Research Article

Bo Wei, Gui-rong Xiao, Cheng-long Wu, Yi-qin Xu*

HAGLR promotes neuron differentiation through the miR-130a-3p-MeCP2 axis

https://doi.org/10.1515/med-2021-0301
received November 19, 2020; accepted April 28, 2021

Abstract: Parkinson’s disease (PD) is a prevalent neurodegenerative disease. Currently, the molecular mechanisms underlying the progressions of PD are not fully understood. The human neuroblastoma cell line SH-SY5Y has been widely used as an in vitro model for PD. This study aims to investigate the molecular mechanisms of the non-coding RNA-mediated SH-SY5Y differentiation induced by retinoic acid (RA). By microArray analysis, lncRNA HAGLR was observed to be significantly upregulated during the RA-induced SH-SY5Y differentiation. Silencing HAGLR blocked the RA-induced SH-SY5Y differentiation. Moreover, bioinformatical analysis illustrated that miR-130a-3p contains binding sites for HAGLR. The RNA pull down assay and luciferase assay demonstrated that HAGLR functioned as a ceRNA of miR-130a-3p in SH-SY5Y cells. Overexpression of miR-130a-3p effectively inhibited SH-SY5Y differentiation. We identified MeCP2, a vital molecule in neuronal diseases, to be a direct target of miR-130a-3p in SH-SY5Y cells. Knockdown of MeCP2, or overexpression of HAGLR, could successfully overcome the RA-induced SH-SY5Y differentiation by targeting MeCP2. In summary, this study reveals a potential molecular mechanism for the lncRNA-HAGLR-promoted in vitro neuron differentiation by targeting the miR-130a-3p-MeCP2 axis, contributing to the understanding of the pathogenesis and progression of PD.

Keywords: PD, neuron differentiation, lncRNA-HAGLR, MeCP2, miR-130a-3p

1 Introduction

Parkinson’s disease (PD), a neurodegenerative disorder, is one of the most prevalent neurodegenerative diseases worldwide [1]. Symptoms of PD generally develop over years, resulting in socioeconomic burden [1,2]. The molecular mechanisms underlying PD and the effective disease-modifying treatments are still under clinical investigation [3]. The human neuroblastoma SH-SY5Y cell line is an in vitro model for PD research since it synthesizes both dopamine (DA) and noradrenaline (NA) [4]. In addition, retinoic acid (RA) has been used to induce the in vitro neuronal phenotypes of SH-SY5Y cells by inducing terminal neural differentiation [5]. However, the precise molecular mechanisms for the RA-induced SH-SY5Y differentiation are not fully understood.

lncRNAs, which are non-coding RNAs with relatively larger sizes (>200 nucleotides), have been extensively studied as important regulators for diverse diseases [6,7]. Accumulating evidence revealed that lncRNAs function by specifically binding to DNA, RNA, or proteins to regulate gene expressions [8]. Moreover, studies uncovered that lncRNAs act as competitive endogenous RNAs (ceRNA) of miRNAs by sponging them to de-repress the expressions of miRNA target genes [9]. lncRNA HAGLR (HOXD antisense growth-associated long non-coding RNA) has been reported to be positively associated with various diseases [10–13]. Currently, the biological roles of HAGLR in neuronal diseases such as PD have not been elucidated.

Methyl CpG binding protein 2 (MeCP2), which specifically binds to methylated cytosines on DNA, is a member of the methyl-CpG-binding domain (MBD) protein family [14]. Studies reported that MeCP2 was the most abundant molecule in the adult brain and tightly correlated with diverse neuronal processes during neurodevelopment [15]. Moreover, mutations in the MeCP2 gene cause the Rett syndrome (RTT), which is a neurologic condition affecting young girls [16] and lead to the MeCP2 duplication syndrome (MDS) [17]. These studies suggest MeCP2 is an important diagnostic biomarker and therapeutic target for neuronal diseases. However, the precise roles and molecular mechanisms of MeCP2 in PD are still under investigation.

* Corresponding author: Yi-qin Xu, Department of Neurology, Shaoxing People’s Hospital, Shaoxing, Zhejiang, 312000, China, e-mail: tkyf888@163.com, tel: +86-18557949316
Bo Wei, Gui-rong Xiao, Cheng-long Wu: Department of Neurology, Shaoxing People’s Hospital, Shaoxing, Zhejiang, 312000, China

Open Access. © 2021 Bo Wei et al., published by De Gruyter.
In this study, we investigated the biological functions of HAGLR and MeCP2 during the RA-induced neuron differentiation of the SH-SY5Y neuroblastoma cell line. From IncRNA-microarray analysis, for the first time, we discovered HAGLR was upregulated from the in vitro neuronal differentiation model. The IncRNA–miRNA ceRNA network and the targets of miRNA will be identified.

2 Materials and methods

2.1 Cell culture and treatment

Human SH-SY5Y and SK-N-MC cells were cultured in Dulbecco’s modified Eagle’s medium/ F12 medium (DMEM/F12) (Thermo Fisher Scientific, Shanghai, China) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Shanghai, China) with 100 units/mL penicillin and 100 µg/mL streptomycin (Thermo Fisher Scientific, Shanghai, China) at 37°C in a humidified atmosphere with 5% CO2. RA induction was performed as previously described [18]. Cells were treated with 5 µM RA for 3–7 days. Morphological changes were monitored under a brightfield microscope. RA was purchased from Sigma-Aldrich (Shanghai, China). Mouse anti-MeCP2 (#M6818) and β-actin antibodies (#A5441) were purchased from Sigma-Aldrich (Shanghai, China).

2.2 Transfections of shRNA, miRNA, and plasmid DNA

Transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocols. SH-SY5Y cells (1 × 10⁶) were plated in 60 mm wells for 24 h. siRNA, miRNAs, shRNA, and their negative controls were synthesized by GenePharma Inc. (Shanghai, China) and transfected at 50 nM for 48 h. Plasmid DNA was transfected at 2 µg/well for 48 h. Cells were then treated for 48 h with RA. Experiments were conducted in triplicate and repeated at least three times.

2.3 IncRNA–miRNA and miRNA–mRNA interaction analysis

The HAGLR-miR-130a-3p and miR-130a-3p-MeCP2 3’UTR bindings were predicted from Targetscan.org and starBase of ENCORI http://starbase.sysu.edu.cn/ according to previous reports [19]. The online non-coding RNA service predicts binding of miRNA with IncRNAs or mRNA targets of miRNA by searching for the presence of conserved 8mer, 7mer, and 6mer sites that match the seed region of each miRNA. Prediction of starBase was from 108 CLIP-Seq (PAR-CLIP, HITS-CLIP, iCLIP, and CLASH) data sets generated by 37 independent studies.

2.4 Quantitative RT-PCR

Total RNA was extracted from SH-SY5Y cells using the RNeasy Mini Kit (Qiagen, Shanghai, China) according to the manufacturer’s instructions. The quality and concentration of RNA samples were determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Shanghai, China). Total RNA was treated with DNase I, followed by reverse transcription into cDNA using the PrimeScript RT Master Mix (TaKaRa, Dalian, China) according to the manufacturer’s instructions. The qRT-PCRs were performed using the SYBR Green PCR Master Mix (ThermoFisher Scientific, Waltham, MA, USA) in an ABI PRISM 7500 Real-Time System. A total of 40 amplification cycles were applied with the following settings: denaturation at 95°C for 15 s, annealing at 58°C for 30 s, and extension at 72°C for 42 s. The relative gene expression was determined by the 2^(-ΔΔCt) method. β-Actin was an internal control for IncRNA and MeCP2 mRNA detection, and U6 was an internal control for miRNA detection. Experiments were performed in triplicate and repeated three times.

2.5 RNA pull-down assay

The RNA pull-down assay was performed as described previously. Briefly, biotin-labeled scramble, sense, or antisense IncRNA HAGLR DNA oligomers (RiboBio) were incubated with the SH-SY5Y cell lysate followed by incubation for 1 h; then streptavidin-coupled agarose beads (Thermo Fisher Scientific, Shanghai, China) were added to pull down the RNA–RNA complexes. The miR-130a-3p expressions were analyzed from the RNA–RNA complexes by quantitative RT-PCR (qRT-PCR). Experiments were repeated three times.

2.6 Luciferase assay

The wild-type (WT) and mutated (Mut) HAGLR or MeCP2 3’UTR were amplified and cloned into the pGL3-control luciferase reporter vector system (Promega, Madison, WI, USA). SH-SY5Y cells were co-transfected with control
miRNA or miR-130a-3p with WT or Mut HAGLR or MeCP2 3′ UTR using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) for 72 h. Luciferase activity was measured using a Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) according to the protocol from the kit. The experiment was performed in triplicate and repeated three times.

2.7 Microarray analysis

Total RNA was extracted from SH-SY5Y cells using the RNeasy Mini Kit (Qiagen, Shanghai, China) according to the manufacturer’s instructions. RNA was quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Shanghai, China). Integrities of RNA samples were assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). RNA was amplified and labeled. The Cy3-labeled complementary RNA was hybridized using the Gene Expression Hybridization Kit (Agilent Technologies, Santa Clara, CA, USA). Slides were scanned using an Agilent Microarray Scanner (Agilent Technologies, Santa Clara, CA, USA). The MicroArray results were analyzed using Agilent Feature Extraction software (Agilent Technologies, Inc.) and GeneSpring GX v11.5.1 software (Agilent Technologies, Inc.).

2.8 Western blot

Proteins from SH-SY5Y cells were extracted using RIPA buffer (ThermoFisher Scientific, Inc.) with a 1x protease inhibitor cocktail (Thermo Fisher Scientific Inc.). After 20 min incubation on ice, lysates were centrifuged at 10,000 g for 10 min at 4°C. An equal amount of protein from each sample was separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (PVDFs) (Millipore, Bedford, MA, USA) followed by blockage for 1 h at room temperature with 4% bovine serum albumin (BSA). Membranes were incubated with primary antibodies at 1:1,000 overnight at 4°C. After washing with phosphate buffered saline with tween 20 (PBST), membranes were incubated with secondary antibody (1:3,000) for 1 h at room temperature. Blots were visualized with enhanced chemiluminescence (Millipore, Bedford, MA, USA). β-Actin was used as a loading control. Experiments were repeated three times.

3 Results

3.1 HAGLR is upregulated during the RA-induced neuron differentiation

We evaluated the cellular mechanisms of neuron differentiation using RA-induction of SH-SY5Y cells as an in vitro model, which has been widely used in PD research [4]. The results showed that the RA treatment (5 µM) of SH-SY5Y cells for 4 days induced remarkable morphologic differentiation with neurite extension compared with control cells that showed no morphological differentiation (Figure 1a and Figure S1a). Moreover, the neuron differentiation marker, GAP43, was induced under RA treatments (Figure S1b), indicating successful neuronal induction by RA. Accumulating studies revealed that non-coding RNAs play vital roles during neuron differentiation [7,8]. To investigate the molecular mechanisms, we performed an lncRNA microArray analysis to profile lncRNA expressions using SH-SY5Y cells with or without RA induction. Among the differentially expressed lncRNAs in SH-SY5Y cells with RA induction, we observed that lncRNA HAGLR was significantly upregulated by the RA treatment (Figure 1b), suggesting that HAGLR is positively associated with neuron differentiation. The results from microArray were further validated by qRT-PCR in SH-SY5Y cells with RA induction at 3, 5, and 7 days. In addition, the RA-induced HAGLR upregulation at 5 and 7 days was observed in another neuroblastoma cell line, SK-N-MC (Figure S2a). To assess the biological functions of HAGLR in neuron differentiation, HAGLR was knocked down in SH-SY5Y cells (Figure 1d). As we expected, SH-SY5Y and SK-N-MC cells with HAGLR silencing displayed little response to RA treatments compared with the control shRNA transfected cells (Figure 1e and Figure S2b). Taken together, these results consistently demonstrated that HAGLR promotes neuron differentiation from an RA-induced in vitro SH-SY5Y model.

3.2 HAGLR sponges miR-130a-3p as a ceRNA in SH-SY5Y cells

We next explored the molecular mechanisms underlying the HAGLR-promoted neuron differentiation. Previous
Cell morphologies were examined under a microscope, and the neurite length was measured. *PCR.

(1) qRT analysis was performed in duplicate.

(a) | RA induction |
---|---|
Control | Sh-SY5Y |

(b) | RA induction |
---|---|
Control | RA induction |

(c) | HAGLR |
---|---|

(d) | Expressions of HAGLR |
---|---|
SH-SY5Y | Ctrl shRNA |

(e) | RA induction |
---|---|
Control | RA 5 day |

Figure 1: Roles of HAGLR in RA-induced neuron differentiation. (a) Effects of RA treatment on cell morphology. SH-SY5Y cells were treated with 5 µM RA for 5 days. (b) SH-SY5Y cells were treated with RA using the above protocol. RNAs were isolated and IncRNA-microArray analysis was performed in duplicate. (c) SH-SY5Y cells were treated with 5 µM RA for 3, 5, and 7 days. HAGLR expressions were examined by qRT-PCR. (d) SH-SY5Y cells were transfected with control shRNA or HAGLR shRNA for 48 h, and expression of HAGLR was determined by qRT-PCR. (e) SH-SY5Y cells were transfected with control shRNA or HAGLR shRNA for 48 h, followed by treatments with 5 µM RA for 5 and 7 days. Cell morphologies were examined under a microscope, and the neurite length was measured. *p < 0.05; **p < 0.01; and ***p < 0.001.

studies uncovered that IncRNAs function by interfering with target miRNAs as molecular sponges [9]. The downregulation of miRNAs by IncRNA leads to de-repression of their target miRNAs [20]. To identify the potential miRNA targets of HAGLR, we analyzed the HAGLR-miRNA interaction through the non-coding RNA database, starBase2.0. We observed miR-130a-3p, which plays an important role during neuron development and neuronal diseases [21], contains HAGLR binding sites (Figure 2a).

We then evaluated the functional roles of miR-130a-3p during RA-induced neuron differentiation. qPCR results remarkably showed a reverse phenotype between HAGLR and miR-130a-3p, which was significantly downregulated in SH-SY5Y and SK-N-MC cells with RA induction at 3, 5, and 7 days (Figure 2b and Figure S3a). Moreover, although RA treatments suppressed miR-130a-3p expressions, exogenous overexpression of miR-130a-3p (Figure S3b and c) rendered cells to maintain relatively high miR-130a-3p expression levels (Figure S3d and e). Expectedly, SH-SY5Y and SK-N-MC cells with exogenous overexpression of miR-130a-3p showed no effective morphological changes in the neurite extension under RA treatments (Figure 2c and Figure S3f). To assess whether HAGLR downregulates miR-130a-3p, HAGLR was overexpressed or silenced in SH-SY5Y cells. Expectedly, overexpression of HAGLR effectively blocked the miR-130a-3p expressions, while knockdown of HAGLR significantly upregulated miR-130a-3p expressions (Figure 2d). To validate the binding of miR-130a-3p on HAGLR, we performed an RNA pull-down assay. The biotin-labeled scramble, sense, or antisense DNA probe of HAGLR was incubated with SH-SY5Y cell lysates. The qRT-PCR results demonstrated that only the antisense HAGLR DNA probe pulled down the enriched miR-130a-3p (Figure 2e). The endogenous miR-130a-3p could not be effectively pulled down by the scramble or sense HAGLR probe (Figure 2e), suggesting that HAGLR specifically interacts with miR-130a-3p in neuroblastoma cells. To validate whether HAGLR directly binds on the seeding region of miR-130a-3p, SH-SY5Y cells were co-transfected with a luciferase vector containing the wild-type HAGLR (WT-HAGLR) or the binding site mutant HAGLR (Mut-HAGLR) and control miRNA or miR-130a-3p. Luciferase activity of cells with WT-HAGLR and miR-130a-3p co-transfection was significantly suppressed compared with that from Mut-HAGLR and miRNA-130a-3p co-transfection (Figure 2f).
summary, these results demonstrated that HAGLR sponges mir-130a-3p by forming a ceRNA network.

3.3 mir-130a-3p directly targets MeCP2 and inhibits the RA-induced neuron differentiation

Since miRNAs function via targeting mRNA 3′UTR to down-regulate target mRNA expressions [20], we performed miRNA–mRNA interaction analysis from the non-coding RNA database, starBase 2.0. Interestingly, the 3′UTR of MeCP2, which is an important molecule in neuron differentiation and neuronal diseases, contains conserved miR-130a-3p binding sites through multiple species (Figure 3a and b). To examine whether miR-130a-3p could suppress protein expression of MeCP2, miR-130a-3p or control miRNA was transfected into SH-SY5Y cells. The results from western blot showed that overexpression of miR-130a-3p effectively blocked MeCP2 protein expression in SH-SY5Y cells (Figure 3d). To validate the direct binding of miR-130a-3p on 3′UTR of MeCP2 mRNA, SH-SY5Y cells were co-transfected with luciferase vector containing WT-MeCP2 3′UTR or the miR-130a-3p binding site mutant MeCP2 3′UTR (Mut-MeCP2) and control miRNA or miR-130a-3p. Luciferase activity of the vector containing WT-MeCP2 was remarkably blocked by miR-130a-3p (Figure 3d). Expectedly, the luciferase activity of the Mut-MeCP2 vector has no significant change by miR-130a-3p overexpression (Figure 3d). Together, these results demonstrated that miR-130a-3p could directly target 3′UTR of MeCP2.

3.4 MeCP2 is induced and promotes the RA-induced neuron differentiation

We continue to evaluate the roles of MeCP2 in RA-induced neuron differentiation. Under RA treatments, protein and mRNA expressions of MeCP2 were significantly upregulated (Figure 4a and b and Figure S4a). Furthermore, MeCP2 was knocked down by siRNA in SH-SY5Y cells. The results in Figure 4c and Figure S4b illustrate that SH-SY5Y cells with downregulated MeCP2 expression exhibited unchanged neurite extension in
Figure 3: MeCP2 is directly targeted by miR-130a-3p. (a) Bioinformatics analysis of potential binding of miR-130a-3p on 3’UTR of MeCP2. (b) The conserved binding sites of miR-130a-3p on 3’UTR of MeCP2 in multiple species. (c) SH-SY5Y cells were transfected with control or miR-130a-3p for 48 h; the protein expression of MeCP2 was detected by western blot. (d) Dual-luciferase reporter assay was performed in SH-SY5Y cells with co-transfection of WT-MeCP2 or Mut-MeCP2 with control miRNA, or miR-130a-3p. **p < 0.01.

Figure 4: Roles of MeCP2 in the RA-induced neuron differentiation. (a) SH-SY5Y cells were treated with 5 µM RA for 3, 5, and 7 days. Protein and (b) mRNA expressions of MeCP2 were detected. (c) SH-SY5Y cells were transfected with control siRNA or MeCP2 siRNA for 48 h, followed by treatments with 5 µM RA for 5 and 7 days. Cell morphologies were examined under a microscope, and the neurite length was measured. *p < 0.05; **p < 0.01; and ***p < 0.001.
response to RA induction compared with control cells, indicating that MeCP2 promotes the process of neuron differentiation.

3.5 Restoration of MeCP2 in miR-130a-3p overexpressing SH-SY5Y cells rescues the RA-induced neuron differentiation

Rescue experiments were performed to validate whether miR-130a-3p blocked the RA-induced neuron differentiation through targeting MeCP2. SH-SY5Y cells were transfected with control miRNAs, miR-130a-3p alone or with MeCP2 overexpression plasmid. Co-transfection of miR-130a-3p with MeCP2 successfully recovered MeCP2 protein levels compared with miR-130a-3p overexpression cells (Figure 5a). Moreover, the expected results demonstrated that SH-SY5Y cells with the restoration of MeCP2 rescued the RA-induced neuron differentiation phenotype compared with that from HAGLR overexpressing cells (Figure 5b). In summary, these mechanism rescue experiments consolidated a HAGLR-miR-130a-3p-MeCP2 axis that contributes to the processes of neuron differentiation.

3.6 HAGLR promoted neuron differentiation by targeting the miR-130a-3p-MeCP2 axis

Finally, we assessed whether neuron differentiation was regulated by the HAGLR-miR-130a-3p-MeCP2 axis. SH-SY5Y cells were transfected with control, HAGLR overexpression plasmid alone, or with miR-130a-3p. qRT-PCR and western blot results demonstrated that co-transfection of HAGLR with miR-130a-3p successfully restored miR-130a-3p and MeCP2 protein levels compared with HAGLR overexpressing cells (Figure 6a and b). As expected, SH-SY5Y cells with co-transfection of HAGLR and miR-130a-3p re-suppressed the RA-induced neuron differentiation phenotype compared with that from HAGLR overexpressing cells (Figure 6c). In summary, these mechanism rescue experiments consolidated a HAGLR-miR-130a-3p-MeCP2 axis that contributes to the processes of neuron differentiation.

4 Discussion

PD is one of the most prevalent neurodegenerative diseases worldwide [1,2]. Currently, the molecular mechanisms underlying PD and the effective disease-modifying approaches are not fully understood. The human neuroblastoma SH-SY5Y cell line is widely studied for investigating the molecular and cellular mechanisms underlying PD pathogenesis and progression since it synthesizes both DA and NA [4]. Moreover, although the SH-SY5Y cell line displays a number of cancerous characteristics, most genes regulations and signal pathways in PD pathogenesis are intact [4]. It is difficult to obtain and maintain human dopaminergic neurons as primary cells, which are mainly affected in PD [4]. Thus, the neuroblastoma SH-SY5Y cell line is a widely used in vitro model for PD research. Currently, the biological roles of lncRNA HAGLR in neuronal diseases remain unclear. In this study, we first reported the
non-coding RNA-regulated neuron differentiation using SH-SY5Y cells as an *in vitro* model. Under RA induction, we detected that HAGLR was significantly upregulated in SH-SY5Y cells from both microArray analysis and qPCR results. In addition, silencing HAGLR effectively blocked the RA-induced SH-SY5Y differentiation based on the observations that the neurite extension of SH-SY5Y cells with lower HAGLR expression showed little response to RA treatments, suggesting an essential role of HAGLR in SH-SY5Y cell differentiation.

Accumulating studies demonstrated that miRNAs play regulatory roles in neuron development and neuronal diseases [20]. Moreover, the lncRNA–miRNA interaction has been shown to control vital functions during the molecular and cellular processes of human malignancies, including neuronal diseases [8,9]. A recent study reported that miR-130a-3p regulates VEGFR-2 expression in sensory and motor neurons during development [22]. Moreover, the miR-130a-3p/DAPK1 axis was known to regulate the pathophysiology of neonatal hypoxic-ischemia encephalopathy [23], suggesting miR-13-3p to be a potential therapeutic target for the hypoxic ischemia encephalopathy treatment. However, the miR-130a-3p-mediated neuron differentiation has not been investigated. Here, we show that miR-130a-3p was remarkably downregulated in SH-SY5Y cells under RA induction. Overexpression of miR-130a-3p inhibited the morphological changes of SH-SY5Y cells under RA treatments. Bioinformatics analysis revealed that HAGLR contains miR-130a-3p binding sites. The predicted ceRNA network was further validated by the RNA pull-down assay and luciferase assay.

MeCP2 is the most abundant methyl-DNA binding domain family member in the adult brain and is tightly correlated with diverse neuronal processes during neurodevelopment [15]. It was known that mutations in the MeCP2 gene cause Rett syndrome (RTT), a neurologic condition affecting primarily young girls [16]. Interestingly, girls with RTT exhibiting motor deficits showed similar phenotypes to those in PD [24], suggesting MeCP2 is involved in the defects of the nigrostriatal pathway. A recent study used the 6-hydroxydopamine-induced human neuroblastoma cell (SH-SY5Y cell) injury as a cell model of PD [25]. They described that overexpression of MeCP2 was able to ameliorate the 6-hydroxydopamine-induced apoptosis of SH-SY5Y cells, suggesting that MeCP2 is a potential therapeutic target for the treatment of PD [25]. Currently, the precise roles and molecular mechanisms of MeCP2 in PD have not been elucidated. Here, we described that the expressions of MeCP2 were significantly upregulated in SH-SY5Y cells by RA induction. One advantage of this study is that we illustrated the direct binding of miR-130a-3p on 3’UTR of MeCP2 in SH-SY5Y cells. Furthermore, the rescue experiments validated that the miR-130a-3p-inhibited SH-SY5Y cell differentiation was by direct targeting of MeCP2. Thus, it was possible to target the above signaling pathways in neuronal disease to improve the clinical therapeutic outcomes. These strategies still have limitations owing to the fact that the neuroblastoma cell line is not purely neuron cells since it is oncogenically transformed with catecholaminergic, resulting in different physiological characteristics compared to those from the normal DAergic neuronal features. In addition, the above *in vitro* signaling pathway needs to be verified in animal models.

In summary, this study reports a non-coding RNA-based molecular mechanism for the PD using SH-SY5Y cells as an *in vitro* model. HAGLR positively regulates SH-SY5Y cell differentiation, which is induced by RA via targeting the miR-130a-3p-MeCP2 axis, contributing to an extensive understanding of the pathogenesis and progression of PD.
Acknowledgement: The authors appreciate the contributions from all medical doctors and research scientists from the Department of Neurology, Shaoxing People's Hospital, for generously supporting this study.

Funding information: No funds.

Author contributions: B.W. and Y.Q.X. designed the experiments and wrote the manuscript. B.W., G.R.X., Ch.L.W., and Y.Q.X. carried out the experiments and analyzed the data.

Conflict of interest: The authors declare that there are no conflicts of interest.

Data availability statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Reich SG, Savitt JM. Parkinson's disease. Med Clin North Am. 2019;103:337–50.
[2] Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. Lancet Neurol. 2016;15:1257–72.
[3] Raza C, Anjum R, Shakeel NUA. Parkinson's disease: mechanisms, translational models and management strategies. Life Sci. 2019;226:77–90.
[4] Xicoy H, Wieringa B, Martens GJ. The SH-SYSY cell line in Parkinson's disease research: a systematic review. Mol Neurodegener. 2017;12:10.
[5] Gonulalan EM, Bayazide O, Yalcin FN, Demirezer LO. The roles of valeric acid on BDNF expression in the SH-SY5Y cell. Saudi Pharm J. 2018;26:960–4.
[6] Peng WX, Koirala P, Mo YY. lncRNA-mediated regulation of cell signaling in cancer. Oncogene. 2017;36:5661–7.
[7] Lyu Y, Bai L, Qin C. Long noncoding RNAs in neurodevelopment and Parkinson's disease. Animal Model Exp Med. 2019;2:239–51.
[8] Riva P, Ratti A, Venturini M. The long non-coding RNAs in neurodegenerative diseases: novel mechanisms of pathogenesis. Curr Alzheimer Res. 2016;13:1219–31.
[9] Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing miRNA-lncRNA Interactions. Methods Mol Biol. 2016;1402:271–86.
[10] Yang C, Shen S, Zheng X, Ye K, Sun Y, Lu Y, et al. Long non-coding RNA HAGLR acts as a microRNA-143-5p sponge to regulate epithelial-mesenchymal transition and metastatic potential in esophageal cancer by regulating LAMP3. FASEB J. 2019;33:10490–504.
[11] Lu C, Ma J, Cai D. Increased HAGLR expression promotes non-small cell lung cancer proliferation and invasion via enhanced de novo lipogenesis. Tumour Biol. 2017;39:1010428317697574.
[12] Sun W, Nie W, Wang Z, Zhang H, Li Y, Fang X. Lnc HAGLR promotes colon cancer progression through sponging miR-185-5p and activating CDK4 and CDK6 in vitro and in vivo. Onco Targets Ther. 2020;13:5913–25.
[13] Zakany J, Darbellay F, Mascalz B, Nesculea A, Duboule D. Control of growth and gut maturation by HoxD genes and the associated IncRNA Haglr. Proc Natl Acad Sci USA. 2017;114:E9290–9.
[14] Horvath PM, Monteggia LM. MeCP2 as an activator of gene expression. Trends Neurosci. 2018;41:72–4.
[15] Sharma K, Singh J, Frost EE, Pillai PP. MeCP2 in central nervous system glial cells: current updates. Acta Neurobiol Exp (Wars). 2018;78:30–40.
[16] Shah RR, Bird AP. MeCP2 mutations: progress towards understanding and treating Rett syndrome. Genome Med. 2017;9:17.
[17] Gulmez Karaca K, Brito DVC, Oliveira AMM. MeCP2: A critical regulator of chromatin in neurodevelopment and adult brain function. Int J Mol Sci. 2019;20:4577.
[18] Teppola H, Sarkanen JR, Jalonen TO, Linne ML. Morphological differentiation towards neuronal phenotype of SH-SYSY neuroblastoma cells by estradiol, retinoic acid and cholesterol. Neurochem Res. 2016;41:731–47.
[19] Li JH, Liu S, Zhou H, Qu LH, Yang JH. StarBase v2.0: decoding miRNA-ceRNA, miRNA-mRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res. 2014;42:D92–7.
[20] Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis. 2015;35:3–11.
[21] Glaesel K, May C, Marcus K, Matschke V, Theiss C, Theis V. miR-129-5p and miR-130a-3p regulate VEGFR-2 expression in sensory and motor neurons during development. Int J Mol Sci. 2020;21:3839.
[22] Glaesel K, May C, Marcus K, Matschke V, Theiss C, Theis V. miR-129-5p and miR-130a-3p regulate VEGFR-2 expression in sensory and motor neurons during development. Int J Mol Sci. 2020;21:3839.
[23] Feng M, Zhu X, Zhuo C. H19/miR-130a-3p/DAPK1 axis regulates the pathophysiology of neonatal hypoxic-ischemia encephalopathy. Neurosci Res. 2021;163:52–62.
[24] Gantz SC, Ford CP, Neve KA, Williams JT. Loss of Mecp2 in substantia nigra dopamine neurons compromises the nigrostriatal pathway. J Neurosci. 2001;31:12629–37.
[25] Xie T, Zhang J, Yuan X, Yang J, Ding W, Huang X, et al. Is X-linked methyl-CpG binding protein 2 a new target for the treatment of Parkinson's disease. Neural Regen Res. 2013;8:1948–57.
Appendix

Figure S1: Effects of retinoic acid treatment on neuron differentiation. (a) SH-SYS cells were treated with RA at 5 µM for 5 days, the relative neurite length was calculated. (b) Expressions of GAP43 in SH-SYS cells under RA treatments at 5 days and 7 days were determined by qRT-PCR. **, $p < 0.01$; ***, $p < 0.001$.

Figure S2: Effects of retinoic acid treatment on SK-N-MC cells. (a) Expressions of HAGLR in SK-N-MC cells under RA treatments for 3, 5 and 7 days were determined by qRT-PCR. (b) The relative neurite length was calculated in SK-N-MC cells without or with HAGLR silencing under RA treatments for 5 and 7 days. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. 
Figure S3: miR-130a-3p suppresses RA-induced neuron differentiation. (a) Expressions of miR-130a-3p in SK-N-MC cells under RA treatments at 3, 5 and 7 days were determined by qRT-PCR. (b) Effects of miR-130a-3p overexpression in SH-SYSY and (c) SK-N-MC cells. (d) Detection of miR-130a-3p expressions in SH-SYSY and (e) SK-N-MC cells without or with miR-130a-3p overexpression under RA treatments for 5 and 7 days. (f) The relative neurite length was calculated in SK-N-MC cells without or with miR-130a-3p overexpression under RA treatments for 5 and 7 days. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Figure S4: Silencing MeCP2 suppresses RA-induced neuron differentiation. (a) SK-N-MC cells under RA treatments for 3, 5 and 7 days, expressions of MeCP2 were determined by qRT-PCR. (b) The relative neurite length was calculated in SK-N-MC cells without or with MeCP2 silencing under RA treatments for 5 and 7 days. *, p < 0.05; **, p < 0.01; ***, p < 0.001.