Original Article

Up-regulation of NCAPG mediated by E2F1 facilitates the progression of osteosarcoma through the Wnt/β-catenin signaling pathway

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Background: In recent years, there are few reports on non-SMC condensin I complex subunit G (NCAPG) in osteosarcoma. Our study aims to explore the biological role of NCAPG in osteosarcoma and its underlying molecular mechanism and to further clarify the reasons for the abnormal expression of NCAPG in osteosarcoma.

Methods: Here, we mined The Cancer Genome Atlas (TCGA) Program public database through bioinformatics methods, analyzed the differential expression of NCAPG in sarcoma tissue and normal tissue, and explored the relationship between NCAPG expression level and sarcoma tissue differentiation, including tumor recurrence, metastasis, and patient survival. Next, the transcription factors responsible for the abnormal expression of NCAPG in osteosarcoma tumors were predicted by multiple online website tools and verified via cellular experiments. Subsequently, loss of function and cell phenotype experiments were performed to confirm the effect of NCAPG on the malignant biological behavior of osteosarcoma cells. Mechanistically, by reviewing the literature, we found that NCAPG can affect the malignant progression of many solid tumors by regulating the Wnt/β-catenin signaling pathway. Therefore, we preliminarily investigated the potential effect of NCAPG on this pathway via western blot experiments in osteosarcoma.

Results: Increased expression of NCAPG was found in sarcoma compared to normal tissues, which was positively correlated with poor differentiation, metastasis, and poor prognosis. Combining the transcription factor prediction results, correlation analysis, and expression level in the TCGA public database with validation outcomes of *in vitro* cell assays, we found that E2F transcription factor 1 (E2F1) regulated the increased expression of NCAPG in osteosarcoma. The results of cell phenotype experiments showed that silencing NCAPG could inhibit the proliferation, migration, and invasion of osteosarcoma cells. The preliminary mechanistic investigation suggested that NCAPG may affect osteosarcoma progression through the Wnt/β-catenin pathway.

Conclusions: Our data reveal that E2F1 facilitates NCAPG expression in osteosarcoma by regulating the transcription of the NCAPG gene. Up-regulation of NCAPG promotes osteosarcoma progression via the Wnt/β-catenin signaling axis.

Keywords: Non-SMC condensin I complex subunit G (NCAPG); E2F transcription factor 1 (E2F1); osteosarcoma; Wnt/β-catenin signaling pathway

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Introduction

Osteosarcoma is a malignant tumor that occurs in adolescents and the elderly. Owing to the driving factors of osteosarcoma being complex and diverse, and the pathogenesis being unclear, it brings great challenges to the clinical treatment of patients. At present, the treatment methods for osteosarcoma mainly include surgery combined with chemotherapy and neoadjuvant chemotherapy. In addition, immunotherapy, gene therapy, and stem cell therapy are occasionally used clinically. However, some patients still have the risk of metastasis and recurrence, and the prognosis of patients is poor. Therefore, it is urgent to search for new biomarkers and therapeutic targets for osteosarcoma to improve the efficiency of diagnosis and treatment.

As a regulatory subunit, the protein encoded by the non-SMC condensin I complex subunit G (NCAPG) gene participates in the formation of the condensin complex, which plays a role in condensing and stabilizing chromosomes during mitosis and meiosis. In addition to its significant role in normal cellular physiological activities, increasing evidence has shown that NCAPG is also closely related to tumors. It has been found that NCAPG expression levels are associated with poor prognosis in a series of solid tumors including hepatocellular carcinoma, gastric cancer, ovarian cancer, etc. (1-7). Mechanistically, Wu *et al.* demonstrated that NCAPG could promote the progression of lung adenocarcinoma via the TGF-β signaling pathway (8). In cardia adenocarcinoma and colorectal cancer, studies indicated that overexpression of NCAPG facilitates epithelial-mesenchymal transition (EMT) through the Wnt/β-catenin signaling pathway (9,10). Interestingly, researchers revealed that NCAPG participates in trastuzumab resistance in human epidermal growth factor receptor 2 (HER2)-positive breast cancer via activating SRC/STAT3 signaling axis (11). In osteosarcoma, many studies have found that the Wnt/β-catenin signaling pathway is closely related to osteosarcoma progression and chemotherapy resistance, among others. Targeting this pathway can significantly inhibit tumor malignant progression while enhancing patient sensitivity to chemotherapy. However, whether NCAPG is involved in osteosarcoma progression by regulating the Wnt/β-catenin signaling axis needs to be further elucidated (12-14).

As a protein-coding gene, the expression of NCAPG is also regulated by a variety of molecules. MicroRNA (miRNA) is a class of non-coding single-stranded RNA molecules of about 22 nucleotides in length encoded by endogenous genes and involved in post-transcriptional gene expression regulation. Studies have clarified that a variety of miRNAs, including miR-99a-3p, miR-378-3p, miR-23b-3p, miR-214-3p, etc., are involved in regulating the expression of NCAPG in a series of malignant neoplasms (15-18). E2F transcription factor 1 (E2F1), a transcription factor closely related to cell cycle and tumor regulation, has been reported to be involved in the progression of cervical cancer, breast cancer, gastric cancer, and other tumors via regulating the expression of downstream target genes (19-21).

However, the regulatory relationship between NCAPG and its regulating transcription factors, especially E2F1, has not been reported yet.

Here, we utilized public databases and online website tools to explore the effect of NCAPG on the malignant biological behavior of osteosarcoma cells and its underlying molecular mechanism combined with *in vitro* experiments. Further, we have initially revealed the regulating mechanism that leads to the abnormal expression of NCAPG in osteosarcoma, hoping to provide a certain theoretical basis for the clinical diagnosis and precisely targeted therapy for osteosarcoma patients. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2175/rc).

Highlight box

Key findings

- Non-SMC condensin I complex subunit G (NCAPG) expression is abnormally upregulated in osteosarcoma;
- E2F transcription factor 1 (E2F1) binding in the NCAPG promoter region mediates its elevated expression in osteosarcoma;
- NCAPG promotes osteosarcoma progression via the Wnt/β-catenin signaling axis.

What is known and what is new?

- Previous studies indicated that NCAPG is associated with the prognosis and progression of a series of solid malignant tumors.
- Our study reveals that NCAPG expression is upregulated in osteosarcoma. We also elucidated that E2F1 binds to the promoter region of NCAPG to promote its transcription, and elevated NCAPG promotes osteosarcoma progression through the Wnt/β-catenin pathway.

What is the implication, and what should change now?

- The potential application value is to provide some theoretical support for the further exploration of diagnostic biomarkers for osteosarcoma in the future, as well as for precision-targeted therapies.

Methods

Cell culture and lentivirus transfection

Human osteosarcoma cell lines involved in this paper, including the hFOB 1.19, U2OS, and MG-63, were maintained in DMEM (A culture medium containing various amino acids and glucose) supplemented with 10% fetal bovine serum-containing 100 U/mL penicillin and streptomycin. HEK293 cells were used as tool cells and maintained in the same medium as osteosarcoma cell lines. The human osteoblast hFOB1.19 cells, as control normal cells, were maintained in DMEM/F-12 supplemented with 10% fetal bovine serum. All cell lines were cultured in a 37 ℃ incubator with a concentration of 5% CO_2. Lentivirus small hairpin RNA (shRNA) targeting NCAPG was obtained from Shanghai Gene Pharma Company. The detailed steps of transfection were performed according to the operating manuscript. The target sequence of NCAPG shRNA was as follows: sense: 5'-GCUGAAACAUUGCAGAAAUTT-3' and antisense: 5'-AUUUCUGCAAUGUUUCAGCTT-3'. Cell lines were obtained from National Collection of Authenticated Cell Cultures (hFOB 1.19, Organism: human, Gender: female, Morphology: epithelial cell and adherent, Serial: GNHu14, Identifier: CSTR:19375.09.3101HUMGNHu14; U2OS, Organism: human, Gender: female, Morphology: epithelial cell and adherent, Serial: TCHu 88, Identifier: CSTR:19375.09.3101HUMTCHu88; MG-63, Organism: human, Gender: male, Morphology: Fibroblast-like and adherent, Serial: TCHu124, Identifier: CSTR:19375.09.3101HUMTCHu124).

RNA isolation, reverse transcription, and reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Firstly, 1 mL TRIZOL was added to lyse the cells, then transferred the cells to a 1.5 mL EP tube. Chloroform of 200 μL was added to the tube, and it was then let stand at 4 ℃ for 15 minutes. The tube was centrifuged at 12,000 rpm for 15 minutes. The supernatant was transferred to another new 1.5 mL EP tube following adding an equal volume of isopropanol. The tube was placed at 4 ℃ for 10 minutes. After centrifuging at 14,000 rpm for 10 minutes, the EP tube was taken out, and the supernatant was discarded. Next, 1 mL of 75% absolute ethanol was added to the tube, and the tube was centrifuged at 7,500 rpm for 5 min. Finally, the supernatant was discarded, and 20 μL of enzyme-free water was added to the tube to dissolve the RNA. After measuring the RNA concentration, reverse transcription was performed with RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo-fisher, USA) according to the instruction manual. Then the cDNA obtained through reverse transcription was utilized for the RT-qPCR experiment. GAPDH was used as an internal control. Finally, calculate the relative quantitative value of each group through the $2^{-\Delta\Delta Ct}$ method. The primers related to our research were listed as follows: GAPDH: forward 5'-CTGGGCTACACTGAGCACC-3', reverse 5'-AAGTGGTCGTTGAGGGCAATG-3'; NCAPG forward 5'-ATCCAGAAGTTAGACGGGCAG-3', reverse 5'-GTGCGCCCTACAATTTTTGGC-3'; E2F1: forward 5'-ACGTGACGTGTCAGGACCT-3', reverse 5'-GATCGGGCCTTGTTTGCTCTT-3'.

Western blot

Osteosarcoma cells and normal osteoblast were lysed using cell lysis RIPA buffer and protease inhibitor at a ratio of 100:1. Subsequently, western blot was performed as described previously (22). Antibodies related to our research included anti-NCAPG (ab226805, Abcam, Cambridge, UK), anti-GAPDH (ab8245, Abcam, Cambridge, UK), anti-E2F1 (ab245308, Abcam), anti-Snail (ab216347, Abcam), anti-E-cadherin (#3195T, CST, Boston, USA), anti-Vimentin (#5741T, CST), anti-Wnt (#2721T, CST), anti-GSK3β (ab32391, Abcam), anti-p-GSK3β (ab68476, Abcam), anti-β-catenin (ab246504, Abcam), anti-p-β-catenin (ab75777, Abcam).

MTT

Cells were seeded in a 96-well plate at a density of 5×10^3 per well, with 6 replicates in each group. The cells were cultured in the incubator for 1 day, 2 days, and 3 days respectively. Then, cells were fixed with 4% paraformaldehyde and stained with crystal violet dye. Finally, the optical density (OD) value of the cells in each well was measured in a microplate reader.

Colony formation assay

1×10^3 cells were planted in a 6-well plate. After placing the cells in an incubator for 2 weeks, take out the six-well plate, and fix the cells with 4% paraformaldehyde for 30 minutes at 4 ℃. Subsequently, cells were stained with crystal violet

at room temperature for 30 minutes. Capture images and analyze the statistical results with Image J software.

Transwell and wound healing assay

Transwell assay and wound healing assay were performed as described previously (8). Differently, in the transwell experiment, the number of osteosarcoma cells planted in the upper chamber was about 8×10^3. In the wound healing assay, we seeded cells in a 6-well plate at a density of 2×10^4 per well.

Chromatin immunoprecipitation (CHIP)

Firstly, the crosslinking reaction was annulled by glycine in osteosarcoma cells treated with formaldehyde. Subsequently, sonication was utilized to process protein-DNA complexes into about 200–1,000 bp small fragments. Then, 5 μg of specific IP grade anti-E2F1 antibody was used so that the E2F1 antibody-NCAPG protein-DNA complex could be formed smoothly. Protein A/Protein G Sepharose beads were added to form an immunoprecipitated complex. After washing and reversing the cross-links, the enriched DNA was purified and then subjected to an RT-qPCR experiment.

Luciferase reporter assay

The human NCAPG luciferase reporter plasmid was constructed by inserting the NCAPG promoter sequence containing E2F1 binding sites or corresponding mutant sites into pGL3-Basic. The overexpression plasmid of E2F1 and the NCAPG luciferase reporter plasmid were co-transfected into HEK293 cells. Next, the biological activity of luciferase was detected by adding the luciferase substrate to the cells, and the transcriptional regulation of NCAPG by E2F1 was finally determined.

Online database

Osteosarcoma patients' clinical information was obtained from the The Cancer Genome Atlas (TCGA) public database (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga). Three online databases, JASPER (https://jaspar.genereg.net/), PROMO (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3/), and hTFtarget (http://bioinfo.life.hust.edu.cn/hTFtarget#!/), were used to predict transcription factors in the NCAPG promoter region (23-26). GEPIA (http://gepia.cancer-pku.cn/index.html) was used to analyze the relationship between NCAPG and survival in sarcoma patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

We downloaded the transcriptome-count raw data for sarcomas from the TCGA public database, which includes 2 normal and 263 sarcoma samples. We performed log2 (count+1) processing on the count data and performed normality tests (Anderson-Darling test, Shapiro-Wilk test, and Kolmogorov-Smirnov test) on the log2(count+1) data in GraphPad Prism software. Student *t*-test was finally used to compare the differential expression of NCAPG, MAZ, and E2F1 between normal and sarcoma patients. For cellular experiments differences in mean values between the two groups were compared using a *t*-test. The correlation between groups was assessed with the Pearson correlation coefficient. Kaplan-Meier method was utilized for survival analysis and the log-rank test was used to determine statistical significance. P value of less than 0.05 was considered statistically significant. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Results

NCAPG was up-regulated in sarcoma and was associated with a poor prognosis

Many studies have suggested that NCAPG is abnormally up-regulated in various malignant tumors (4,5,7-10). At the same time, among tumor patients, patients with higher NCAPG expression often indicate a poor prognosis. Considering that there are few studies on NCAPG in osteosarcoma, we analyzed the expression level of NCAPG in the tissues of patients with sarcoma in the TCGA public database by employing bioinformatics method. The results showed that the expression of NCAPG was significantly increased in sarcoma compared with normal tissues (*Figure 1A*). Further mining of the clinical information of sarcoma patients in the TCGA public database, we found that the expression of NCAPG was positively correlated with poor differentiation of sarcoma tissue (*Figure 1B*), but was not significantly correlated with tumor recurrence

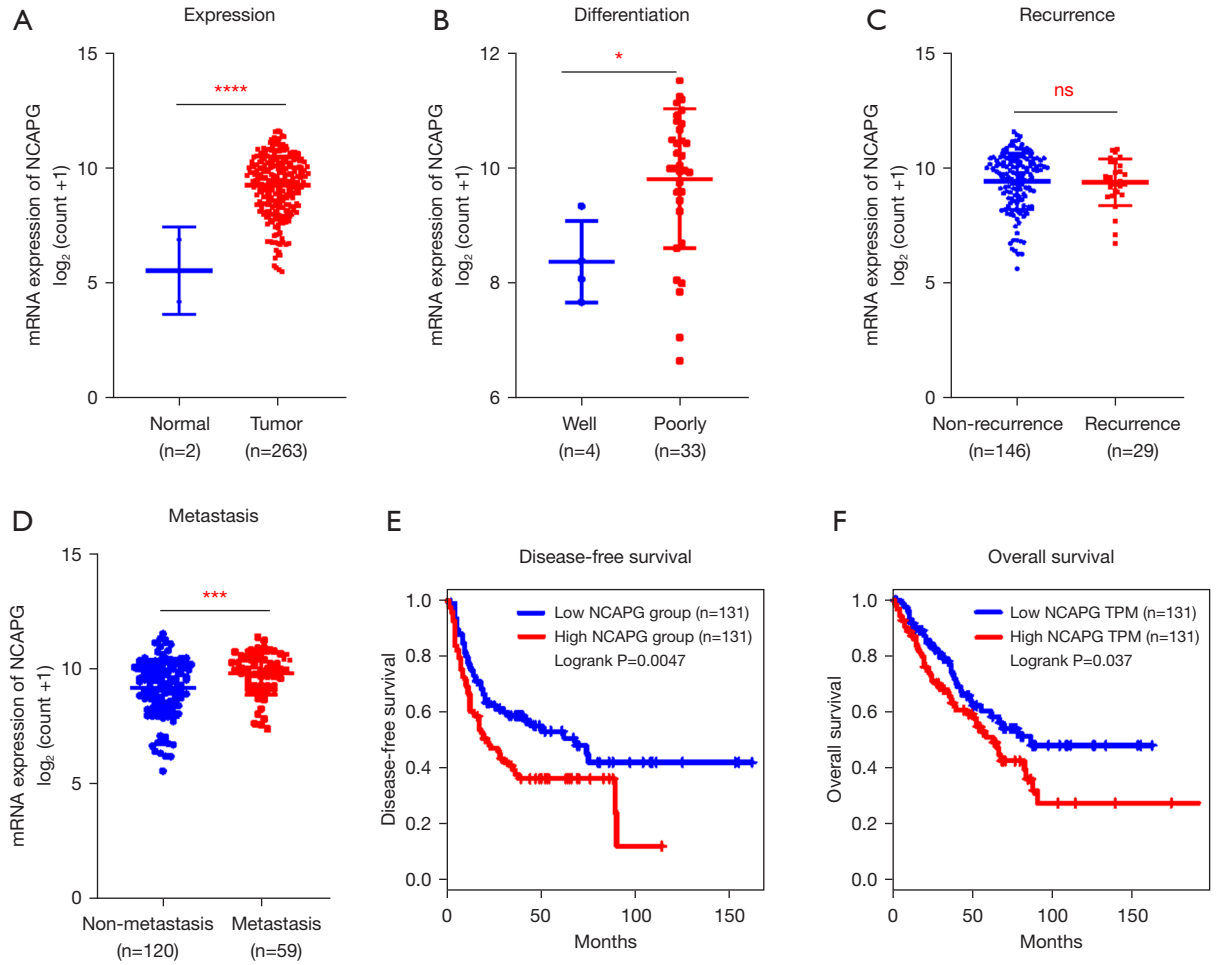

Figure 1 NCAPG was up-regulated in sarcoma and was associated with a poor prognosis. (A) Expression levels of NCAPG gene between sarcoma patients and normal patients via analyzing TCGA public database. (B-D) Relationship between NCAPG expression and sarcoma differentiation, tumor recurrence, and metastasis in TCGA database. (E) the disease-free survival time of sarcoma patients with the high or low expression level of NCAPG was analyzed via GEPIA. Log rank P=0.005. (F) the overall survival time of sarcoma patients with the high or low expression level of NCAPG was analyzed by GEPIA. Log rank P=0.04. *, P<0.05; ***, P<0.001; ****, P<0.0001; ns, no significance. NCAPG, non-SMC condensin I complex subunit G; TPM, transcripts per million; TCGA, The Cancer Genome Atlas; GEPIA, Gene Expression Profiling Interactive Analysis.

(*Figure 1C*). NCAPG is expressed at higher levels in patients with metastatic sarcoma compared to patients with non-metastatic sarcoma (*Figure 1D*). Combining the survival time and survival status of cases with sarcoma, we found that compared with patients with low NCAPG expression, patients with high NCAPG expression had worse disease-free survival and overall survival, which indicated that NCAPG expression was associated with a poor prognosis of patients (*Figure 1E,1F*).

E2F1 may act as a potential transcription factor for NCAPG via bioinformatics analysis

In the early stage, we found that the expression of NCAPG was increased in sarcoma through bioinformatics analysis. It is well known that transcriptional regulation is an important mechanism leading to increased gene expression. To further explore the molecular mechanism that causes the abnormal increase of NCAPG in sarcoma,

we first predicted the transcription factors in the promoter region of the NCAPG gene through the public databases JASPAR, PROMO, and hTFtarget, and comprehensively analyzed the transcription factors predicted from the three databases and found that the three transcription factors MYC associated zinc finger protein (MAZ), GATA binding protein 2 (GATA2), and E2F transcription factor 1 (E2F1) were common to the three database predictions (*Figure 2A*). Subsequently, we performed a correlation analysis between these three transcription factors and NCAPG, respectively, and found that in sarcoma, MAZ was positively correlated with NCAPG expression (r=0.3678) (*Figure 2B*), GATA2 was negatively correlated with NCAPG expression (r=–0.2880) (*Figure 2C*). E2F1 was also positively correlated with NCAPG (r=0.7415) (*Figure 2D*). Next, we analyzed the expression of MAZ and E2F1 in sarcoma and their relationship with tumor metastasis in the TCGA database. The results indicated that MAZ was up-regulated in sarcoma (*Figure 2E*), while it did not yet show a significant difference between metastatic and non-metastatic sarcoma (*Figure 2F*). E2F1 was elevated in sarcoma (*Figure 2G*). Moreover, E2F1 expression was higher in metastatic tumors compared to non-metastatic sarcoma (*Figure 2H*). The analysis results above indicated that E2F1 may be a potential transcription factor leading to the increased expression of NCAPG in sarcoma.

E2F1 regulated NCAPG transcription by binding to the promoter region of the NCAPG gene in osteosarcoma cells

To confirm the transcriptional regulation effect of E2F1 on NCAPG in osteosarcoma cells, we first detected the expression level of E2F1 by RT-qPCR and western blot. The results showed that compared with osteoblast, E2F1 expression was increased in osteosarcoma cells (*Figure 3A*,*3B*). We constructed stable NCAPG-silenced cell lines in U2OS and MG-63 cells and examined the influence of E2F1 knockdown on NCAPG expression. RT-qPCR and western blot results indicated that the expression of NCAPG was decreased after the knockdown of E2F1 (*Figure 3C-3E*). Next, we predicted the recognition motif of E2F1 by JASPAR (*Figure 3F*) and found that there were two E2F1 binding sites in the NCAPG promoter region (*Figure 3G*). Subsequently, we found via CHIP experiments that E2F1 mainly bound at site1 but not at site2 in the NCAPG promoter region (*Figure 3H*,*3I*). Finally, we performed luciferase reporter experiments by co-transfecting the E2F1 overexpression plasmid and NCAPG wild-type/mutant-type luciferase reporter plasmid in HEK-293 cells, and the results suggested that the luciferase activity of the wild-type group was higher than that of the mutant group, which further confirmed that E2F1 activated NCAPG transcription by binding at E2F1 binding site 1 in the NCAPG promoter region (*Figure 3J*).

Silencing of NCAPG inhibited the proliferation ability of osteosarcoma cells

To explore the effect of NCAPG on the malignant biological behavior of osteosarcoma cells, we first verified the elevated expression of NCAPG in osteosarcoma cells by RT-qPCR and western blot experiments (*Figure 4A-4C*). Subsequently, we used shRNA targeting NCAPG to inhibit the expression of NCAPG in U2OS and MG-63 cells (*Figure 4D-4F*). The results of MTT experiments showed that compared with the control group, the growth of NCAPG knockdown cells was slowed down (*Figure 4G*,*4H*). Clonal formation experiments indicated that knockdown of NCAPG reduced the ability of U2OS and MG-63 cells to form clonal colonies (*Figure 4I*,*4J*). These results suggested that the proliferation of osteosarcoma cells was attenuated after NCAPG inhibition.

Knockdown of NCAPG suppressed the invasion and migration of osteosarcoma via restraining the Wnt/β-catenin pathway

By performing wound healing experiments on U2OS and MG-63, we found that after 24 hours of scratching, the wound repairability of osteosarcoma cells in the knockdown NCAPG group was significantly inferior to that in the control group (*Figure 5A-5D*). The results of transwell assays also suggested that inhibiting the expression level of NCAPG could weaken the invasive ability of U2OS and MG-63 (*Figure 5E*,*5F*). EMT is a known pathway significantly associated with tumor cell invasion and migration. Therefore, we examined the effect of NCAPG on EMT-related molecular markers, and the results showed that after knocking down NCAPG, the expression of E-cadherin protein was increased, and the levels of Snail, N-cadherin, and Vimentin were decreased (*Figure 5G*). This suggested that inhibition of NCAPG can repress the EMT process. Wnt/β-catenin is a switch for the EMT process, so we would like to know whether NCAPG has a certain

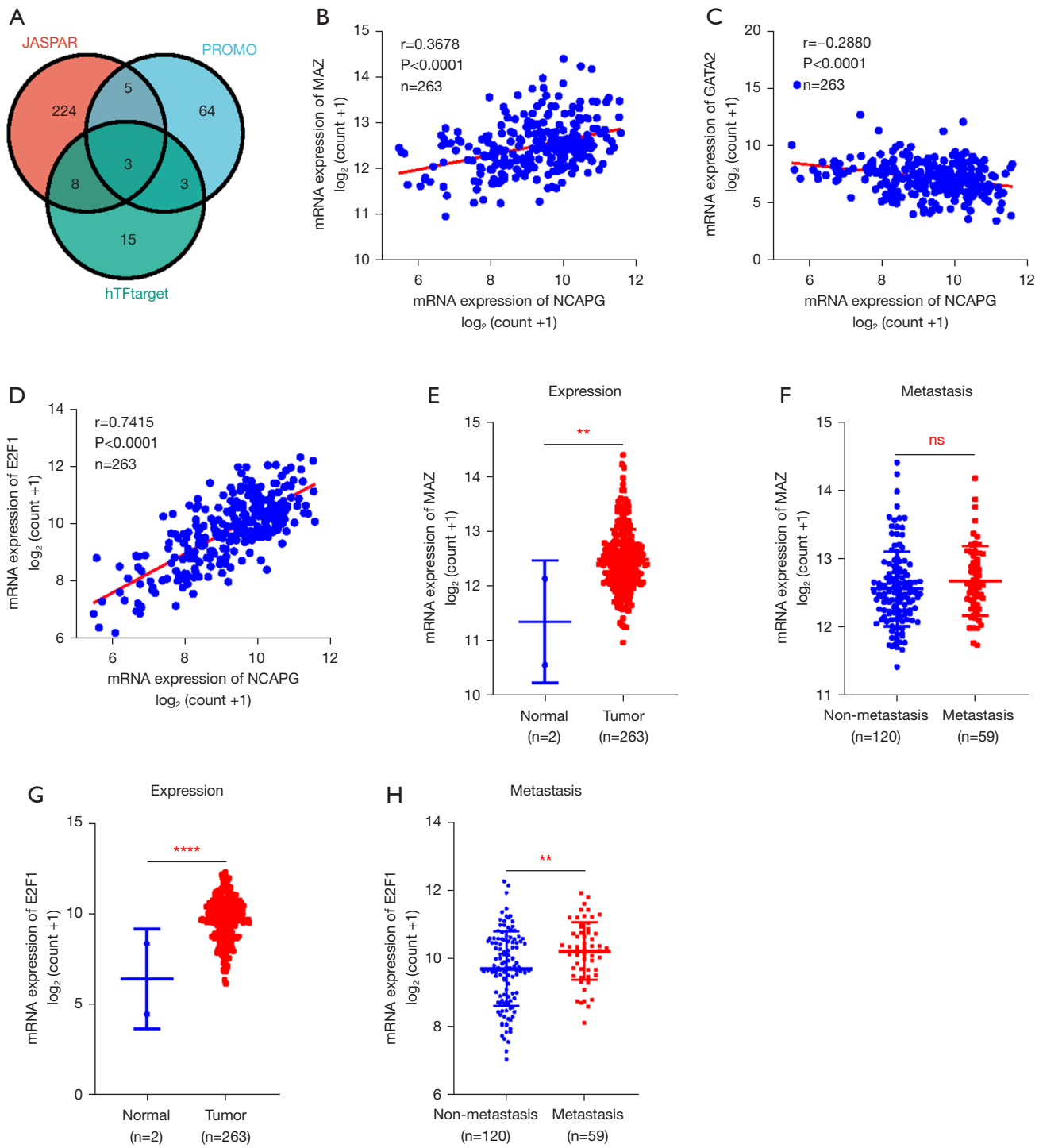

Figure 2 E2F1 may act as a potential transcription factor for NCAPG via bioinformatics analysis. (A) Venn diagram showing the results of three online databases (JASPAR, PROMO, and hTFtarget) to predict NCAPG common transcription factors. (B-D) Correlation analysis of transcription factors MAZ, GATA2, and E2F1 with NCAPG in TCGA public database. (E-H) Expression levels of transcription factors MAZ and E2F1 in sarcoma patients and normal patients and their relationship with metastasis. **, P<0.01; ****, P<0.0001; ns, no significance. MAZ, MYC associated zinc finger protein; NCAPG, non-SMC condensin I complex subunit G; GATA2, GATA binding protein 2; E2F1, E2F transcription factor 1.

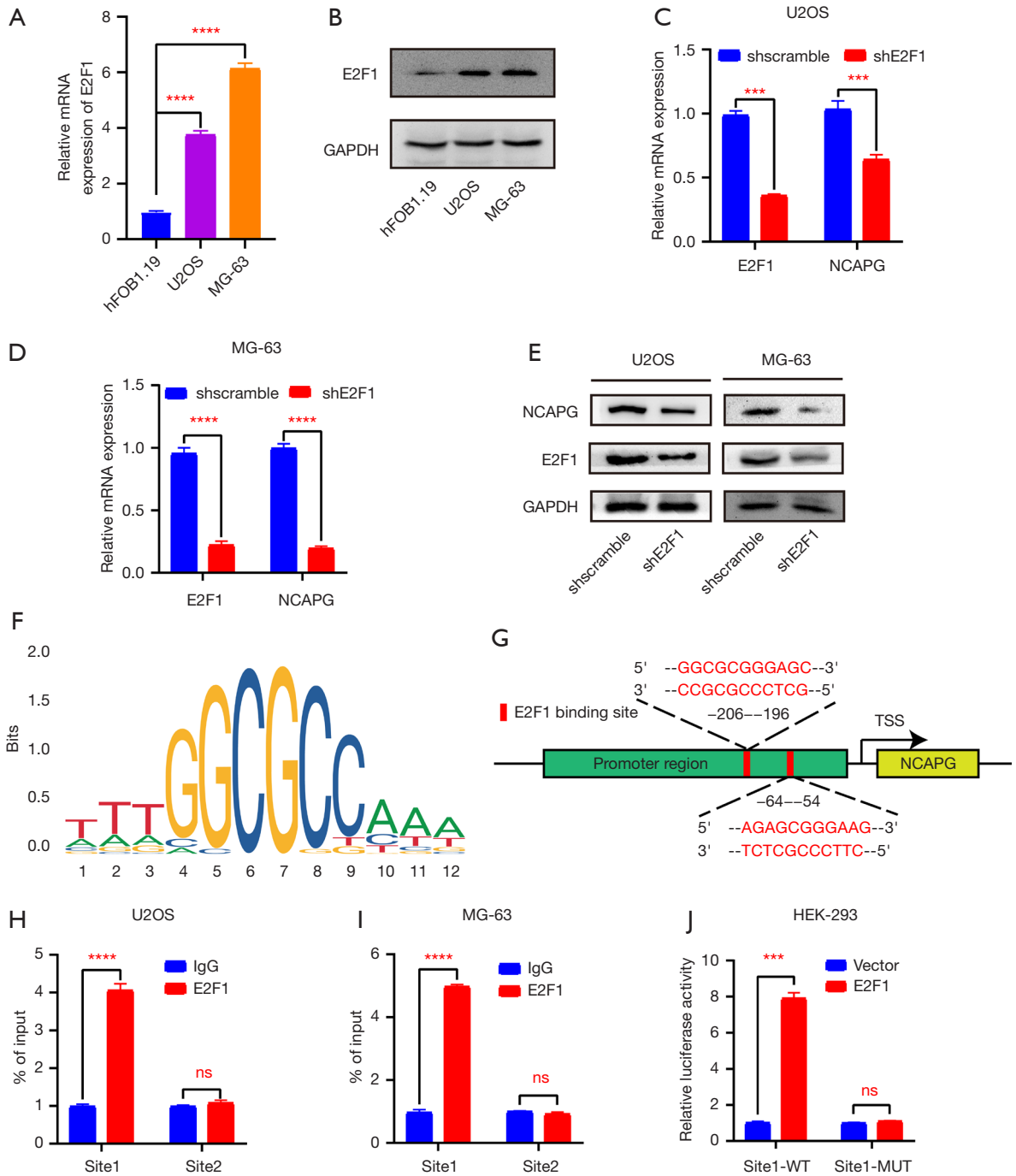

Figure 3 E2F1 regulated NCAPG transcription by binding to the promoter region of the NCAPG gene in osteosarcoma cells. (A,B) Detection of E2F1 expression at the mRNA and protein levels in osteoblast and osteosarcoma cell lines. (C-E) The effect of the knockdown of E2F1 on NCAPG expression was examined in U2OS and MG-63 cell lines. (F) Prediction of binding motifs of transcription factor E2F1 by JASPAR online tool. (G) Two E2F1 binding sites may exist in the promoter region of NCAPG. (H,I) CHIP-qPCR verifies the binding site of E2F1 in the NCAPG promoter region in U2OS and MG-63 cells. (J) The binding site of E2F1 in the NCAPG promoter region was further confirmed by CHIP-qPCR experiments after site 1 was mutated. IgG was used as negative control. ***, P<0.001; ****, P<0.0001; ns, no significance. E2F1, E2F transcription factor 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NCAPG, non-SMC condensin I complex subunit G; CHIP-qPCR, chromatin immunoprecipitation-quantitative polymerase chain reaction.

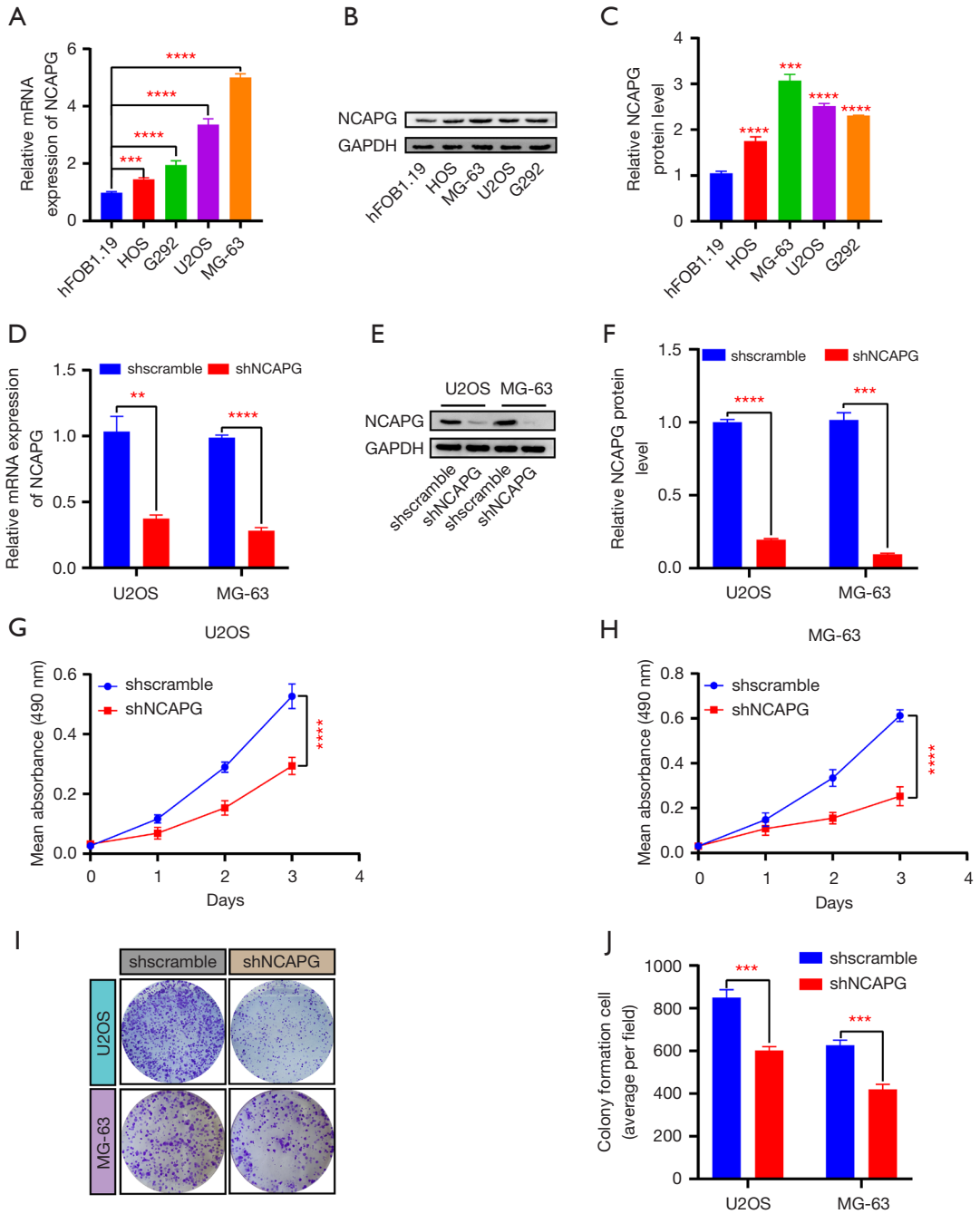

Figure 4 Silencing of NCAPG inhibited the proliferation ability of osteosarcoma cells. (A,B) The expression difference of NCAPG in normal osteoblasts and osteosarcoma cells was explored by RT-qPCR and western blot experiments. (C) Quantitative analysis of western blot in (B). Differential expression was analyzed based on osteosarcoma cells versus normal osteoblasts (hFOB1.19). (D-F) Construction of cell lines with stable knockdown of NCAPG in U2OS and MG-63. Validation of the efficiency of constructing knockdown NCAPG cell lines in U2OS and MG-63 cells at the mRNA (D) and protein (E,F) levels, respectively. (G-J) MTT (G,H) and colony formation (I,J) assays were used to detect the proliferation ability after NCAPG was knocked down. Colony formation experiments were performed in six-well plates (crystal violet staining). Each image of colony formation experiments is a representation of one of the six wells (non-local). ***, P<0.001; ****, P<0.0001. NCAPG, non-SMC condensin I complex subunit G; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

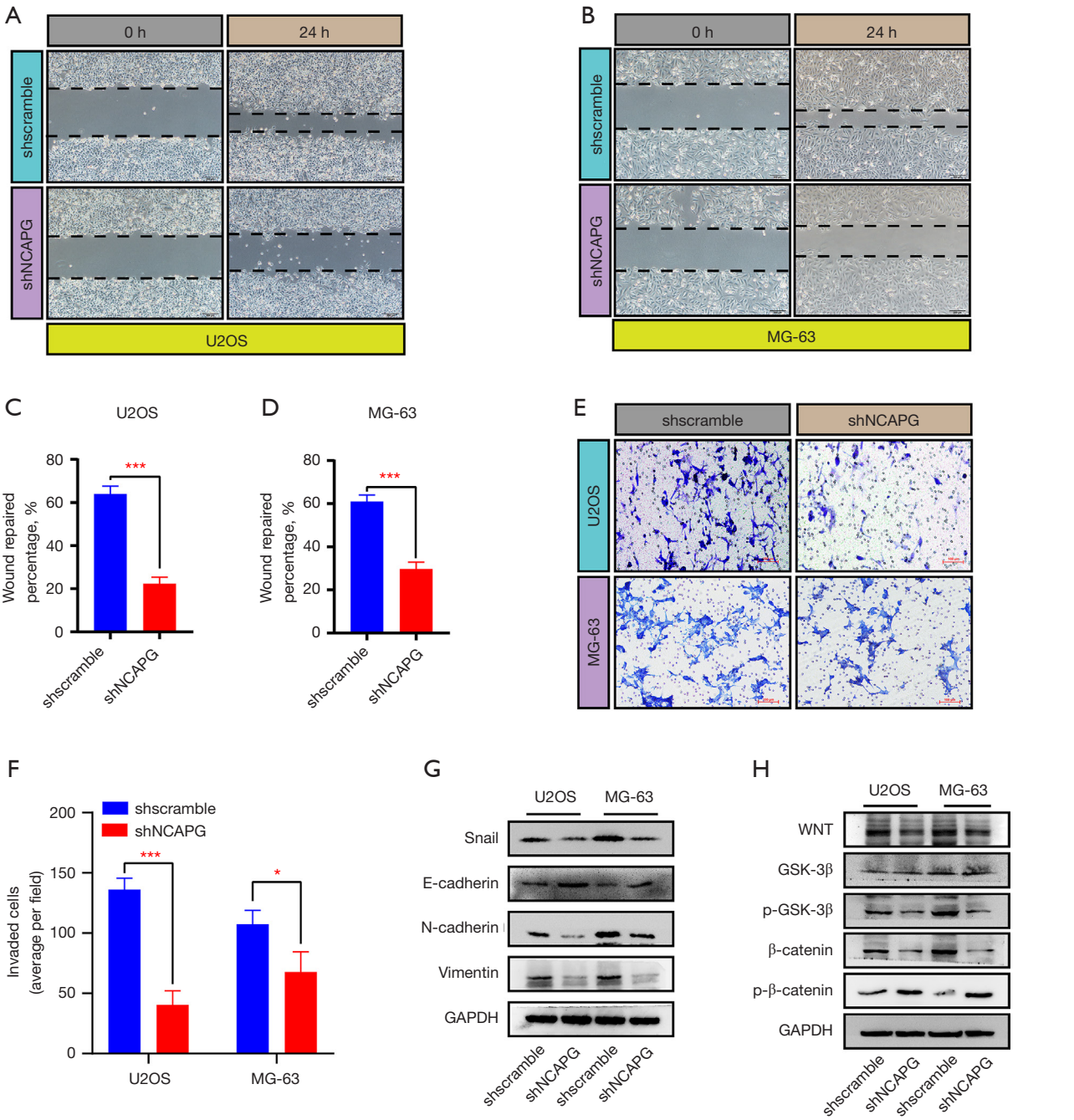

Figure 5 Knockdown of NCAPG suppressed the invasion and migration of osteosarcoma via restraining the Wnt/β-catenin pathway. (A-D) Wound healing experiments were carried out to explore the effect of NCAPG knockdown on the migration of U2OS and MG-63 cells. The results of wound healing experiments were observed under a normal light microscope (no staining required), magnification: 40× (E,F) Transwell (crystal violet staining) was used to detect the invasion ability after NCAPG was knocked down. Magnification: 100×. (G) EMT-related molecular markers were detected after silencing of NCAPG via western blot. (H) Explore the effect of NCAPG on the expression levels of Wnt/β-catenin pathway-related proteins. *, P<0.05; ***, P<0.001. NCAPG, non-SMC condensin I complex subunit G; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EMT, epithelial-mesenchymal transition.

regulatory effect on the Wnt/β-catenin pathway. Western Blot results indicated that Wnt/β-catenin was inhibited by silencing NCAPG (*Figure 5H*), which preliminarily proved that NCAPG may regulate the invasion and migration of osteosarcoma through the Wnt/β-catenin pathway, and ultimately accelerate the progression of osteosarcoma.

Discussion

Osteosarcoma is the most common primary bone malignancy with a low five-year survival rate. According to statistics, about 20% of patients with osteosarcoma have lung metastases at the time of diagnosis, thus losing an excellent opportunity for treatment. Therefore, early diagnosis is of great significance for the treatment and prognosis of patients with osteosarcoma. Current studies have shown that molecules including Snail, MICA, RASSF1A, HER2, MMP-2, MMP-9, and SATB2 have a certain significance in the diagnosis of osteosarcoma (27-33). However, specific biomarkers for the diagnosis of osteosarcoma are still lacking. In our study, we preliminarily found that NCAPG expression was abnormally elevated in osteosarcoma through bioinformatics analysis and cell experiment validation. At the same time, the results of the public database indicated that there was a certain correlation between the high expression level of NCAPG and the differentiation and tumor recurrence of osteosarcoma. Nonetheless, it should be noted that our study has examined only the expression level in the database and in vitro. Our results were not validated by sufficient tissue samples due to the limited access to tissue samples, which is where we need to work to supplement in the future.

Existing studies have shown that a variety of genes and related signaling pathways including receptor tyrosine-related pathways, PI3K/AKT signaling pathway, MAPK signaling pathway, TP53 mutation, PTEN mutation, etc. are involved in the occurrence and progression of osteosarcoma. Therapeutic modalities targeting the above-mentioned signaling axes and mutated genes have achieved considerable efficacy. However, due to tumor heterogeneity, different patients may respond significantly to the same treatment modality. This prompts us to search for new targeting molecules for osteosarcoma, which can provide certain theoretical guidance for the subsequent development of new clinical drugs.

Previous studies indicated that NCAPG is associated with the prognosis and progression of a series of solid malignant tumors including gastric cancer, breast cancer, oral squamous cell carcinoma, prostate cancer, etc. Loss of NCAPG inhibits tumor cell proliferation, migration, and invasion (1,11,18,21). Mechanistically, the knockdown of NCAPG induces the G0/G1 cell cycle arrest and accordingly inhibits cell division in gastric cancer (1). In non-small cell lung cancer, the function of NCAPG in promoting tumor initiation and progression is closely related to LGALS1 (4). Moreover, in HER2-positive breast cancer, NCAPG could facilitate trastuzumab resistance via mediating the SRC/STAT3 signaling pathway. Inhibition of NCAPG expression in breast cancer cells enables drug-resistant patients to regain trastuzumab-sensitive characteristics (11). In our research, we initially found that NCAPG could regulate tumor progression by regulating the Wnt/β-catenin pathway. Inhibition of NCAPG expression can suppress the proliferation and invasion of osteosarcoma cells to a certain extent. These results suggested that the development of small-molecule drugs targeting MCAPG would have great clinical application prospects. As mentioned earlier, many non-coding miRNAs can bind to NCAPG mRNA molecules, reduce the expression level of NCAPG in tumors, and then achieve the effect of inhibiting tumor progression. In this study, we confirmed for the first time through bioinformatics analysis and cell experiments that E2F1 can regulate the transcription of the NCAPG gene in osteosarcoma, and then play a role in promoting tumor malignant progression. This suggests that E2F1 has certain feasibility as a target for the treatment of osteosarcoma. Nonetheless, our research on the detailed molecular mechanisms by which NCAPG affects osteosarcoma progression is not deep enough, which is also the direction we need to continue to explore in the future.

Conclusions

In summary, our study found that NCAPG was abnormally highly expressed in sarcoma, and its high expression level was significantly associated with the poor prognosis of patients. Further exploration indicated that the transcription factor E2F1 can bind to the promoter region of the NCAPG gene to promote the transcription of NCAPG, which is one of the mechanisms leading to the up-regulation of NCAPG in osteosarcoma. Next, we expounded through phenotypic experiments that silencing of NCAPG restricted the proliferation and invasion of osteosarcoma

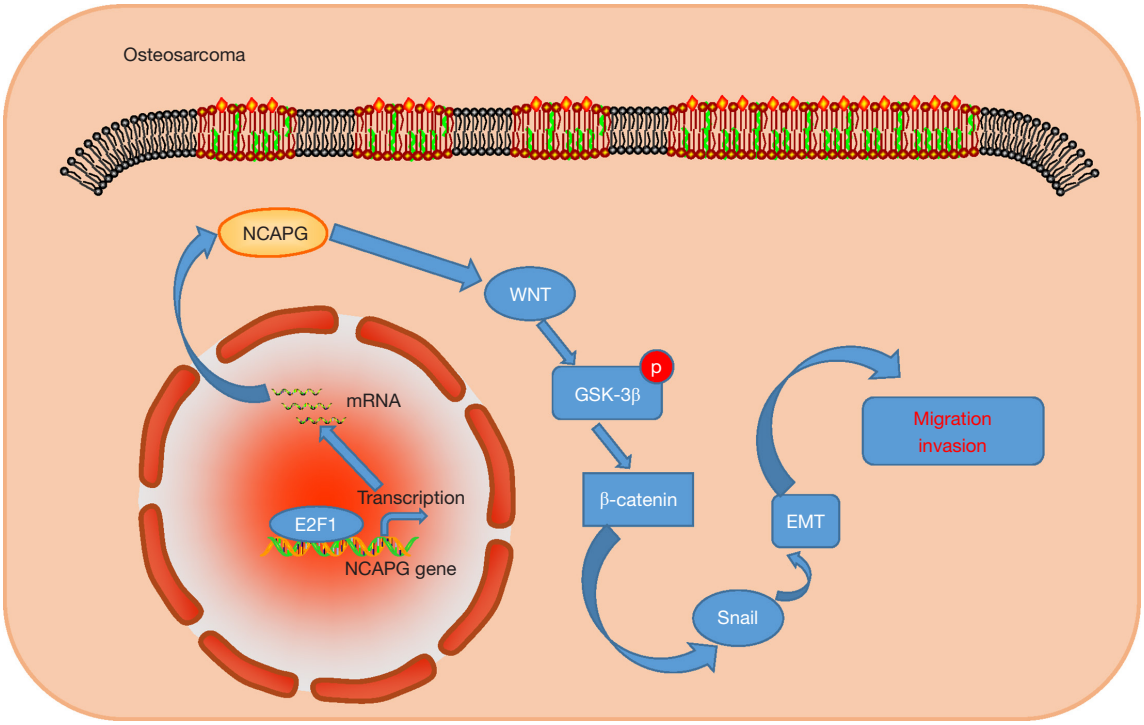

Figure 6 Mechanism diagram of NCAPG regulating the progression of osteosarcoma. E2F1, E2F transcription factor 1; NCAPG, non-SMC condensin I complex subunit G; EMT, epithelial-mesenchymal transition.

cells. Preliminary exploration results suggested that the promoting effect of NCAPG on osteosarcoma may be related to the activation of the Wnt/β-catenin signaling pathway (*Figure 6*).

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2175/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2175/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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