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LncRNA-TMPO-AS1 promotes apoptosis of osteosarcoma cells by targeting miR-329 and regulating E2F1

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Abstract. – OBJECTIVE: This study aims to explore the clinical value and mechanism of lncRNA-TMPO-AS1 in osteosarcoma.

PATIENTS AND METHODS: We collected 51 samples of cancer tissues and 51 samples of matched adjacent tissues from 51 patients with osteosarcoma undergoing surgery in our hospital from February 2018 to February 2019. The expression of TMPO-AS1 in tissue samples was tested to analyze its value in the diagnosis and prognosis prediction of osteosarcoma. We transfected osteosarcoma cells with stable and transient vectors containing overexpressed or inhibited genes. Then, we measured cell proliferation by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, cell invasion by transwell assay, and cell apoptosis by flow cytometry (FCM). The relationship between TMPO-AS1 and miR-329 or between miR-329 and E2F1 was determined by the Dual-Luciferase reporter (DLR) assay.

RESULTS: TMPO-AS1 was up-regulated in osteosarcoma cells. Serum TMPO-AS1 could work as a diagnostic marker for osteosarcoma, with an area under the receiver operating characteristic (ROC) curve (AUC) of more than 0.8. Osteosarcoma cells were transfected with siRNA-TMPO-AS1, pcDNA3.1-TMPO-AS1, miR-329-mimics, and miR-329-inhibitor. The results revealed that inhibited TMPO-AS1/overexpressed miR-329/inhibited E2F1 suppressed the proliferation and invasion of osteosarcoma cells and enhanced cell apoptosis. The DLR assay demonstrated that TMPO-AS1 could target miR-329 and miR-329 could target E2F1. *In vitro* experiments revealed that TMPO-AS1 could regulate the progression of epithelial-mesenchymal transition (EMT) in osteosarcoma through the miR-329/E2F1 axis.

CONCLUSIONS: TMPO-AS1 can function as a diagnostic and prognostic marker for osteosarcoma. LncRNA-TMPO-AS1 can promote apoptosis of osteosarcoma cells by targeting miR-329 and regulating E2F1, which is a potent treatment option for osteosarcoma.

Key Words:
Osteosarcoma, TMPO-AS1, MiR-329, E2F1, Biological function.

Introduction

Osteosarcoma is the most prevalent primary bone tumor, featured with invasive growth and distant organ metastasis[1,2]. Chemotherapy, a predominant treatment method for osteosarcoma, however, can cause a poor prognosis in patients due to severe side effects of anti-tumor drugs[3]. With a limited understanding of the molecular mechanism of osteosarcoma, the treatment and prognosis of osteosarcoma have become major clinical challenges, making it urgent to identify new biomarkers for osteosarcoma[4]. The development of specific targeted therapeutic agents for metastatic osteosarcoma depends on the understanding of its molecular mechanism[5]. In recent years, some studies have noted a relationship between the development and progression of osteosarcoma and long non-coding RNAs (lncRNAs)[6,7].

LncRNAs can modulate tumor proliferation, migration, and apoptosis[8,9]. They are abnormally expressed in various cancers[10,11]. LncRNA TMPO-AS1 can suppress the progression of many tumors and may function as a potential biomarker for tumor diagnosis and prognosis, as well as a therapeutic target for tumor progression[12,13]. LncRNA TMPO-AS1 can sponge miR-140-5p and induce SOX4-mediated EMT to boost cell invasion and migration in gastric cancer[14]. But to date, lncRNA targeted therapy has not been used to treat osteosarcoma. So, we designed this study to provide a new theoretical basis for the diagnosis

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and treatment of osteosarcoma by lncRNA targeted therapy.

Here we quantified the expression of related lncRNAs in osteosarcoma. We explored how lncRNA-TMPO-AS1 promotes the progression of osteosarcoma through the miR-329/E2F1 axis, aiming to identify accurate markers for the diagnosis and prognosis of osteosarcoma and potential drug targets.

Patients and Methods

We collected 51 samples of cancer tissues and 51 samples of matched adjacent tissues from 51 patients with osteosarcoma undergoing surgery in our hospital from February 2018 to February 2019. Inclusion criteria: patients diagnosed with osteosarcoma by pathology, cytology, and imaging[15]; patients with no history of chemotherapy, immunotherapy, or radiation prior to this study. Exclusion criteria: patients with cirrhosis or coagulation disorders; patients with incomplete general clinical data; patients who did not cooperate with the follow-up; patients with an expected survival of less than 1 month; patients who got lost during the follow-up. This study was approved by the Ethics Committee of our hospital and obtained informed consent from all patients.

Main Instruments and Reagents

Saos-2 human osteosarcoma cells and human calvarial osteoblasts (HCOs) were purchased from BeNa Culture Collection (item numbers: BNCC338485, BNCC341176). SYBR Green PCR Master Mix was manufactured by Applied Biosystems (Waltham, MA, USA). DR5000 UV/Vis Spectrophotometer was from Bio-Rad (Hercules, CA, USA). The 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay kit was from ABSCI (Vancouver, WA, USA, item number: AB452). Penicillin-streptomycin mixed solution and 10% fetal bovine tissue were provided by Upstate (Temecula, CA, USA). Dulbecco's Modified Eagle's Medium (DMEM) and transwell kit were from Gibco (Waltham, MA, USA).

Super ECL Detection Reagent was provided by Yeasen Biotech Co, Ltd. (Shanghai, China, item number: 36208ES60). Bicinchoninic acid assay (BCA) protein kit was from AmyJet Scientific Inc. (Wuhan, China, item number: K812-1000). ABI StepOnePlus™ Real-Time PCR System with Tower and Multiskan GO Microplate Spectrophotometer was from Thermo Fisher Scientific Inc. (Waltham, MA, USA, item number: 51119200). FACSCanto flow cytometer was manufactured by Becton Dickinson (Franklin Lakes, NJ, USA). The design of all primer sequences was performed by GenePharma Co., Ltd. (Shanghai, China).

Detection Method

DLR assay

The Dual-Luciferase reporter (DLR) assay results revealed that lncRNA-TMPO-AS1 regulates E2F1 by targeting miR-329. The relationship between lncRNA-TMPO-AS11 and miR-329 was analyzed on Starbase3.0. The prediction of potential targets of miR-329 was performed on TargetScan Release 7.2 (www.targetscan.org/vert_72) and the binding sites between miR-329 and the 3'-untranslated region (3'-UTR) of E2F1 were noted.

Cell culture and transfection

Saos-2 cells were cultured in a DMEM medium supplemented by 10% fetal bovine tissue and penicillin-streptomycin mixed solution at 37°C, with 5% CO_2 and saturated humidity. Saos-2 cells were transfected with si-TMPO-AS1-#1, si-TMPO-AS1-#2, and si-TMPO- AS1-#3 (GenePharma Co., Ltd., Shanghai, China) using the Lipofectamine 3000 kit (Invitrogen, Carlsbad, CA, USA, item number: L3000015). To construct the overexpression plasmids of TMPO-AS1, its coding sequence (CDS) were amplified and then inserted into the pcDNA3.1 vector. The pcDNA3.1-TMPO-AS1, miR-329-inhibitor, miR-329-mimics, and miR-NC were designed by GenePharma Co. Ltd (Shanghai, China). Cell transfection efficiency was measured by the Quantitative real-time polymerase chain reaction (qRT-PCR).

QRT-PCR detection

The expression of related mRNAs in tissues, serums, and cells was tested by the qRT-PCR. The total RNA was extracted from tissues with the TRIzol reagent and dissolved in 20 μL Diethyl pyrocarbonate (DEPC) water, followed by the reverse transcription. PCR reaction conditions: Pre-denaturation 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 15 minutes. We designed 3

Table I. Primer sequences of miR-329, TMPO-AS1, and their internal references.

Gene	Sense	Antisense
E2F1	5'-CATACTAGTTTCCAGAGATGCTCACCTTGT-3'	5'-CTTAAGCTTAAGACAGAAGTGCTCTCACCGTC-3'
TMPO-AS1	5'-GAGCCGAACUACGAACCAATT-3'	5'-UUGGUUCGUAGUUCGGCUCTT-3'
miR-329	5'-CAAGGGAGCATGGTATTAGGTTT-3'	5'-CTAACGTGAGGAACGCCTTTT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
GAPDH	5'-CAAAGGTGGATCAGATTCAAG-3'	5'-GGTGAGCATTATCACCCAGAA-3'

replicate wells for each sample. After the PCR reaction, the amplification curve and melting curve were plotted, and data were analyzed using the 2-ΔΔCt method. The detection was repeated five times (Table I).

Western Blot (WB) assay

Cells were lysed by radioimmunoprecipitation assay (RIPA) lysis buffer to collect the total proteins. The proteins were subjected to electrophoretic separation by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to polyvinylidene difluoride (PVDF) membrane. The membrane was incubated together with the specific primary antibody at 4°C overnight. Then, horseradish peroxidase (HRP)-labeled goat anti-mouse secondary antibody (1:5000) was added to the membrane and incubated at 37°C for 1 hour, followed by 3 Tris-Buffered Saline and Tween-20 (TBST) washing, 5 minutes each time. The excessive liquid on the membrane was absorbed, and color development was performed on the enhanced chemiluminescence (ECL) system in a dark room.

Cell proliferation assay

Cell proliferation was tested by the MTT assay. Cells were harvested 24 hours after the transfection and seeded in 96-well plates ($5*10^4$ cells/well), and incubated at 37°C for 24 hours, 48 hours, and 72 hours. At the 24th, 48th, and 72nd hours, 20 μL of MTT solution (5 μmg/mL) was added to the plate and inoculated at 37°C for 4 hours. Then, 200 μL of dimethyl sulfoxide was added to each well. Finally, the OD value of each well was measured at 480 nm.

Transwell invasion assay

We applied the Matrigel glue to the transwell chambers and kept the set still at 37°C for 30 minutes. Cells were resuspended in tissue-free DMEM medium at a cell density of 4×10^5 cells/mL. 200 μL of the cell suspension was added to the apical chamber and 800 μL of DMEM medium supplemented by 10% fetal bovine tissue was added to the basolateral chamber. After culture for 24-48 hours, the chamber was taken out and the cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 20 minutes. We removed non-invading cells and randomly counted cells passing across the basement membrane of the chamber under an optical microscope to analyze cell invasion.

Cell apoptosis assay

Cells in the Petri dish were digested by trypsin digestion, washed in cold phosphate-buffered saline (PBS), and fixed with 80% cold ethanol. Then, cells were resuspended in AnnexinV combined with 100 μL of buffer solution to prepare cell suspension at a density of 1×10^6 cells/mL. Cell suspension was mixed with 5 μL of Annexin-V/FITC solution and incubated at 4°C for 15 minutes. Then, 5 μL of propidium iodide (PI) staining solution was added for incubation at 4°C for 5 minutes. Finally, cell apoptosis was measured by the flow cytometry (FCM) assay. The test was repeated 3 times to obtain the mean value.

Statistical Analysis

Data were subjected to the statistical analysis on SPSS 20.0 (SPSS IBM, Armonk, NY, USA). The measurement data following normal distribution was expressed by the mean ± standard deviation (mean ± SD) and compared between the two groups using the independent sample *t*-test. The comparison between multiple time points was analyzed by the repeated measurement ANOVA and the Bonferroni post-hoc test. The comparison of the means between multiple groups was analyzed by the one-way ANOVA and the LSD post-hoc test. The diagnostic value was assessed by the receiver operating characteristic (ROC) curve. The correlation analysis was performed by the Pearson test. The difference was statistically significant when $p < 0.05$.

Results

Expression of LncRNA-TMPO-AS1 in Osteosarcoma

The results of qRT-PCR detection revealed that the expression levels of TMPO-AS1 were higher in osteosarcoma tissues than in normal bone tissues. On the ROC curve, the area under the curve (AUC) of TMPO-AS1 for diagnosing osteosarcoma was more than 0.8. AUC value ranges from 0.1 to 1 and a higher AUC value suggests a better result. We divided patients into the high TMPO-AS1 expression group and the low TMPO-AS1 expression group according to the median expression level of TMPO-AS1, and then we found that the expression of TMPO-AS1 was related to the differentiation of osteosarcoma. Compared with HCOs, the expression levels of TMPO-AS1 in Saos-2 cells were markedly higher (Figure 1).

Effect of LncRNA-TMPO-AS1 on the Biological Function of Saos-2 Cells

We selected Saos-2 cells for transfection. TMPO-AS1 expression levels were notably lower in the siRNA-TMPO-AS1 group than in the siRNA-NC group ($p < 0.001$). The expression levels of TMPO-AS1 were markedly increased in the pcDNA3.1-TMPO-AS1 group ($p < 0.001$). Cells in the siRNA-TMPO-AS1 group showed marked-

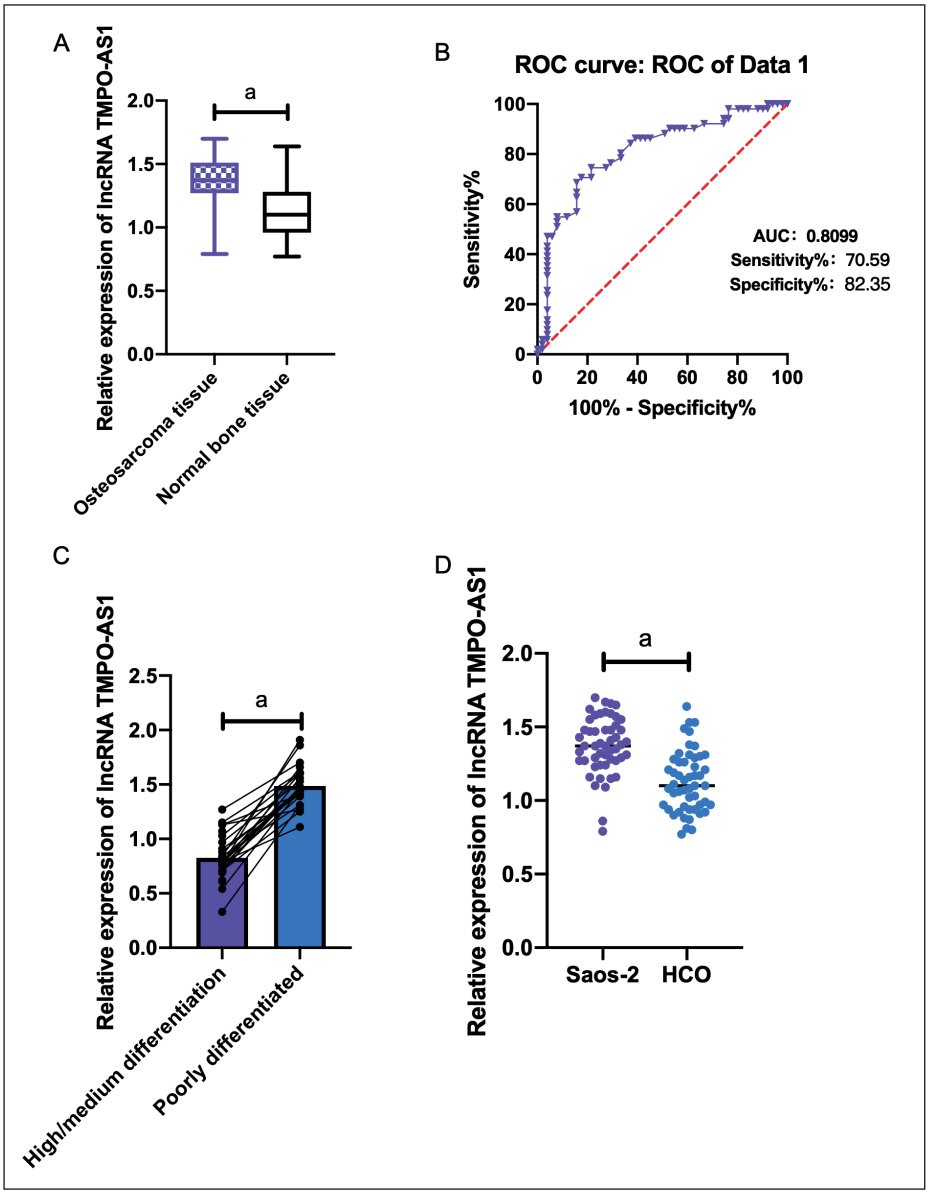

Figure 1. Expression of lncRNA-TMPO-AS1 in osteosarcoma. **A**, TMPO-AS1 expression was markedly higher in osteosarcoma tissues than in normal bone tissues. **B**, According to the ROC curve, the AUC of TMPO-AS1 for diagnosing osteosarcoma was more than 0.8. **C**, TMPO-AS1 expression was markedly lower in osteosarcoma tissues with high/moderate differentiation than in tumor tissues with poor differentiation. **D**, TMPO-AS1 expression was markedly higher in Saos-2 cells than in HCOs. Note: "a" indicates $p<0.001$.

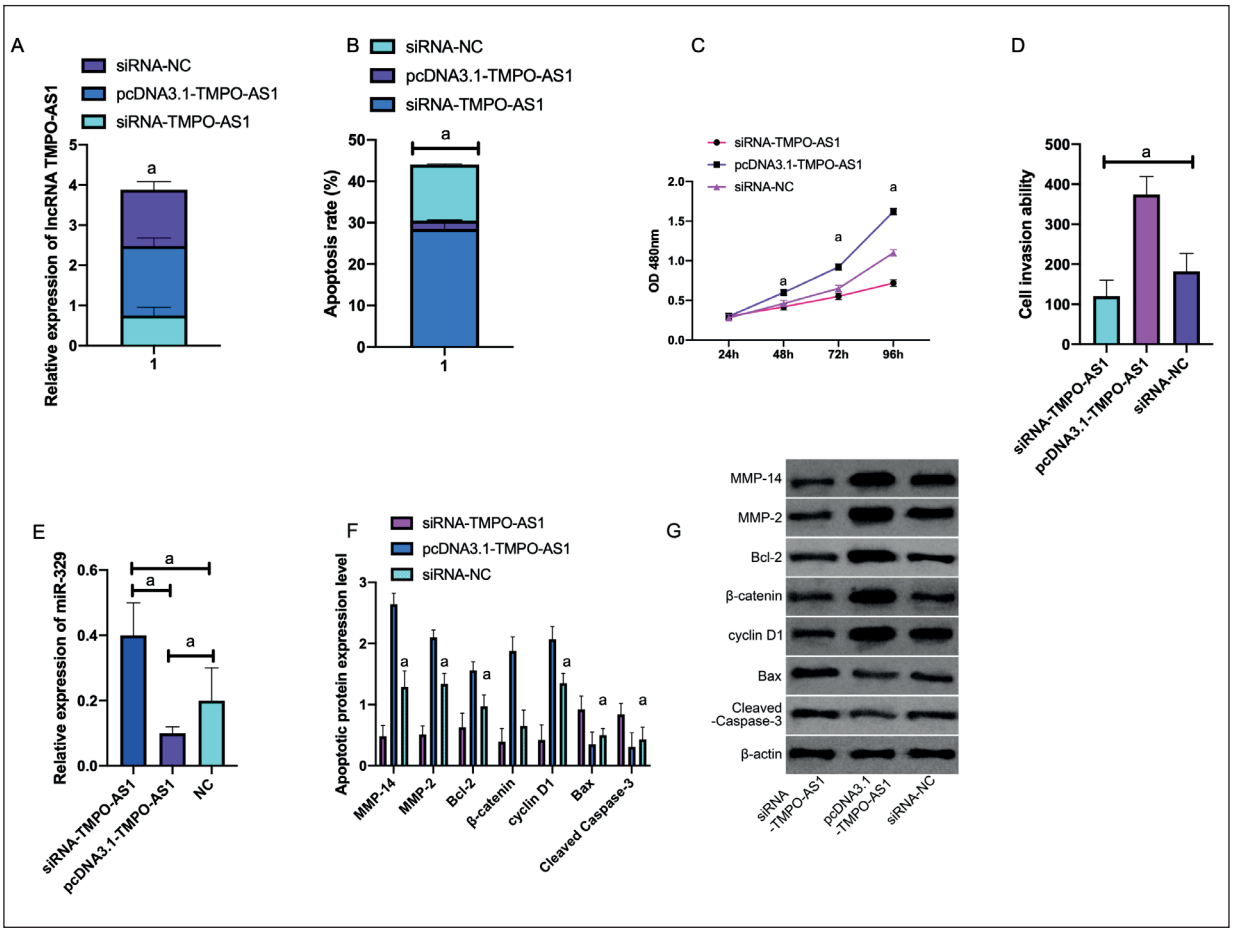

Figure 2. Expression of TMPO-AS1 in cells with transfection and its effect on the biological function of Saos-2 cells. **A**, TMPO-AS1 expression in Saos-2 cells after transfection. **B**, Saos-2 cell apoptosis after transfection. **C**, Saos-2 cell proliferation after transfection. **D**, Saos-2 cell invasion after transfection; **E**, MiR-329 expression in Saos-2 cells after TMPO-AS1 transfection. **F**, Expression of apoptosis-related proteins in Saos-2 cell after transfection. **G**, The WB imaging of apoptosis-related proteins in Saos-2 cells. Note: "a" indicates $p<0.001$.

ly weaker proliferation and invasion and stronger apoptosis than cells in the siRNA-NC group ($p < 0.001$). Cells in the pcDNA3.1-TMPO-AS1 group showed markedly stronger proliferation and invasion and weaker apoptosis than cells in the siRNA-NC group ($p < 0.001$; Figure 2).

Target Binding Between LncRNA-TMPO-AS1 and MiR-329

The qRT-PCR results showed that miR-329 expression levels were markedly lower in Saos-2 cells than in HCOs ($p < 0.001$). According to the DLR assay results, pmirGLO-TMPO-AS1-WT and miR-329 mimics remarkably inhibited the Luciferase activity of Saos-2 cells. We selected Saos-2 for transfection. Compared with the miR-NC group, the miR-329 expression levels were markedly higher in the miR-329-mimics group and markedly lower in the miR-329-inhibition group. Compared with cells in the miR-NC group, Saos-2 cells in the miR-329-mimics group showed markedly weaker proliferation and invasion and stronger apoptosis ($p < 0.001$), while cells in the miR-329-inhibition group showed markedly stronger proliferation and invasion and weaker apoptosis ($p < 0.001$; Figure 3).

LncRNA-TMPO-AS1/miR-329/E2F1 Molecular Axis

The qRT-PCR results showed that E2F1 expression levels were markedly lower in Saos-2 cells than in HCOs ($p < 0.001$). We selected Saos-2 for transfection. According to the DLR assay results, pmirGLO-E2F1-WT and miR-329 mimics remarkably inhibited the luciferase activity of Saos-2 cells. The qPCR test results showed that

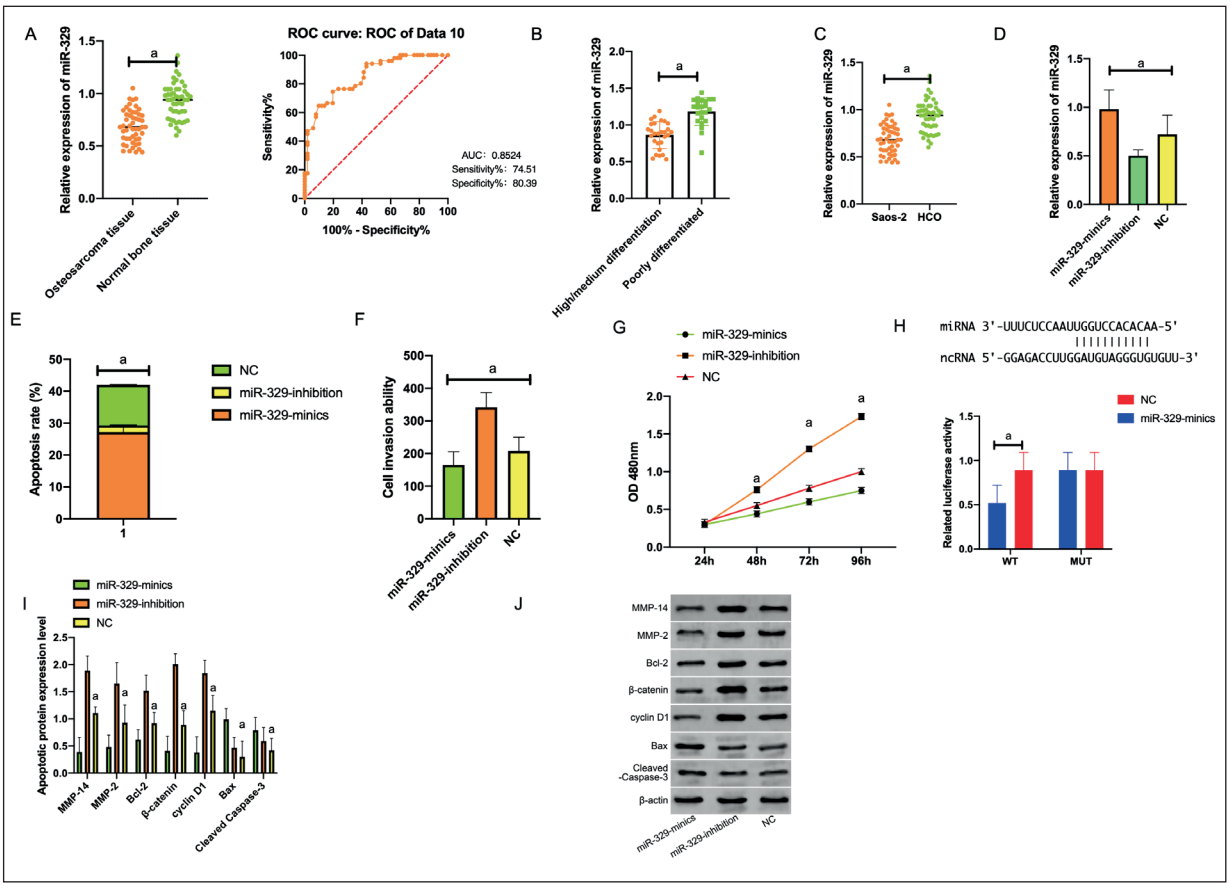

Figure 3. Target binding between lncRNA-TMPO-AS1 and miR-329. **A**, MiR-329 expression in osteosarcoma tissues; the AUC of miR-329 for diagnosing osteosarcoma was more than 0.8. **B**, MiR-329 expression in tumor tissues with high/moderate/poor differentiation. **C**, MiR-329 expression in HCOs and Saos-2 cells. **D**, MiR-329 expression in Saos-2 cells after transfection. **E**, Saos-2 cell apoptosis after transfection. **F**, Saos-2 cell invasion after transfection. **G**, Saos-2 cell proliferation after transfection. **H**, The luciferase activity of Saos-2 cells. **I**, Expression of apoptosis-related proteins in Saos-2 cells after transfection. **J**, The WB image. Note: "a" indicates $p<0.001$.

down-regulated lncRNA TMPO-AS1 markedly inhibited the expression of E2F1, but the inhibition of E2F1 was reversed by miR-329-inhibitor. Correlation analysis results revealed a negative correlation between TMPO-AS1 and miR-329, a negative correlation between miR-329 and E2F1, and a positive correlation between TMPO-AS1 and E2F1 (Figure 4).

In vitro Experiments Verified that TMPO-AS1 Promotes the Occurrence and Metastasis of Osteosarcoma by Inhibiting MiR-329/E2F1 Molecular Axis

Up-regulated TMPO-AS1 remarkably promoted the proliferation of Saos-2 cells, while the down-regulation of E2F1 or overexpression of miR-144-3p at the same time reversed the promotion caused by up-regulated TMPO-AS1. WB assay results showed that the up-regulation of TMPO-AS1 led to markedly increased expression levels of β-catenin, Bcl-2, cyclin D1, vimentin, ILK, Twist, and N-cadherin, as well as markedly decreased expression levels of Bax, Cleared Caspase-3, E-cadherin, and Cytokeratin. However, the down-regulation of E2F1 or overexpression of miR-144-3p reversed the promotion of EMT caused by up-regulated TMPO-AS1. Such results suggest that TMPO-AS1 can inhibit the miR-329/E2F1 molecular axis to promote the occurrence and metastasis of osteosarcoma (Figure 5).

Discussion

At present, a competitive endogenous RNA (ceRNA) network composed of lncRNA, miRNA, and mRNA has attracted much clinical attention[16].

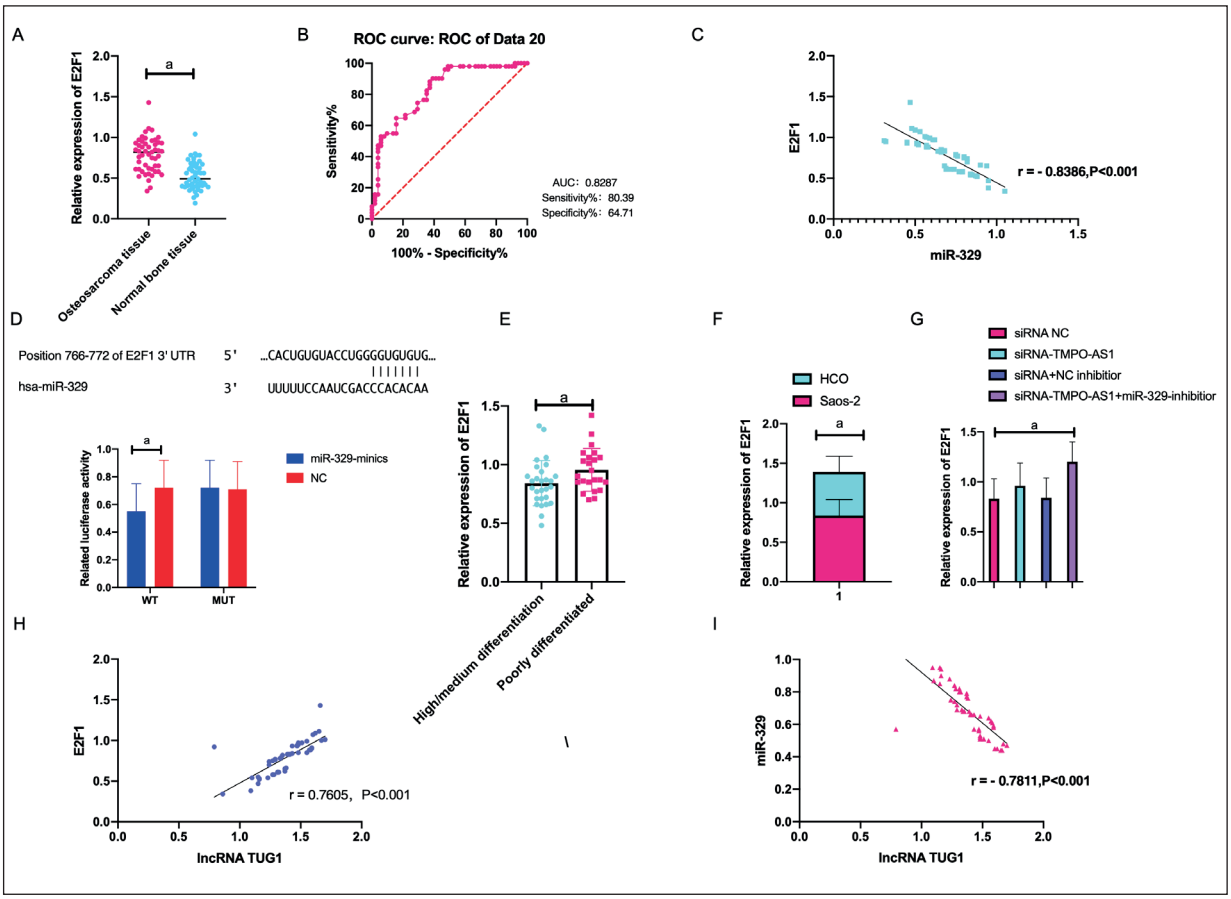

Figure 4. LncRNA-TMPO-AS1 targets miR-329 to regulate E2F1 expression. **A**, E2F1 expression in cancer tissues and adjacent tissues. **B**, The AUC of E2F1 for diagnosing osteosarcoma was more than 0.8. **C**, MiR-329 was negatively correlated with E2F1. **D**, Luciferase activity of Saos-2 cells. **E**, E2F1 expression was markedly lower in cancer tissues with high/moderate differentiation than in cancer tissues with poor differentiation. **G**, E2F1 expression in HCOs and Saos-2 cells. **G**, E2F1 expression in Saos-2 cells after transfection. **H**, LncRNA-TMPO-AS1 was positively correlated with E2F1. **I**, LncRNA-TMPO-AS1 was negatively correlated with miR-329. Note: "a" indicates $p<0.001$.

LncRNA can sponge miRNA by changing the chromatin structure and thus affect the carcinogenic process[17,18]. Cui et al[19] suggest that lncRNA TMPO-AS1 can sever as a ceRNA to regulate the miR-199a-5p/WNT7B axis to promote osteosarcoma tumor development. This study aimed to explore the role of lncRNA-TMPO-AS1 in targeting miR-329 and regulating E2F1 to boost osteosarcoma cell apoptosis.

First, we conducted the qRT-PCR test and detected highly expressed lncRNA-TMPO-AS1 in osteosarcoma tissues and Saos-2 osteosarcoma cells. We also noted a correlation between the high expression of TMPO-AS1 and the osteosarcoma differentiation. Peng et al[20] suggests that lncRNA-TMPO-AS1 is notably overexpressed in breast cancer, liver cancer, gastric cancer, and retinoblastoma. Also, lncRNA TMPO-AS1 is significantly up-regulated in lung adenocarcinoma cell lines. Silencing TMPO-AS1 can induce apoptosis of lung adenocarcinoma cell lines and suppress cell invasion[21]. We analyzed data from the TargetScan database and discovered a targeting relationship between lncRNA-TMPO-AS1 and miR-329 and a targeting relationship between miR-329 and E2F1. E2F1, a crucial intracellular transcription factor, is the first cloned protein in the E2F family. It regulates cell proliferation, differentiation, and apoptosis[22]. Correlation analysis results revealed a negative correlation between TMPO-AS1 and miR-329, a negative correlation between miR-329 and E2F1, and a positive correlation between TMPO-AS1 and E2F1. We also found that miR-329 and E2F1 were correlated with osteosarcoma differentiation. MiR-329 is lowly expressed in malig-

nant tumors such as liver cancer, cervical cancer, and bladder cancer[22]. Wang et al[23] showed that miR-329 inhibitors can reverse the knockdown of lncRNA-ZFAS1 to boost the invasion of bladder cancer cells. Fei et al[24] revealed that lncRNA-AFAP1-AS1 stimulates osteosarcoma progression by regulating the miR-497/IGF1R axis. Whether lncRNA-TMPO-AS1 can work as a ceRNA to regulate osteosarcoma cell genes via the miR-329/E2F1 axis is unclear.

Here in cell experiments, we detected highly expressed TMPO-AS1 and E2F1 and lowly expressed miR-329 in Saos-2 cells. Then, we interfered with the expression of TMPO-AS1, miR-329, and E2F1 in Saos-2 cells. The results showed that down-regulated lncRNA TMPO-AS1 markedly inhibited the expression of E2F1, while the transfection with miR-329-inhibitor at the same time reversed such inhibition. LncRNA-SNHG12/miR--326/E2F1 can promote the progression of oral squamous cell carcinoma cells. In the rescue experiments by Zhou et al[25], they stimulated the expression of E2F1 to induce the expression of silenced SNHG12. A former study suggests

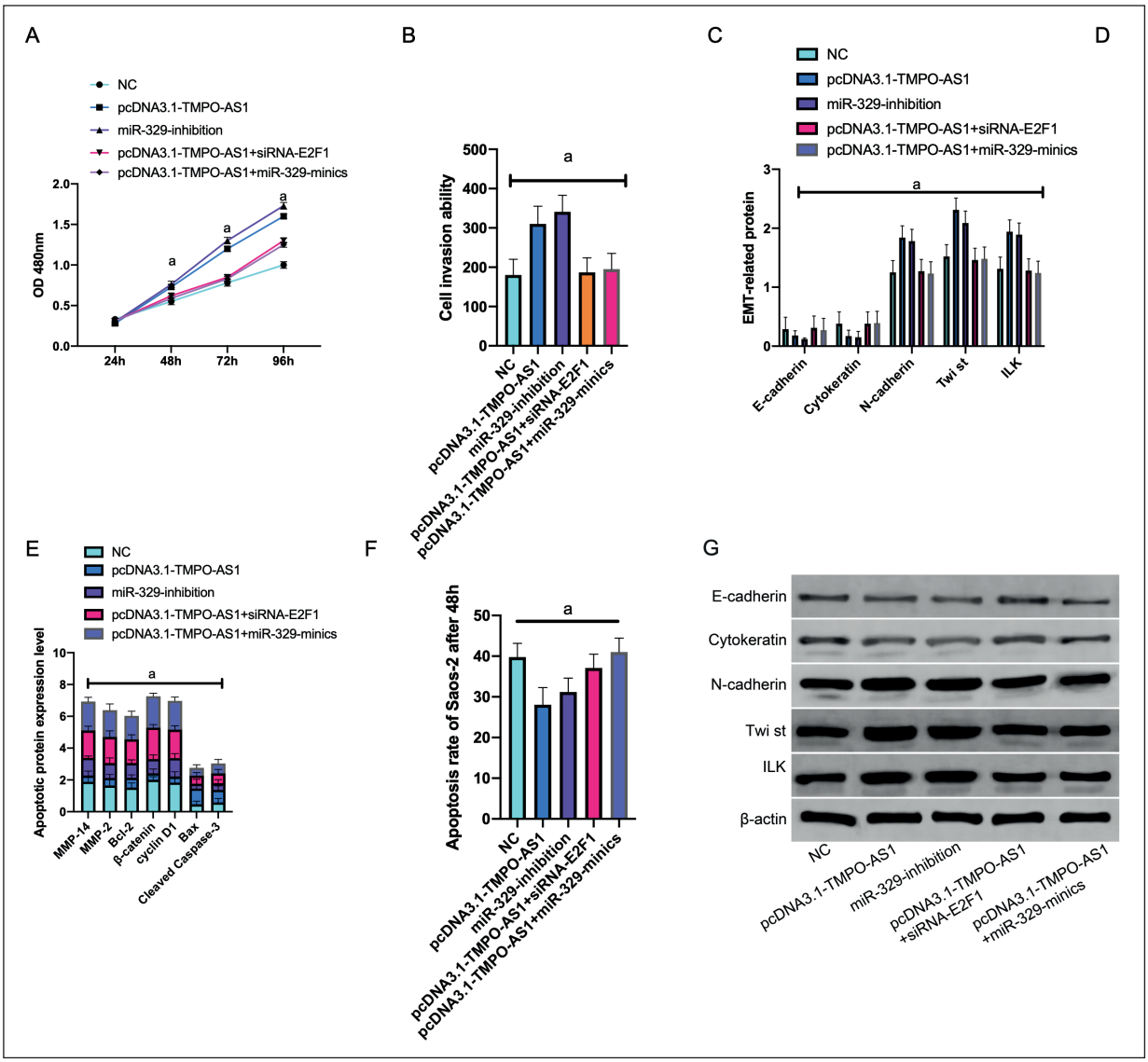

Figure 5. *In vitro* experiments verified that TMPO-AS1 promotes the occurrence and metastasis of osteosarcoma by inhibiting miR-329/E2F1 molecular axis. **A**, Saos-2 cell proliferation. **B**, Saos-2 cell invasion. **C**, Expression of EMT-related proteins in Saos-2 cell cytoplasm. **D**, The WB image of EMT-related proteins. **E**, Expression of apoptosis-related proteins in Saos-2 cell cytoplasm. **F**, The WB image of apoptosis-related proteins. **G**, Saos-2 cell apoptosis.

that lncRNA-RHPN1-AS1 positively regulates the expression of SNAI2 by sponging miR-506. Up-regulated miR-506-SNAI2 axis can suppress osteosarcoma by enhancing the malignant EMT of osteosarcoma cells *in vitro* and *in vivo*[26]. Such results indicate that lncRNA-TMPO-AS1 may inhibit the EMT of osteosarcoma by regulating the miR-329/E2F1 axis.

We conducted *in vitro* experiments and observed that TMPO-AS1 inhibits the miR-329-E2F1 molecular axis to promote the EMT process of osteosarcoma cells. EMT is a pathological process that leads to tumor progression and facilitates tumor cell invasion and metastasis. Dong et al[27] found that the lncRNA PGM5-AS1 promotes EMT, invasion, and metastasis of osteosarcoma cells by weakening the miR-140-5p-mediated FBN1 inhibition. Also, inhibited PGM5-AS1 and FBN1 or overexpressed miR-140-5p can inhibit osteosarcoma cell migration, invasion, and EMT *in vitro*. Silenced PGM5-AS1/FBN1 or overexpressed miR-140-5p can notably inhibit tumorigenesis in nude mice *in vivo*. Based on such results, PGM5-AS1 can negatively regulate miR-140-5p to inhibit FBN1 expression and hence control the progression of osteosarcoma[28]. In this study, down-regulated TMPO-AS1 remarkably inhibited the expression of E2F1 mRNAs in osteosarcoma and suppressed the proliferation and EMT of osteosarcoma cells, while overexpressed miR-144-3p or down-regulated E2F1 reversed the inhibition of EMT caused by down-regulated TMPO-AS1. A similar study suggests that silencing TMPO-AS1 in ovarian cancer cells can inhibit the miR-200c/TMEFF2 molecular axis to destroy the PI3K/Akt signaling pathway in ovarian cancer cells and ultimately promote the EMT of ovarian cancer cells[29]. This study is novel because it proposed that related lncRNAs in osteosarcoma cells can regulate miRNAs to target and regulate downstream mRNA to affect cell proliferation and apoptosis, and verified that TMPO-AS1 can inhibit the miR-329/E2F1 molecular axis to promote the development and metastasis of osteosarcoma. But this research has some limitations. For example, we did not conduct animal experiments. Besides, we did not figure out the regulatory network of TMPO-AS1, so it is necessary to further explore whether it can affect the occurrence and development of tumors in other ways. We will conduct bioinformatics analysis in the future to analyze the regulatory network of TMPO-AS1, seeking to provide more references for our experiments.

Conclusions

In summary, lncRNA-TMPO-AS1 can promote apoptosis of osteosarcoma cells by targeting miR-329 and regulating E2F1, which is a potent treatment option for osteosarcoma.

Conflict of Interests

The authors declare that they have no conflict of interests.

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