Role of LncMALAT1-miR-141-3p/200a-3p-NRXN1 Axis in the Impairment of Learning and Memory Capacity in ADHD

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Summary

As a prevalent neurodevelopmental disease, attention-deficit hyperactivity disorder (ADHD) impairs the learning and memory capacity, and so far, there has been no available treatment option for long-term efficacy. Alterations in gene regulation and synapse-related proteins influence learning and memory capacity; nevertheless, the regulatory mechanism of synapse-related protein synthesis is still unclear in ADHD. LncRNAs have been found participating in regulating genes in multiple disorders. For instance, lncRNA Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) has an essential regulatory function in numerous psychiatric diseases. However, how MALAT1 influences synapse-related protein synthesis in ADHD remains largely unknown. Here, our study found that MALAT1 decreased in the hippocampus tissue of spontaneously hypertensive rats (SHRs) compared to the standard controls, Wistar Kyoto (WKY) rats. Subsequent experiments revealed that MALAT1 enhanced the expression of neurexin 1 (NRXN1), which promoted the synapse-related genes (SYN1, PSD95, and GAP43) expression. Then, the bioinformatic analyses predicted that miR-141-3p and miR-200a-3p, microRNAs belonging to miR-200 family and sharing same seed sequence, could interact with MALAT1 and NRXN1 mRNA, which were further confirmed by luciferase report assays. Finally, rescue experiments indicated that MALAT1 influenced the expression of NRXN1 by sponging miR-141-3p/200a-3p. All data verified our hypothesis that MALAT1 regulated synapse-related proteins (SYN1, PSD95, and GAP43) through the MALAT1-miR-141-3p/200a-3p-NRXN1 axis in ADHD. Our research underscored a novel role of MALAT1 in the pathogenesis of impaired learning and memory capacity in ADHD and may shed more light on developing diagnostic biomarkers and more effective therapeutic interventions for individuals with ADHD.

Key words

MALAT1 • NRXN1 • Synapse • Hippocampus • ADHD

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Introduction

Attention-deficit hyperactivity disorder (ADHD) is a kind of neurodevelopmental disorders (NDDs), marked by inattention, motor hyperactivity, impulsivity, or a combination of them [1]. A complicated interplay between environmental and genetic factors is implicated etiologically in ADHD, affecting brain networks' structure and functional capacity associated with cognition [2]. As a result, individuals with ADHD tend to show neurocognitive deficits, creating a substantial burden for individuals and the community [3]. Although some medication-based treatment options show excellent short-term efficacy for current clinical practice, the evidence for long-term efficacy on aspects relating to the

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quality of life, such as academic and vocational achievements, is less clear [4]. Therefore, exploring the etiology of ADHD, hoping to find more effective clinical therapeutic approaches to improve the living quality of patients, is of great significance.

Next-generation sequencing (NGS) has identified a wide range of genes correlated to NDDs, among which the most representative components are synaptic genes [5]. The synapse is the basic unit of the nervous system and is responsible for long-term memory; therefore, synapse is becoming a core focus of studies to understand the mechanism of learning and memory [6]. However, research on cognitive impairments of ADHD is relatively scarce. Therefore, we focused on synaptic genes in ADHD with the hope of finding potential therapeutic targets to improve the capacity of learning and memory.

A myriad of evidence pointed out that long non-coding RNAs (lncRNAs) are correlated with neurological disorders and could serve as novel targets for cognitive impairments [7]. As a highly conservative lncRNA belonging to mammalian species with considerable abundance in neurons [8], lncRNA Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) has captured accumulating interests in cognitive disorders, such as Alzheimer's disease [9]. However, the role of MALAT1 in the impairment of learning and memory capacity of ADHD remains unknown and needs to be further elucidated.

Herein, first of all, we found that MALAT1 expression declined in the hippocampus of spontaneously hypertensive rats (SHRs) compared with the controls. Subsequent experiments demonstrated that MALAT1 regulated the expression of synapsin-1 (SYN1), postsynaptic density 95 (PSD95), and growth-associated protein-43 (GAP43), which belong to synapse-related proteins, through neurexin 1 (NRXN1). Finally, we discovered that MALAT1 modulated NRXN1 expression by sponging miR-141-3p/200a-3p. Our research yields more insights into the function of the MALAT1-miR-141-3p/200a-3p-NRXN1 axis in the pathogenesis of impaired learning and memory capacity in ADHD and might uncover promising diagnostic biomarkers and targets for ADHD.

Materials and Methods

Animals

8 male Wistar Kyoto (WKY) rats and 8 male SHRs from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) were engaged in experiments. All animals were 4 weeks of age upon arrival in the animal facility and were reared individually in ventilated cages on a cycle of 12 h light and dark, provided *ad libitum* with ample food and water. The house temperature kept 22±2 °C, and relative humidity remained 40±10 %. All the rats acclimated to their new circumstances for 1 week in advance of performing the study. All procedures were approved by the Ethics Committee of Nanjing Medical University with the approval number IACUC-2202044.

Tissue preparation

After 7-day adaptation to the new circumstances, all the rats were sacrificed under anesthesia. Then pull off the skull and cerebral cortex to expose the hippocampus. All the hippocampus tissue was quickly removed from the surrounding tissue, which was operated on the ice to prevent the degradation of RNA and protein. Then an Ultra-Turrax homogenizer (IKA Labortechnik, Staufen, Germany) was used to homogenize samples for the subsequent biological tests.

Cell culture and transfection

Rat primary hippocampal neurons, obtained from ScienCell Research Laboratories (CA, USA), were incubated in the neuronal medium (ScienCell, CA, USA), which contained 1 % neuron growth additive (ScienCell, CA, USA), and 1 % penicillin-streptomycin (Beyotime, Shanghai, China). Cells were transfected with plasmid DNAs (GeneChem, Shanghai, China and Miaolingbio, Wuhan, China) and small interfering RNAs (siRNAs, Miaolingbio, Wuhan, China), which were strictly under the Lipofectamine 3000 manufacturer's protocol. The transfection efficiencies were validated by qRT-PCR. The overexpression efficiency of plasmid overexpressing MALAT1 (OE-MALAT1) was around 16.2 times and that of plasmid overexpressing NRXN1 (OE-NRXN1) was approximately 9.2 times, whereas the maximum interference efficiency of MALAT1 small interfering RNAs (si-MALAT1) was about 36 % and that of NRXN1 small interfering RNAs (si-NRXN1) was around 46 %. All cell lines were grown at standard conditions of temperature (37 °C) and atmosphere (5 % CO_2).

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

RNA of hippocampus and cultured cells was extracted with TRIzol reagent (Invitrogen, CA, USA) following the user guides. A NanoDrop 2000 system (NanoDrop Technologies, Wilmington, USA) was

applied to ensure the RNA samples were pure and to measure the concentrations. cDNA was synthesized with the usage of PrimeScript^{TM}RT reagent Kit (Takara, Tokyo, Japan) and analyzed by performing qRT-PCR utilizing SYBR Premix Ex Taq^{TM} II (Takara, Tokyo, Japan) under the manufacturer's protocols. The primers were generated by RiboBio Co., Ltd. (Guangzhou, China), and the sequences of the primers are shown below (Table 1).

Table 1. Sequences of primers used in this study.

Gene	**Primer Sequence (5' to 3')**
MALAT1	Forward: TAGACCAGCATGTCAATGTGCAA Reverse: CAAGTTACCATCCTCAAGTTACAGA
NRXN1	Forward: CATGCAGTCCGAGATGTCCA Reverse: GTGGTCTGGCTGATAGGCTC
SYN1	Forward: TCCACTCCTTGCCTGGACTTG Reverse: TTTGCTTCCCGACTCTTCTCT
PSD95	Forward: GATGGTGAAGGCATCTTCATCT Reverse: TGACCGTCTGACCCGCATT
GAP43	Forward: ATACCACCATGCTGTGCTGTATG Reverse: GGTTGCAGCCTTATGAGCCTT
miR-141-3p	Forward: ACACTCCAGCTGGGCATCTTCCAG Reverse: CTCAACTGGTGTCGTGGAGTCGGC
miR-200a-3p	Forward: GAGAGAAACGGUACAAA Reverse: GCAGGGTCCGAGGTATTC
GAPDH	Forward: GCGAGATCCCGCTAACATCA Reverse: CTCGTGGTTCACACCCATCA

Protein extraction and western blot

The tissue samples and cultured cells were lysed in the RIPA buffer with protease inhibitors (Beyotime, Shanghai, China) and boiled after mixing with loading buffer (Beyotime, Shanghai, China). After being run on an SDS-PAGE gel, proteins were transferred into polyvinylidene fluoride membranes (Thermo Fisher Scientific, MA, USA). The antibodies used included anti-synapsin 1 (ab64581, Abcam, Cambridge, UK), anti-NRXN1 (A10066, ABclonal, Wuhan, China), anti-GAP43 (A19055, ABclonal, Wuhan, China), anti-PSD95 (ab18258, Abcam, Cambridge, UK), anti-GAPDH (10494-1-AP, Proteintech, Rosemont, USA), and goat anti-rabbit antibody IgG (ab97051, Abcam, Cambridge, UK). The densitometry of bands were measured by Image J (National Institutes of Health, Bethesda, USA).

Target gene prediction

Targeted miRNAs that could interact with MALAT1 were identified by performing StarBase [10] (http://starbase.sysu.edu.cn/) and miRNet [11] (https://www.mirnet.ca/miRNet/upload/NcRNAUploadView.xhtml). StarBase was set with strict CLIP data stringency (≥5) and high degradome data stringency (≥3). miRNet was set to limit potential miRNAs in the brain. TargetScan [12] (www.targetscan.org) and miRDB [13] (http://www.mirdb.org/) were used to examine whether the candidate miRNAs could bind to 3' untranslated region (3'UTR) of NRXN1.

Luciferase reporter assays

The wild type and mutant NRXN1 and MALAT1 plasmids were constructed by Miaolingbio (Wuhan, China). The HEK293T cells from Stem Cell Bank (Chinese Academy of Science, Shanghai, China) were grown in 24-well plates and then co-transfected with target plasmids along with candidate miRNAs mimics or negative controls the next day. 48 h post transfection, the luciferase activities were tested under the manufacturer's protocol (Beyotime, Shanghai, China).

Statistical analysis

All experiments were repeated in triplicates. GAPDH was applied as an internal control. Quantitative data were exhibited as mean value ± standard deviation.

GraphPad Prism 9 (La Jolla, USA) was operated to calculate the statistical significance between two groups by Student's *t*-test. Differences were considered to be of statistical significance when $P<0.05$.

Results

MALAT1 is downregulated in hippocampus of SHRs and upregulates SYN1, PSD95, and GAP43 expression

To preliminarily explore whether MALAT1 has regulatory functions associated with the impaired hippocampus-related learning and memory capacity in ADHD, firstly we assessed the MALAT1 expression in the hippocampus tissue of 8-pair SHRs and controls, WKY rats. The analysis showed that the MALAT1 expression decreased in the hippocampus of SHRs in contrast with those of WKY rats ($P<0.01$) (Fig. 1A), which aroused our interests and facilitated the subsequent investigation. Recently, a study found that three synapse-related proteins, SYN1, PSD95, and GAP43, have decreases in expression in the hippocampus of SHRs and lead to poorer performance in Morris water maze tests [14]. Then, to figure out whether there is a relationship between the expression of MALAT1 and these synapse-related genes, the expression of SYN1, PSD95, and GAP43 was measured in the hippocampus of SHRs and WKY rats. As expected, SYN1, PSD95, and GAP43 expression levels declined in SHRs (Fig. 1B-F) which were consistent with the expression change trend of MALAT1. To take a step further to elucidate whether MALAT1 had a biological function on regulating SYN1, PSD95, and GAP43 expression, OE-MALAT1 and si-MALAT1s were constructed and transfected into rat primary hippocampal neurons, whose transfection efficiencies were validated using qRT-PCR (Fig. 1G, H). Then expression of SYN1, PSD95, and GAP43 was measured. As expected, the expression was elevated after MALAT1 overexpression plasmid transfected into the primary hippocampal neurons (Fig. 1I-K), while silencing MALAT1 led to the opposite effects (Fig. 1L-N). With all the above results, we proved that the overexpression of MALAT1 could enhance the levels of SYN1, PSD95, and GAP43 expression, whereas silence of MALAT1 had an opposite effect, manifesting the regulatory role of MALAT1 in SYN1, PSD95, and GAP43 expression.

NRXN1 mediates the effect of MALAT1 on SYN1, PSD95, and GAP43 expression

Previous study revealed that NRXN1 modulates the SYN1, PSD95, and GAP43 expression levels through the synapse-related signaling pathways mediated by 5-HT_6R in rat primary hippocampal neurons, which aroused our attention [14]. Therefore, we put forward a hypothesis that MALAT1 might regulate the expression levels of SYN1, PSD95, and GAP43 through NRXN1. As a result, subsequent experiments were conducted to verify if the hypothesis was correct. Firstly, NRXN1 expression levels in the hippocampus samples of SHRs and WKY rats were measured. Results showed that the NRXN1 expression decreased in SHRs compared with WKY rats ($P<0.05$) (Fig. 2A-C). Then OE-MALAT1 and si-MALAT1s were correspondingly transfected into primary hippocampus neurons to further verify the association between MALAT1 and NRXN1. As we noted above, NRXN1 mRNA and protein levels increased when MALAT1 was overexpressed, and the converse results were acquired after MALAT1 was silenced (Fig. 2D-F), which suggested that MALAT1 positively regulated the expression of NRXN1. Then rescue experiments were carried out to further explore whether NRXN1 mediates the influence of MALATI on SYN1, PSD95, and GAP43 expression. The transfection efficiencies were validated through qRT-PCR (Fig. 2G, H). Results showed that overexpressing MALAT1 enhanced the expression of SYN1, PSD95, and GAP43, and this promotive effect could be rescued by silencing NRXN1 (Fig. 2I-M). In contrast, knocking down MALAT1 attenuated the expression of SYN1, PSD95, and GAP43, whereas this inhibitive effect could be reversed by overexpressing NRXN1 (Fig. 2N-R). In summary, these findings substantiated that NRXN1 mediated the influence of MALAT1 on SYN1, PSD95, and GAP43 expression in rat primary hippocampal neurons.

MALAT1 regulates NRXN1 expression via directly sponging miR-141-3p/200a-3p

Pieces of evidence have indicated that MALAT1 achieves its biological functions through interacting with miRNAs in a mountain of diseases, such as Alzheimer's disease [9], and cerebral ischemia-reperfusion injury [15]. Taking the positive regulation of MALAT1 on NRXN1 into account, we conceivably hypothesized that MALAT1 regulated NRXN1 expression by serving as a competitive endogenous RNA (ceRNA) to competitively sponge miRNAs which could bind to the mRNA of NRXN1.

Firstly, we searched online databases for the potential miRNAs targeting MALAT1, and the predictions of StarBase and miRNet revealed two common miRNAs, miR-141-3p and miR-200a-3p. Then bioinformatic prediction utilizing TargetScan and

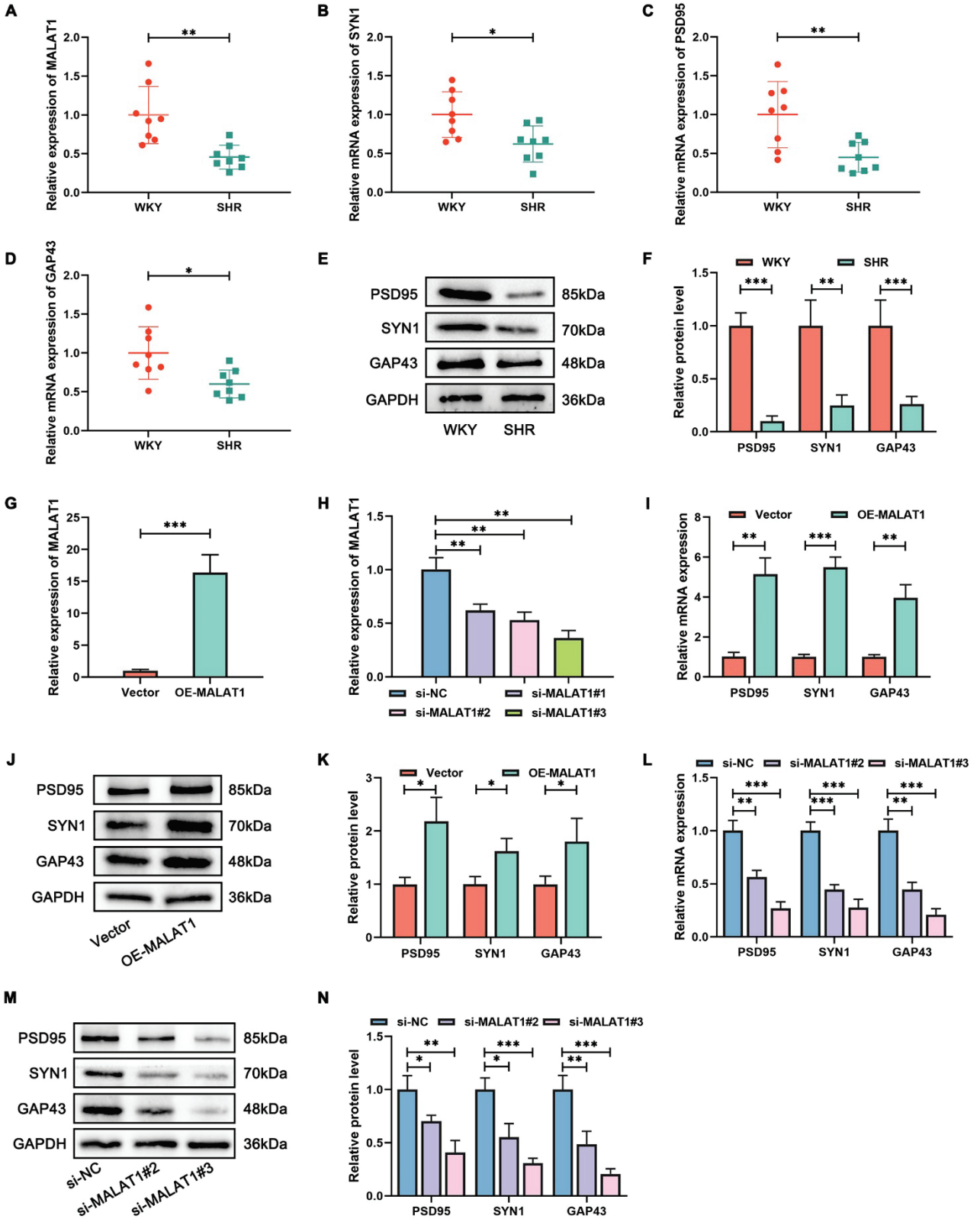

Fig. 1. MALAT1 is downregulated in hippocampus of SHRs and upregulates SYN1, PSD95, and GAP43 expression. (**A, B, C, D**). Relative mRNA expression of MALAT1, SYN1, PSD95, and GAP43 in hippocampus samples of WKY rats (n=8) and SHRs (n=8) was measured. (**E**). The protein levels of SYN1, PSD95, and GAP43 in the hippocampus of WKY rats and SHRs were detected. (**F**). The densitometry of western blot bands of (E). (**G**). The overexpression efficiency of MALAT1 overexpression plasmid (OE-MALAT1) was tested by qRT-PCR. (**H**). The interference efficiencies of three siRNAs targeting MALAT1 (si-MALAT1) were verified *via* qRT-PCR. (**I, J, K**). Rat primary hippocampal neurons were transfected with OE-MALAT1. Then SYN1, PSD95, and GAP43 expression was quantified. (**L, M, N**). SYN1, PSD95, and GAP43 expression of the indicated cells was examined by performing qRT-PCR and western blot. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

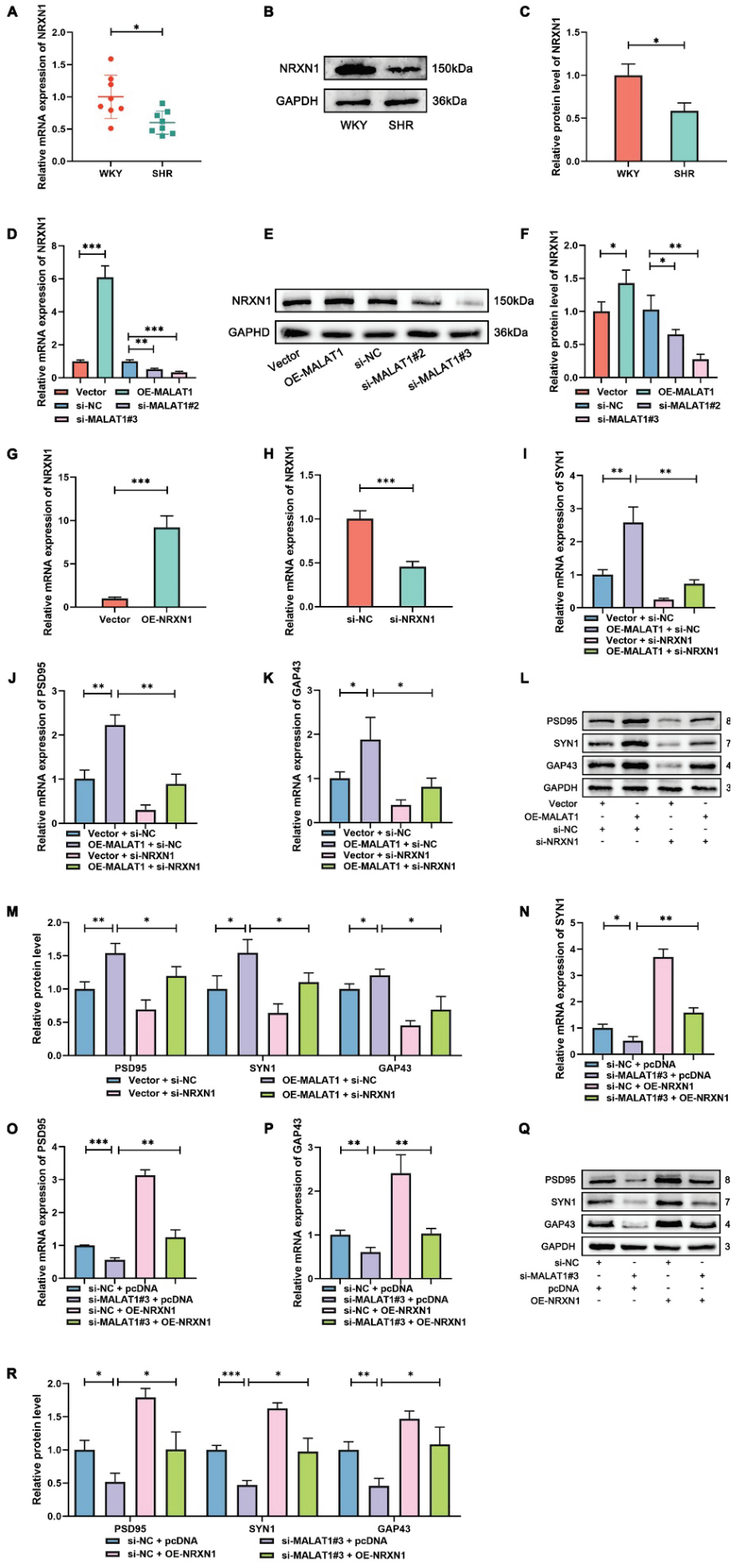

Fig. 2. NRXN1 mediates the effect of MALAT1 on SYN1, PSD95, and GAP43 expression. (**A, B**). The expression of NRXN1, extracted from the hippocampus of WKY rats (n=8) and SHRs (n=8), were measured. (**C**). The densitometry of western blot bands of (B). (**D, E, F**). The NRXN1 expression were quantified in the indicated rat primary hippocampus neurons. (**G, H**). The overexpression efficiency of NRXN1 overexpression plasmid (OE-NRXN1) and the interference efficiency of siRNA targeting NRXN1 (si-NRXN1) were verified *via* qRT-PCR. (**I, J, K, L, M**). Rat primary hippocampal neurons were transfected with different combinations of OE-MALAT1, si-NRXN1 and corresponding negative controls, and then the expression of SYN1, PSD95, and GAP43 of the indicated cells were detected. (**N, O, P, Q, R**). Rat primary hippocampal neurons were transfected with si-MALAT1#3 and OE-NRXN1 or corresponding negative controls. The levels of SYN1, PSD95, and GAP43 expression were quantified. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

miRDB was performed, and intriguingly the results showed that the seed sequences of both miR-141-3p and miR-200a-3p could couple with the 3'UTR of NRXN1 (Fig. 3A). On the other side, RNAhybrid [16] (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid) was operated to calculate the minimum free energy (MFE) of these interactions to further test these predictions from the point of binding stability. The measured MFE of miR-141-3p positioned at MALAT1 or 3'UTR of NRXN1 was low and similar to that of miR-200a-3p positioned at MALAT1 or 3'UTR of NRXN1, supporting that miR-141-3p/200a-3p were the potentially functional miRNAs sponged by MALAT1 and NRXN1. Then, the two miRNAs expression was examined in hippocampus tissue of WKY rats and SHRs. The results showed that both the two miRNAs had a higher expression in SHRs compared with WKY rats (Fig. 3B, C). Next, luciferase reporter assays were carried out to validate the authenticity of bioinformatic analyses. Results demonstrated that miR-141-3p/200a-3p reduced luciferase activity of the HEK293T cells transfected with wild MALAT1 or 3'UTR of NRXN1 with no difference in those transfected with mutant MALAT1 or 3'UTR of NRXN1, suggesting that both the two miRNAs could physically couple with MALAT1 and 3'UTR of NRXN1 (Fig. 3D-F). Finally, to further explore the regulation network of MALAT1, cells stably overexpressing MALAT1 were transfected with miR-141-3p/200a-3p mimics or mimic negative controls, and then NRXN1 expression was detected. The results revealed that overexpressing miR-141-3p or miR-200a-3p could reverse MALAT1-induced high expression of NRXN1 (Fig. 3G-L). Consistent with these findings, the impact of downgrading MALAT1 could be rescued by repressing expression of the two miRNAs (Fig. 3M-R). On the basis of the above findings, our results confirmed that MALAT1 altered expression of NRXN1 *via* directly sponging miR-141-3p/200a-3p.

Discussion

The prevalence of ADHD in children and adolescents lies at approximately 5 %, with a likelihood of 50 % to persist into adulthood [17]. Individuals diagnosed with ADHD show functional deficits relating to long-term impaired cognition with no targeted therapy so far, generally affecting the quality of life of the affected populations [4]. Treatments for individuals with ADHD can be pharmacologic, non-pharmacologic, or both. Although there have been widely used available treatment options, whose short-term efficacy and safety have been soundly confirmed in lots of clinical trials, there are still evidence gaps in their long-term effects on education, vocation, and other aspects related to the quality of life [4]. These treatment limitations highlight the importance of further research to develop new therapeutic strategies for ADHD. Nowadays, scientific and clinical advancements in the etiology and pathophysiology of ADHD have fundamentally altered our current concepts of understanding the disorder, and much more efforts are underway to find out better biomarkers and therapies of ADHD. For example, variations in particular brain regions may lead to dysregulated neural network activities, which are the critical biological basis for neuropsychiatric disorders. With the application of neuroimaging, monitoring variations in a specific brain network, including the hippocampus, could accurately distinguish ADHD from other psychiatric groups and predict symptom severity [18]. Inspired by these previous achievements, we selected the hippocampus, a fundamental brain structure involving learning and memory [14] and having morphological changes in individuals with ADHD [18], as our study target. Meanwhile, SHRs, which exhibit behavioral phenotypes such as impaired cognition resemble to those in ADHD patients, are the most widely used animal models for ADHD research [19]. Above all, our study collected hippocampus samples and analyzed the difference in gene expression between SHRs and WKY rats.

Synaptic plasticity is the most distinguishing feature of the brain and is considered as the cellular mechanism of learning and memory. The forms of plasticity rely on new proteins synthesis in order to reinforce the synaptic connections whose mechanisms are conserved in different types of hippocampal neurons [20]. SYN1, a part of the Synapsin protein family, is believed to manage the distribution of synaptic vesicles (SVs) [21]. The mice with genetic deletion of SYN1 display deficits in cognition and social behavior [22]. PSD95 belongs to postsynaptic scaffolding proteins and is linked to NDDs such as autism spectrum disorder [23]. GAP43 is an essential factor ensuring axons to grow towards correct directions and then form synaptic connections and GAP43 knockout mice behave as impaired hippocampal-dependent memory [24]. Recently, a study found that these synapse-related proteins (SYN1, PSD95, and GAP43) decline in hippocampus of SHRs and are associated with performance in Morris water maze [14].

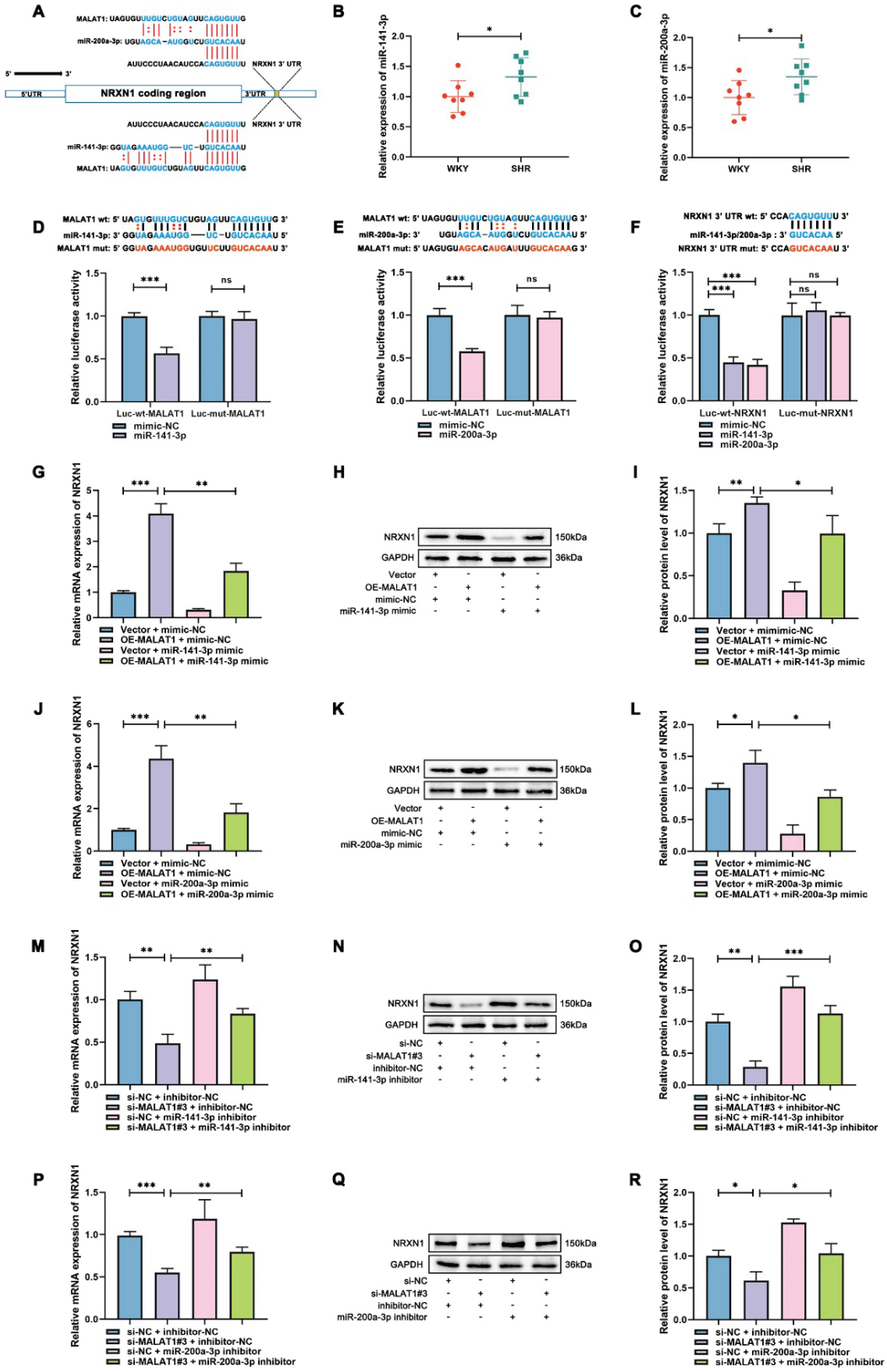

Fig. 3. MALAT1 regulates NRXN1 expression *via* directly sponging miR-141-3p/200a-3p. (**A**). Schematic diagram showed the miR-141-3p/200a-3p binding sites to MALAT1 and NRXN1. (**B, C**). Relative mRNA expression of miR-141-3p/200a-3p in hippocampus samples of WKY rats (n=8) and SHRs (n=8). (**D, E, F**). Luciferase report assay suggested that miR-141-3p/200a-3p bind with MALAT1 and 3'UTR of NRXN1. (**G, H, I, J, K, L**). Rat primary hippocampal neurons were transfected with different combinations of OE-MALAT1 and miR-141-3p/200a-3p mimics or negative controls (mimic-NC). NRXN1 expression was measured in the indicated rat primary hippocampal neurons. (**M, N, O, P, Q, R**). Rat primary hippocampal neurons with or without si-MALAT1#3 were transfected respectively with miR-141-3p/200a-3p inhibitors or inhibitor control (inhibitor-NC). NRXN1 expression of the indicated primary hippocampus neurons was tested. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

As a result, we selected SYN1, PSD95, and GAP43 to represent the capacity of learning and memory in animal models. In line with previous findings, our study proved that the expression of SYN1, PSD95, and GAP43 descended in hippocampus tissue of SHRs in comparison with WKY rats.

Recently numerous studies have proved that lncRNAs participate in regulating gene expression in the occurrence and development of multiple diseases, such as diseases with cognitive impairments. For example, lncRNA PTCHD1-AS plays a vital role in maintaining miniature excitatory postsynaptic current frequency, and deletions of PTCHD1-AS could result in synaptic impairment associated with autism spectrum disorders [25]. LncRNA MALAT1 is highly evolutionary conservative among mammals and abundant in brain tissue [9], suggesting its imperative role in neuron development. Studies have revealed that the dysregulation of MALAT1 has considerable influence on cell viability [15], and neuron excitability [26]. To be specific, Li *et al.* [9] identified that MALAT1 could enhance neuronal viability and decrease neuronal injury in SHSY-5Y (Human neuroblastoma) cell lines and Alzheimer's disease animal models. MALAT1 displays massive functions in cognition. However, to our knowledge, there has been no research investigating the regulatory processes of MALAT1 in the impaired learning and memory ability of ADHD so far. In this study, we identified that MALAT1 was of lower abundance in hippocampus of SHRs than WKY rats, and MALAT1 regulated synapse-related genes (SYN1, PSD95, and GAP43) through modulating NRXN1. In accordance with previous research, our study confirmed the regulatory role of MALAT1 in maintaining synapse-related proteins (SYN1, PSD95, and GAP43), filling the evidence gap of MALAT1 in ADHD.

NRXN1 is a kind of synaptic neuronal adhesion proteins and belongs to neurexin family which can maintain the structure and function of synapses [27]. Meanwhile, for several extracellular conjugated partners, like neuroligins, neurexins, as presynaptic receptors, become a hub for the presynaptic organization [28], suggesting that neurexins play a pivotal role in trans-synaptic cell adhesion, as well as signaling pathways related to synapse formation and maintenance [29]. Variations in neurexin family, especially the copy number variants (CNVs) of NRXN1, have been identified to be in connection with a broad series of neurodevelopmental disorders, such as ASD [30]. Recently, leveraging Morris water maze, Zhang *et al.* [14] demonstrated that NRXN1 enhances the capacity of spatial learning and memory of the ADHD model rats through overexpressing synapse-related genes (SYN1, PSD95, and GAP43), which is consistent with our findings.

Previous research has revealed that MALAT1 is mainly located in cytoplasm and could serve as a ceRNA *via* sponging miRNAs [9]. miRNAs are small non-coding RNAs modulating gene expression at the post-transcriptional level and could function as diagnostic and therapeutic targets [31]. miRNAs can identify and bind with the sites in the 3'UTR of mRNAs, and therefore repress protein productions by either inhibiting translation or minimizing the stability of mRNAs [32]. Importantly, each miRNA has vast target RNAs, including non-coding RNAs (ncRNAs) and mRNAs. ncRNAs could bind to miRNAs in competition with mRNAs, thus altering the quantities of translation productions, which is called “ceRNA hypothesis” [31]. Up to now, the role of MALAT1 as a ceRNA in the biological functions of human diseases has been broadly reported. However, there has been no associated study on ADHD. As our research found that the expression trend of NRXN1 agreed with that of MALAT1, we conceivably put forward the hypothesis that MALAT1 upregulated NRXN1 by sponging miRNAs. Therefore, online bioinformatics databases were utilized and identified miR-141-3p and miR-200a-3p as potential targets of MALAT1 and the 3'UTR of the NRXN1 mRNA. Gong *et al.* [33] have confirmed that MALAT1 could interact with the sequences of miR-141-3p and miR-200a-3p, thus decreasing miRNAs expression, which corresponds with our prediction. miR-141-3p and miR-200a-3p are a part of miR-200 family, which is made up of five members and is divided into two groups on the basis of the resemblance of seed sequences [34]. miR-141-3p and miR-200a-3p share the same seed sequence (AACACUG), indicating that they target a train of collective genes, thus heightening the effects of regulation [35]. Subsequently, luciferase reporter assays were conducted and further verified that the two miRNAs could physically combine with MALAT1 and 3'UTR of NRXN1 mRNA. Finally, rescue experiments revealed that overexpressing miR-141-3p/200a-3p led to the opposite effects which upregulating MALAT1 expression brought about on NRXN1 expression. Collectively, we proved that MALAT1 regulated NRXN1 expression by sponging miR-141-3p and miR-200a-3p.

Conclusions

In summary, our study revealed that MALAT1, NRXN1, and synapse-related genes (SYN1, PSD95, and GAP43) were of low abundance in hippocampus of SHRs. The low expression of MALAT1 released more miR-141-3p/200a-3p to depress NRXN1 expression, which finally downregulated the expression levels of SYN1, PSD95 and GAP43. Our study confirmed the MALAT1-miR-141-3p/200a-3p-NRXN1 axis and may aid in understanding the pathogenesis of impaired learning and memory capacity in ADHD and uncovering diagnostic targets and therapeutic interventions.

Conflict of Interest

There is no conflict of interest.

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