Research article

Therapeutic role of long non-coding RNA TCONS_00019174 in depressive disorders is dependent on Wnt/β-catenin signaling pathway

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Abstract

Chronic stress is one of the major causes that lead to major depressive disorder, which is a prevalent mood disorder worldwide. Many patients with major depressive disorder do not benefit from available medication due to the complex etiology of the condition. Recently, long non-coding RNAs, molecular switches of downstream genes expression, have been reported to be involved in the pathogenesis of major depressive disorder. The long non-coding RNA TCONS_00019174 has been implicated in major depressive disorder risk and antidepressant effects, However, the effect of long non-coding RNA TCONS_00019174 on antidepressant responses has not been investigated. This study is designed to determine whether altered expression of long non-coding RNA TCONS_00019174 contributes to depression-like behaviors associated with chronic stress. We found that mice exposed to chronic ultra-mild stress displayed apparent depression-like behaviors and decreased expression of long non-coding RNA TCONS_00019174 in hippocampus. Both changed behaviors and long non-coding RNA TCONS_00019174 expression level were rescued by chronic treatment with imipramine. Viral-mediated long non-coding RNA TCONS_00019174 overexpression in hippocampal neurons improved the behaviors of mice exposed to chronic ultra-mild stress. Further, it was found long non-coding RNA TCONS_00019174 overexpression upregulated phosphorylated-GSK3β(p-GSK3β) protein and β-catenin in the hippocampus. These findings suggest that long non-coding RNA TCONS_00019174 exerts antidepressant-like effect in mice by activating a Wnt/β-catenin pathway, and that long non-coding RNA may serve as a potential therapeutic target for major depressive disorder in clinical application.

Keywords

long non-coding RNA; long non-coding RNA TCONS_00019174; depression; chronic stress mouse model; GSK3β; β-catenin pathway

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1. Introduction

Stress is a potent negative factor that can lead to psychiatric disease such as depression and anxiety disorders [1, 2]. Major depressive disorder (MDD) is one of the most common mental disordersassociated with depressed mood, anhedonia, and low self-esteem [3]. Nowadays, more than 350 million people suffer from MDD worldwide. It has been reported that MDD might become the second world's leading causes of disease burden by 2020 [4]. Although the pathological mechanisms underlying depression remain to be fully clarified, stress-induced aberrant synaptic functions, neuronal plasticity, and nerve cell metabolisms may be key participants in the pathological changes of major depression [1–3, 5, 6]. Great efforts have been made to examine metabolic dysregulation [7] and structural and functional changes in depression-related brain regions [8]. Growth factors, pro-inflammatory cytokines, and endocrine factors [9] have been found, and that presumably lead to major depression. Recent research has reported that either the environment or behavior could cause epigenetic changes at specific gene loci, such as dysregulated transcription of genes expression, contributing to the pathogenesis of depression [10].

Initially, studies focused on the differential expression of microRNAs (miRNAs) in subjects with depression. MiRNAs are a class of small non-coding RNAs that mediate cleavage or translational repression of target mRNAs [11, 12]. Other evidence shows that miRNAs such as miRNA-26b, miRNA-1972, miRNA-4743, miRNA-4485, and miRNA-4498 are involved in the pathophysiology of depression and antidepressant treatment [6, 13–17]. Long non-coding RNAs (lncRNAs), defined as non-protein-coding RNAs, play an important role in the epigenetic regulation of the human genome, such as DNA methylation, histone modification, and chromatin remodeling, that result in gene activation or silencing. LncRNAs participate in various biological processes, such as human cancer, cardiovascular diseases, and diseases of the central nervous system[18, 19]. Recently,it has been found that six lncRNAs (TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000517573, NONHSAT034045, and NONHSAT142707) were significantly downregulated in patients with MDD compared with healthy controls, suggesting the diagnostic and therapeutic value of lncRNAs for MDD [20].

To reveal the potential mechanism of lncRNAs in the process of depression, the behavioral changes and expression level of lncRNA TCONS_00019174 was evaluated in the hippocampus of mice exposed to chronic unpredictable mild stress (CUMS). It was found that lncRNA TCONS_00019174 significantly decreased in mice with depression. Further, depressive behavioral effects and the downstream signaling pathway were examined by manipulating hippocampal lncRNA TCONS_00019174 expression. Results suggested that CUMS-induced downregulation of hippocampal lncRNA TCONS_00019174 level and concomitant dysregulation of glycogen synthase kinase 3β(GSK3β) contribute to depressive behaviors and antidepressant-like effects. It is well documented that the activation of the Wnt pathway leads to inhibition of GSK-3βphosphorylation, stabilization of cytosolic β-catenin, subsequent nucleus translocation, and further activation of downstream target gene transcription [21]. Previous study has shown that a heterozygous GSK-3β deletion manifested an anti-depression effect in a forced swimming test (FST) of mutant mice [21], while infusion of one GSK-3 selective inhibitorinduced the same change for a FST [22]. Therefore, upregulated β-catenin signaling has been viewed as a marker for antidepression-like behavior [22].

In this study, the potential antidepressant effect of lncRNA TCONS_00019174 was explored in mice with depression, as well as the antidepressant-like effect of β-catenin signaling.Results confirmed the diagnostic and therapeutic value of lncRNAs for depression.

2. Materials and methods

2.1. Animals

8 weeks old adult male BALB/c mice (SLAC Laboratory Animal) were maintained on a 12 h/12 h light/dark cycle with *ad libitum* access to normal food and water. All experimental procedures were performed according to the "Principles of laboratory animal care" (National Institutes of Health 1985). The mice were randomly divided into groups for the behavioral experiments. Experimenters were blinded from the groups when collecting behavioral data.

2.2. Chronic stress

The procedure used in this study was a revised version of the induction of chronic mild stress. The stress was based on environmental and social stressors without food/water deprivation or any nociceptive event [23]. Briefly, mice were subjected to various mild stressors such as paired housing, cage tilt (30°), a soiled cage (50 ml of water/L of sawdust bedding), and confinement to small cages ($11 \times 8 \times 8$ cm). A reversed light/dark cycle was used from Friday evening to Monday morning. This procedure was scheduled over a one week period and repeated six times, but the reversed light/dark cycle was removed during the weekend of the last session. Mice were subjected to a one hour period of morning stress, a two hour period of afternoon stress, and overnight stress.

2.3. Drug treatments

Imipramine (IMI; Sigma-Aldrich) was dissolved in drinking water at a concentration of 160 mg/L for 3 weeks [23].

2.4. Behavioral procedures

Behavioral tests were performed during the light phase (9:00 A.M. to 3:00 P.M.) as described previously [23, 24].

2.4.1. Social interaction (SI) test

A single mouse was placed in a measuring cage for 120 min. A male juvenile (4–5 weeks old) was then introduced into the cage and the amount of time spent in SI such as sniffing, licking, grooming, or crawling over or under the other mouse was recorded during a 3-min session.

2.4.2. Sucrose preference test (SPT)

Mice were subjected to water deprivation for 16 h and then two preweighed bottles, one containing 1.5% sucrose solution and the other containing tap water, were presented for 1.5 h. The positions of the sucrose and water bottles were switched every 30 min. To calculate the volume consumed from each bottle, bottles were weighed and the weight difference during the last 60 min was recorded. The total water and sucrose intake was defined as the total intake and sucrose preference was expressed as the percentage of sucrose intake relative to the total intake.

2.4.3. Forced swim test (FST)

Experiments were carried out in a transparent plexiglass cylinder (diameter 20 cm, height 30 cm). Before the experiment, the tank was filled with water to a depth of about 25 cm Water temperature was maintained at 23$\backsim$25°C. First day, mice were placed in the cylinder to adapt to swimming for 5 min. Second day, mice were placed in the cylinders to swim for adaptation times of six minutes and two minutes. The duration of any immobility of the mice in the water during the 2–6 min swimming period was recorded. A mouse was considered to be immobile when it floated on the the surface of the water, do nothing except making tiny movements of its head to keep it out of the water. Following each experiment, the mice were removed from the water, towel dried, and returned to normal temperature in warm air. Total duration of aquatic murine immobility was analyzed by video recording.

2.4.4. Novelty suppressed feeding test

One cm thick sawdust was placed at the bottom of a plastic box (76 $\times$ 76 $\times$ 46 cm) where 12 similarly sized food pellets were evenly distributed in the center. The mice were fasted for 24 hours (h), placed in the experimental apparatus, and the incubation period was calculated. The criterion for eating was that mice began chewing rather than just sniffing or playing with the food. The incubation period was employed as a parameter to determine the behavioral activity effects of the drug. In the experiment, the test environment was different from the feeding environment as the light intensity was greater in the former. Each time, mice should be placed at the same position and orientation.

2.5. Quantitative real-time PCR

Adult mouse hippocampi were isolated as described with modification (Sabine, 2011). In brief, the brain was dissected out and placed on ice dorsal side up in petri dish. Two parallel cuts about two mm apart were made on the cortex from the midpoint of the midline to the caudal-lateral side (about 40° to the midline), the cortical tissue in-between was removed, and the 'C'-shaped hippocampus was exposed. After dissociating the convex side with fine forceps, the hippocampus was dissected out for RNA isolation or protein extraction. Total RNA was extracted using TRIzol Reagent (Life Technologies)

and digested with DNase (DNA-free; Life Technologies). one μg of total RNA was reverse-transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was performed using the Applied Biosystems Step One Real-Time PCR System with SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's protocol. PCR conditions were 15 min at 95°C, followed by 45 cycles of 15 s at 95°C and 30 s at 60°C. The relative quantification method following the manufacturer's protocol was used for the estimation of target mRNA expression. All measurements were duplicated. Gapdh mRNA or U6 snRNA were used to normalize the relative expression levels of target mRNAs.

2.6. Western blotting

Western blotting was performed as previously described. Equal amounts of protein were separated on 10% SDS-PAGE gels and transblotted onto polyvinylidene difluoride membranes (GE Healthcare). After blocking with 5% skim milk, membranes were incubated with the indicated primary antibody (β-actin 1:5000, p-GSK-3β1:1000, GSK-3β1:1000, β-catenin 1:1000). After incubation with appropriate HRP-conjugated secondary antibodies (1:4000; Cell Signaling Technology), blots were developed using Pierce ECL Western Blotting Substrate Plus.

2.7. Construction of viral vectors

AAV-mediated gene transfer was performed as previously described [23, 24]. A DNA fragment of mouse Camk2a promoter (1.3 kb) was amplified from BALB/c mouse genomic DNA and inserted into the pAAV-CMV-MCS vector, yielding the pAAV-Camk2a-MCS vector. EGFP cDNA was then inserted into the pAAV-Camk2a-MCS vector, yielding the pAAVCamk2a-EGFP vector. To generate the lncRNA TCONS_00019174 expression plasmids, the DNA fragment of lncRNA TCONS_00019174 was PCR amplified from mouse genomic DNA using the forward primer 5'-TCGTGGGGGGAGCGCTCC-3'and the reverse primer 5'-ACCATCCCCTTGAGGTGAA-3'. The fragments were inserted in front of an EGFP-coding region within the pAAV-Camk2a-EGFP vector, yielding the pAAV–Camk2a-lncRNA TCONS_00019174 vector. Recombinant viruses (AAV serotype 8) were generated at Vector Laboratories. The genomic titer of each virus was determined using real-time PCR. The titers of AAV8-Camk2a-EGFP (AAV–GFP) and AAV8–Camk 2a-lncRNA TCONS_00019174-EGFP (AAV–Lnc) were measured as 6.0×10^{13} and 6.7×10^{13} viral genomes/ml, respectively. For viral vector injections, mice were anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. The skull was exposed and a small portion of the skull over the hippocampus was removed bilaterally with a dental drill. Subsequently, AAV vectors were dissolved in physiological saline (0.5 μl) and injected bilaterally into the hippocampus (AP, -2.0 mm; ML, $\pm$ 1.5 mm; DV, -2.0 mm) at 0.1 μl/min. After surgery, the mice were first maintained in cages with a heat lamp until fully recovered from anesthesia, then transferred to normal housing conditions for three weeks for maximum transgene induction. Successful transduction in the hippocampus was confirmed by immunohistochemistry with an antibody against GFP.

2.8. Cell cultures

Primary hippocampal neurons were isolated from embryonic day 17 (E17) mouse embryos and cultured with minor modification as previously reported [25]. In brief, the entire E17 mouse brain was dissected out and placed on sterile gauze under a laminar flow hood. After removing the cerebellum, the brain was separated into two hemispheres along the midline. Under a dissection microscope, the meninges were carefully removed and the hippocampus was fully exposed as a curved structure at the distal part of the hemisphere. The hippocampus was dissected out; cut into pieces and incubated in 2.5% trypsin (Life Technologies) for 20 min at 37°C, then incubated with a trypsin inhibitor (Worthington), triturated into single cells with polished Pasteur pipette and subsequently resuspended in DMEM supplemented with 10% fetal bovine serum. Viable cells were seeded on poly-D-lysine-coated 24-well dishes. After four hours, the medium was replaced with Neurobasal medium (Life Technologies) containing 1% B27 supplement (Life Technologies) and 50 μg/ml streptomycin. The cultures were maintained at 37°C in a 5% CO_2 humidified atmosphere. On day 2 *in vitro* (2 DIV), hippocampal neurons were treated with 2 μM 1-β-D-arabinofuranosylcytosine (Sigma-Aldrich) to remove any proliferating non-neuronal cells.

siRNA oligos targeting TCONS_00019174 were designed and synthesized by GenePharma (Shanghai).Five DIV hippocampal neurons were treated with control or TCONS_00019174 targeting siRNA together with Lipofectamine^{TM} RNAiMAX Transfection Reagent according to the manufacturer's instructions.

2.9. Statistical analyses

Multiple groups were compared using ANOVA (one-way or two-way). Unpaired t-tests were used for two-group comparisons. Tests were two-tailed and considered significant when $p < 0.05$. All data are presented as mean $\pm$ SEM.

3. Results

3.1. Chronic stress reduces hippocampal lncRNA TCONS_00019174 expression level

It has been reported that BALB/c mice are more vulnerable to a CUMS challenge [23], thus BALB/c mice were chosen for this study. BALB/c mice were either exposed to a 6-week CUMS environment or not, with or without IMI, and assessed for depressive behavior. It was shown that mice exposed to CUMS had a reduced SI time compared with nonstressed (NS) mice; this change could be rescued given IMI treatment (Fig. 1a). In the FST test, the CUMS group increased their immobility time, whereas, following NS and CUMS exposure, IMI treatment decreased immobility time for the mice This indicated the antidepressant efficacy of IMI [23, 26, 27] (Fig. 1b). In the SPT test, mouse anhedonia (diminished interest or satisfaction) was verified and mice exposed to CUMS displayed decreased sucrose preference, which was rescued by IMI [Fig. 1c]. In another novelty-suppressed feeding (NSF) test, which used the latency to eating food in the center of an open field to test anxiety and antidepressant-like response [24, 26, 28], the CUMS group showed increased latency to feeding compared with NS mice, which also was rescued by IMI treatment (Fig. 1d). The preceding behavioral data demonstrates that stressed BALB/c mice manifested depression-like behavior. The hippocampal expression level of lncRNA TCONS_00019174 was then examined in these groups by Northern blotting, it was found that lncRNA TCONS_00019174 level was significantly reduced in stressed mice compared with NS mice and the expression level of lncRNA TCONS_00019174 improved after IMI treatment (Fig. 1e–1f).

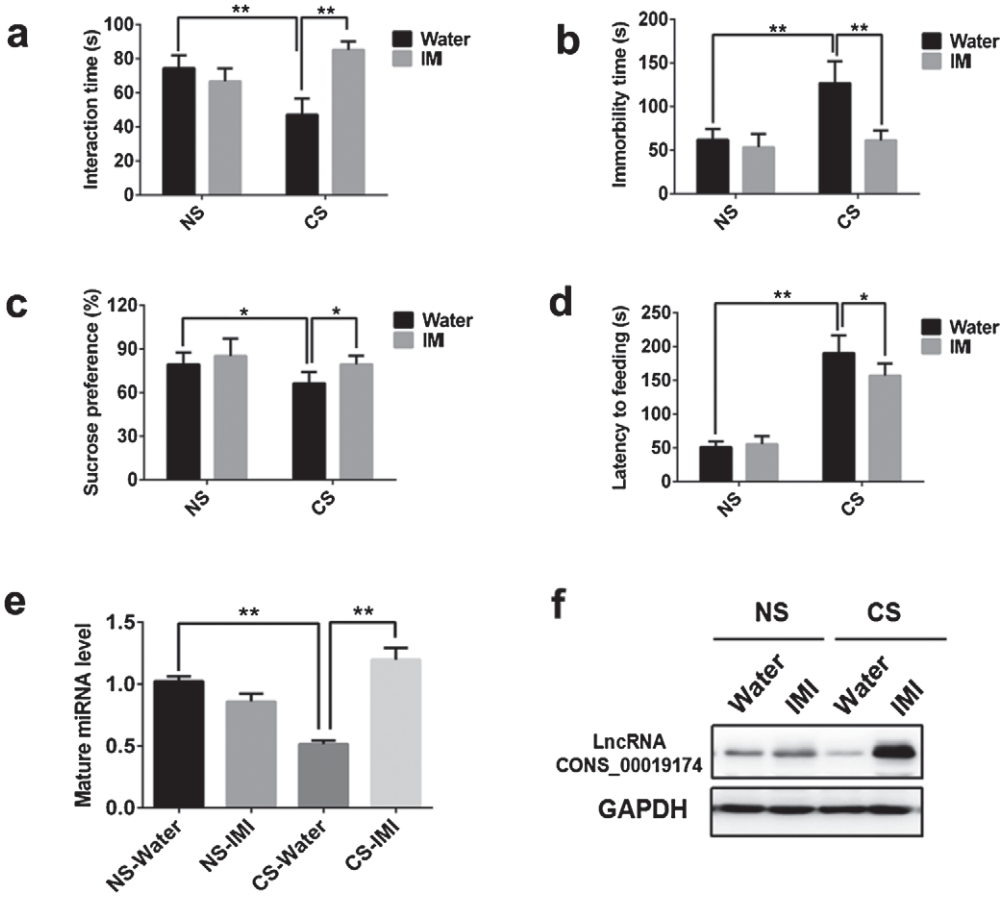

Fig. 1. Chronic stress induces depression-like behavior and lncRNA TCONS_00019174 downregulation in the hippocampus. Results of depression-like behavioral tests. a, SI time during SI test. b, Immobility time during FST test. c, Sucrose during SPT test. d, Latency to feeding during NSF test. $n = 15$ mice per group. $**p < 0.01$. e–f, LncRNA TCONS_00019174 level in hippocampus of mice exposed to CUMS or the NS and receiving either water or IMI treatment for the last three weeks. LncRNA TCONS_00019174 expression level was down-regulated by CUMS. This effect was blocked by IMI treatment. n= 10 mice per group. $**p < 0.01$.

3.2. *Effect of lncRNA TCONS_00019174 on depression suppression in mice*

To explore the role of hippocampal lncRNA TCONS_00019174 in the pathogenesis of depression, a mouse model was constructed by bilateral hippocampus injection of AAV vector expressing pre-lncRNA TCONS_00019174 and GFP under the control of Camk2a promoter, which limited expression to excitatory neurons. Mice injected with AAV vector expressing GFP alone was used as the control treatment (Fig. 2a and 2b). Mice were grouped for behavioral experiments.

It was found that NS mice overexpressing lncRNA TCONS_00019174 showed no significant behavioral changes compared with NS control mice (Fig. 2c–2f), while CUMS mice overexpressing lncRNA TCONS_00019174 developed resistance to depression-like behavior compared with CUMS control mice. In the SI test, lncRNA TCONS_00019174 overexpressed mice exposed to CUMS spent more time than CUMS-stressed mice infected with control vector (Fig. 2c). In FST, there was no significant difference between CUMS-exposed mice with lncRNA TCONS_00019174 overexpression and control mice (Fig. 2d). But similar antidepression-like phenotypes were found in the SPT and NSF tests as CUMS mice exhibited decreased sucrose preference and increased latency to feeding (Fig. 2e and 2f) when compared with NS control mice, while CUMS-exposed mice overexpressing lncRNA TCONS_00019174 showed no significant difference in these tests when compared with the NS control mice.

3.3. *lncRNA TCONS_00019174 exerts antidepressant-like effect in mice by activating Wnt/β-catenin pathway*

The activation of the Wnt pathway, which leads to inhibition of GSK-3β and upregulation of β-catenin signaling, has been interpreted as a marker for antidepressive-like behavior. It was hypothesized here that alteration of lncRNA TCONS_00019174 expression level by chronic stress and/or chronic IMI treatment would mediate these behaviors through the GSK-3β pathway, based on findings that chronic stress significantly decreased β-catenin mRNA and protein, while increased GSK-3βmRNA and protein levels in the hippocampus of mice exposed to CUMS compared with NS control (Fig. 3a–3c). However, these expression variations were reversed by IMI treatment (Fig. 3a–3c), suggesting that the lncRNA TCONS_00019174–GSK-3β–β-catenin pathway may contribute to the behavioral responses to stress, whereas the depression-like behaviors could be rescued by IMI treatment. Subsequently, it was investigated whether the expression levels of pGSK-3β and β-catenin are regulated by

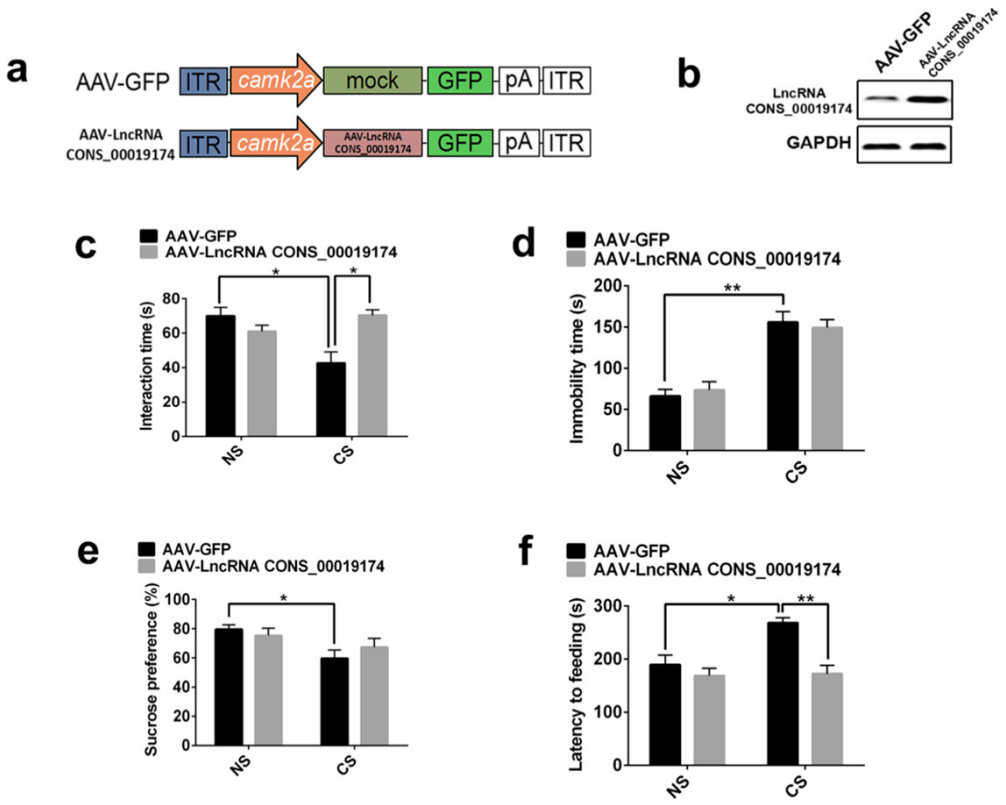

Fig. 2. Hippocampal lncRNA TCONS_00019174 play an important role in regulating behavior under chronic stress. a, Schematics of AAV engineered to overexpress either lncRNA TCONS_00019174 or mock under control of the Camk2a promoter. b, Northern blotting shows overexpression of lncRNA TCONS_00019174 in hippocampus. c–f, lncRNA TCONS_00019174 overexpression blocked CUMS-induced depression-like behavior. Behavioral results: c, SI time in the SI test. d, Immobility time in the FST test. e, Sucrose preference in the SPT test. f, Latency to feeding in the NSF test. n= 15 mice per group. $*p < 0.05$.

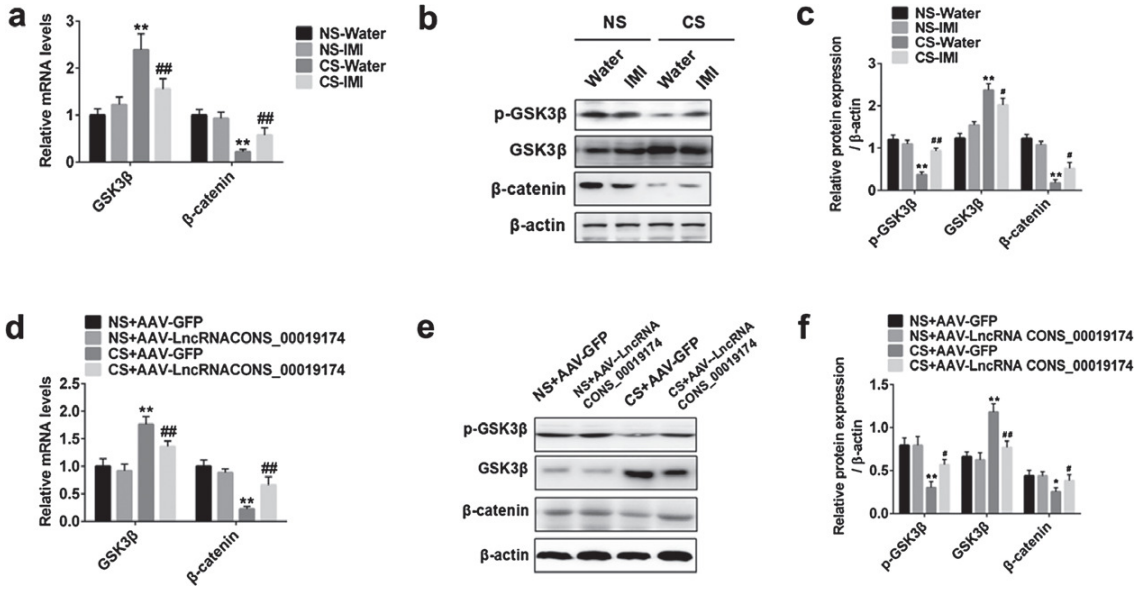

Fig. 3. lncRNA TCONS_00019174 regulates β-catenin/GSK-3βsignaling pathway. a, Level of mRNAs for GSK3$\beta$and β-catenin in hippocampus of mice subjected to 6-week CUMS or NS challenge receiving either water or IMI treatment. b and c, Protein levels of p-GSK3β, GSK3β, and β-catenin in hippocampus of mice subjected to 6-week CUMS or NS receiving either water or IMI treatment. d, mRNA levels of GSK3$\beta$and β-catenin in hippocampus of CUMS mice injected with AAV–LncRNA TCONS_00019174 or control vector. e and f, Protein levels of p-GSK3β, GSK3$\beta$and β-catenin in hippocampus of CUMS mice injected with AAV–GFP of AAV–LncRNA TCONS_00019174. $**p < 0.01$ compared to the untreated model group (NS-Water). $\#\#p < 0.01$ compared to the AAV–GFP-injected CUMS group. All data presented as mean $\pm$ SEM.

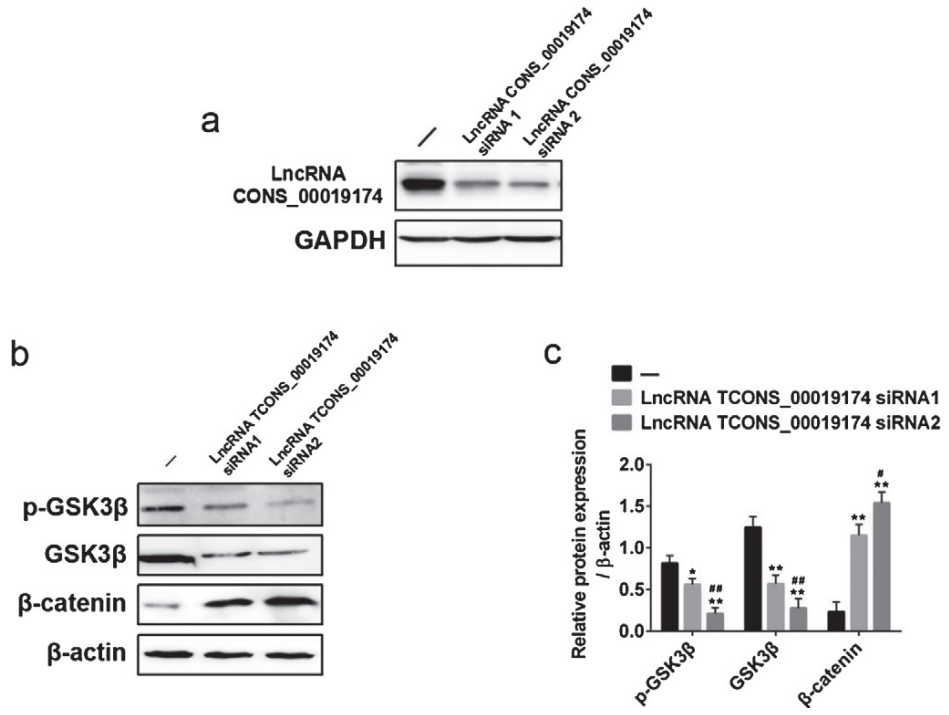

Fig. 4. Effect of lncRNA TCONS_00019174 on activation of the canonical Wnt pathway in hippocampus. Western blots and quantitative analysis of p-GSK3β, GSK3$\beta$and β-catenin are presented. a, LncRNA TCONS_00019174 knock-down after siRNA treatment. b, Elevated GSK3β and decreased β-catenin protein levels observed in mice injected with lncRNA TCONS_00019174 siRNAs. c, Quantitative statistical results of WB. $*p < 0.05$, $**p < 0.01$. The data presented as mean $\pm$ SEM.

lncRNA TCONS_00019174 *in vivo*. It was found that the pattern of the Wnt signaling pathway resembled IMI treatment in CUMS mice. Decreased pGSK-3β level and β-catenin level by CUMS was reversed by lncRNA TCONS_00019174 overexpression (Fig. 3e–3f). Overall, these results suggest that lncRNA TCONS_00019174 regulates depression-like changes in mice through the Wnt/β-catenin signaling pathway under CUMS stimulation.

3.4. *lncRNA TCONS_00019174 knock-down inhibits activation of the canonical Wnt pathway in hippocampal neurons*

To further explore the effects of lncRNA TCONS_00019174 on the Wnt pathway, RNAi experiments were undertaken in cultured hippocampal neurons with two different siRNA oligonucleotides targeting TCONS_00019174. As shown in Fig. 4a, compared with control siRNA, the TCONS_00019174 knock-down dramatically reduced GSK-3β phosphorylation. Consequently, protein levels of β-catenin in both RNAi groups were significantly up-regulated, confirming the activation of canonical Wnt pathway when TCONS_00019174 expression was blocked. It was of interest to note that, contrary to the p-GSK-3β trend, total GSK-3β protein levels were elevated after TCONS_00019174 knock-down, which might be the result of a negative feed-back effect.

4. Disscussion

The antidepressant effect of lncRNA TCONS_00019174 in mice was explored in this study. It was found that elevated lncRNA TCONS_00019174 expression level alleviated depression-like behaviors in CUMS mice, as indicated by increased time of communication in SI, decreased immobility in FST, reduced latency of time to feeding in NSF, and an elevated sucrose preference index in SPT. Moreover, lncRNA TCONS_00019174 overexpression induced the activation of the canonical Wnt/β-catenin pathway in hippocampus of CUMS mice, which was mainly responsible for the observed anti-depressive effects of lncRNA TCONS_00019174 in mice.

Currently, there is accumulating evidence about epigenetic biomarkers for MDD, such as DNA methylation, miRNAs and lncRNAs, that indicates a significant contributions of miRNAs and lncRNAs to depression, anxiety, and antidepressant actions [29]. miRNAs (miR-16) [30, 31], miR-221-3p, miR-34a-5p, let-7d-3p, and miR-451a [32], miRNA-26b, miRNA-1972, miRNA-4743, miRNA-4498, and miRNA-4485 [33] were found to be involved in the occurrence and development of MDD, and changes of miRNAs expression have been closely associated with the improvement of MDD symptoms [34, 35]. More research attention has been focused on the expression profiles of another non-coding RNA, lncRNAs, in MDD. The biological functions of the differentially regulated lncRNAs in the body have shown the important role of lncRNAs in multiple biological processes including translational elongation, protein transport and localization, protein complex biogenesis and assembly. LncRNAs are also involved in central nervous system diseases such as Alzheimer's disease, Parkinson's disease, and even depression. Six lncRNAs (TCONS_00019174, ENST00000566208, NONHSAG045500, ENST00000517573, NONHSAT034045, and NONHSAT142707) were significantly downregulated in MDD patients compared to controls, indicating the potential diagnostic and therapeutic biomarker roles of lncRNAs for MDD [20]. Thus, post-transcriptional regulation by miRNAs and lncRNAs networks in de-

pression and antidepressant drug actions attract increasing attention.

It has been demonstrated here that hippocampal lncRNA TCONS_00019174 influences behavioral responses to chronic stress in mice. Inhibition of hippocampal lncRNA TCONS_00019174 enhanced behavioral preference to depression under mild stress stimulation. These behavioral effects were paralleled by changes in downstream gene expression as it was found that lncRNA TCONS_00019174 would regulate a Wnt/β-catenin signaling pathway associated with mediating p-GSK3β/GSK3β level. It is known that both genetic background and social stress may have effects on gene expression profiles. Further, studies have been conducted to clarify how gene–environment interaction change lncRNA expression in depression. AAV vectors with the Camk2a promoter were used to overexpress lncRNA TCONS_00019174 specifically in excitatory neurons, and it was found that lncRNA TCONS_00019174 overexpression markedly improved depression-like behaviors exposed to chronic stress. Though chronic stress decreased both the mRNA and protein level of p-GSK3β/GSK3β,this effect was blocked by lncRNA TCONS_00019174 overexpression as one therapeutic antidepressant treatment. In contrast, lncRNA TCONS_00019174 overexpression did not affect mRNA and protein levels of GSK3β levels in the control condition. LncRNAs participate in mediating transcription or translational repression of target genes, and lncRNAs have been involved in biological processes of the central nervous system, especially hippocampal development, which has been associated with the development of depression. Data suggests that lncRNA TCONS_00019174 might increase GSK3β expression in response to chronic stress and suppresses β-catenin signaling under conditions of stress. Intriguingly, both GSK3β and β-catenin have been associated with depression and antidepressant drug effects. Enhancement of GSK-3β activity helps to reduce synaptic spine density in response to stress [1, 36] and patients with MDD have increased GSK-3β activity [37]. Increasing evidence suggests that inhibition of GSK-3β may contribute to antidepressant treatment [38]. Consistent with this, lower p-GSK-3β and p-GSK-3β/GSK-3β levels in the hippocampus of SCH rats, leads to depression-like behavior [39]. These results suggest a role for GSK-3β in anti-depressive effects and highlight GSK-3β as a potential target in the treatment of depression. β-catenin, a substrate of GSK-3β [40], has been implicated in brain development and growth [41]. Phosphorylation of β-catenin by GSK-3β induces the degradation of the protein, while phosphorylation of GSK-3β would stabilize β-catenin in the cell cytoplasm. In depressed subjects, the level of β-catenin was decreased in postmortem prefrontal cortices compared with controls [42], thus β-catenin level could be viewed as an index for antidepressant behaviors [43]. Above all, depression would bring about a high GSK-3β activation state and low β-catenin levels.

In conclusion, It is proposed that lncRNA TCONS_00019174 acts as an important regulator of behavioral responses to chronic stress. Detailed investigation of the lncRNA TCONS_00019174-associated gene networks involved in activating Wnt/β-catenin signaling pathway should be carried out. Behavioral changes and stress-induced epigenetic gene regulations reported here reveal new aspects of depression pathophysiology and treatment. At least and in part, direct modulation of lncRNAs expression and the Wnt/β-catenin pathway may be an effective therapeutic strategy for depression.

Acknowledgments

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Conflict of Interest

All authors declare no conflicts of interest.

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