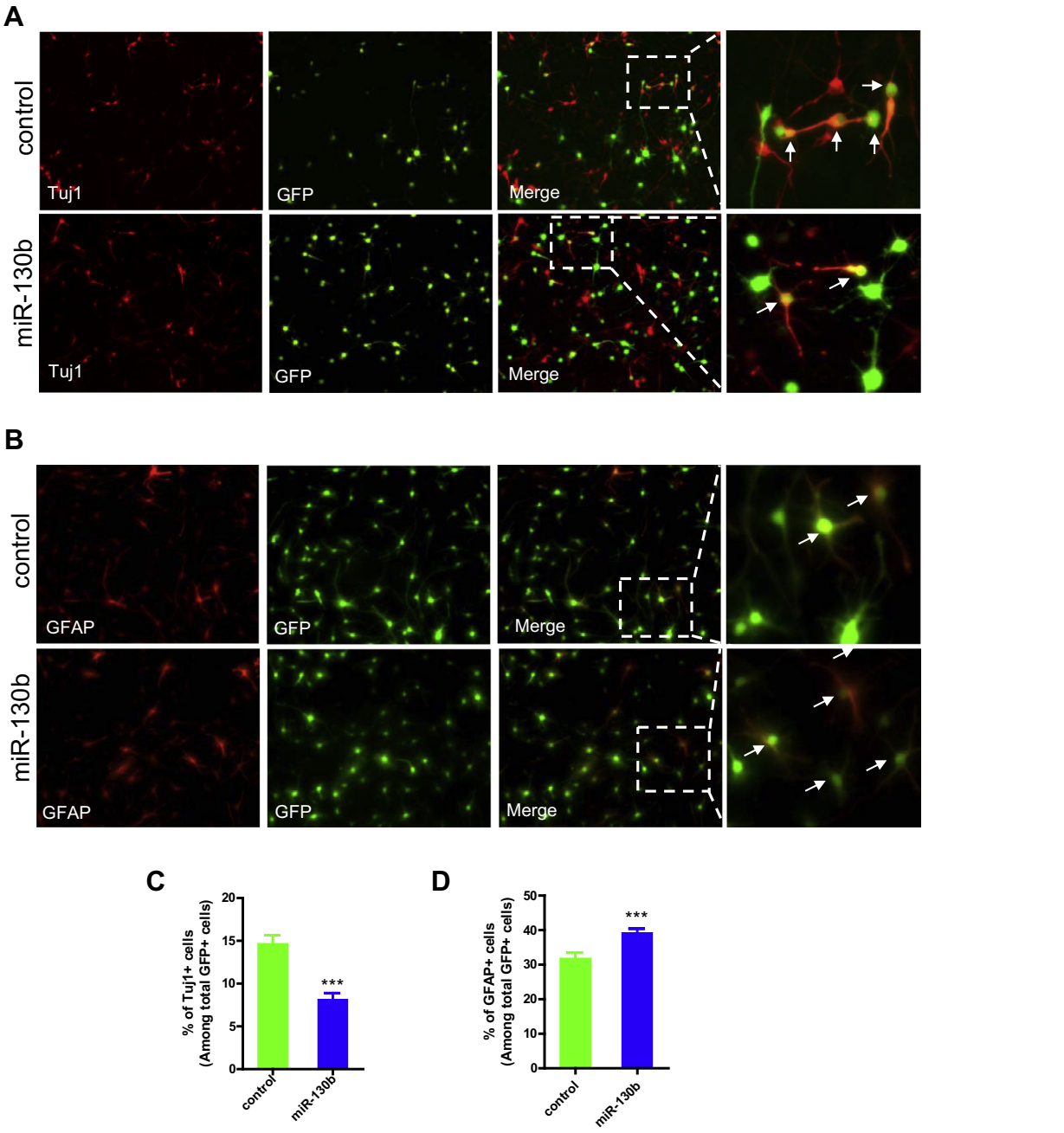

Fig. 4. MiR-130b alters eNPC fate specification. (A) Immunocytochemistry shows that miR-130b-over-expressing eNPCs and controls could differentiate into Tuj1+ (red) neurons. (B) Immunocytochemistry shows that miR-130b-over-expressing eNPCs and controls could differentiate into GFAP+ (red) astrocytes. (C) and (D) Quantitative analyses of differentiated eNPCs demonstrate that miR-130b-over-expressing eNPCs differentiated into fewer Tuj1+ neurons (44.57%) but more GFAP+ astrocytes (23.03%) compared to the negative control. Statistical analyses were performed using a two-tailed unpaired Student's *t*-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

downstream of a luciferase reporter construct and cotransfect it with the miRNA of interest for dual-luciferase assays. Therefore, we cloned the 3′ UTR of mouse *Fmr1* into the psiCHECK-2 reporter vector, expressing both *Renilla* and *Firefly* luciferase, at the 3′ end of the *Renilla* luciferase coding sequence. This reporter vector, together with miR-130b or negative control miRNA, was transfected into HEK-293T cells. We found that miR-130b significantly reduced luciferase activity compared to the negative control (Fig. 1C), which suggests that miR-130b could target the 3′ UTR of *Fmr1* mRNA and impair its translation. To further confirm this result, we generated a deleted mutant *Fmr1* 3′ UTR sequence that lacks the binding site of miR-130b, TTGCACT (Fig. 1D), and used it to replace the wild-type *Fmr1* 3′ UTR sequence at the 3′ end of the *Renilla* luciferase coding sequence in the psiCHECK-2 reporter vector. We observed that the mutant 3′ UTR of *Fmr1* increased the relative luciferase activity by four-fold when compared to the wild-type 3′ UTR (Fig. 1E), suggesting that *Fmr1* mRNA was an authentic target of mmu-miR-130b.

3.2. MiR-130b suppresses Fmr1 expression in eNPCs

FMRP was expressed in Sox2 and Nestin double-positive adult and embryonic neural progenitor cells and in either NeuroD1-positive or doublecortin (DCX)-positive newly generated neurons [14]. To investigate the effect of miR-130b on *Fmr1* expression in mouse eNPCs, we isolated eNPCs from fetal brains of ICR mice at

embryonic day 12.5. Nearly all cultured eNPCs were Sox2 and Nestin double-positive (Fig. 2A), suggesting a relative homogeneity of these primary eNPCs. We then established miR-130b-over-expressing eNPCs using a lentiviral pMDL system and found that miR-130b expression was upregulated approximately 40-fold compared to control eNPCs (Fig. 2B). In the miR-130b-over-expressing eNPCs, both *Fmr1* mRNA levels (Fig. 2C) and protein levels (Fig. 2D) were significantly reduced compared to the control. These results suggest that miR-130b could suppress *Fmr1* expression in eNPCs.

To further confirm the relationship between endogenous miR-130b and FMRP expression, we infected anti-miR-130b which complemented endogenous miR-130b in mouse eNPCs, and found increased FMRP protein levels in these eNPCs compared with the negative control (Fig. 2E), suggesting that *Fmr1* is regulated by endogenous miR-130b in eNPCs.

3.3. MiR-130b alters eNPC proliferation

A previous study has shown that FMRP controls proliferation of adult neural stem cells in mice [14] and neural stem cells and the germline in *Drosophila* [28,29]. The loss of mouse FMRP and *Drosophila* FMRP led to a significant increase in the number of mitotic NPCs [14,28,29]. Because miR-130b can regulate FMRP expression, we sought to determine whether miR-130b could have an impact on eNPC proliferation. To tackle this question, we used BrdU, a pyrimidine analogue of thymidine that selectively incorporates into cell DNA at the S phase of the cell cycle, to pulse-label proliferating cells for 4 h before paraformaldehyde fixation to assess the proliferation of eNPCs. We found that miR-130b-over-expressing eNPCs exhibited twice as much BrdU incorporation as the negative control (Fig. 3A and B).

3.4. MiR-130b alters eNPC fate specification

The effect of FMRP on neural differentiation has been previously studied, and the results were controversial. Fragile X human embryonic stem cells (FX-hESCs) showed abnormal neurogenesis during neural differentiation, leading to poorer neuronal maturation and higher gliogenic development when compared to normal human embryonic stem cells [30]. Similarly, the loss of FMRP from mouse adult neural progenitors was shown to generate more glial cells at the expense of neurons [13,14]. However, it was also reported that mouse and human FMRP-deficient neurospheres generated more Tuj1-positive cells than the control neurospheres generated from normal mouse and human brains [31]. To determine whether miR-130b could affect neuronal and astrocyte differentiation in eNPCs, we performed spontaneous differentiation by removing the growth factors. eNPCs with or without exogenous miR-130b were differentiated for 3 days, and the phenotypes of differentiated cells were stained using cell lineage-specific antibodies: β-III tubulin (Tuj1) for neurons and glial fibrillary acidic protein (GFAP) for astroglia. The cultured cells were induced to differentiate into neurons (Fig. 4A) and astrocytes (Fig. 4B); however, miR-130b-over-expressing eNPCs exhibited a 44.57% decrease in neuronal differentiation (Fig. 4C) and a 23.03% increase in astrocyte differentiation (Fig. 4D) compared to the negative control. This result was consistent with previous studies [13,14,30] and suggested that miR-130b alters embryonic neurogenesis by inhibiting FMRP expression.

4. Discussion

Fragile X syndrome is a neurodevelopmental disorder caused by the selective loss of FMRP production. Recently, an increasing number of studies showed that NPCs lacking FMRP undergo aberrant neural differentiation that could contribute to the pathogenesis of fragile X mental retardation. Some experimental evidence has demonstrated that the loss of functional FMRP in aNPCs leads to reduced neurogenesis both in vitro and in vivo and markedly impairs hippocampus-dependent learning in mice [13,14]. Eadie et al. reported that loss of *Fmr1* expression can produce region-specific alterations in hippocampal adult neurogenesis and alter anxiety-related behaviors in mice [32]. Moreover, Telias et al. showed that neural differentiation of fragile X human embryonic stem cells reveals abnormal neurogenesis and aberrant gene expression, leading to poor neuronal maturation and high gliogenic development [30]. Another study [31] reported that FMRP-deficient NPC cultures from the brains of *Fmr1*-KO mice and human fragile X fetuses showed an altered ratio of Tuj1-positive to glial fibrillary acidic protein (GFAP)-positive cells. Despite the known relationship between loss of functional FMRP and aberrant neural differentiation, the consequences of finely tuned modulation of *Fmr1* expression on NPC neuronal differentiation are still poorly understood.

To further examine this issue, we down-regulated *Fmr1* expression in mouse eNPCs using microRNA and analyzed fate specification. We found that miR-130b, one of the brain-expressed microRNAs, has two binding sites in the 3′ UTR of *Fmr1* and can negatively modulate the expression of *Fmr1*. Our study has demonstrated that miR-130b promoted mouse eNPC proliferation, decreased neuronal differentiation and increased glial differentiation via downregulation of *Fmr1* expression, suggesting that the finely tuned modulation of *Fmr1* expression appears to be crucial to neurogenesis.

miRNAs are endogenously encoded single-stranded RNAs that can post-transcriptionally regulate gene expression via either translational inhibition or mRNA degradation [33]. One mRNA can be targeted by multiple miRNAs, and therefore, miRNAs often act as fine-tuning devices rather than primary gene regulators. Previous studies showed that miR-125b, miR-132, miR-101, miR-129-5p and miR-221 could target the 3′ UTR of Fmr1 mRNA and are involved in the molecular pathology of Fragile X syndrome at the synaptic structure and function [12,34]. In this study, we found that miR-130b is an important regulator of Fmr1 and is responsible for NPC fate determination. Because miR-130b can negatively modulate the expression of Fmr1, antagonizing miR-130b may serve as a new therapeutic entry point for treating Fragile X syndrome.

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References

[1] B. Bardoni, L. Davidovic, M. Bensaid, E.W. Khandjian, The fragile X syndrome: exploring its molecular basis and seeking a treatment, Expert Rev. Mol. Med. 8 (2006) 1–16.
[2] S. Jacquemont, R.J. Hagerman, P.J. Hagerman, M.A. Leehey, Fragile-X syndrome and fragile X-associated tremor/ataxia syndrome: two faces of FMR1, Lancet Neurol. 6 (2007) 45–55.
[3] E.W. Loomis, J.S.P. Eid, et al., Sequencing the unsequenceable: expanded CGG-repeat alleles of the fragile X gene, Genome Res. 23 (2013) 121–128.
[4] C.T. Ashley Jr., K.D. Wilkinson, D. Reines, S.T. Warren, FMR1 protein: conserved RNP family domains and selective RNA binding, Science 62 (1993) 563–566.
[5] H. Siomi, M.C. Siomi, R.L. Nussbaum, G. Dreyfuss, The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein, Cell 4 (1993) 291–298.
[6] E.W. Khandjian, A. Fortin, A. Thibodeau, et al., A heterogeneous set of FMR1 proteins is widely distributed in mouse tissues and is modulated in cell culture, Hum. Mol. Genet. 4 (1995) 783–789.

[7] C. Bagni, W.T. Greenough, From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome, Nat. Rev. Neurosci. 6 (2005) 376–387.
[8] G.J. Bassell, S.T. Warren, Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function, Neuron 60 (2008) 201–214.
[9] S. De Rubeis, C. Bagni, Fragile X mental retardation protein control of neuronal mRNA metabolism: insights into mRNA stability, Mol. Cell. Neurosci. 43 (2010) 43–50.
[10] J.C. Darnell, K.B. Jensen, P. Jin, et al., Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function, Cell 107 (2001) 489–499.
[11] V. Brown, P. Jin, S. Ceman, et al., Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome, Cell 107 (2001) 477–487.
[12] D. Edbauer, J.R. Neilson, K.A. Foster, et al., Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132, Neuron 65 (2010) 373–384.
[13] W. Guo, A.M. Allan, R. Zong, et al., Ablation of FMRP in adult neural stem cells disrupts hippocampus-dependent learning, Nat. Med. 17 (2011) 559–565.
[14] Y. Luo, G. Shan, W. Guo, et al., Fragile X mental retardation protein regulates proliferation and differentiation of adult neural stem/progenitor cells, PLoS Genet. 6 (2010) e1000898.
[15] S. Bagga, J. Bracht, S. Hunter, et al., Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation, Cell 122 (2005) 553–563.
[16] R.S. Pillai, S.N. Bhattacharyya, C.G. Artus, et al., Inhibition of translational initiation by Let-7 microRNA in human cells, Science 309 (2005) 1573–1576.
[17] H.M. Heneghan, N. Miller, M.J. Kerin, Role of microRNAs in obesity and the metabolic syndrome, Obes. Rev. 11 (2010) 354–361.
[18] M. Åkerblom, R. Sachdeva, I. Barde, et al., MicroRNA-124 is a subventricular zone neuronal fate determinant, J. Neurosci. 32 (2012) 8879–8889.
[19] R. Schickel, B. Boyerinas, S.M. Park, M.E. Peter, MicroRNAs: key players in the immune system, differentiation, tumorigenesis and cell death, Oncogene 27 (2008) 5959–5974.
[20] C. Zhao, G. Sun, S. Li, Y. Shi, A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination, Nat. Struct. Mol. Biol. 16 (2009) 365–371.
[21] K.E. Szulwach, X. Li, R.D. Smrt, et al., Cross talk between microRNA and epigenetic regulation in adult neurogenesis, J. Cell Biol. 189 (2010) 127–141.
[22] R.D. Smrt, K.E. Szulwach, R.L. Pfeiffer, et al., MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1, Stem Cells 28 (2010) 1060–1070.
[23] P.J. Paddison, A.A. Caudy, E. Bernstein, et al., Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells, Genes Dev. 16 (2002) 948–958.
[24] A. Krek, D. Grün, M.N. Poy, et al., Combinatorial microRNA target predictions, Nat. Genet. 37 (2005) 495–500.
[25] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell 120 (2005) 15–20.
[26] X. Wang, I.M. El Naqa, Prediction of both conserved and nonconserved microRNA targets in animals, Bioinformatics 24 (2008) 325–332.
[27] O.A. Burmistrova, A.Y. Goltsov, L.I. Abramova, et al., MicroRNA in schizophrenia: genetic and expression analysis of miR-130b (22q11), Biochemistry (Mosc) 72 (2007) 578–582.
[28] A.M. Epstein, C.R. Bauer, A. Ho, et al., *Drosophila* Fragile X protein controls cellular proliferation by regulating cbl levels in the ovary, Dev. Biol. 330 (2009) 83–92.
[29] M.A. Callan, C. Cabernard, J. Heck, et al., Fragile X protein controls neural stem cell proliferation in the *Drosophila* brain, Hum. Mol. Genet. 19 (2010) 3068–3079.
[30] M. Telias, M. Segal, D. Ben-Yosef, Neural differentiation of fragile X human embryonic stem cells reveals abnormal patterns of development despite successful neurogenesis, Dev. Biol. 374 (2013) 32–45.
[31] M. Castrén, T. Tervonen, V. Kärkkäinen, et al., Altered differentiation of neural stem cells in fragile X syndrome, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 17834–17839.
[32] B.D. Eadie, W.N. Zhang, F. Boehme, et al., *Fmr1* knockout mice show reduced anxiety and alterations in neurogenesis that are specific to the ventral dentate gyrus, Neurobiol. Dis. 36 (2009) 361–373.
[33] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[34] S. Zongaro, R. Hukema, S. D'Antoni, et al., The 3′ UTR of *Fmr1* mRNA is a target of miR-101, miR-129-5p and miR-221: implications for the molecular pathology of FXTAS at the synapse, Hum. Mol. Genet. 22 (2013) 1971–1982.