Knockdown of LincRNA PADNA Promotes Bupivacaine-Induced Neurotoxicity by miR-194/FBXW7 Axis

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Abstract

The aim of the study was to explore the function and mechanism of lincRNA PADNA in bupivacaine-induced neurotoxicity. Mouse DRG neurons were cultured in vitro and treated with bupivacaine to establish the neurotoxicity model. Caspase3 activity, cell viability, tunel assay were analyzed to assess the role of lincRNA PADNA. Dual-luciferase reporter assay was used to determine the binding target of lincRNA PANDA. The expression of lincRNA PADNA was significantly increased with the increasing concentration of bupivacaine. Functional analysis revealed that knockdown of lincRNA PADNA accelerated the caspase3 activity and inhibited the cell viability. Western blot showed that knockdown of lincRNA PADNA promoted the occurrence of cleaved-caspase3. We also proved that lincRNA PADNA may bind with miR-194. Overexpression of miR-194 could rescued the function of lincRNA PADNA, suggesting that lincRNA PADNA may sponge miR-194. In addition, we provided new evidences that lincRNA PADNA/miR-194/FBXW7 axis play an important role in the neurotoxicity process. We performed comprehensive experiments to verify the function and mechanism of lincRNA PADNA in bupivacaine-induced neurotoxicity. Our study provided new evidences and clues for prevention of neurotoxicity.

Introduction

Bupivacaine is one of the most commonly used anesthetics for local infiltration anesthesia\(^1\)–\(^3\). Researches have demonstrated that the adverse drug reactions (ADRs) of bupivacaine are mainly limited the in central nervous system (CNS) and cardiovascular system because of the systemic absorption of bupivacaine\(^4\),\(^5\). During the past decades, bupivacaine has been found neurotoxicity in the setting of local injection, causing symptoms like paralysis, paresthesia, hypoventilation, fecal and urinary incontinence\(^6\)–\(^9\). The side effects of bupivacaine have aroused enormous interests and attention and a great number of researches have been conducted to elucidate the mechanism of bupivacaine-induced neurotoxicity and to find the way to prevent or target these side effects\(^6\). However, the results are hard to conclude and mechanism of bupivacaine-induced neurotoxicity remains unclear.

Efforts have been made in investigating the association between bupivacaine-induced neurotoxicity and non-coding RNAs (ncRNAs)\(^10\). The non-coding RNAs (ncRNAs) is identified that the RNA molecules cannot be translated into a protein\(^11\). There are different types of non-coding RNAs including transfer RNAs, ribosomal RNAs, microRNAs, and the long ncRNAs\(^12\),\(^13\). The microRNAs are the small ncRNAs usually containing about 22 nucleotides\(^14\), while long non-coding RNAs is type of ncRNAs, defined as being transcripts with lengths exceeding 200 nucleotides\(^15\). They have been both widely studied and found abundantly and functionally important in regulating cell cycle\(^16\), cell metabolism\(^17\) and the related diseases such as malignant tumor\(^18\), immune response\(^19\), tissue injury and repair\(^20\). However, the role of ncRNA, especially miRNA and IncRNA in bupivacaine-induced neurotoxicity is barely researched. Jiang R found miR-489-3p and bupivacaine could synergistically inhibited neurite growth and miR-489-3p could inhibited proliferation of neurons and promoted apoptosis by regulating PI3K/AKT pathway\(^21\). Chen L found microRNA-137 could target lysine (K)-specific demethylase 1A (LSD1) and inversely regulate
anesthetics-induced neurotoxicity in dorsal root ganglion neurons. In current research, we investigated the long non-coding RNA Gm14012 (named linc PADNA, promote cell death RNA, for its role in promoting cell death) which has been found up-regulated in bupivacaine-induced neurotoxicity model in our previous research. We demonstrated the expression of lincRNA PADNA is in accordance with the elevation of bupivacaine's concentration and treatment extension which seems lincRNA PADNA takes part in the process of bupivacaine-induced neurotoxicity. To further investigate the inner mechanism of how lincRNA PADNA participating in bupivacaine-induced neurotoxicity, we conducted the bioinformatics analysis and the results revealed that lincRNA PADNA may sponge miR-194 by complementary pairing, which has been reported playing a protective role tumor progress. And the miR-194 has been found targeting the 3'UTR of the cancer-related protein, F-box and WD repeat domain containing 7 (FBXW7) by searching in starbase2.0.

To be brief, by conducting these comprehensive experiments, we want to explore the inner association between lincRNA PADNA and bupivacaine-induced neurotoxicity and demonstrate that lincRNA PADNA functioned as protective role in inhibiting the progress of bupivacaine-induced neurotoxicity by sponging miR-194 who can induce cell death by antagonizing FBXW7 in the setting of bupivacaine-induced neurotoxicity. The current research may explore the new target for inhibiting or reversing bupivacaine-induced neurotoxicity.

**Materials And Methods**

**Cell culture and treatment**

HEK293 cell line were stored in our laboratory, while primary dorsal root ganglion (DRG) neurons were extracted from 5-week old C57BL/6 mice as previously described. Briefly, 5-week old C57BL/6 mice were anesthetized and sacrificed by cervical dislocation. L4-L5 portion of spinal cord was extracted. The dorsal root ganglia were retrieved and dissociated with 0.25% trypsin (Invitrogen, USA). The cells were washed with 2.5% bovine serum albumin (BSA, Invitrogen, USA), re-suspended in serum-free neurobasal medium (Invitrogen, USA) supplemented with penicillin/streptomycin (40,000 unit/L, Invitrogen, USA) and B-27 serum-free supplement (Invitrogen, USA). To induce neurotoxicity, the DRG neurons were treated with various concentration of bupivacaine (0.5, 1.0, 1.5 or 2.0 mM) for 6 h, 12 h, 24 h, 48 h.

**Transfection**

The knockdown vectors of lincRNA PADNA were constructed by Gene Pharma (Shanghai, China). Empty vectors and vectors with the wild-type (WT) or mutant (mut) binding sites for miR-194 were constructed by Gene Pharma (Shanghai, China). The 3'-untranslated region (UTR) of FBXW7, with wild-type (WT) or mutant (mut) binding sites for miR-194, was amplified and cloned into the pGL3 vector (Promega, Madison, WI) to generate the vector pGL3-WT-FBXW7-3'-UTR or pGL3-mut-FBXW7-3'-UTR. The miR-194 mimic, miR-194 inhibitor, mimic NC, inhibitor NC were purchased from Shanghai Gene Pharma (Shanghai,
China). Briefly, the DRG neurons were transfected with vectors, miR-194 mimic, miR-194 inhibitor, mimic NC and inhibitor NC by lipo3000 reagent (Invitrogen) according to the manufacturer's protocol. Cells were incubated for 48 h before further researches.

**Cell Viability Assay**

The DRG neurons were seeded into 96-well plates and treated with bupivacaine (0.5, 1.0, 1.5 or 2.0 mM) for 24 h to establish bupivacaine-induced neurotoxicity model. Each well was added 10 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL, Beyotime, Shanghai, China) and incubated for 4 hours at 37 °C. Cell viability was measured by a microplate reader at 570 nm (Bio-Tek, Winooski, VT).

**Caspase-3 Activity**

A caspase-3 activity assay kit (Beyotime, Shanghai, China) was used to assess caspase-3 activity according to the manufacturer's protocol. DRG neurons were lysed and centrifuging. A final concentration of 0.2 mM ADEVD-pNA (caspase-3 substrate) were added into the cell supernatant and incubated for one hour. The caspase-3 activity was measured by a microplate reader at 405 nm (Bio-Tek, Winooski, VT).

**Tunel Assay**

A terminal deoxynucleotidy1 transferase- mediated dUTP nick end-labeling (TUNEL) assay were conducted to evaluate the apoptosis of DRG neurons. DRG neurons were incubated with TdT and fluorescein-labelled dUTP for 45 min at 37 °C. Then FACSCalibur flow cytometer was used to measure the percentage of apoptotic of cells.

**Dual-luciferase Reporter Assay**

Empty vectors or vectors with the wild-type(WT) or mutant (mut) binding sites for miR-194 were co-transfected with miR-194 mimic or mimic NC. Luciferase activity was analyzed using dual-luciferase reporter system following the manufacture's protocol. Firefly luciferase activity and Rinilla luciferase activity were measured with Multiskan Spectrum (Thermo Fisher, USA). Similarly, empty vectors or vectors of 3’-UTR of FBXW7 with their wild-type or mutant binding sites for miR-194 were co-transfected with miR-194 mimic or mimic NC. Luciferase activity was analyzed using dual-luciferase reporter system. Firefly luciferase activity and Rinilla luciferase activity were measured with Multiskan Spectrum (Thermo Fisher, USA).

**Real-time Pcr**
Total RNA was extracted by TRIzol reagent (Thermo Fisher Scientific). RNA reverse transcription was performed using a PrimeScript™ RT reagent Kit with gDNA eraser (RR047A; Takara, Tokyo, Japan) and cDNA was performed using SYBR® Premix Ex Taq™ (RR420A; Takara, Tokyo, Japan). The data were normalized using β-actin levels and further analyzed by the 2 − ΔΔCT method.

**Western Blotting**

DRG neurons were harvested and lysed by RIPA lysis buffer containing proteinase inhibitor (Roche, USA). Total protein was quantified using BCA protein assay kit (Pierce, Rockford, IL, USA). Protein samples were resolved by 10% SDS-PAGE gel and transferred to polyvinylidene difluoride membrane. After blocked, they were incubated with primary antibodies against caspase3 (1:1000, Abcam, MA, USA), FBXW7 (1:1000, Abcam, MA, USA) and Actin (1:1000, Abcam, MA, USA) at 4 °C overnight, followed by incubation with a peroxidase-conjugated goat anti-rabbit (or mouse) IgG antibody. Immunopositively bands were analyzed using a FluorChem M system (ProteinSimple, San Jose, CA, USA).

**Data analysis**

We used SPSS 23.0 to calculate the values (means ± standard error of the mean). Statistical analyses were analyzed using two-sided Student’s t-test or one-way ANOVA. The statistical significance was P < 0.05.

**Results**

**The establishment of bupivacaine-induced neurotoxicity model**

To analyze the bupivacaine-induced neurotoxicity process, we firstly verified the model. Apoptosis and cell viability were analyzed respectively via caspase3 activity and MTT. The caspase3 activity was increased with the increasing of concentration of bupivacaine and reached the highest at 2.0 mM (Fig. 1A). The cell viability was significantly reduced compared with blank group. And 2.0 mM bupivacaine can reduced cell viability almost 50 percent (Fig. 1B). Thus, our results suggested that bupivacaine could indeed result cell apoptosis.

**Expression Of Lincrna Gm14012 In Bupivacaine-induced Neurotoxicity**

We firstly detected the expression of lincRNA Gm14012 to investigate the possible change. As shown in Fig. 1C, the expression of lincRNA Gm14012 was increased with the elevation of bupivacaine's concentration and reached highest almost 16-fold times at 2.0 mM. Similar, we also detected the
expression of lincRNA Gm14012 exposed to different times at 1.0 mM. Our results showed that the expression of lincRNA Gm14012 was increased with the time development (Fig. 1D).

Function Of Lincrna Padna In Bupivacaine-induced Neurotoxicity

We constructed knockdown vector to assess the function of lincRNA Gm14012. As shown in Fig. 2A, the expression of lincRNA Gm14012 was significantly downregulated. Next, we used MTT assay to assess function of lincRNA Gm14012. Interestingly, knockdown of lincRNA Gm14012 significantly promoted cell death and reduced cell viability exposed to bupivacaine (Fig. 2B). To date, there is no report of lincRNA Gm14012 in bupivacaine-induced neurotoxicity. Thus, we named this lincRNA PADNA (Promote cell death RNA). To further explore the function of lincRNA PADNA in bupivacaine-induced neurotoxicity, we performed comprehensive analysis such as tunel assay, caspase3 activity, western blot. Tunel assay was to assess the cell apoptosis. There was no significant difference between the two groups under normal conditions, however, when cells were exposed to 1.0 mM bupivacaine, knockdown of lincRNA PADNA significantly increased the cell apoptosis rates, suggesting that lincRNA PADNA promoted cell death (Fig. 2C). In addition, we measured the caspase3 activity using the kit. Our results suggested that down-regulated of lincRNA PADNA significantly increased the caspase3 activity (Fig. 2D). Similar results could be achieved via western blot (Fig. 2E). Overall, our experiments demonstrated that lincRNA PADNA served a protective role in the bupivacaine-induced neurotoxicity.

Preliminary Analysis Of Lincrna Padna Involved Mechanism

Long non coding RNA often worked as sponge RNA to bind with miRNA so that they can possessed different function in numerous processes. To investigate the possible mechanism of lincRNA PADNA, first of all, we used miRDna to predict the potential binding targets of lincRNA PADNA. As shown in Fig. 3A, miR-194 was predicted to bind with lincRNA PADNA. Next, we used dual-luciferase reporter analysis to verify whether lincRNA PADNA can bind with miR-194. Our results suggested that the relative luciferase activity of miR-194 was significantly reduced in the wt-lincRNA PADNA group and no significant difference was detected in mut-lincRNA PADNA group (Fig. 3B). Thus, the above results were preliminarily identified the miR-194 was the target of lincRNA PADNA. Next, we detected the expression of lincRNA PADNA under different conditions. Overexpression of miR-194 distinctly reduced the expression of lincRNA PADNA and vice versa (Fig. 3C). Similar results could also be achieved in HEK293 cells (Fig. 3D). Moreover, we also analyzed the expression of miR-194 in bupivacaine-treated cells. Our results revealed that the expression of miR-194 was manifestly reduced with the increase of concentration of bupivacaine, and reached the lowest at 2.0 mM bupivacaine (Fig. 3E). The above results revealed that lincRNA PADNA could negatively regulate the expression of miR-194.
Fbxw7 Is The Direct Target Of Mir-194

To further study the mechanism of miR-194 involved in bupivacaine-induced neurotoxicity, we used starbase2.0 to predict the target of miR-194. We found that FBXW7 was predicted as the direct target of miR-194. Previous studies have also shown that FBXW7 was regulated by miR-194. However, the role of miR-194 and FBXW7 in bupivacaine-induced neurotoxicity still remains unknown. Thus, we performed comprehensive analysis to analyze their relationship. The binding sequence was shown in Fig. 4A. Dual-luciferase reporter assay revealed that the relative density was significantly reduced in wt-FBXW7 group. The level of miR-194 was not changed in mut-FBXW7 group (Fig. 4B). Overexpression of miR-194 manifestly reduced the expression of FBXW7 and vice versa (Fig. 4C). We also performed western blot assay to analyze the protein level of FBXW7. Our results suggested that the protein level of FBXW7 was decreased in the miR-194 overexpression group (Fig. 4D). Figure 4E shown the negative correlation between miR-194 and FBXW7. In addition, we also analyzed the expression of FBXW7 in bupivacaine-treated cells. The expression of FBXW7 was increased with the increase of concentration and time course of bupivacaine and reached the highest level at 2.0 mM bupivacaine (Fig. 4F, Fig. 4G). Thus, our study provided the evidence that miR-194 negatively regulate the expression of FBXW7.

Lincrna Padna/mir-194/fbxw7 Axis In Bupivacaine-induced Neurotoxicity

We performed comprehensive analysis to further verify the lincRNA PADNA/miR-194/FBXW7 pathway. The expression of FBXW7 was downregulated in lincRNA PADNA knockdown group via qPCR (Fig. 5A). Similar results could achieve via western blot, suggesting that lincRNA PADNA positively regulate FBXW7 (Fig. 5B). Considering the above results, we overexpressed miR-194 and knockdown lincRNA PADNA and detect the expression of FBXW7. Interestingly, the expression of FBXW7 was increased in co-transfection group via qPCR and western blot assay (Fig. 5C, Fig. 5D). Thus, miR-194 could blocked the effect of lincRNA PADNA. Thus, rescue experiment was performed to verify the relationship between miR-194 and FBXW7. Tunel assay revealed that overexpression of miR-194 and knockdown of lincRNA PADNA could reduce the apoptosis effect compared with knockdown of lincRNA PADNA group (Fig. 5E). Besides, the caspase3 activity and cleaved-caspase3 band was decreased (Fig. 5F, Fig. 5H). Moreover, the cell viability was increased in co-transfection group compared with knockdown of lincRNA PADNA group (Fig. 5G). Through rescue experiment, we have proved that lincRNA PADNA/miR-194/FBXW7 axis play an important role in bupivacaine-induced neurotoxicity.

Discussion

Anesthetic-induced neurotoxicity may cause spinal cord injury or even induce permanent and irreversible neurological complications. Long non-coding RNAs have been found playing a role in anesthetic-induced neurotoxicity. For example, Propofol was reported inducing neuroapoptosis in the hippocampus,
with differential expression of 159 lncRNAs and 100 mRNAs (fold change $\pm$ 2.0, $P < 0.05$). Wang J indicated suppression of lncRNA-ATB prevents amyloid-$\beta$-induced neurotoxicity in PC12 cells via regulating miR-200/ZNF217 axis. Pang X demonstrated Long non coding RNA SNHG16 reduced ketamine-induced neurotoxicity in human embryonic stem cell-derived neurons. In current research, we established the bupivacaine-induced neurotoxicity model by treating the DRG neurons with various concentration of bupivacaine(0.5, 1.0, 1.5 or 2.0 mM) and different extension(6 h, 12 h, 24 h, 48 h) and we found that caspase 3 activity and the expression of lincRNA PADNA was significantly increased in a time and dose dependent manner. To further investigate the role of lincRNA PADNA, we constructed knockdown vector to assess the function of lincRNA PADNA and found knockdown of lincRNA PADNA significantly promoted cell death and reduced cell viability exposed to bupivacaine. Our experiments demonstrated that lincRNA PADNA served a protective role in the bupivacaine-induced neurotoxicity.

Long non-coding RNAs are a type of RNA, defined as being transcripts with lengths exceeding 200 nucleotides that are not translated into protein. LncRNAs are extensively reported to be involved in the regulation of gene transcription, in epigenetic regulation and especially in post-transcriptional regulation. Previous researches have reported that lncRNA could worked as a competing endogenous RNA to sponge miRNAs in various diseases. For example, Kallen AN and Imig J found lincRNA H19 could bind let-7 family members and miR-106a respectively in their researches. Cesana M found linc-MD1 could sponge miR-133 and miR-135. In current research, we used bioinformatics analysis to predict the possible target of lincRNA PADNA and found that miR-194 which has been reported playing a role in inhibiting malignant tumor progress may be the direct target of lincRNA PADNA. Though research has indicated that miR-194 could inhibit intervertebral disc degeneration, the role of miR-194 in anesthetic-induced neurotoxicity remains unclear. To further explore the possible molecular biological function of miR-194, we firstly conducted the dual-luciferase reporter assay and found the relative luciferase activity was significantly reduced in DRG neurons co-transfected with wt-lincRNA PADNA vector and miR-194 mimic than in DRG neurons co-transfected with mut-lincRNA PADNA vector or mimic NC. Thus, the above results were preliminarily identified the miR-194 was the target of lincRNA PADNA. Next, we detected the expression of lincRNA PADNA in DRG neurons and HEK293 cells treated with miR-194 mimic or miR-194 inhibitor, and found overexpression of miR-194 distinctly reduced the expression of lincRNA PADNA and vice versa. Moreover, we also analyzed the expression of miR-194 in bupivacaine-treated cells and our results revealed that the expression of miR-194 was manifestly reduced with the increase of concentration of bupivacaine and reached the lowest at 2.0 mM bupivacaine. The above results revealed that lincRNA PADNA could negatively regulate the expression of miR-194 in the setting of bupivacaine-induced neurotoxicity.

Secondly, we searched in starbase2.0 and found, the cancer-related protein, F-box and WD repeat domain containing 7 (FBXW7) may be the target of miR-194. To prove this hypothesis, we conducted dual-luciferase reporter assay and found the relative luciferase activity was significantly reduced in DRG neurons co-transfected with pGL3-WT-FBXW7-3′-UTR vector and miR-194 mimic than in DRG neurons co-transfected with pGL3-mut-FBXW7-3′-UTR vector or mimic NC which means the FBXW7-3′-UTR is the
target of miR-194. We also performed western blot and qPCR assay to analyze the protein level of FBXW7 and found overexpression of miR-194 manifestly reduced the expression of FBXW7 and vice versa.

To further verify the role of lincRNA PADNA/miR-194/FBXW7 pathway in bupivacaine-induced neurotoxicity, we conducted the comprehensive analysis and found the expression of FBXW7 was downregulated in lincRNA PADNA knockdown group. Considering the above results, we overexpressed miR-194 in lincRNA PADNA knockdown group and found the expression of FBXW7 was reversed in co-transfection group. Tunel assay revealed that overexpression of miR-194 in lincRNA PADNA knockdown treated cells reduced the apoptosis effect compared with knockdown of lincRNA PADNA group. Similar results were found in caspase-3 activity and cell viability assay. As a consequence, the results of comprehensive researches are in accordance with our hypothesis and it seems quite reliable to believe that lincRNA PADNA could prevent from bupivacaine-induced neurotoxicity by sponging miR-194 and inhibiting its function of antagonizing FBXW7.

In brief, we demonstrated that lincRNA PADNA could prevent from bupivacaine-induced neurotoxicity through miR-194/FBXW7 axis.

**Conclusion**

Anesthetic-induced neurotoxicity is one of the adverse drug reactions (ADRs) of bupivacaine which can cause permanent and irreversible neurological complications. The current research demonstrated that the expression of lincRNA PADNA was significantly increased with the increasing concentration and treatment extension of bupivacaine and lincRNA PADNA could prevent from bupivacaine-induced neurotoxicity through miR-194/FBXW7 axis which may be used as the new target for inhibiting or reversing bupivacaine-induced neurotoxicity.

**Declarations**

**Conflict of Interest**

Authors declare no conflict of interest existed.

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Figures

Figure 1

Expression of lincRNA PADNA in bupivacaine-induced neurotoxicity (A) Caspase3 activity of DRG neurons was significantly up-regulated with the elevation of concentration of bupivacaine. (B) Cell viability of DRG neurons was significantly decreased with the elevation of concentration of bupivacaine. (C) The expression of lincRNA PADNA was significantly up-regulated with the elevation of concentration of bupivacaine. (D) The expression of lincRNA PADNA was significantly up-regulated with the extension of bupivacaine treatment.
Function of lincRNA PADNA in bupivacaine-induced neurotoxicity (A) The expressions of lincRNA PADNA were significantly decreased in DRG neurons treated with siRNA3 or siRNA4 when compared with blank group, NC group, DRG neurons treated with siRNA1 or siRNA2. (B) Cell viability was significantly decreased in DRG neurons treated with knockdown vector of lincRNA PADNA. (C) Percentage of apoptotic of cells was significantly increased in DRG neurons treated with knockdown vector of lincRNA PADNA. (D) Caspase3 activity was significantly increased in DRG neurons treated with knockdown vector of lincRNA PADNA. (E) (F) The expression of Caspase3 protein was significantly increased in DRG neurons treated with knockdown vector of lincRNA PADNA.
miR-194 is the direct target of lincRNA PADNA (A) The predicted binding sequence of lincRNA PADNA and miR-194. (B) The relative luciferase activity was significantly reduced in DRG neurons co-transfected with wt-lincRNA PADNA vector and miR-194 mimics than in DRG neurons co-transfected with mut-lincRNA PADNA vector or empty vector or mimic NC. (C) Overexpression of miR-194 distinctly reduced the expression of lincRNA PADNA in DRG neurons. (D) Overexpression of miR-194 distinctly reduced the expression of lincRNA PADNA in HEK293 cells. (E) The relative expression of miR-194 was manifestly reduced with the increase of concentration of bupivacaine.
Figure 4

FBXW7 is the direct target of miR-194 (A) The predicted binding sequence of the 3'UTR of FBXW7's mRNA and miR-194. (B) The relative luciferase activity was significantly reduced in DRG neurons co-transfected with wt-lincRNA PADNA vector and miR-194 mimics than in DRG neurons co-transfected with mut-lincRNA PADNA vector or empty vector or mimic NC. (C) miR-194 negatively regulate the expression of FBXW7. (D) The expression of FBXW7 protein was significantly decreased in DRG neurons treated with miR-194 than in DRG neurons treated with mimic NC; while the expression of FBXW7 protein was significantly increased in DRG neurons treated miR-194 inhibitors than in DRG neurons treated with inhibitor NC. (E) RT-PCR revealed the expression of FBXW7 mRNA is negatively correlated with the expression of miR-194. (F) The expression of FBXW7 mRNA was significantly up-regulated with the elevation of concentration of bupivacaine. (G) The expression of FBXW7 mRNA was significantly up-regulated with the extension of bupivacaine treatment.
Figure 5

LincRNA PADNA/miR-194/FBXW7 axis in bupivacaine-induced neurotoxicity (A) Knockdown of lincRNA PADNA significantly reduced the expression of FBXW7 mRNA in DRG neurons. (B) Knockdown of lincRNA PADNA significantly reduced the expression of FBXW7 protein in DRG neurons. (C) (D) Overexpression of miR-194 reversed the expression of FBXW7 in DRG neurons which is inhibited by knockdown of lincRNA.
PADNA. (E) Overexpression of miR-194 reversed the up-regulated percentage of apoptotic of DRG neurons which is induced by the knockdown of lincRNA PADNA. (F) Overexpression of miR-194 reversed the up-regulated caspase3 activity of DRG neurons which is induced by the knockdown of lincRNA PADNA. (G) Overexpression of miR-194 reversed the down-regulated cell viability of DRG neurons which is induced by the knockdown of lincRNA PADNA. (H) Overexpression of miR-194 reversed the up-regulated expression of caspase3 protein in DRG neurons which is induced by the knockdown of lincRNA PADNA.