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Knockdown of lncRNA MALAT1 alleviates bupivacaine-induced neurotoxicity via the miR-101-3p/PDCD4 axis

Yanling Zhao, Yanqiu Ai*

Department of Anesthesiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China

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ABSTRACT

Aims: Bupivacaine, a common local anesthetic, can cause neurotoxicity and abnormal neuro-disorders. However, the precise underlying mechanisms have not been fully elucidated. In this study, we investigated the function of lncRNA MALAT1 in the bupivacaine-induced neurotoxicity process.

Materials and methods: SH-SY5Y cells and neonatal mouse DRG neurons were cultured in vitro and treated with bupivacaine to establish a neurotoxicity model. Caspase3 activity and cell survival rates were detected to evaluate the function of lncRNA MALAT1. Western blotting was used to detect the expression levels of PDCD4 and cleaved-caspase-3. A dual-luciferase reporter assay was used to explore the potential binding target of lncRNA MALAT1.

Results: We found that the expression of lncRNA MALAT1 was upregulated upon exposure to bupivacaine. Knockdown of lncRNA MALAT1 significantly increased the cell death rates, and Caspase3 activity assays revealed that the apoptosis rates were manifestly increased in the MALAT1 downregulation group. In addition, we screened the possible target and found that miR-101-3p is the direct target of MALAT1 using a dual-luciferase reporter assay; these results suggest that lncRNA MALAT1 may function as a decoy to sponge miR-101-3p. Furthermore, we demonstrated that activation of the MALAT1/miR-101-3p/PDCD4 axis protected cells against bupivacaine treatment.

Conclusion: We elucidated the function and mechanism of MALAT1 in bupivacaine-induced neurotoxicity. Targeting MALAT1 might provide new methods to prevent neurotoxicity.

1. Introduction

Bupivacaine, a local anesthetic, is commonly used for clinical treatment [1–3] and for epidural or spinal anesthesia [4] and nerve blockade [5]. The concentration of bupivacaine used in clinical treatment depends on the anesthesia method, which varies from 1.5 mm for sympathetic ganglion block to 15.0 mM for local infiltration. It is widely believed that bupivacaine can cause neurotoxicity and abnormal neuro-disorders, such as cauda equina syndrome [6,7], sensory disturbance [8,9], and motor paralysis [10,11]. Bupivacaine exposure resulted in a reduction in cell viability and increases in lactate dehydrogenase release, apoptosis rates and cleaved caspase-3 levels in SH-SY5Y cells [12]. However, the precise mechanisms have not been fully elucidated.

Recently, numerous studies have identified the role of long non-coding RNA in different diseases. Long noncoding RNA is a specific noncoding RNA that is longer than 200 nt [13]. Previous studies have shown the role and function of lncRNA in various biological and pathological processes, such as apoptosis [14], cell cycle [15], necroptosis [16], chromosome recombination [13] and posttranslational protein stability [17]. For example, GUARDIN is a p53-responsive long non-coding RNA that is essential for genomic stability [14]. LncRNA H19 binds directly to miR-103/107 and regulates FADD expression and necrosis [16]. Sirt1 antisense long noncoding RNA promotes cardiomyocyte proliferation by enhancing the stability of Sirt1 [18]. However, the role of lncRNA in bupivacaine-induced neurotoxicity remains largely unknown. Thus, exploring more lncRNAs will undoubtedly provide new information for clinical applications.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), a long noncoding RNA (lncRNA), is also called nuclear-46 enriched autosomal transcript 2 (NEAT2) [19]. Numerous studies have reported the various functions of lncRNA MALAT1 in different diseases. For example, hematopoietic deficiency of MALAT1 promotes atherosclerosis and plaque inflammation [20]. Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth [21]. To date,

* Corresponding author at: Department of Anesthesiology, The First Affiliated Hospital of Zhengzhou University, No. 1 Jianshe East Road, Erqi District, Zhengzhou, Henan, China.
E-mail address: yanqiuaizz@163.com (Y. Ai).

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there have been no reports on the function of MALAT1 in the bupivacaine-induced neurotoxicity process.

In this study, we investigated the function of lncRNA MALAT1 in the bupivacaine-induced neurotoxicity process. We found that the expression of lncRNA MALAT1 was increased upon exposure to bupivacaine. Knockdown of lncRNA MALAT1 significantly promoted cell death rates, and a caspase3 activity assay revealed that the apoptosis rates were manifestly increased in the MALAT1 downregulation group. In addition, we screened the possible target and found that miR-101-3p is its direct target using a dual-luciferase reporter assay; these results suggest that lncRNA MALAT1 may function as a decoy to sponge miR-101-3p. Furthermore, we demonstrated that activation of the MALAT1/miR-101-3p/PDCD4 axis protected cells from bupivacaine treatment. In summary, we elucidated the function and mechanism of MALAT1 in bupivacaine-induced neurotoxicity. Targeting MALAT1 might provide a new method for preventing neurotoxicity.

2. Materials and methods

2.1. Cell culture and treatment

The human neuroblastoma cell line SH-SY5Y was obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences. Primary dorsal root ganglion (DRG) neurons were extracted from neonatal (postnatal 1–2 days old) mice as previously described [22]. SH-SY5Y cells and DRG neurons were cultured in DMEM/F12 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Life Technologies, Waltham, MA, USA) in a 5% CO_2 incubator at 37 °C. Bupivacaine, a local anesthetic reagent, was added to the culture medium to induce neurotoxicity. To explore the dose-dependent response, the cells were treated with 0.5, 1.0, 1.5 or 2.0 mM bupivacaine for 24 h as previously described [22].

2.2. Gene transfection

Small interfering RNA targeting MALAT1 (si-MALAT1) and a negative control (NC) were synthesized by GenePharma (Shanghai, China). miR-101-3p mimics, NC mimics, miR-101-3p inhibitor, and NC inhibitor were purchased from Shanghai GenePharma (Shanghai, China). PDCD4 and MALAT1 mRNA 3′-UTR vectors and mutant vectors were constructed by Shanghai GenePharma (Shanghai, China). SH-SY5Y cells and DRG neurons were transfected using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol. After 48 h of infection, the cells were harvested for research.

2.3. Cell viability assay

SH-SY5Y cells and DRG neurons were seeded into 96-well plates and incubated overnight. A total of 2000 cells were seeded into each well. After bupivacaine treatment (0.5, 1.0, 1.5 or 2.0 mM bupivacaine for 2 h), 10 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL, Beyotime, Shanghai, China) was added to each well of the 96-well plates and incubated for 4 h at 37 °C. Cell viability was measured by a microplate reader at 570 nm (Bio-Tek, Winooski, VT).

2.4. Caspase-3 activity

A caspase-3 activity assay kit (Beyotime, C1116) was used to assess Caspase-3 activity according to the manufacturer's protocol. The cell supernatants of SH-SY5Y cells and DRG neurons were obtained after lysis and centrifugation. A final concentration of 0.2 mM ADEVD-pNA (caspase-3 substrate) was added to the cell supernatants and incubated for 1 h. The caspase-3 activity was measured by a microplate reader at 405 nm (Bio-Tek, Winooski, VT).

Table 1
Primers used in this study.

Primer	Sequence	Application
MALAT1	F: GAATTGCGTCATTTAAAGCCTAGTT R: GTTTCATCCTACCACTCCCAATTAAT	qPCR
miR-101-3p	F: GGGGCATTAAGCACCCAGTAA R: TACGTTGCTAATCCAGCGGGA	qPCR
PDCD4	F: GCCGAGCGCACAACTAGAAAG R: CCAATCATGCCCCAAGAGCA	qPCR
GAPDH	F: TGTTGCCATCAATGACCCCTT R: CTCCACGACGTACTCAGCG	qPCR

2.5. Dual-luciferase reporter assay

The 3′-UTRs of MALAT1 and PDCD4 and their wild-type and mutant binding sites for miR-101-3p were amplified and cloned into the pGL3 vector (Promega, Madison, WI, USA) to generate pGL3-wt-MALAT1-3′-UTR, pGL3-mut-MALAT1-3′-UTR, pGL3-wt-PDCD4-3′-UTR, and pGL3-mut-PDCD4-3′-UTR plasmids. SH-SY5Y cells and DRG neurons were used to perform the luciferase reporter assays. miR-101-3p mimics or inhibitors and plasmids were transfected using Lipofectamine 2000. Luciferase activity was analyzed using a dual-luciferase system following the manufacturer's protocol.

2.6. Real-time PCR

Total RNA was extracted and lysed using TRIzol reagent (Thermo Fisher Scientific). RNA reverse transcription was performed using a PrimeScript™ RT reagent kit with gDNA Eraser (RR047A; Takara, Tokyo, Japan) according to the manufacturer's instructions, and cDNA was subjected to qRT-PCR using SYBR® Premix Ex Taq™ (RR420A; Takara, Tokyo, Japan). All primer sequences are listed in Table 1. The data were normalized to the GAPDH levels and further analyzed by the $2^{-\Delta\Delta CT}$ method.

2.7. Western blotting

SH-SY5Y cells and DRG neurons were harvested and lysed with RIPA lysis buffer containing proteinase inhibitor (Roche, USA). Total protein was quantified using a BCA protein assay kit (Pierce, Rockford, IL, USA). Protein samples were resolved on 10% SDS-PAGE gels (120 V, 120 mins) and transferred to polyvinylidene difluoride (PVDF) membranes (300 mA, 90 mins). The membranes were blocked with 5% pure milk for 1 h. After that, they were incubated with primary antibodies against Bax (1:1000, Abcam, ab32503, MA, USA), Bcl-2 (1:500, Abcam, ab196495, MA, USA), PDCD4 (1:1000, Abcam, ab80590, MA, USA) and GAPDH (1:1000, Abcam, ab181602, MA, USA) at 4 °C overnight, followed by incubation with a peroxidase-conjugated goat anti-rabbit (or mouse) IgG antibody (CST, 1:5000, 7074). Immunopositive bands were analyzed using a FluorChem M system (Protein Simple, San Jose, CA, USA).

2.8. Data analysis

All experiments were performed at least 3 independent times, and data were shown as means + S.E.M. When only 2 groups compared, statistical differences were analyzed with unpaired 2-tailes Student's *t*-test. We used SPSS 21.0 to calculate the values (mean ± standard error of the mean (SEM)).Otherwise statistical significance was assessed using 1- or 2-way ANOVA followed by Bonferroni multiple comparison test. Statistical significance was indicated by $P < 0.05$.

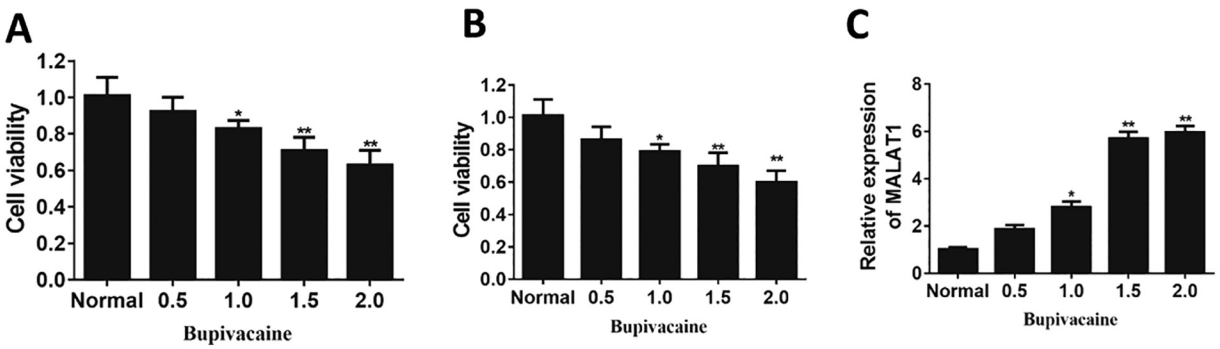

Fig. 1. Bupivacaine induced neurotoxicity model. (A) The cell viability was significantly decreased with the elevation of concentration of bupivacaine in SH-SY5Y cells. (B) The cell viability was significantly decreased with the elevation of concentration of bupivacaine in DRG neurons. (C) The expression of lncRNA MALAT1 was upregulated by bupivacaine in a dose-dependent manner in DRG neurons. Data represent the mean ± SD of 3 independent measurements. Data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$ compared to control group.

3. Results

3.1. MALAT1 was upregulated by a local anesthetic in DRG neurons

Previous studies have demonstrated that the application of local anesthetics, including bupivacaine, induced neurotoxicity in DRG neurons. In this study, we first wondered whether endogenous expression of lncRNA MALAT1 in neonatal mouse DRG neurons was affected by the in vitro application of bupivacaine. As shown in Fig. 1A, SH-SY5Y cell viability was significantly decreased with increasing concentrations of bupivacaine. We also treated DRG neurons in vitro with bupivacaine. Similar results were detected and are shown in Fig. 1B. The expression of lncRNA MALAT1 was upregulated by bupivacaine in a dose-dependent manner in DRG neurons (Fig. 1C). Thus, lncRNA MALAT1 may function in local anesthetic-induced injury in DRG neurons.

3.2. MALAT1 downregulation promoted cell apoptosis in local anesthetic-injured DRG neurons

To examine the function of MALAT1 in local anesthetic-injured DRG neurons, we first used lncRNA MALAT1 siRNA to knockdown the expression of MALAT1. We found that lncRNA MALAT1 expression was significantly lower in the group treated with MALAT1 siRNA than in the control group (Fig. 2A). We next wondered whether MALAT1 had a functional role in bupivacaine-induced neurotoxicity in DRG neurons. First, cell viability was measured via MTT assay. Our results revealed that knockdown of lncRNA MALAT1 significantly decreased cell viability in SH-SY5Y cells and DRG neurons (Fig. 2B, C). When comparing the caspase3 activity of siRNA-NC- and siRNA-MALAT1-transfected DRG neurons, MALAT1 downregulation significantly promoted apoptosis after bupivacaine-induced neurotoxicity in SH-SY5Y cells and DRG neurons (Fig. 2D, E). In addition, we detected that the apoptosis markers Bax and Bcl-2 were increased in the lncRNA MALAT1 downregulation group, suggesting that MALAT1 knockdown exerts a pro-apoptosis effect in local anesthetic-injured DRG neurons (Fig. 2F). Thus, our results suggested that MALAT1 exerts a protective role in local anesthetic-injured DRG neurons.

MALAT1 negatively regulated miR-101-3p in local anesthetic-injured DRG neurons.

Previous studies have reported that MALAT1 could function as a competing endogenous lncRNA to sponge miRNAs in different diseases [23–25]. To further study the mechanism of MALAT1, we screened miRNAs to identify whether MALAT1 can regulate miRNA in local anesthetic-injured DRG neurons. We used bioinformatics analysis and found that miR-101-3p may be a direct target of MALAT1. The potential binding sequence between miR-101-3p and MALAT1 is shown in Fig. 3A. A dual-luciferase reporter assay was performed to identify whether miR-101-3p was a direct target of lncRNA MALAT1. Our results revealed that miR-101-3p was decreased in the wt lncRNA MALAT1 group, whereas no difference was detected in the mut lncRNA MALAT1 group (Fig. 3B). We detected the expression of miR-101-3p after exposure to bupivacaine and found that it was decreased with increasing concentrations of bupivacaine (Fig. 3C). Overexpression of miR-101-3p significantly reduced the expression of lncRNA MALAT1 and vice versa (Fig. 3D). LncRNA MALAT1 can negatively regulate the expression of miR-101-3p (Fig. 3E). Thus, our data proved that MALAT1 negatively regulated miR-101-3p in local anesthetic-injured DRG neurons.

3.3. PDCD4 is a direct target of miR-101-3p

To further study the mechanism of miR-101-3p, we used bioinformatics software to analyze the potential target of miR-101-3p. We searched an online database (miRDB) and found that the apoptosis-related protein programmed cell death protein 4 (PDCD4), which is also increased dose-dependently in bupivacaine-treated cells, is a target of miR-101-3p. Thus, we wondered whether miR-101-3p can regulate the expression of PDCD4 in local anesthetic-injured DRG neurons. The potential binding sequence between miR-101-3p and PDCD4 is shown in Fig. 4A. miR-101-3p significantly reduced the luciferase activity of PDCD4, and there was no significant difference for the mut PDCD4 group (Fig. 4B). Next, we measured the expression of PDCD4 in the miR-101-3p overexpression group and miR-101-3p knockdown group. The expression of PDCD4 was reduced in the miR-101-3p overexpression group via qPCR, and the opposite results were found in the miR-101-3p knockdown group (Fig. 4C). Similar results were also detected via Western blotting (Fig. 4D). Next, we detected the expression of PDCD4 after exposure to bupivacaine and found that it was increased with increasing concentrations of bupivacaine (Fig. 4E). Thus, our data proved that PDCD4 was a direct target of miR-101-3p.

3.4. MALAT1/miR-101-3p/PDCD4 axis in local anesthetic-injured DRG neurons

To further study the mechanism of lncRNA MALAT1/miR-101-3p/PDCD4 axis involvement in local anesthetic-injured DRG neurons, we performed a rescue experiment to verify the relationship among lncRNA MALAT1, miR-101-3p and PDCD4. First, qPCR revealed that the expression of PDCD4 was decreased in the lncRNA MALAT1 knockdown group (Fig. 5A). Similar results were achieved via Western blotting (Fig. 5B). We found that overexpression of miR-101-3p blocked the effect of lncRNA MALAT1. qPCR also revealed that the expression of PDCD4 was increased in the lncRNA MALAT1 and miR-101-3p co-transfection group (Fig. 5C). Similar results were achieved via Western blotting (Fig. 5D). A rescue experiment was performed to verify that

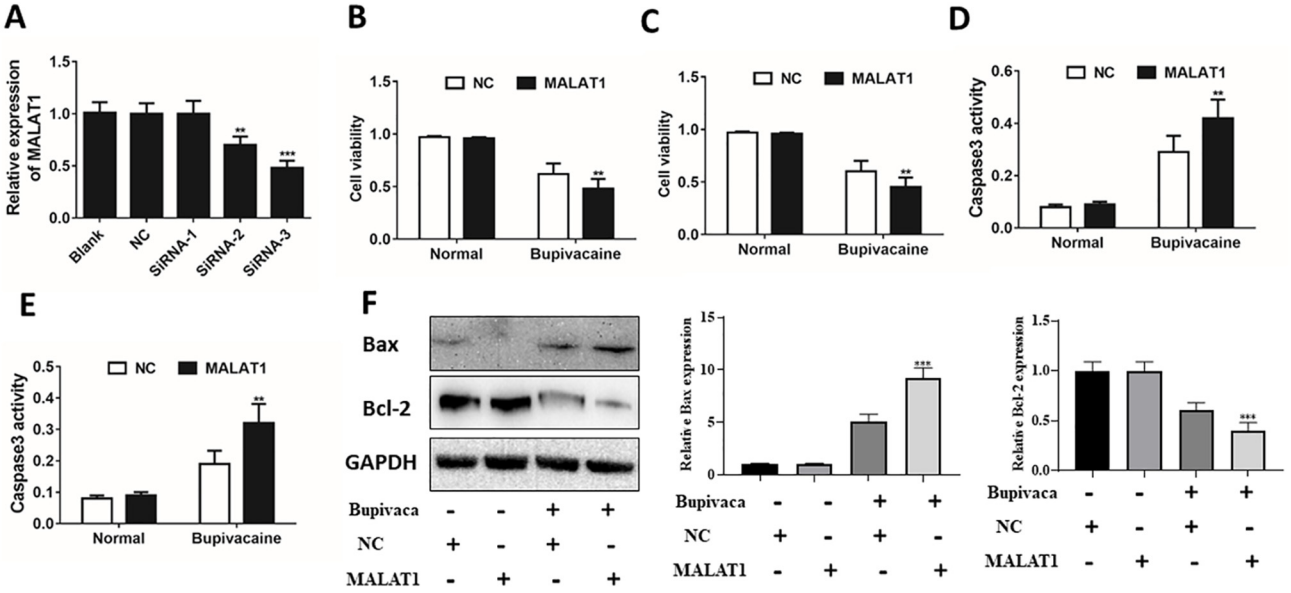

Fig. 2. Functional analysis of lncRNA MALAT1 in bupivacaine-induced neurotoxicity. (A) The expression of lncRNA MALAT1 was significantly decreased compared with control group. (B) knockdown of lncRNA MALAT1 significantly decreased the cell viability in SH-SY5Y cells. (C) knockdown of lncRNA MALAT1 significantly decreased the cell viability in DRG neurons. (D) The caspase3 activity was significantly increased in lncRNA MALAT1 downregulation group in SH-SY5Y cells. (E) The caspase3 activity was significantly increased in lncRNA MALAT1 downregulation group in DRG neurons. (F) The apoptosis marker Bax and Bcl-2 were detected. The expression of Bax was increased in lncRNA MALAT1 downregulation group, suggesting that knockdown of MALAT1 exerts a pro-apoptosis effect in local anesthetic injured DRG neurons. All the cells were treated with 1.0 mM bupivacaine for 24 h. Data represent the mean ± SD of 3 independent measurements. Data are presented as the mean ± SD. $*p < 0.05$, $**p < 0.01$ compared to control group.

lncRNA MALAT1 protects cells from bupivacaine-induced neurotoxicity by regulating miR-101-3p and PDCD4. Cell viability was decreased in the lncRNA MALAT1 knockdown group, whereas it was increased in the lncRNA MALAT1 and miR-101-3p co-transfection group, suggesting that miR-101-3p could partially block the protective effect of lncRNA MALAT1 (Fig. 5E). Caspase3 activity was significantly increased in the MALAT1 downregulation group and decreased in the lncRNA MALAT1 downregulation and miR-101-3p group (Fig. 5F). In addition, the expression of Bax was significantly increased in the lncRNA MALAT1 downregulation group in DRG neurons and decreased in the lncRNA MALAT1 and miR-101-3p co-transfection group (Fig. 5G). Thus, we demonstrated that lncRNA MALAT1 protects cells from bupivacaine-induced neurotoxicity by regulating miR-101-3p and PDCD4. Fig. 6 shows a schematic diagram of the lncRNA MALAT1/miR-101-3p/

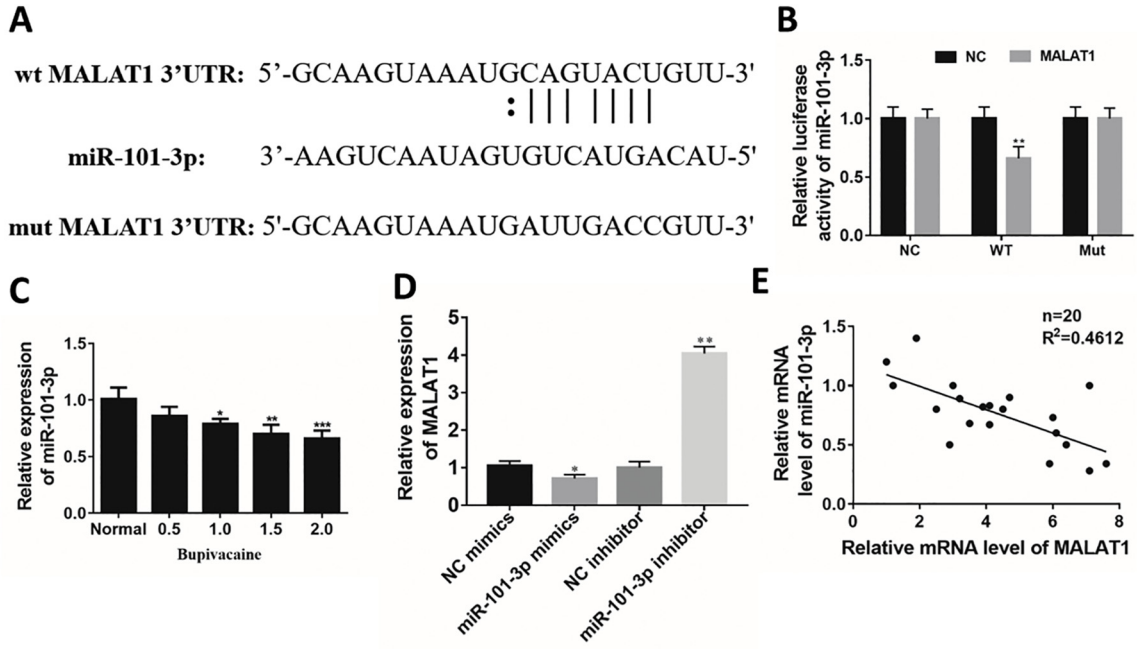

Fig. 3. miR-101-3p is the direct target of lncRNA MALAT1. (A)The potential binding sequence between miR-101-3p and lncRNA MALAT1. (B) The dual-luciferase activity was significantly decreased in wt lncRNA MALAT1 group, and no difference detected in mut lncRNA MALAT1 group. (C) The expression of miR-101-3p was downregulated by bupivacaine in a dose-dependent manner in DRG neurons. (D) Overexpression of miR-101-3p inhibited the expression of lncRNA MALAT and vice versa. (E) lncRNA was negatively regulated by miR-101-3p. Data represent the mean ± SD of 3 independent measurements. Data are presented as the mean ± SD. $*p < 0.05$, $**p < 0.01$ compared to control group.

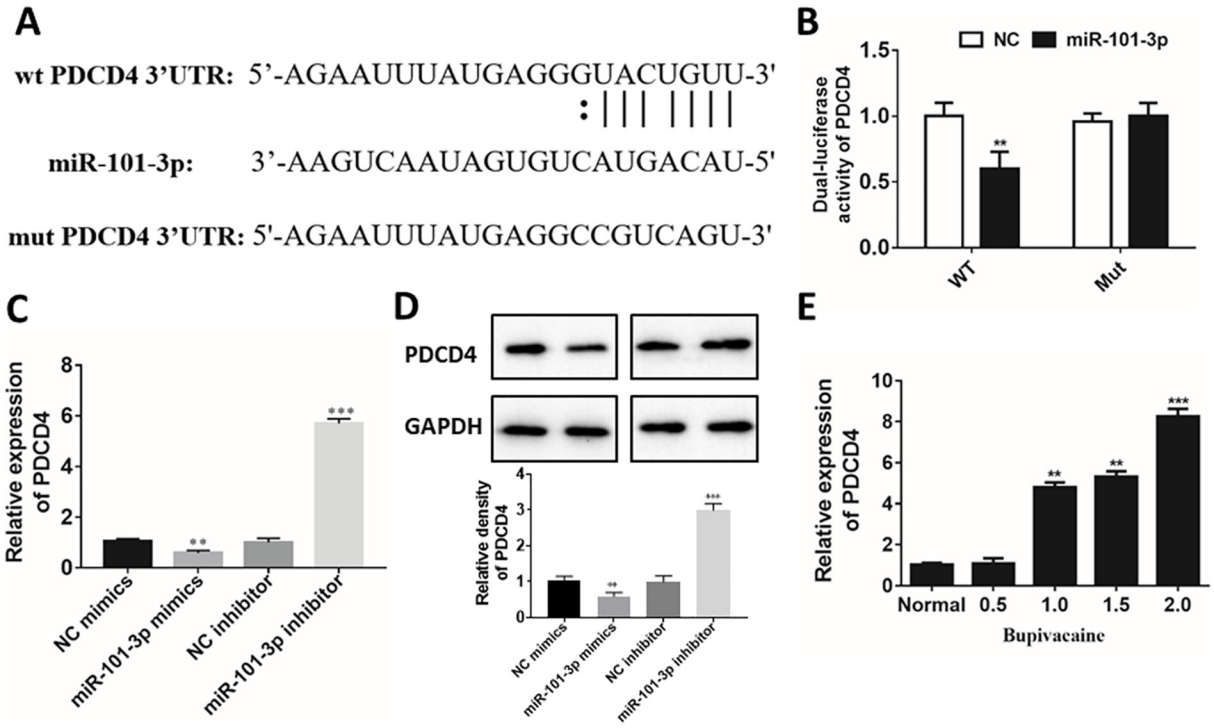

Fig. 4. PDCD4 is the direct target of miR-101-3p. (A) The potential binding sequence between PDCD4 and miR-101-3p. (B) The dual-luciferase activity was significantly decreased in wt PDCD4 group, and no difference detected in mut PDCD4 group. (C) Overexpression of miR-101-3p inhibited the expression of PDCD4 and vice versa by qPCR. (D) Overexpression of miR-101-3p inhibited the expression of PDCD4 and vice versa by Western blotting. (E) The expression of PDCD4 was increased by bupivacaine in a dose-dependent manner in DRG neurons. Data represent the mean ± SD of 3 independent measurements. Data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$ compared to control group.

PDCD4 axis in our study.

4. Discussion

In this study, we investigated the function of lncRNA MALAT1 in the bupivacaine-induced neurotoxicity process. We found that the expression of lncRNA MALAT1 was increased upon exposure to bupivacaine. Knockdown of lncRNA MALAT1 significantly promoted cell death rates, and a caspase3 activity assay revealed that apoptosis rates were manifestly increased in the MALAT1 downregulation group. In addition, we screened the possible target and found that miR-101-3p is a direct target using a dual-luciferase reporter assay; these results suggest that lncRNA MALAT1 may function as a decoy to sponge miR-101-3p. Furthermore, we demonstrated that activation of the MALAT1/miR-101-3p/PDCD4 axis protected cells from bupivacaine treatment. In summary, we elucidated the function and mechanism of MALAT1 in bupivacaine-induced neurotoxicity. Targeting MALAT1 might provide a new method for preventing neurotoxicity.

Bupivacaine is one of the most commonly used local anesthetics, but its possible side effects include sleepiness [26], muscle twitching [27], ringing in the ears and changes in vision [28]. It has been reported that bupivacaine can cause neurotoxicity and permanent neurological disorders, and there has been mounting evidence of growth cone collapse, neurite degeneration and other histopathologic damage in neurons in animal models [29,30]. Cell apoptosis is a representative change in bupivacaine-induced injured neurons in vivo. Although the possible molecular mechanisms have been widely investigated, little attention has been directed to the non-coding RNAs that indeed play a vital role in neural pathology and regeneration in spinal cord DRG neurons.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), a long noncoding RNA (lncRNA) that is highly expressed in neurons and acts as a gene expression regulator involved in synapse formation and maintenance [31], is upregulated in neurological disorders and increases neuronal proliferation and survival via attenuating cell apoptosis [31,32]. In the current study, we demonstrated that cell viability was significantly decreased after treatment with various concentrations of bupivacaine in both the human neuroblastoma cell line SH-SY5Y and neonatal mouse DRG neurons; the endogenous expression of lncRNA MALAT1 was also upregulated. To examine the potential function of MALAT1 in local anesthetic-injured DRG neurons, we downregulated the expression of lncRNA MALAT1 by siRNA and found that the viability of SH-SY5Y cells and neonatal mouse DRG neurons was significantly decreased, and caspase3 activity was increased. In addition, we detected the apoptosis markers Bax and Bcl-2 and found that Bax was increased in the lncRNA MALAT1 downregulation group, suggesting that MALAT1 exerts a protective role in local anesthetic-injured DRG neurons.

MicroRNAs (miRNAs) are highly conserved non-coding RNAs (~19–23 nucleotides in length) that can post-transcriptionally inhibit the expression of specific genes by binding to the 3′-untranslated region (3′-UTR) of target mRNAs [33]. Previous studies have demonstrated that the aberrant expression of miRNAs plays a vital role in neural pathology [34], while MALAT1 has been indicated as a competing endogenous lncRNA that sponges miRNAs in different diseases. Using bioinformatics analysis, we predicted that miR-101-3p, which was also downregulated with increasing concentrations of bupivacaine, may be a direct target of MALAT1. Our results revealed that MALAT1 could negatively regulate miR-101-3p. miR-101-3p has also been reported to promote apoptosis. To elucidate how miR-101-3p modulates cell apoptosis, we searched an online database (miRDB) and found that the apoptosis-related protein programmed cell death protein 4 (PDCD4), which is also dose-dependently increased in bupivacaine-treated cells, is a target of miR-101-3p. Our further studies proved that PDCD4 is a direct target of miR-101-3p. Taken together, we demonstrated that bupivacaine can upregulate the expression of lncRNA MALAT1 to protect cells from apoptosis by regulating miR-101-3p and PDCD4.

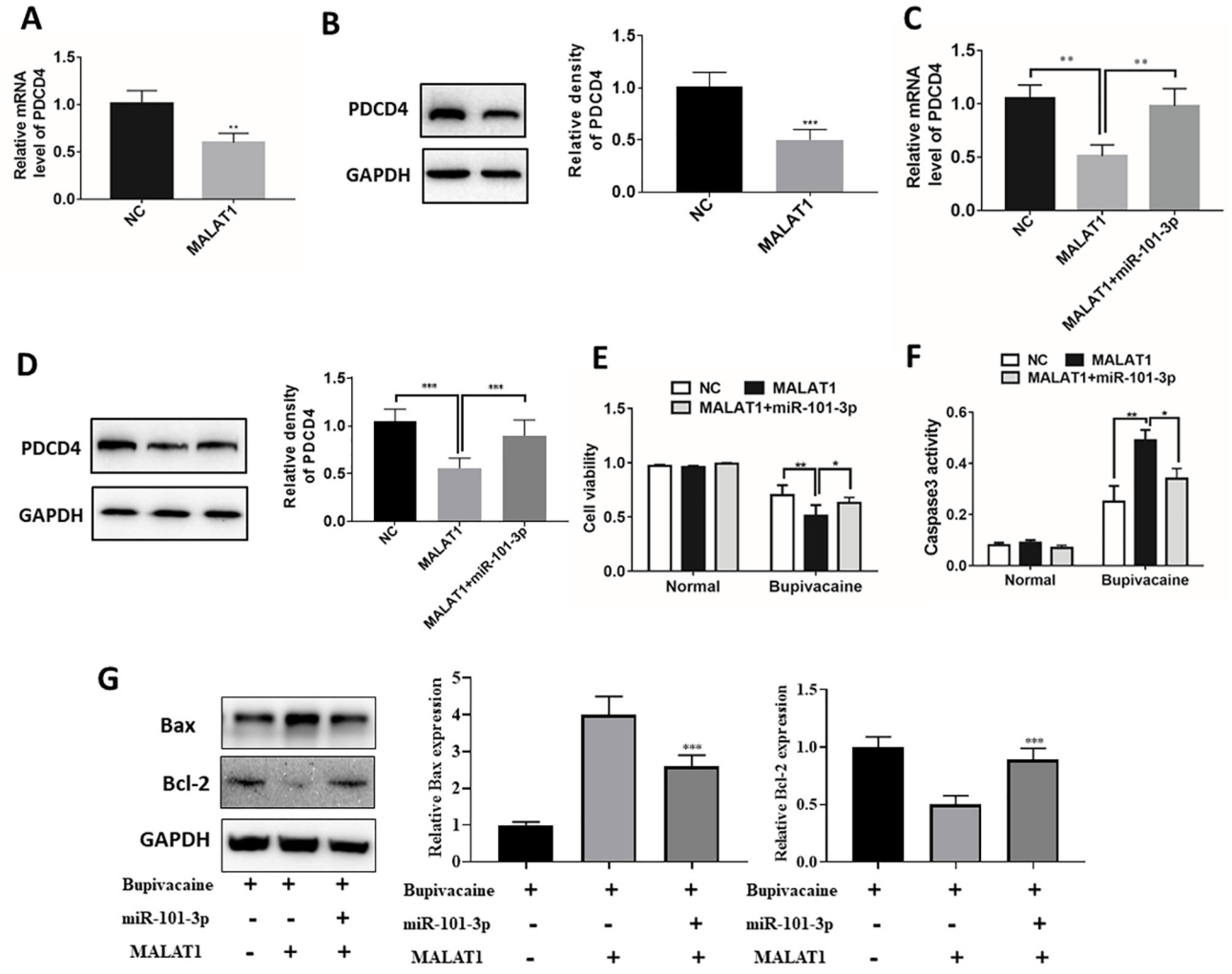

Fig. 5. MALAT1/miR-101-3p/PDCD4 axis in bupivacaine-induced neurotoxicity. (A) The expression of PDCD4 was decreased in lncRNA MALAT1 downregulated group via qPCR. (B) The expression of PDCD4 was decreased in lncRNA MALAT1 downregulated group via Western blotting. (C) Overexpression of miR-101-3p blocked the effect of lncRNA MALAT1. The expression of PDCD4 was increased in lncRNA MALAT1 and miR-101-3p co-transfection group via qPCR. (D) The expression of PDCD4 was increased in lncRNA MALAT1 and miR-101-3p co-transfection group via Western blotting. (E) co-transfection of lncRNA MALAT1 and miR-101-3p significantly increased the cell viability in DRG neurons. (F) The caspase3 activity was significantly decreased in lncRNA MALAT1 downregulation and miR-101-3p group in DRG neurons. (G) The expression of Bax was significantly increased in lncRNA MALAT1 downregulation group in DRG neurons and decreased in co-transfection of lncRNA MALAT1 and miR-101-3p group. All the cells were treated with 1.0 mM bupivacaine for 24 h. Data represent the mean ± SD of 3 independent measurements. Data are presented as the mean ± SD. *p < 0.05, **p < 0.01 compared to control group.

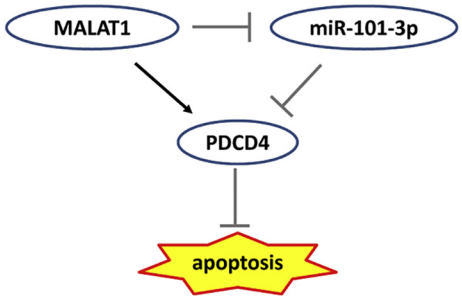

Fig. 6. Schematic diagram of lncRNA MALAT1/miR-101-3p/PDCD4 axis.

In conclusion, we proved that bupivacaine treatment induced not only cell apoptosis but also lncRNA MALAT1 expression, which plays a protective role against cell apoptosis in local anesthetic-injured DRG neurons. Our findings not only elucidate the potential effect of the MALAT1/miR-101-3p/PDCD4 axis in local anesthetic-injured DRG neurons but also provide a new target for the prevention of local anesthetic injury in DRG neurons by mediating the MALAT1/miR-101-3p/PDCD4 axis.

Declaration of Competing Interest

All authors declare no conflicts of interest.

Acknowledgments

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