

Long non-coding RNA SNHG16 activates USP22 expression to regulate colorectal cancer progression by sponging miR-132-3p

Xiaowen He (✉ xiaolin4628197@163.com)

Liu Zhou People's Hospital

Jun Ma

Liu Zhou People's Hospital

Mingming Zhang

Liu Zhou People's Hospital

Jianhua Cui

Liu Zhou People's Hospital

Hao Yang

Liu Zhou People's Hospital

Research

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Abstract

Colorectal cancer (CRC) is the most common cause of cancer-related mortality in the world. Long non-coding RNAs (lncRNAs) were involved in the development of many cancers. However, studies on the effect of lncRNA small nucleolar RNA host gene 16 (SNHG16) on the proliferation, metastasis and apoptosis of CRC cells were still few. SNHG16 was highly expressed in CRC tissues and cells. Knockdown of SNHG16 reduced the proliferation, migration, invasion, and promoted the apoptosis of CRC cells. MiR-132-3p could interact with SNHG16, and its inhibitor recovered the suppression effects of silenced-SNHG16 on CRC cell progression. Besides, USP22 was a target of miR-132-3p, and its overexpression restored the inhibition effects of miR-132-3p mimic on CRC cell progression. Interference of SNHG16 reduced the tumor growth in vivo. LncRNA SNHG16 played an oncogene in CRC progression. The discovery of SNHG16/ miR-132-3p/ USP22 pathway provided new thinking for the treatment of CRC.

Introduction

Colorectal cancer (CRC) has become a common type of cancer worldwide and progresses rapidly [1, 2]. According to statistics, in 2015, the incidence and mortality rate of CRC in China were 376.3 and 191.0 per 100,000, respectively, which was the fifth leading cause of all cancer-related deaths [3]. In recent decades, significant progress have been made in the prevention, diagnosis and treatment of CRC [4, 5]. However, due to the complex pathogenesis of CRC, the 5-year survival rate was still very low [6]. Therefore, it is urgent to elucidate the molecular mechanism of CRC and explore new approaches to treat CRC.

Studies have shown that many pro-cancer and anti-cancer genes, long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) were correlated with the occurrence of CRC [7, 8]. Both lncRNAs and miRNAs belong to non-coding RNAs, with a length of about 20 to 200 nucleotides (nts), respectively [9]. Also, lncRNAs and miRNAs played the role of oncogenes or tumor suppressor genes in CRC development process by regulating cell proliferation, invasion, apoptosis and prognosis [10–12]. For instance, lncRNA Malat1 induced CRC cell proliferation and inhibited apoptosis through sponging miR-101, hence promoted the CRC progression [13]. Du et al. discovered that lncRNA DUXAP8 served as competing endogenous RNA (ceRNA) to modulate miR-577 to promote the metastasis of CRC cells [14]. Besides, Zhao et al. reported that lncRNA LINC02418 regulated CRC tumorigenesis by absorbing miR-1273 g-3p [15]. Therefore, to explore the mechanism of lncRNAs in CRC was beneficial to provide more new evidences for CRC genomic therapy.

Small nucleolar RNA host gene 16 (SNHG16) has been shown to be highly expressed in many cancers, including hepatocellular carcinoma, breast cancer and glioma [16–18]. Similarly, Li et al. found that SNHG16 expression was also improved in CRC and correlated with poor prognosis of CRC patients [19]. Furthermore, Christensen et al. confirmed that SNHG16 was related to the lipid metabolism of CRC [20]. However, at present, few studies have been conducted on the regulation of CRC cell proliferation and metastasis by SNHG16, which were not enough to support the existence of SNHG16 as an oncogene.

In our research, we investigated the mechanism of SNHG16 promoting the CRC progression through bioinformatics prediction and experimental verification, and verified the function of SNHG16 on CRC tumor

growth in vivo. The discovery of SNHG16/ miR-132-3p/ USP22 axis established the effect of SNHG16 as an oncogene and provided a new possibility for targeted therapy of CRC.

Materials And Methods

Tissue samples collection

Tumor and adjacent normal tissue samples of 50 CRC patients admitted to Liu Zhou People's Hospital from July 2016 to January 2018 without any treatment were collected.

Cell culture and transfection

CRC cell lines (SW480 and SW620) and normal human colonic epit CON) were bought from the American Type Culture Collection (ATCC, Manassas, VA, USA). Culture medium consists of RPMI-1640 (Gibco, Waltham, MA, USA), 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin and 0.1 mg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) in 5% CO₂ at 37°C. Small interfering RNA (siRNA) against SNHG16 (si-SNHG16) and SNHG16 overexpression plasmid (pcDNA-SNHG16) or their negative controls (si-NC and pcDNA), ubiquitin specific peptidase 22 (USP22) overexpression plasmid (pcDNA-USP22), miR-132-3p mimic (miR-132-3p), miR-132-3p inhibitor and their negative controls (miR-NC and inhibitor-NC) were purchased from RiboBio (Guangzhou, China). Lentiviral short hairpin RNA (shRNA) targeting SNHG16 (sh-SNHG16) and negative control (sh-NC) were synthesized by Genechem (Shanghai, China). All oligonucleotides or plasmid vectors were transfected into SW480 and SW620 cells using Lipofectamine 3000 (Invitrogen).

Quantitative real-time polymerase chain reaction (qRT-PCR)

TRIzol reagent (Invitrogen) was utilized for extracting the total RNA. The Reverse Transcription Kit (Takara, Dalian, China) was used to synthesize cDNA. SYBR Green (Takara) was used to quantify the levels of SNHG16, miR-132-3p and USP22 using ABI7500 system (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used as internal controls. The primers were listed as below: SNHG16: F, 5'-CAGAATGCCATGGTTTCCCC-3', R, 5'-TGGCAAGAGACTTCCTGAGG-3'; USP22: F, 5'-GGCGGAAGATCACCACGTAT-3', R, 5'-TTGTTGAGACTGTCCGTGGG-3'; GAPDH: F, 5'-CCACATCGCTCAGACACCAT-3', R, 5'-ACCAGGCGCCAATACG-3'; miR-132-3p: F, 5'-GCGCGGTAACAGTCTACAGG-3', R, 5'-GTCGTATCCAGTGCAGGGTCC-3'; U6: F, 5'-CTCGCTTCGGCAGCACATATACT-3', R, 5'-CGCTTCACGAATTTGCGTGT-3'. The fold-change was calculated using $2^{-\Delta\Delta C_t}$ method.

Cell vitality assay

The proliferation of CRC cells was determined using 3-(4, 5-dimethyl-2 thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) Assay Kit (Beyotime, Shanghai, China). The transfected SW480 and SW620 cells were cultured with MTT solution for 4 h. After the cells were washed with phosphate buffer saline (PBS), dimethylsulfoxide (DMSO) was added for shock dissolution for 10 min, and the absorbance at 490 nm was tested.

Flow cytometry

After digestion with trypsin, SW480 and SW620 cells were re-suspended with PBS and collected into the centrifuge tube. Then, the cells were stained using Annexin V-FITC/PI Apoptosis Detection Kit (Yeasen, Shanghai, China) away from light for 20 min. The rate of apoptosis was detected by Flow cytometry (Merck KGaA, Darmstadt, Germany).

Transwell assay

Transwell assay was performed using 8 μm polycarbonate membrane of chambers (Corning Inc., Corning, NY, USA) coated with Matrigel (BD Biosciences, San Jose, CA, USA) to detect cell invasion, but chambers without Matrigel were utilized to detect cell migration. The transfected SW480 and SW620 cells were collected and seeded into the upper chambers containing the serum-free medium. The lower chambers were filled with RPMI-1640 medium containing 10% FBS. After 24 h, the lower chamber cells were fixed with paraformaldehyde and stained with crystal violet. Finally, images were taken under a microscope (Shoif, Shanghai, China) to observe and count the number of metastasis of cells ($\times 200$).

Dual-luciferase reporter assay

The SNHG16 wild-type (SNHG16-WT) and) reporter vectors containing the miR-132-3p binding sites and mutant binding sites were synthesized by General Biosystems (Anhui, China). Similarly, USP22 3'UTR-WT/MUT reporter vectors were constructed in the same way. The above reporter vectors were co-transfected with miR-132-3p mimic and miR-NC into SW480 and SW620 cells. After 48 h, Dual-luciferase Reporter Assay Kit (Promega, Madison, WI, USA) was performed to detect the luciferase activity.

Western Blot (WB) analysis

SW480 and SW620 cells were lysed with lysis buffer (Beyotime). Protein concentration was measured using the BCA Assay Kit (Yeasen). Protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blockage with non-fat milk, the membranes were cultured with the primary antibodies against USP22 (1:2,000, Invitrogen) or β -actin (1:5,000, Invitrogen) overnight at 4°C. Then, the membranes were cultured with horseradish peroxidase-coupled secondary antibody (1:2,000, Invitrogen). Protein signals were observed with an enhanced chemiluminescence (ECL) solution (Beyotime).

Mice xenograft models

Twelve male BALB/c-nude mice (5-week-old) were bought from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). SW620 cells transfected with sh-SNHG16 or sh-NC ($n = 6$ per group) were subcutaneously injected into the mice. From d 7, the tumor length and width were recorded to calculate tumor volume every 5 d. After 27 d, the tumor was removed for weight measurement and qRT-PCR.

Statistical analysis

Data were presented as mean \pm standard deviation (SD). Statistical analysis was performed using GraphPad 5.0 software (GraphPad Software, La Jolla, CA, USA). The Student's *t*-test and one-way analysis of variance (ANOVA) were used to analyze the differences between the paired groups and multiple groups, respectively. Significant difference was defined as $P < 0.05$.

Results

SNHG16 was highly expressed in CRC

To investigate the effect of SNHG16 on CRC, we first detected the expression of SNHG16 in CRC. As shown in Fig. 1A, compared with adjacent normal tissues, SNHG16 expression was markedly increased in CRC tumor tissues. Besides, compared with CCD841 CON cells, the level of SNHG16 was also up-regulated in SW480 and SW620 cells (Fig. 1B). These indicated that SNHG16 had an abnormal expression in CRC.

Silencing of SNHG16 suppressed the proliferation, migration, invasion and promoted apoptosis of CRC cells

Subsequently, we verified the effect of SNHG16 on CRC cell progression by reducing the expression of SNHG16. The efficiency test results showed that si-SNHG16 had an excellent inhibitory effect on SNHG16 expression, which could be used for follow-up experiments (Fig. 2A-B). MTT assay revealed that knockdown of SNHG16 impaired proliferation ability of SW480 and SW620 cells (Fig. 2C-D). Flow cytometry confirmed that si-SNHG16 improved the apoptosis rate of CRC cells (Fig. 2E). Besides, Transwell assay results further demonstrated that the migration and invasion capacities of SW480 and SW620 cells were decreased by SNHG16 silencing (Fig. 2F-G). These results confirmed that SNHG16 played a pro-cancer role in CRC progression.

MiR-132-3p had a targeting relationship with SNHG16

To investigate the effect of SNHG16 on the proliferation and metastasis of CRC cells, we used Starbase3.1 to predict the target of SNHG16 and found that miR-132-3p had complementary binding sites with SNHG16. Subsequently, we constructed SNHG16-WT and SNHG16-MUT reporter vectors to verify the functional relationship between SNHG16 and miR-132-3p (Fig. 3A). Dual-luciferase reporter assay demonstrated that miR-132-3p mimic could significantly repress the luciferase activity of SNHG16-WT, while had no effect on SNHG16-MUT (Fig. 3B-C). Besides, miR-132-3p expression was increased by knockdown of SNHG16, and reduced by SNHG16 overexpression (Fig. 3D-E). Through qRT-PCR, we found that the expression of miR-132-3p was decreased in CRC tumor tissues and cells (Fig. 3F-G). Also, correlation analysis revealed that the expression of miR-132-3p was negatively correlated with SNHG16 in CRC tissues (Fig. 3H). Therefore, we confirmed that miR-132-3p could interact with SNHG16.

MiR-132-3p inhibitor recovered the suppression effects of SNHG16 knockdown on CRC cell progression

To verify whether SNHG16 regulating CRC cell progression by sponging miR-132-3p, SW480 and SW620 cells were co-transfected with si-SNHG16 and miR-132-3p inhibitor. The efficiency experiment results showed that the miR-132-3p inhibitor significantly blocked miR-132-3p expression (Fig. 4A). Through the test results of MTT, flow cytometry and Transwell assays, we concluded that miR-132-3p inhibitor could reverse the

inhibition effects of silenced-SNHG16 on the proliferation, migration and invasion of SW480 and SW620 cells, as well as the promotion effect on apoptosis (Fig. 4B-F). This indicated that SNHG16 regulated the proliferation, apoptosis, migration and invasion of CRC cells through miR-132-3p.

USP22 was a target of miR-132-3p

To clarify the mechanism of SNHG16, we used TargetScanHuman tool to predict the downstream target genes of miR-132-3p, and the results showed that USP22 had the complementary binding sites with miR-132-3p, so we constructed USP22 3'UTR-WT and USP22 3'UTR-MUT reporter vectors for the validation of Dual-luciferase reporter assay (Fig. 5A). The results showed that overexpression of miR-132-3p remarkably inhibited the luciferase activity of USP22 3'UTR-WT in SW480 and SW620 cells, without affecting USP22 3'UTR-MUT (Fig. 5B-C). Then, the mRNA and protein expression levels of USP22 were down-regulated in SW480 and SW620 cells transfected with miR-132-3p mimic (Fig. 5D-E). Besides, through detecting the expression of USP22, we found that USP22 was increased in CRC tumor tissues and cells (Fig. 5F-G), and was negatively correlated with miR-132-3p level (Fig. 5H). These results confirmed the targeting relationship between USP22 and miR-132-3p in CRC cells.

Overexpressed USP22 restored the inhibition effects of miR-132-3p on CRC cell progression

To confirm whether miR-132-3p regulating CRC cell progression through USP22, we transfected USP22 overexpression plasmid (pcDNA-USP22) with miR-132-3p mimic into SW480 and SW620 cells. The detection results of mRNA and protein expression showed that, compared with miR-132-3p mimic + pcDNA, the addition of pcDNA-USP22 could markedly restore the inhibition effect of miR-132-3p mimic on USP22 level (Fig. 6A-B). Further experimental verification showed that overexpression of USP22 reversed the suppression effects of miR-132-3p overexpression on the proliferation, migration and invasion of SW480 and SW620 cells, as well as the acceleration effect of apoptosis (Fig. 6C-G), further confirming that miR-132-3p participated in the progression of CRC through USP22.

SNHG16 improved USP22 expression via sponging miR-132-3p in CRC cells

Meanwhile, we examined the expression of USP22 under the premise of knocking down SNHG16 and miR-132-3p. QRT-PCR results showed that the inhibition of SNHG16 hindered USP22 level in SW480 and SW620 cells, and miR-132-3p inhibitor could increase the level of USP22 (Fig. 7A-B). Also, WB results confirmed that the inhibition of si-SNHG16 on the expression of USP22 could be alleviated by interfering with miR-132-3p in SW480 and SW620 cells (Fig. 7C-D). Detection of USP22 expression suggested that SNHG16 regulated USP22 level through miR-132-3p in CRC cells.

Interference of SNHG16 reduced tumor growth *in vivo*

We also constructed the mice xenograft models *in vivo* to further verify the effect of SNHG16 on CRC tumor growth. As shown in Fig. 8A, the tumor volume of the SNHG16 knockdown group was significantly smaller than that of the control group with an increase of transplantation days. After tumor weighing, we found that the tumor weight in the SNHG16 knockdown group was obviously decreased (Fig. 8B). According to qRT-PCR analysis, silenced-SNHG16 blocked the expression levels of SNHG16 and USP22 and promoted miR-132-3p

level in tumors (Fig. 8C). Besides, we also found that knockdown of SNHG16 significantly decreased the number of metastasis nodules (Fig. 8D). These data suggested that silencing of SNHG16 impeded the tumor growth of CRC through regulating the expression levels of miR-132-3p and USP22 *in vivo*.

Discussion

Recent studies have shown that SNHG16 was a potential oncogene in many cancers. In CRC, Li et al. reported that SNHG16 improved CRC progression and tumor growth [19]. In our system, consistent with published findings, SNHG16 was enriched in CRC tumor tissues and cells. Also, SNHG16 knockdown restrained the progression of CRC cells *in vitro* and reduced the tumor growth *in vivo*. These revealed that SNHG16 played a vital role in the development of CRC.

MiR-132-3p was a tumor suppressor miRNA in multiple cancers, including mesothelioma, bladder carcinoma and breast cancer [21–23]. Previous studies have covered the low level of miR-132-3p in CRC compared with normal samples, and overexpression of miR-132-3p restrained the proliferation and metastasis of CRC cells [24]. Song et al. suggested that miR-132-3p might involve in the regulation of XIST on CRC cell proliferation [25]. In our researches, we proved that SNHG16 contained binding sites of miR-132-3p through bioinformatics analysis and experimental verification. Also, the level of miR-132-3p was reduced in CRC and negatively correlated with SNHG16, and its inhibition had the opposite effect with silenced-SNHG16 in CRC cells. Besides, in mice xenograft models, the interference of SNHG16 also improved the level of miR-132-3p *in vivo*.

USP22 is a member of the deubiquitinating enzyme (DUB) family, which is related to the occurrence and development of various tumor types, including CRC [26–28]. Depletion of USP22 led to the accumulation in G1 phase and blocked the proliferation of CRC cells [29]. It has been identified that high USP22 expression was markedly associated with poor prognosis of CRC [30]. Consistent with previous research results, here, we found that USP22 expression was improved in CRC tissues and cells. Also, USP22 was a target of miR-132-3p. Moreover, the abnormal expression of USP22 recovered the detracting function of miR-132-3p overexpression on proliferation and metastasis of CRC cells, and promoted the CRC progression. These results confirmed that USP22 functioned as an oncogene, and was involved in the regulation of CRC processes.

To improve the functional relationship of SNHG16/ miR-132-3p/ USP22 axis, we also tested the effects of SNHG16 and miR-132-3p on USP22 level. The results revealed that inhibition of SNHG16 hindered USP22 expression, while silencing of miR-132-3p promoted its expression. Besides, in tumor tissues of mice xenograft models, knockdown of SNHG16 also reduced USP22 level *in vivo*. These results perfected the hypothesis that lncRNA SNHG16 functioned as a ceRNA to regulate the expression of downstream target genes through adsorption of miRNAs [31], and enriched the study on the effect of SNHG16 on CRC.

In summary, we concluded that lncRNA SNHG16 regulated USP22 expression to improve CRC progression through absorbing miR-132-3p. Our results provided a clear recognizing of the relationship between lncRNA SNHG16 and CRC molecular pathogenesis, providing a potential pathway for the treatment of CRC.

Abbreviations

CRC: Colorectal cancer; lncRNAs: Long non-coding RNAs; SNHG16: small nucleolar RNA host gene 16; miRNAs: microRNAs; ceRNA: competing endogenous RNA; FBS: fetal bovine serum; siRNA: Small interfering RNA; USP22: ubiquitin specific peptidase 22; miR-132-3p: miR-132-3p mimic; qRT-PCR: Quantitative real-time polymerase chain reaction; PBS: phosphate buffer saline; DMSO: dimethylsulfoxide; SNHG16-WT: SNHG16 wild-type; SNHG16-MUT: SNHG16 mutant-type;

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Liu Zhou People's Hospital and written informed consents were collected from all patients and hospitals.

The animal experiment was permitted by the Animal Research Committee of The Liu Zhou People's Hospital and performed in accordance with the guidelines of the National Animal Care and Ethics Institution.

Consent for publication

Not applicable

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no financial conflicts of interest

Funding

None

Authors' contributions

Xiaowen He participated in the conception and design of the study. Jun Ma performed the analysis and interpretation of data. Mingming Zhang contributed to drafting the manuscript. Jianhua Cui and Hao Yang reviewed and approved the final submitted manuscript.

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Figures

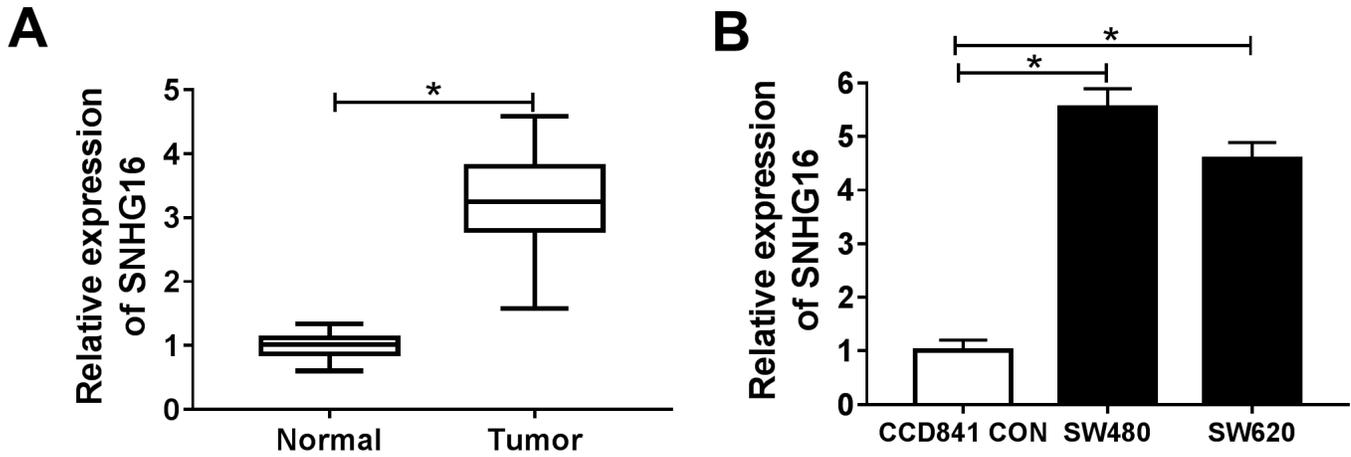


Figure 1

The expression of SNHG16 was higher in CRC tissues and cells. (A) The expression of SNHG16 was measured by qRT-PCR in CRC tumor tissues (Tumor) (n = 50) and adjacent normal tissues (Normal) (n = 50). (B) SNHG16 expression was detected by qRT-PCR in colonic epithelial cells (CCD841 CON) and CRC cells (SW480 and SW620). *P < 0.05.

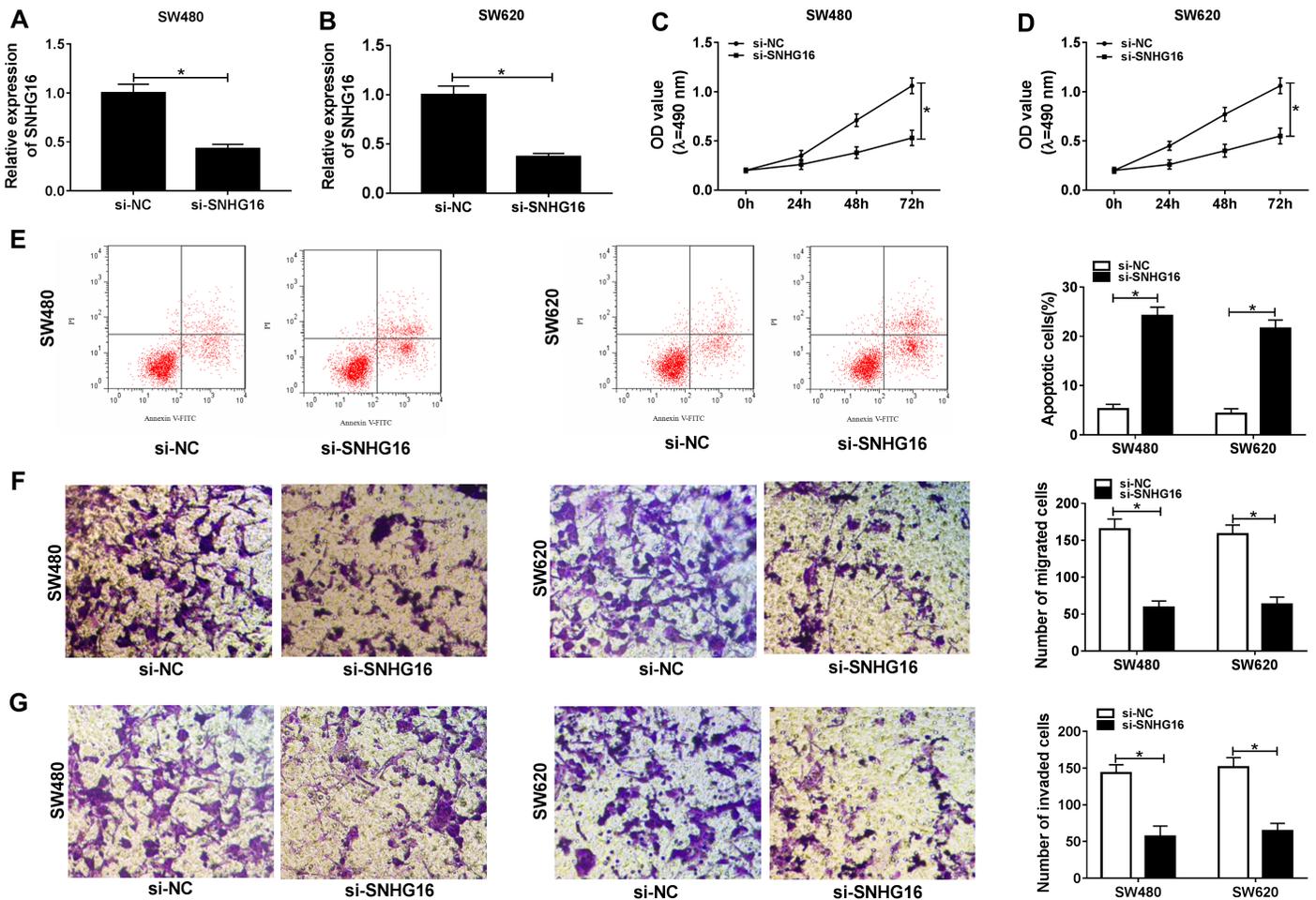


Figure 2

Silenced-SNHG16 regulated the progression of CRC cells. SW480 and SW620 cells were transfected with si-SNHG16 and si-NC. (A-B) The expression of SNHG16 was detected by qRT-PCR to evaluate transfection efficiency. MTT (C-D), flow cytometry (E) and Transwell (F-G) assays were used to measure the abilities of cell proliferation, apoptosis, migration and invasion, respectively. * $P < 0.05$.

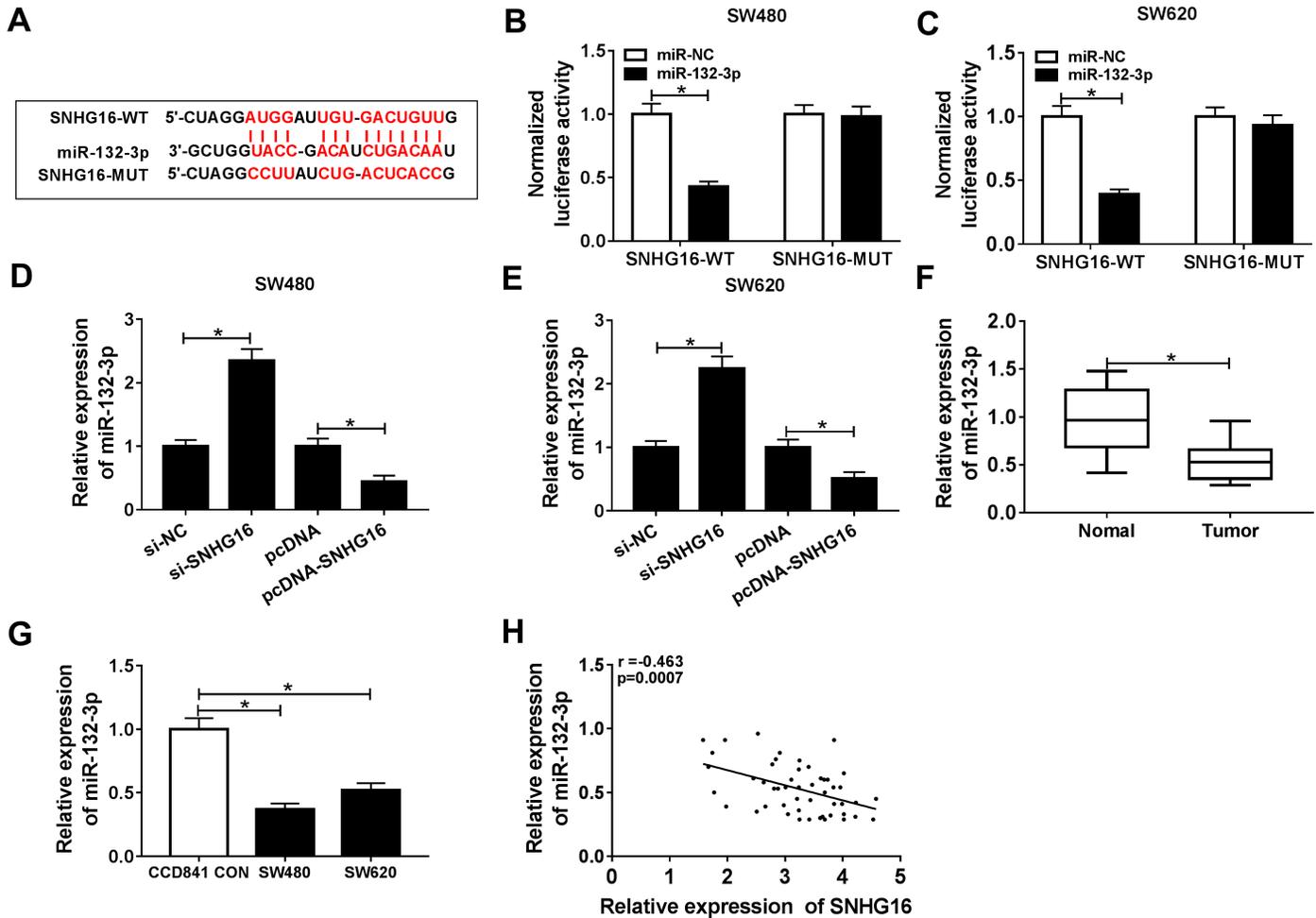


Figure 3

SNHG16 acted as a sponge of miR-132-3p. (A) SNHG16 containing miR-132-3p binding sites (SNHG16-WT) and mutant binding sites (SNHG16-MUT) were shown. (B-C) Dual-luciferase reporter assay was used to detect the interaction between miR-132-3p and SNHG16 in SW480 and SW620 cells. (D-E) The relative expression of miR-132-3p was examined by qRT-PCR in SW480 and SW620 cells transfected with si-SNHG16 and pcDNA-SNHG16. (F-G) The expression of miR-132-3p was decreased in CRC tumor tissues and cells detected by qRT-PCR. (H) The correlation between miR-132-3p and SNHG16 expression was measured by Pearson correlation coefficient analysis. * $P < 0.05$.

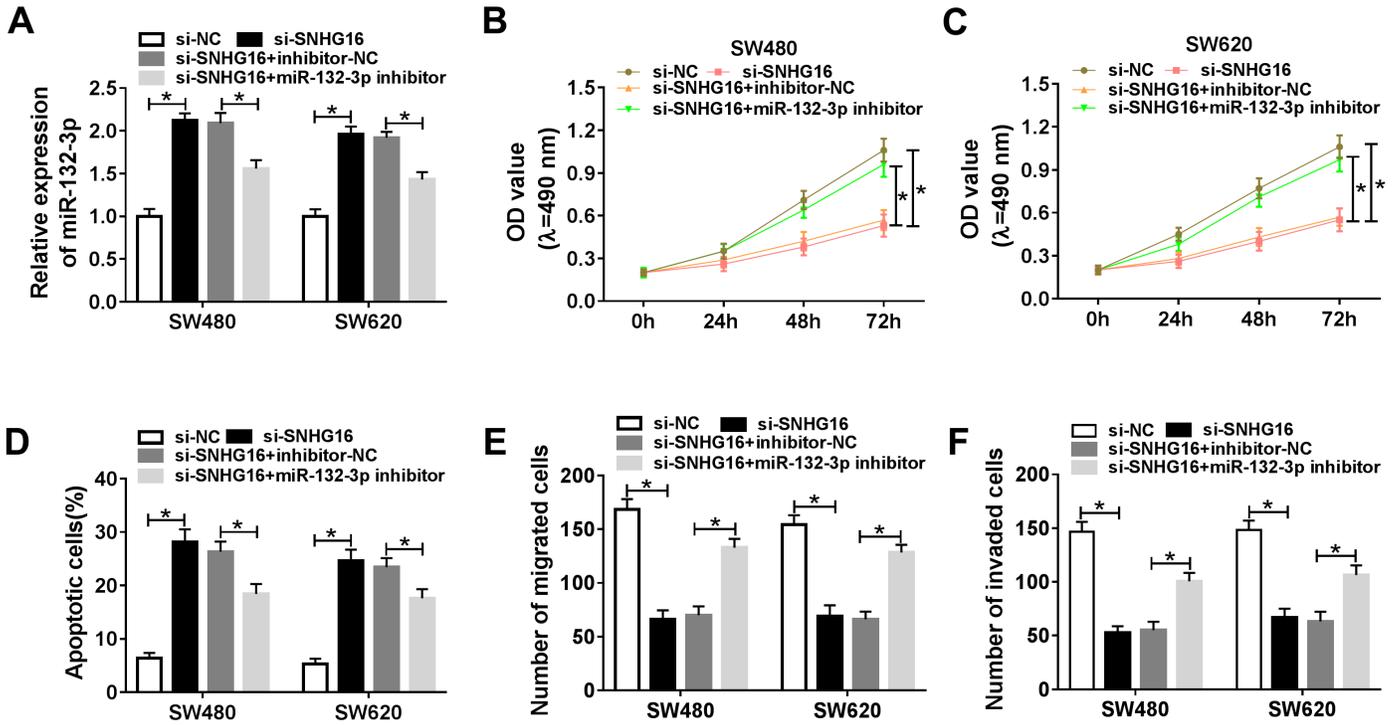


Figure 4

Knockdown of SNHG16 and miR-132-3p regulated the progression of CRC cells. SW480 and SW620 cells were co-transfected with si-SNHG16 and miR-132-3p inhibitor or their corresponding negative controls (si-NC and inhibitor-NC). (A) The expression of miR-132-3p was evaluated by qRT-PCR. (B-F) MTT (B-C), flow cytometry (D) and Transwell (E-F) assays were used to assess the abilities of cell proliferation, apoptosis, migration and invasion, respectively. * $P < 0.05$.

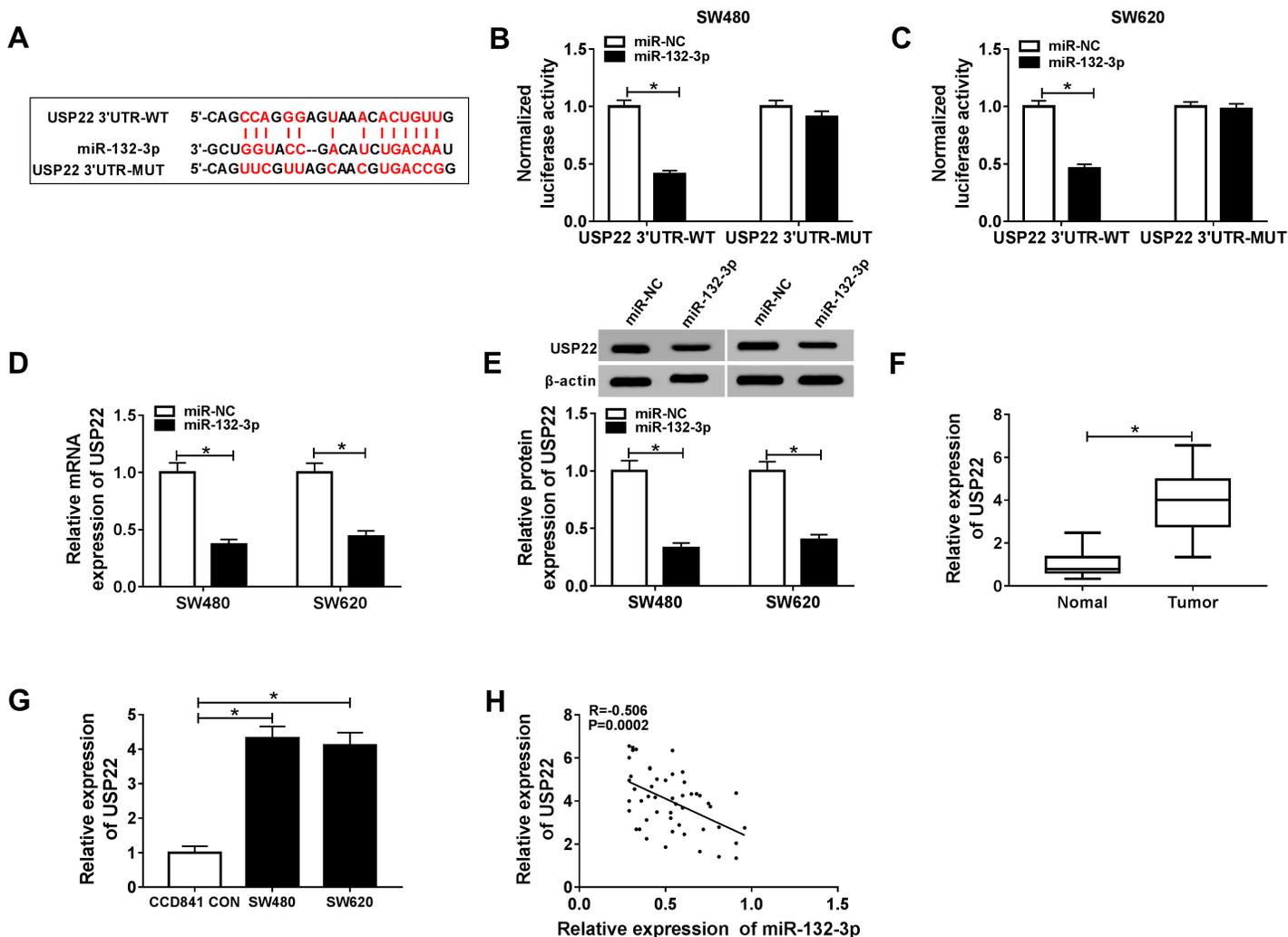


Figure 5

USP22 was a target of miR-132-3p. (A) Wild-type and mutant-type USP22 3'UTR reporter vectors (USP22 3'UTR-WT and USP22 3'UTR-MUT) were established according to the complementary sequences of miR-132-3p. (B-C) The luciferase activity of USP22 3'UTR-WT and USP22 3'UTR -MUT were detected by Dual-luciferase reporter assay. (D-E) The mRNA and protein levels of USP22 were examined by qRT-PCR and WB analysis in SW480 and SW620 cells transfected with miR-132-3p mimic. (F-G) The expression of USP22 was increased in CRC tumor tissues and cells detected by qRT-PCR. (H) The correlation between USP22 and miR-132-3p expression was determined by Pearson correlation coefficient analysis. * $P < 0.05$.

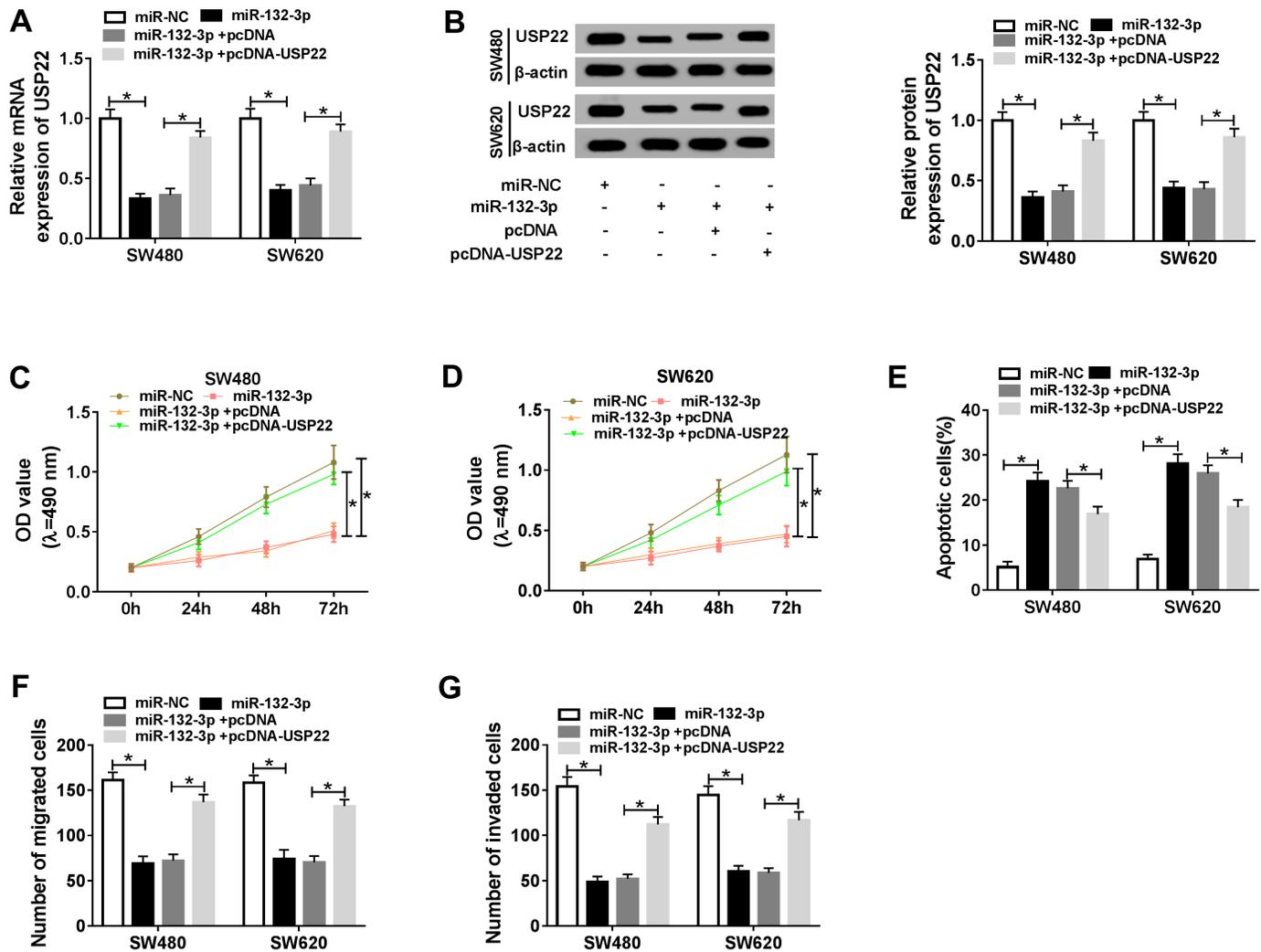


Figure 6

Overexpression of miR-132-3p and USP22 regulated the progression of CRC cells. MiR-132-3p mimic and pcDNA-USP22 or their negative controls (miR-NC and pcDNA) were co-transfected into SW480 and SW620 cells. (A) The expression of USP22 was evaluated by qRT-PCR. (B) The protein level of USP22 was measured by WB analysis. (C-G) MTT (C-D), flow cytometry (E) and Transwell (F-G) assays were used to detect the abilities of cell proliferation, apoptosis, migration and invasion, respectively. * $P < 0.05$.

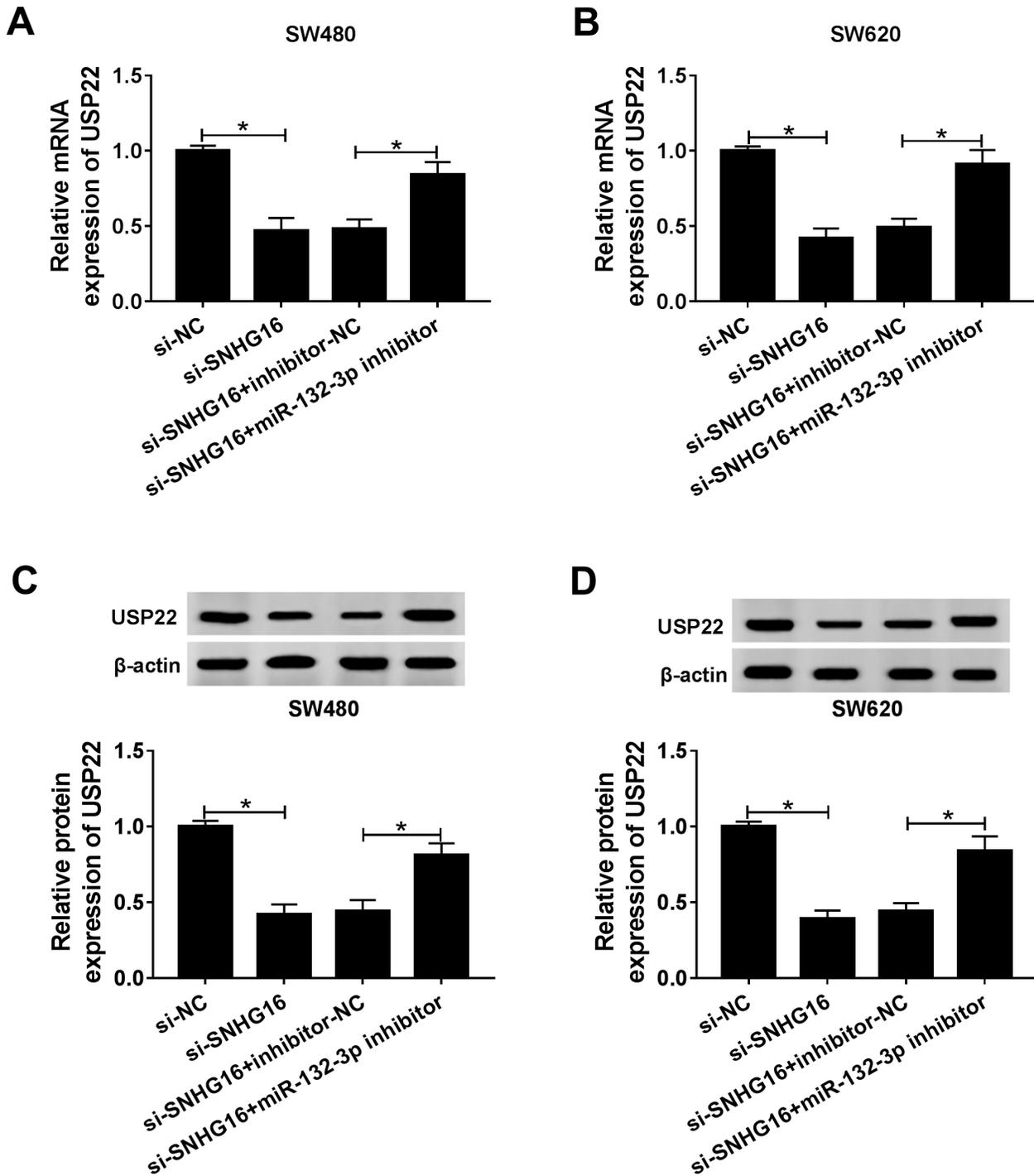


Figure 7

Inhibition of SNHG16 and miR-132-3p regulated the expression of USP22. SW480 and SW620 cells were co-transfected with si-SNHG16 and miR-132-3p inhibitor or their negative controls (si-NC and inhibitor-NC). (A-B) The expression of USP22 was examined by qRT-PCR. (C-D) WB analysis was performed to determine the protein level of USP22. * $P < 0.05$.

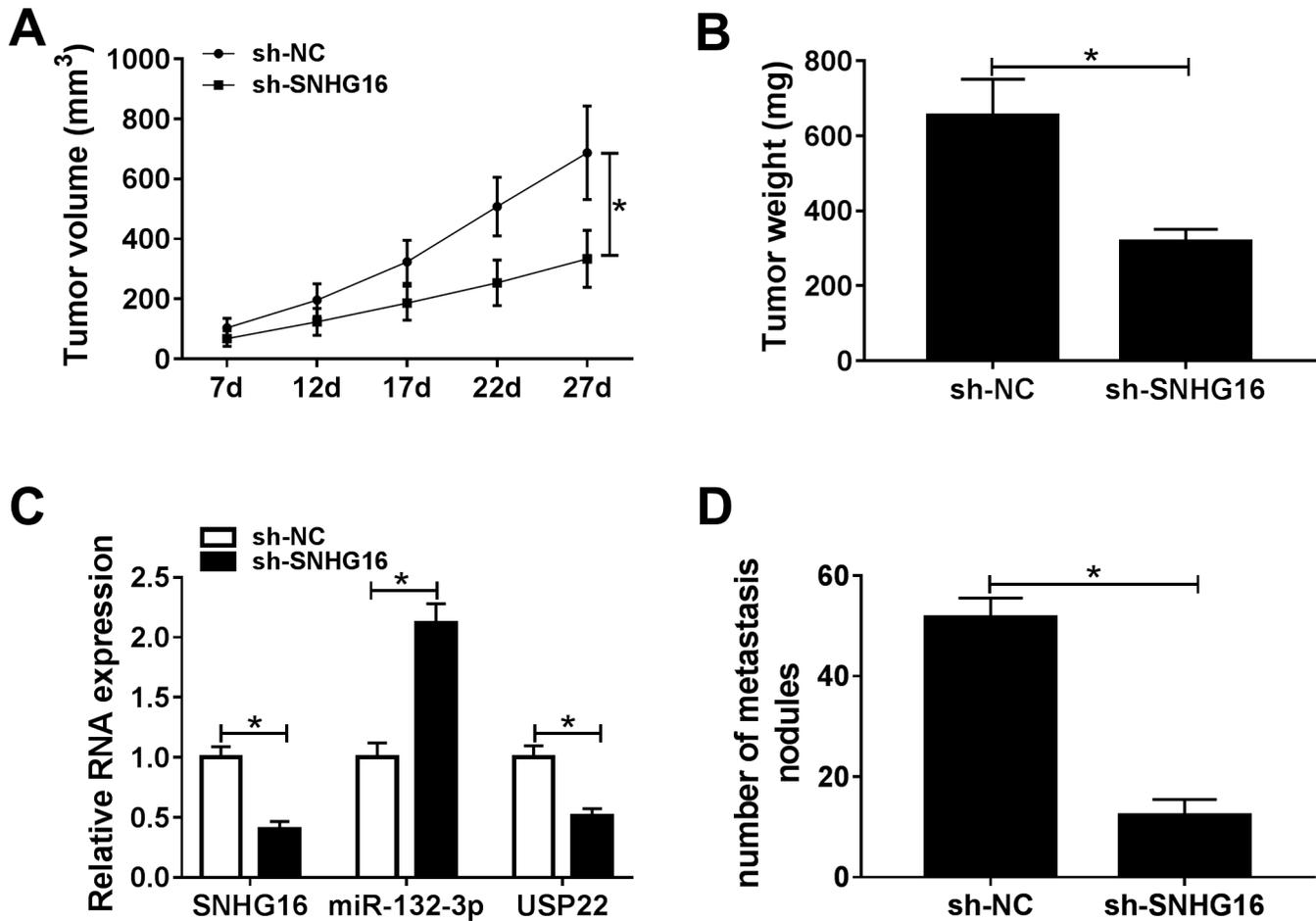


Figure 8

SNHG16 knockdown regulated the tumor growth of CRC in vivo. SW620 cells transfected with sh-SNHG16 or sh-NC were subcutaneously injected into the mice to construct the xenograft models. (A) Tumor volume was calculated with length \times width² / 2 method every 5 d from d 7 to d 27. (B) Tumor weight was measured in mice xenograft models. (C) The expression levels of SNHG16, miR-132-3p and USP22 were detected by qRT-PCR. (D) The number of metastasis nodules in the tumor was counted. *P < 0.05.