Inhibition of lncRNA TMPO-AS1 suppresses proliferation, migration and invasion of colorectal cancer cells by targeting miR-143-3p

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Abstract. Long non-coding RNAs (lncRNAs) are widely studied in cancer pathogenesis. Accumulating evidence has demonstrated that lncRNAs are involved in the cellular progression of colorectal cancer (CRC). However, the regulatory mechanism of lncRNA TMPO-antisense (AS)1 in CRC has not been fully elucidated. The present study aimed to elucidate the role and regulatory mechanisms of lncRNA TMPO-AS1 in CRC. In the present study, the expression levels of TMPO-AS1 and microRNA-143-3p (miR-143-3p) were detected using reverse transcription-quantitative PCR assay. The relative protein expression levels were measured via western blot analysis. MTT and Transwell assays were used to determine cell proliferation, migration and invasion, while a luciferase reporter assay was performed to assess the relationship between TMPO-AS1 and miR-143-3p. In addition, a tumor animal model was used to investigate the effect of TMPO-AS1 on tumor growth in CRC *in vivo*. TMPO-AS1 expression was increased and miR-143-3p expression was decreased in CRC cells. TMPO-AS1 knockdown and miR-143-3p overexpression significantly inhibited cell proliferation, migration and invasion of CRC cells. Luciferase reporter assay results demonstrated that miR-143-3p was a direct target of TMPO-AS1. Inhibition of miR-143-3p could alleviate the suppressive effects of TMPO-AS1 deletion on cell proliferation, migration and invasion of CRC cells. Furthermore, TMPO-AS1 deletion could inhibit tumor growth in CRC *in vivo*. It was concluded that TMPO-AS1 regulated cell proliferation, migration and invasion of CRC cells by targeting miR-143-3p. These findings provided a new regulatory network and therapeutic target for the treatment of CRC.

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Introduction

Colorectal cancer (CRC) is one of the most frequent malignant gastrointestinal tumors that threatens human health (1). CRC has a high incidence rate; the second highest (9.5% of 8.6 million new cases) in females and the third highest (10.9% of 9.5 million new cases) in males worldwide (2). Therefore, investigating the pathogenesis of CRC can contribute to the treatment and diagnosis of the disease.

With the development of sequencing technology, the functions of non-coding RNAs (ncRNAs) in the development, metastasis and drug resistance in multiple cancer types have been increasingly revealed (3-9). Long ncRNAs (lncRNAs) are a type of ncRNAs with a length of >200 nucleotides (nts) and serve important regulatory roles in tumor cell progression, including cell proliferation, migration, invasion and apoptosis in CRC (10-12). For example, Zhang *et al* (13) reported that lncRNA CPS-IT1 is downregulated in CRC tissues and cells, which could inhibit CRC cell proliferation, invasion and metastasis. MicroRNAs (miRNAs/miRs), a type of ncRNAs with a length of ~22 nts, are also closely related to the proliferation, invasion and apoptosis of CRC cells (14-16). Moreover, Luo *et al* (17) demonstrated that miR-17 upregulation contributes to cell proliferation, tumor growth and cycle progression in CRC.

lncRNAs and miRNAs have become widely investigated in the research of cancer pathogenesis. A number of studies have revealed that lncRNAs and miRNAs serve important regulatory roles in tumor occurrence and migration by forming regulatory networks (18-21). For example, lncRNA PVT1 promotes cell proliferation and migration by regulating miR-448 in pancreatic cancer (22). TMPO-antisense (AS)1 is also associated with CRC and may serve a role in CRC (23). Cui *et al* (24) reported that TMPO-AS1 acts as a competing endogenous RNA to regulate osteosarcoma tumorigenesis via the miR-199a-5p/Wnt family member 7B axis. The present study found that TMPO-AS1 expression was increased in CRC cells. However, the underlying regulatory mechanism of TMPO-AS1 has not been fully investigated in CRC.

In the current study, the effects of TMPO-AS1 and miR-143-3p on cell proliferation, migration and invasion were investigated, and the regulatory relationship between TMPO-AS1 and miR-143-3p was further evaluated. The present study demonstrated that the regulatory networks of

TMPO-AS1 and miR-143-3p affected the cellular proliferation of CRC *in vitro* and *in vivo*.

Materials and methods

Cell culture and transfection. Human CRC cell lines (SW480, HCT15, SW1116 and HCT116) and normal connective mucosal epithelial cells (NCM460) were purchased from the Cell Bank of the Chinese Academy of Science. All cells were cultured in DMEM (Thermo Fisher Scientific, Inc.) containing 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.) and 1% penicillin and streptomycin under a 5% CO_2 atmosphere at 37°C.

Small interfering (si)-TMPO-AS1, pcDNA-TMPO-AS1, miR-143-3p, anti-miR-143-3p and their negative controls (si-NC, pcDNA, miR-NC and anti-miR-NC) were purchased from Shanghai GenePharma Co., Ltd. All plasmids (100 nM) and (si)-TMPO-AS1 (50 nM), miR-143-3p (50 nM), anti-miR-143-3p (50 nM) and their controls were transfected into SW480 and HCT15 cells using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The siRNA for TMPO-AS1 (si-TMPO-AS1, 5'-GAAGACUAGUGACCUAUAAUU-3'), miR-143-3p mimic (5'-UGAGAUGAAGCACUGUAG CUC-3'), miR-143-3p mimic inhibitor (anti-miR-143-3p, 5'-GAGCUACAGUGCUUCAUCUCA-3'). Cells ($1x10^5$/well) were collected after 48 h for use in subsequent experiments.

Reverse transcription-quantitative (RT-q) PCR. Total RNA from $5x10^6$ cells was separated using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). TaqMan miRNA Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to reverse transcribe the total RNA into cDNA of miR-143-3p, and the M-MLV Reverse Transcriptase, M-MLV 5X Reaction Buffer, dNTPs (Invitrogen; Thermo Fisher Scientific, Inc.) were used to synthesize first-strand cDNA of TMPO-AS1. The reaction volume was 12 μl. U6 snRNA and GAPDH served as the internal reference genes for normalization of miR-143-3p and TMPO-AS1, respectively. SYBR Green Real-time PCR kit (Invitrogen; Thermo Fisher Scientific, Inc.) was used to perform RT-qPCR on an iQTM5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.). The following thermocycling conditions were used for PCR: 95°C for 10 min, followed by denaturation at 95°C for 10 sec, annealing at 60°C for 60 sec, and extension at 72°C for 30 sec, with a total of 45 cycles. The expression levels of miR-143-3p and TMPO-AS1 were calculated using the $2^{-\Delta\Delta Cq}$ method (25). The following primers were used: miR-143-3p: Forward, 5'-GUC ACGAAGUAGAGU-3' and reverse, 5'-CTCAACTGGTGT CGTGGA-3'; GAPDH: Forward, 5'-TGACTTCAACAGCGA CACCCA-3' and reverse, 5'-CACCCTGTTGCTGTAGCC AAA-3'; TMPO-AS1: Forward 5'-CAGTTTAAAAGGCGC TGGGG-3' and reverse 5'-CCTTATCGGCGTCTAAGGGG'; and U6: Forward, 5'-GCTTCGGCAGCACATATACTAA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'. A total of three independent repeats was performed.

MTT assay. MTT assay was used to detect cell proliferation. Transfected cells in each well ($2x10^3$) were seeded into 96-well plates. After culture for 24, 48 and 72 h at 37°C, 15 μl MTT solution was added into each well. Then, cells were incubated for another 4 h at room temperature. Next, 150 μl DMSO solution was added into each well to dissolve formazan for 10 min at 37°C. The absorbance was detected using SpectraMax 360 pc microplate reader (Molecular Devices, LLC) at a wavelength of 490 nm.

Western blot analysis. Cells were lysed in RIPA buffer (Cell Signaling Technology, Inc.) and the proteins extracted. Then, the protein concentration was detected using a bicinchoninic acid Protein assay kit (Beyotime Institute of Biotechnology). Next, 10% SDS-PAGE was used to separate the proteins (20 mg/lane), which were then transferred onto a PVDF membrane (EMD Millipore). Next, 5% non-fat milk was employed to block the membrane for 2 h at room temperature. The membrane was incubated with the primary antibodies against cyclin D1 (cat. no. 55506), p21 (cat. no. 2947), p27 (cat. no. 3686), matrix metalloproteinase (MMP)-2 (cat. no. 40994), MMP-9 (cat. no. 13667), MMP-14 (cat. no. 13130) and GAPDH (cat. no. 5174) (all 1:2,000; all Cell Signaling Technology, Inc.) at 4°C overnight, and then incubated with the secondary antibody HRP-conjugated goat anti-rabbit IgG; 1:2,000; Cell Signaling Technology, Inc.) for 1 h at room temperature. The protein blots were resolved using enhanced chemiluminescence reagents (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. In addition, Image Studio Lite software (version 3.1; LI-COR Biosciences) was used to quantify the density of protein bands, as previously described (26).

Cell migration and invasion. Transwell migration and invasion assay were used to measure cell migration and invasion. 24-Transwell inserts (8 μm pores; Corning Inc.) were used to assess cell migration, and Transwell inserts coated with Matrigel (BD Biosciences) at 37°C for 6 h were applied for cell invasion measurement. The cells were seeded onto the upper chamber of a Transwell supplemented with the serum-free medium at $5.0x10^4$ for migration and $1.0x10^5$ for invasion and then incubated in a 5% of CO_2 atmosphere at 37°C for 24 h. The lower chamber was filled with complete medium containing 10% FBS. Next, cells in the upper chamber supplemented with medium containing 10% FBS were removed. The numbers of migratory and invasive cells in the lower chambers were fixed with 4% paraformaldehyde for 30 min at 37°C and stained with 0.1% crystal violet for 10 min at room temperature. The cells were counted in 5 random fields under a Leica DM3000 routine light microscope (magnification, x100, Leica Microsystems GmbH).

Luciferase reporter assay. The targeted sequence between TMPO-AS1 and miR-143-3p was predicted using StarBase database (27). Fragments of TMPO-AS1 containing the wild-type (WT) or mutant (MUT) were synthesized and cloned into pMIR-REPORT™ (Thermo Fisher Scientific, Inc.) to construct the vector of WT-TMPO-AS1 or MUT-TMPO-AS1, respectively, according to the manufacturer's protocols. Next, the vector of WT-TMPO-AS1 or MUT-TMPO-AS1 (100 ng) was co-transfected with miR-NC or miR-143-3p into SW480 and HCT15 cells at a final concentration of 50 nM using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher
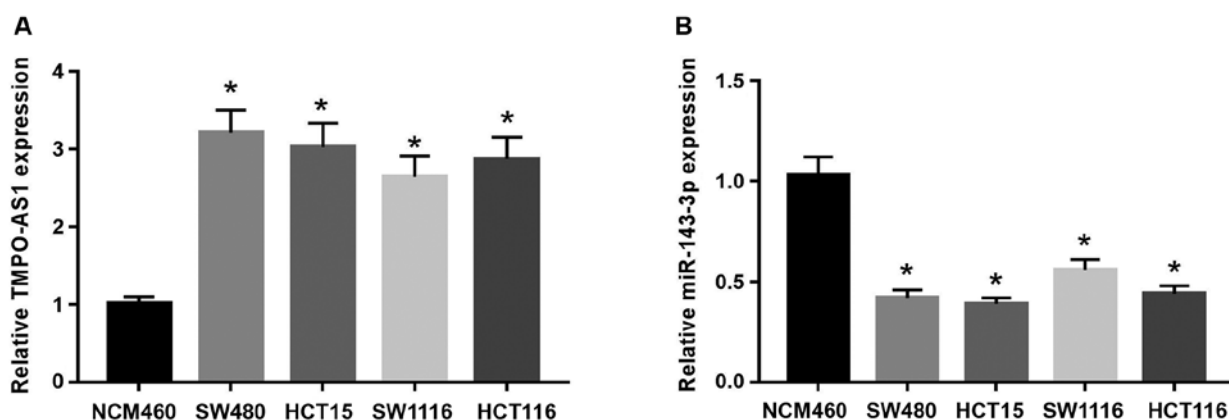
Figure 1. TMPO-AS1 expression is increased, while miR-143-3p expression is decreased in CRC cells. Reverse transcription-quantitative PCR analysis of (A) TMPO-AS1 and (B) miR-143-3p expression levels in CRC cell lines (SW480, HCT15, SW1116 and HCT116) and normal connective mucosal epithelial cells (NCM460). *P<0.05 vs. NCM460. miR, microRNA; CRC, colorectal cancer; AS, antisense.
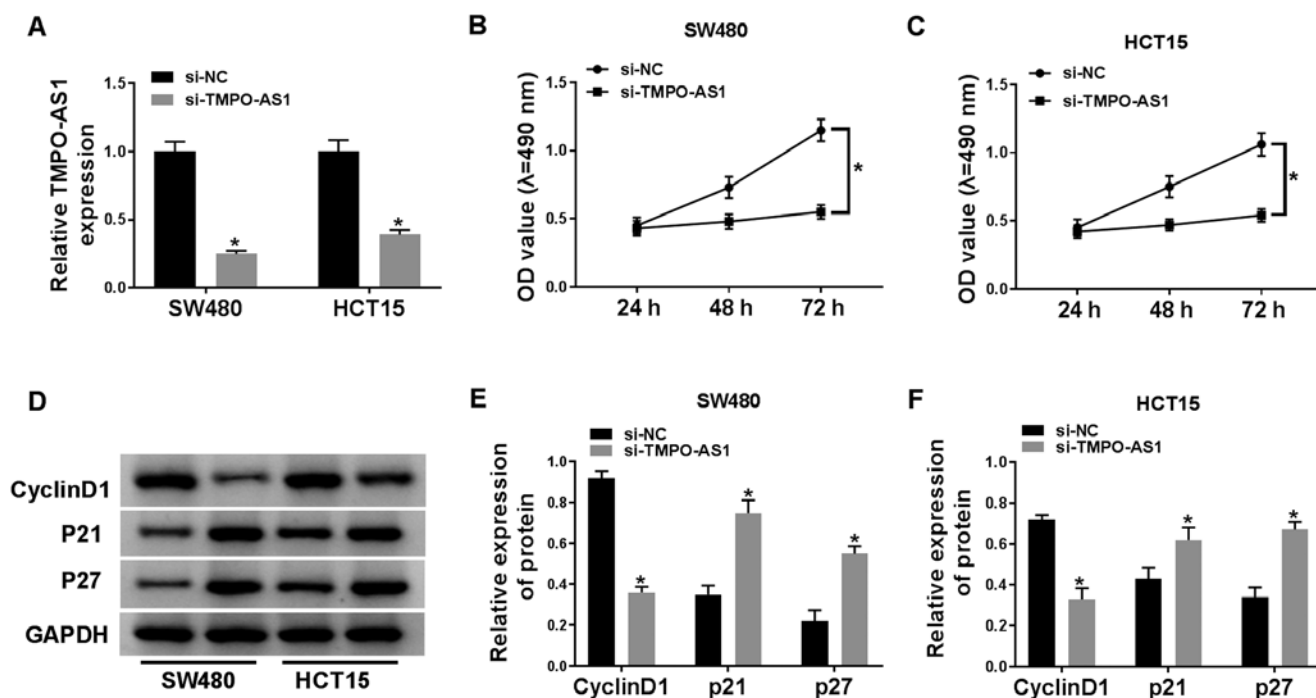

Figure 2. Inhibition of TMPO-AS1 suppresses cell proliferation in CRC cells. (A) Knockdown of TMPO-AS1 represses TMPO-AS1 expression in SW480 and HCT15 cells. TMPO-AS1 knockdown suppresses cell proliferation in (B) SW480 and (C) HCT15 cells. (D) Cyclin D1, p21 and p27 protein expression levels assessed using western blotting in SW480 and HCT15 cells transfected with si-NC and si-TMPO-AS1. Inhibition of lncRNA TMPO-AS1 reduced cyclin D1 protein and enhanced p21 and p27 protein expression in (E) SW480 and (F) HCT15 cells. *P<0.05 vs. si-NC. CRC, colorectal cancer; si, short interfering; lnc, long non-coding; OD, optical density; NC, negative control; AS, antisense.

Scientific, Inc.), according to the manufacturer's instructions. Following 36 h transfection, cells were harvested. The luciferase activities were detected using a Dual-Luciferase Reporter Assay system (Promega Corporation). The normalization of firefly luciferase activity utilized the Renilla luciferase activity as the control.

Tumor xenograft model. All animal experiments were approved by the Animal Ethics Committee of Lanzhou University Second Hospital, China. A total of 12 female BALB/c nude mice (age, 4-5 weeks; weight 15-18 g) were obtained from the Animal Center of Central South University. Mice were maintained at a temperature of 18-23˚C, 12-h light/dark cycle,

45-65% humidity, and free access to water and food. A total of 12 female BALB/c nude mice were randomly divided into two groups (6 mice/group). The flanks of the mice were injected with cells ($1x10^6$ cell/ml) transfected with short hairpin (sh)-NC or sh-TMPO-AS1 (Shanghai GenePharma Co., Ltd.), respectively. Following injection, the tumor size (length and width) was measured every week. After 5 weeks, the mice were sacrificed and the tumor weight was measured. The tumor volume=0.5 x length x width[2]. The volume for the maximum tumor was 729 mm[3] with the length for 18.0 mm and the width for 9.0 mm.

Statistical analysis. Data are presented as the mean ± standard deviation, and analyzed using GraphPad Prism 7.0 (GraphPad
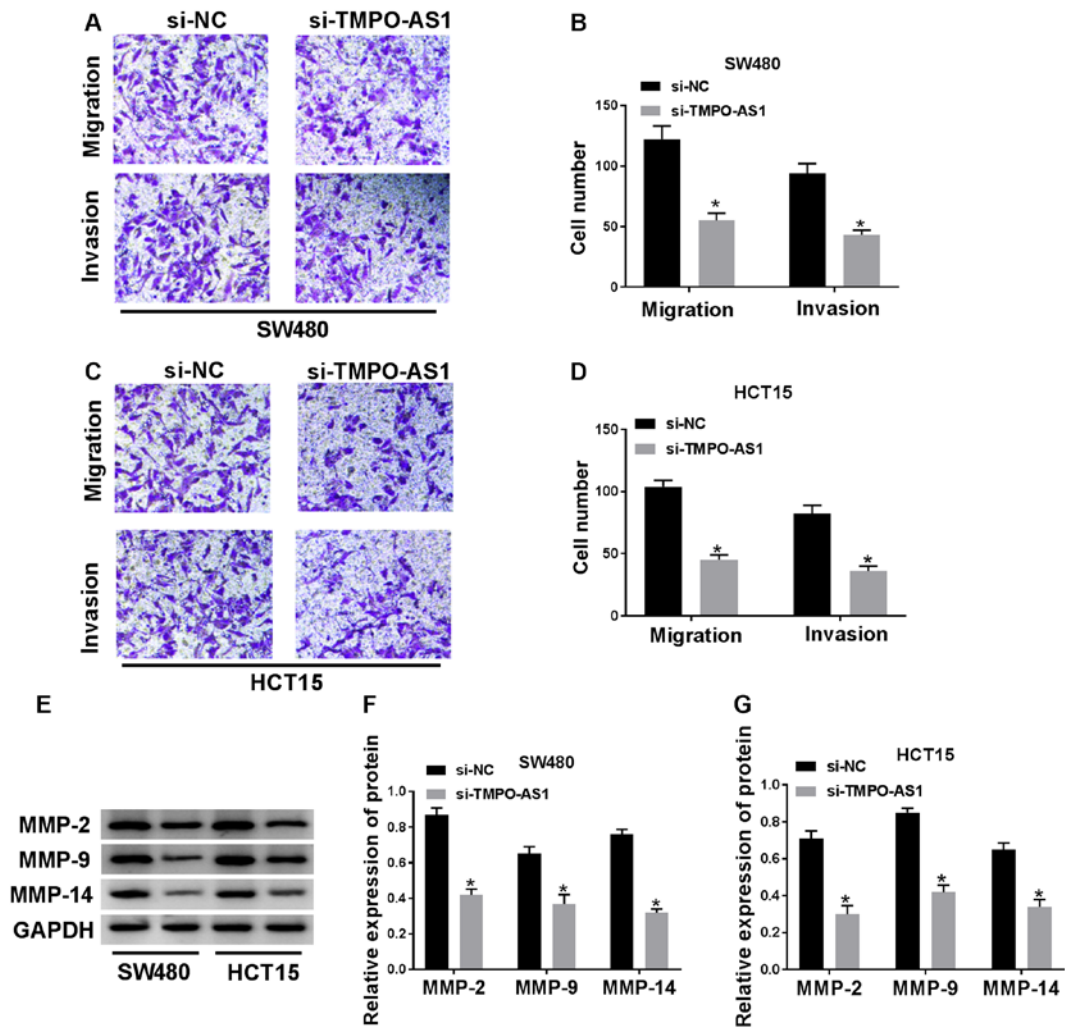
Figure 3. Knockdown of TMPO-AS1 reduces cell migration and invasion in CRC cells. TMPO-AS1 knockdown suppressed (A) cell migration and invasion in (B) SW480 cells. TMPO-AS1 knockdown suppressed (C) cell migration and invasion in and (D) HCT15 cells. (E) Western blotting results of MMP-2, MMP-9 and MMP-14 expression levels in SW480 and HCT15 cells transfected with si-NC and si-TMPO-AS1. The protein expression of MMP-2, MMP-9 and MMP-14 was inhibited by TMPO-AS1 knockdown in (F) SW480 and (G) HCT15 cells. *$P<0.05$ vs. si-NC. si, short interfering; NC, negative control; MMP, matrix metalloproteinase; AS, antisense.

Software, Inc.). All experiments were performed in triplicate. Statistical comparison of data was analyzed with an unpaired Student's t-test and one-way ANOVA followed by Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

TMPO-AS1 expression is increased and miR-143-3p expression is decreased in CRC cells. First, the expression levels of TMPO-AS1 and miR-143-3p in CRC cell lines (SW480, HCT15, SW1116 and HCT116) and normal connective mucosal epithelial cells (NCM460) were determined using RT-qPCR. The data demonstrated that TMPO-AS1 was significantly increased, while the expression of miR-143-3p was significantly decreased, in SW480, HCT15, SW1116 and HCT116 cells compared with NCM460 cells (Fig. 1A and B). Thus, it was indicated that TMPO-AS1 and miR-143-3p may serve crucial roles in CRC cells. To further investigate the functions of TMPO-AS1 and miR-143-3p in CRC cells, SW480 and HCT15 CRC cell lines were selected for subsequent functional studies.

Knockdown of TMPO-AS1 suppresses cell proliferation in CRC cells. si-NC or si-TMPO-AS1 was transfected into SW480 and HCT15 cells. RT-qPCR results indicated that the expression of TOMP-AS1 was significantly reduced in cells transfected with si-TMPO-AS1 (Fig. 2A). Then, cell proliferation was determined using a MTT assay, which was found to be significantly decreased by TMPO-AS1 knockdown in SW480 and HCT15 cells (Fig. 2B and C). In addition, western blot analysis demonstrated that the protein expression of cyclin D1 was inhibited, while the protein expression levels of p21 and p27 were increased, in SW480 and HCT15 cells transfected with si-TMPO-AS1 compared with the si-NC (Fig. 2D-F). Therefore, these data suggested that downregulation TMPO-AS1 inhibited cell proliferation of CRC cells.

Knockdown of TMPO-AS1 reduces cell migration and invasion in CRC cells. Transwell migration and invasion assays demonstrated that cell migratory and invasive capacities of SW480 and HCT15 cells were suppressed by TMPO-AS1 knockdown (Fig. 3A-D). The MMP protein family is closely associated with cell metastasis (28). Thus, MMP-2, MMP-9 and
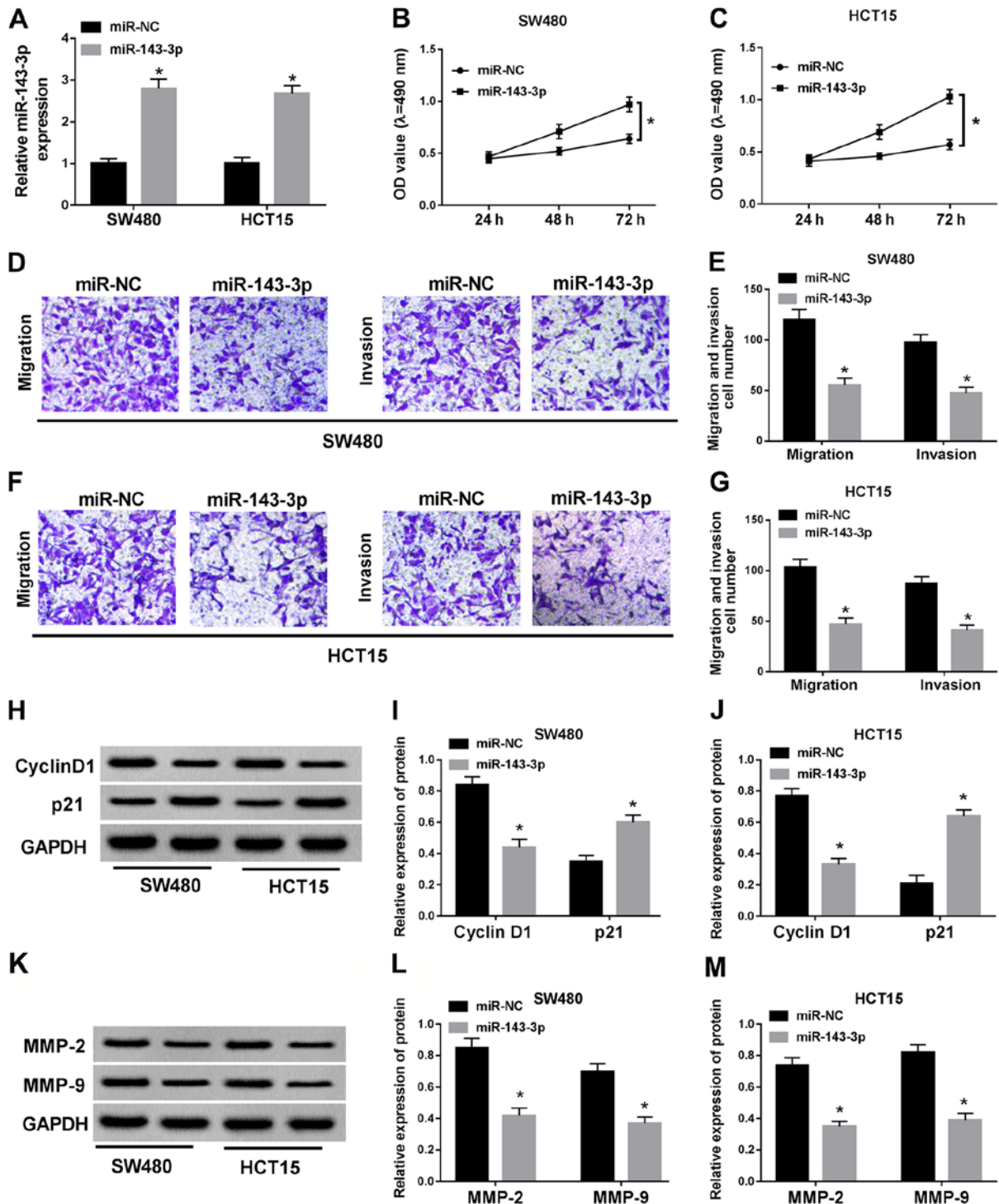
Figure 4. Overexpression of miR-143-3p inhibits cell proliferation, migration and invasion in CRC cells. (A) Expression of miR-143-3p was detected in SW480 and HCT15 cells transfected with miR-NC and miR-143-3p using reverse transcription-quantitative PCR. Overexpression of miR-143-3p inhibited cell proliferation in (B) SW480 and (C) HCT15 cells. Overexpression of miR-143-3p reduced (D) cell migration and invasion in (E) SW480 cells. (F) These cellular processes were also decreased in (G) HCT15 cells, as determined using Transwell migration and invasion assays. (H) Western blotting results of cyclin D1 and p21 expression levels in SW480 and HCT15 cells transfected with miR-NC and miR-143-3p. Protein expression of cyclin D1 and p21 was determined in miR-NC and miR-143-3p groups in (I) SW480 and (J) HCT15 cells. (K) Western blotting results of MMP-2 and MMP-9 expression levels in (L) SW480 and (M) HCT15 cells transfected with miR-NC and miR-143-3p. *$P<0.05$ vs. miR-NC. miR, microRNA; NC, negative control; MMP, matrix metalloproteinase.

MMP-14 protein expression levels were determined in SW480 and HCT15 cells transfected with si-NC or si-TMPO-AS1. It was demonstrated that MMP-2, MMP-9 and MMP-14 protein expression levels were significantly reduced by si-TMPO-AS1 transfection compared with the si-NC group (Fig. 3E-G).

Thus, knockdown of TMPO-AS1 could inhibit cell migration and invasion in CRC cells.

Overexpression of miR-143-3p inhibits cell proliferation, migration and invasion of CRC cells. To confirm the function
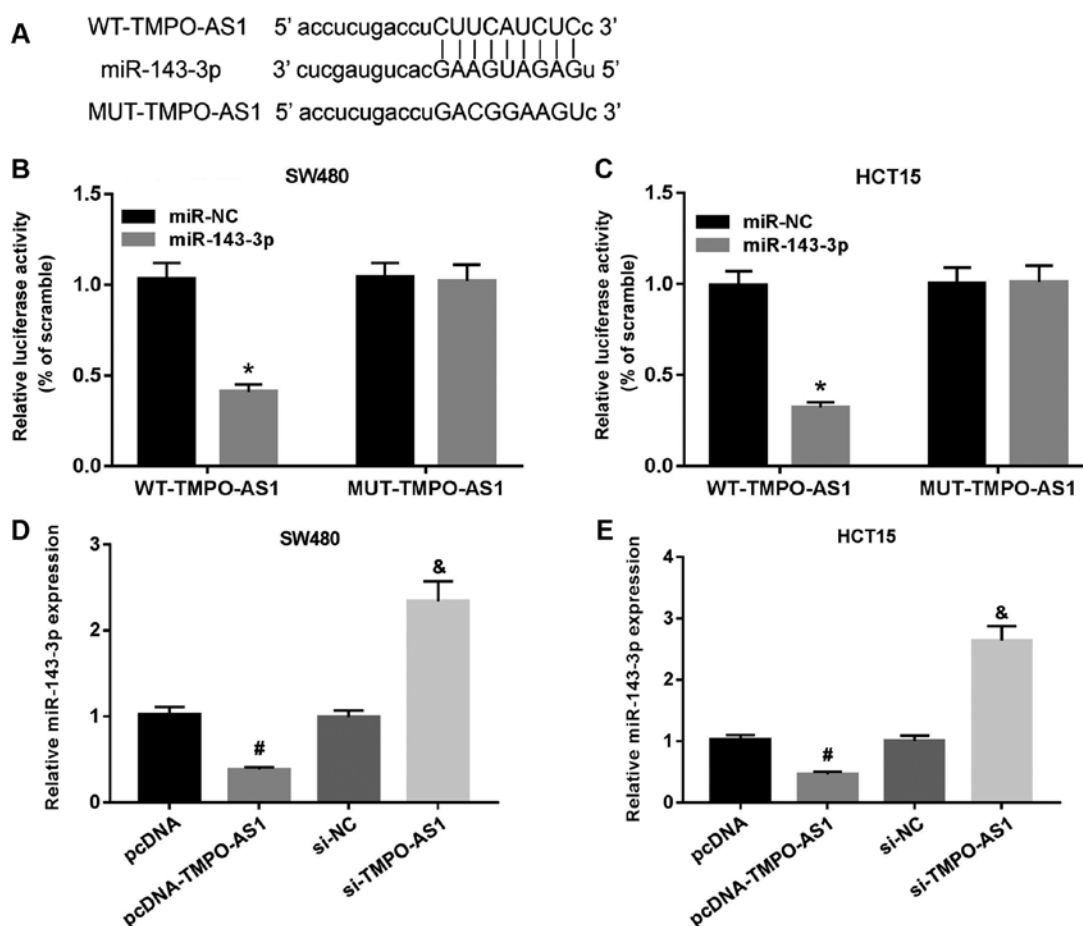
Figure 5. TMPO-AS1 directly targets miR-143-3p in CRC cells. (A) WT or MUT binding sites between TMPO-AS1 and miR-143-3p predicted via bioinformatics analysis. Luciferase activities were detected after (B) SW480 and (C) HCT15 cells were co-transfected with WT-TMPO-AS1 or MUT-TMPO-AS1 and miR-NC or miR-143-3p. Expression of miR-143-3p was detected in (D) SW480 and (E) HCT15 cells transfected with pcDNA, pcDNA-TMPO-AS1, si-NC and si-TMPO-AS1 using reverse transcription-quantitative PCR. *$P<0.05$ vs. miR-NC; #$P<0.05$ vs. pcDNA; &$P<0.05$ vs. si-NC. miR, microRNA; CRC, colorectal cancer; WT, wild-type; MUT, mutant; si, short interfering; NC, negative control; AS, antisense.

of miR-143-3p, miR-143-3p was overexpressed in SW480 and HCT15 cells (Fig. 4A). It was found that miR-143-3p overexpression significantly decreased proliferation of SW480 and HCT15 cells (Fig. 4B and C). In addition, cell migration and invasion were significantly inhibited by overexpression of miR-143-3p in SW480 and HCT15 cells (Fig. 4E-G). The miR-143-3p mimic also reduced the protein expression levels of cyclin D1, MMP-2 and MMP-9, but promoted the protein expression of p21 (Fig. 4H-N). These results indicated that overexpression of miR-143-3p could inhibit cell proliferation, migration and invasion in CRC cells.

TMPO-AS1 directly targets miR-143-3p in CRC cells. Bioinformatics analysis predicted that TMPO-AS1 had complementary binding sites with miR-143-3p (Fig. 5A). A luciferase reporter assay was performed to assess the relationship between miR-143-3p and TMPO1-AS, and it was demonstrated that the miR-143-3p mimic significantly reduced the luciferase activity of WT-TMPO-AS1, while it had no effect on the luciferase activity of MUT-TMPO-AS1 in SW480 or HCT15 cells (Fig. 5B and C). As shown in Fig. S1A, pcDNA-TMPO-AS1 could significantly upregulate the expression of pcDNA-TMPO-AS1 in both SW480 and HCT15 cells. Moreover, miR-143-3p expression was

inhibited by TMPO-AS1 overexpression but promoted by TMPO-AS1 knockdown in SW480 and HCT15 cells (Fig. 5D and E). These results suggested that miR-143-3p was a target of TMPO-AS1 and negatively regulated by TMPO-AS1 in CRC cells.

Knockdown of miR-143-3p reverses the suppressive effects of si-TMPO-AS1 on cell proliferation, migration and invasion in CRC cells. To further investigate the underlying regulatory mechanism between miR-143-3p and TMPO-AS1, si-NC, si-TMPO-AS1, si-TMPO-AS1 + anti-miR-NC or si-TMPO-AS1 + anti-miR-143-3p were transfected into SW480 and HCT15 cells. The data demonstrated a successful knockdown efficiency of anti-miR-143-3p in SW480 and HCT15 cells (Fig. S1B).

RT-qPCR results indicated that miR-143-3p was enhanced by TMPO-AS1 knockdown, which was significantly eliminated by miR-143-3p inhibitor in SW480 and HCT15 cells (Fig. 6A). MTT assay results demonstrated that cell proliferation inhibited by TMPO-AS1 knockdown was partially blocked by miR-143-3p inhibitor (Fig. 6B and C). Moreover, the inhibitory effects of TMPO-AS1 knockdown on cell migration and invasion were eliminated by anti-miR-143-3p in SW480 and HCT15 cells (Fig. 6D-G). The decrease of
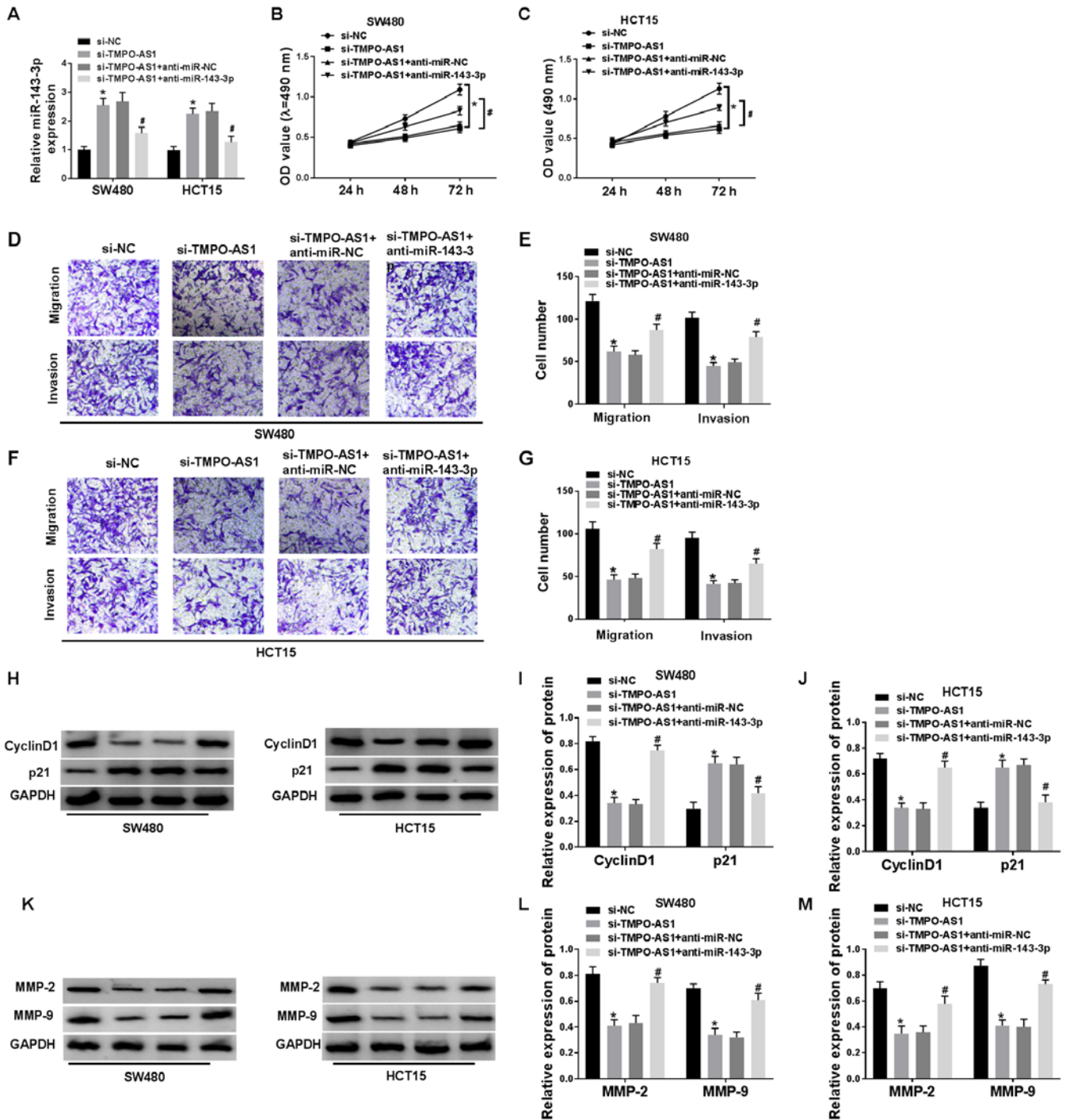
Figure 6. Downregulation of miR-143-3p reverses the suppressive effects of si-TMPO-AS1 on cell proliferation, migration and invasion in CRC cells. (A) Expression of miR-143-3p was detected in SW480 and HCT15 cells transfected with si-NC, si-TMPO-AS1, si-TMPO-AS1 + anti-miR-NC and si-TMPO-AS1 + anti-miR-143-3p via reverse transcription-quantitative PCR. Cell proliferation was measured in si-NC, si-TMPO-AS1, si-TMPO-AS1 + anti-miR-NC and si-TMPO-AS1 + anti-miR-143-3p groups in (B) SW480 and (C) HCT15 cells using a MTT assay. (D) Cell migration and invasion were detected in si-NC, si-TMPO-AS1, si-TMPO-AS1 + anti-miR-NC and si-TMPO-AS1 + anti-miR-143-3p groups in (E) SW480, (F) as well as in and (G) HCT15 cells using Transwell assays. (H) Western blotting results of cyclin D1 and p21 expression levels in (I) SW480 and (J) HCT15 cells transfected with si-NC, si-TMPO-AS1, si-TMPO-AS1 + anti-miR-NC and si-TMPO-AS1 + anti-miR-143-3p. (K) Western blotting results of MMP-2 and MMP-9 in (L) SW480 and (M) HCT15 cells transfected with si-NC, si-TMPO-AS1, si-TMPO-AS1 + anti-miR-NC and si-TMPO-AS1 + anti-miR-143-3p. $^*P<0.05$ vs. si-NC; $^\#P<0.05$ vs. si-TMPO-AS1 + anti-miR-NC. miR, microRNA; si, short interfering; CRC, colorectal cancer; NC, negative control; MMP, matrix metalloproteinase; AS, antisense; OD, optical density.

cyclin D1 protein expression and the increase of p21 protein expression caused by TMPO-AS1 knockdown were partially restored by anti-miR-143-3p transfection in SW480 and HCT15 cells (Fig. 6H-J). In addition, the protein expression levels of MMP-2 and MMP-9 were decreased by the transfection of si-TMPO-AS1, but alleviated by anti-miR-143-3p in SW480 and HCT15 cells (Fig. 6K-M). Collectively, these results suggested that inhibition of TMPO-AS1 reduced cell proliferation, migration and invasion by targeting miR-143-3p in CRC cells.
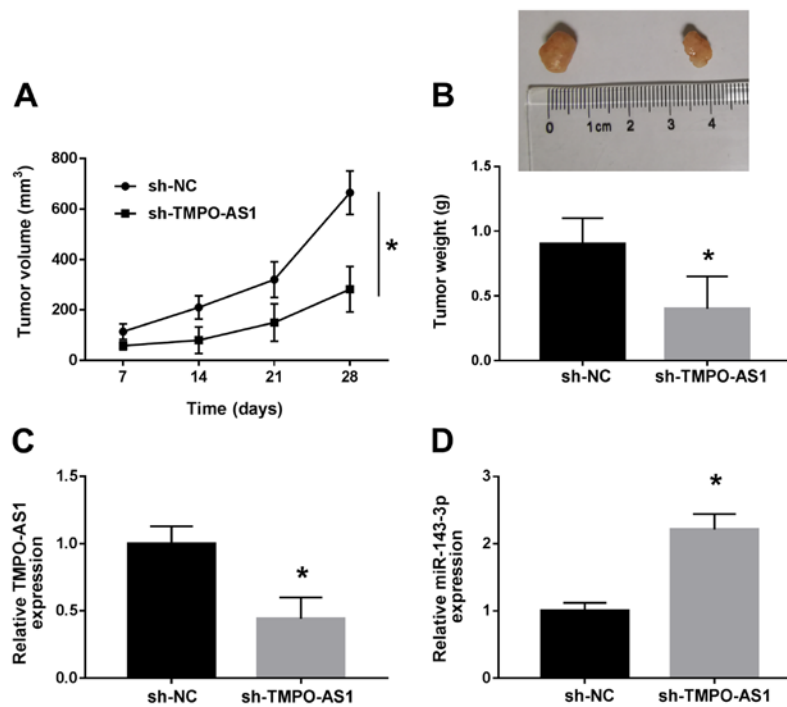
Figure 7. Knockdown of TMPO-AS1 suppresses tumor growth in CRC *in vivo*. TMPO-AS1 deletion inhibited tumor (A) volume and (B) weight *in vivo*. TMPO-AS1 knockdown (C) decreased TMPO-AS1 expression but (D) increased miR-143-3p expression. *P<0.05 vs. sh-NC. CRC, colorectal cancer; miR, microRNA; sh, short hairpin; NC, negative control; AS, antisense.

Knockdown of TMPO-AS1 suppresses tumor growth in CRC in vivo. To further evaluate the effect of TMPO-AS1 on CRC progression *in vivo*, animal experiments were performed. As shown in Fig. 7A, the tumor volume of sh-TMPO-AS1 group was significantly inhibited compared with the sh-NC group. In addition, tumor weight was markedly decreased in sh-TMPO-AS1 group compared with sh-NC group (Fig. 7B). Then, the expression levels of TMPO-AS1 and miR-143-3p were detected in tumor tissues. The results demonstrated that sh-TMPO-AS1 transfection inhibited TMPO-AS1 expression and promoted miR-143-3p expression (Fig. 7C and D). Thus, knockdown of TMPO-AS1 could inhibit tumor growth in CRC *in vivo*.

Discussion

lncRNAs serve important roles in the development and progression of multiple types of cancer (29). As a regulatory factor, lncRNA can inhibit or promote the development of cancer cell and tumor formation (30-33). For instance, upregulated lncRNA DLX6-AS1 enhances cell proliferation and invasion by regulating miR-181b in pancreatic cancer (34), while lncRNA ATB is involved in cell progression in CRC (35). In addition, certain lncRNAs are closely associated with the prognosis of CRC and could serve as biomarkers (36-38). However, the function of TMPO-AS1 in CRC progression remains unknown. A previous study reported that TMPO-AS1 was upregulated in prostate cancer tissues, and that overexpression of TMPO-AS1 promotes the proliferation and migration of prostate cancer cells (39). Moreover, TMPO-AS1 possesses a tumor-promoting action in osteosarcoma (24) Consistent with these studies, the present results demonstrated the promoting effect of TMPO-AS1 in CRC, as evidenced by inhibition on cell proliferation, migration and invasion in CRC cells after TMPO-AS1 knockdown.

lncRNA can target miRNAs to participate in the regulation of cell processes and metabolism (13). The present study found that TMPO-AS1 directly targeted miR-143-3p. Accumulating evidence indicates that miR-143-3p is involved in cell proliferation and apoptosis in various of types of cancer, including breast cancer, hepatocellular carcinoma and esophageal squamous cell carcinoma (40-42). Furthermore, miR-143-3p is downregulated in cervical cancer, colorectal cancer and breast cancer. It has also been shown that overexpression of miR-143-3p inhibits cell progression (40,43,44). In concurrence, the present study found that miR-143-3p was decreased in CRC cells, and that overexpression of miR-143-3p significantly suppressed cell proliferation, migration and invasion in CRC cells.

The results of the present recovery experiments indicated that TMPO-AS1 affected cell proliferation, migration and invasion by targeting miR-143-3p. Therefore, the TMPO-AS1/miR-143-3p axis may be an important regulatory mechanism for CRC cell proliferation and metastasis. Additionally, it was demonstrated that TMPO-AS1 knockdown repressed tumor growth via regulating miR-143-3p *in vivo*.

In conclusion, the current study demonstrated the function and regulatory network of TMPO-AS1 in CRC. The results suggested that TMPO-AS1 expression levels were increased in CRC cells. Knockdown of TMPO-AS1 impeded cell proliferation, migration and invasion in CRC cells, and inhibited tumor growth in CRC *in vivo*, by regulating miR-143-3p. These findings improved the understanding of the regulatory mechanism of CRC and may provide a new target for the treatment of CRC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LZ and YL designed the study and wrote the manuscript. LZ, YL and ALS performed the experiments and analyzed the data. LZ edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Animal Ethics Committee of Lanzhou University Second Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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