Original Article

NCAPG is transcriptionally regulated by *CBX3* and activates the Wnt/β-catenin signaling pathway to promote proliferation and the cell cycle and inhibit apoptosis in colorectal cancer

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Background: Colorectal cancer (CRC) is highly heterogeneous at the genetic and molecular level and a major contributor to cancer-death worldwide. Non-structural maintenance of chromosomes (SMC) condensin I complex subunit G (*NCAPG*) is a subunit of condensin I and has been shown to be associated with the prognosis of cancers. This study investigated the functional role of *NCAPG* in CRC and its mechanism.

Methods: Messenger RNA (mRNA) and protein expressions of *NCAPG* and chromobox protein homolog 3 (*CBX3*) were determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot. The proliferation, cycle, and apoptosis of HCT116 cells were analyzed by Cell Counting Kit-8 (CCK-8), flow cytometry, and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. RT-qPCR and western blot were used to determine the transfection efficacy of short hairpin (sh)-NCAPG and sh-CBX3. Western blot was used to explore cycle-, apoptosis-, and Wnt/β-catenin signaling-related proteins, and the activity of *NCAPG* promoter was evaluated using a luciferase report assay. The expressions of cleaved caspase9 and cleaved caspase3 were assessed by colorimetric caspase activity assay.

Results: The results showed that *NCAPG* expression was elevated in CRC cells. After transfection with sh-NCAPG, *NCAPG* expression was reduced. It was also discovered that *NCAPG* knockdown suppressed proliferation and the cell cycle but induced apoptosis in HCT116 cells. The Human Transcription Factor Database (HumanTFDB; http://bioinfo.life.hust.edu.cn/HumanTFDB#!/) predicted the binding sites of *CBX3* and *NCAPG* promoters. Meanwhile, the Encyclopedia of RNA Interactomes (ENCORI) database (https://starbase.sysu.edu.cn/) revealed that *CBX3* was positively correlated with *NCAPG*. Our results showed that *NCAPG* was transcriptionally regulated by *CBX3*. Additionally, Wnt/β-catenin signaling was discovered to be activated by *CBX3* overexpression. Further experiments showed that *NCAPG* transcriptionally regulated by *CBX3* activated Wnt/β-catenin signaling to regulate the proliferation, cell cycle, and apoptosis of HCT116 cells.

Conclusions: Collectively, the results of our study indicated that *NCAPG* was transcriptionally regulated by *CBX3* and activated the Wnt/β-catenin signaling pathway to facilitate the progression of CRC.

Keywords: Colorectal cancer (CRC); non-structural maintenance of chromosomes condensin I complex subunit G (*NCAPG*); chromobox protein homolog 3 (*CBX3*); Wnt/β-catenin signaling

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Introduction

Colorectal cancer (CRC) develops from the uncontrolled division or abnormal growth of colon or rectum cells (1). As the second most common adult cancer in females and the third most common in males, CRC is the fourth major contributor to cancer deaths, accounting for 9.2% of deaths worldwide (2,3). In 2020, about 1.5 million people were diagnosed with CRC and more than 50,000 people died of it (4). It is well-documented that environmental and genetic factors are critical in the pathogenesis of CRC (1). In recent years, tremendous progresses have been achieved in surgical and medical treatments, and the potential of targeted therapies has shown great promise (5). Nevertheless, CRC is prone to tumor metastasis and distant metastasis is a critical cause of poor prognosis (6). Many studies have found that individualized targeted therapy is effective for the treatment of tumor metastasis. For example, FEM1C upregulation might inhibit the metastasis in CRC (7). Kang and co-workers have evidenced that the downregulation of miR-138 helped to impede the progression of CRC (8). In view of this, the exploration of independent biomarkers involved in the advancement of CRC is of great significance.

Non-structural maintenance of chromosomes (SMC) condensin I complex subunit G (*NCAPG*) is a mitosis-associated chromosomal condensing protein (9). It was reported that high expression of *NCAPG* correlated with immune cell infiltration in the tumor microenvironment (9,10). As a tumor-promoting gene, *NCAPG* is overexpressed in some malignant tumors, including castration-resistant prostate cancer and hepatocellular carcinoma (11,12). Interestingly, Shi and co-workers demonstrated that *NCAPG* expression was upregulated in CRC tissues and cell lines and the knockdown of *NCAPG* exhibited inhibitory effects on the proliferation, migration, and invasion of HCT116 and SW480 cells (13). Besides, the overexpression of *NCAPG* was evidenced to serve as a candidate biomarker for CRC prognosis (14). Nevertheless, the biological function of *NCAPG* in CRC still requires further investigation.

Chromobox protein homolog 3 (*CBX3*) is a member of the heterochromatin-associated protein 1 (HP1) family (15). By inhibiting the expression level of a variety of genes, *CBX3* acts as a critical regulator in many cellular processes, such as cell growth, differentiation, and DNA damage (16). Aberrant expression of *CBX3* has been found to be implicated in the progression of various cancer types, including gastric cancer and glioma (17,18). More importantly, *CBX3* has been shown to be upregulated in colon cancer cells and to promote cell cycle progression and proliferation (19). In addition, according to the Human Transcription Factor Database (HumanTFDB; http://bioinfo.life.hust.edu.cn/HumanTFDB#!/), *CBX3* could bind to *NCAPG* promoters, while data from the Encyclopedia of RNA Interactomes (ENCORI) database (https://starbase.sysu.edu.cn/) revealed that *CBX3* was positively correlated with *NCAPG*.

Wnt/β-catenin signaling has been widely implicated in many human cancers, including gastric cancer (20), breast cancer (21), and prostate cancer (22), and CRC is no exception (23,24). As a core factor of the Wnt signaling pathway, β-catenin is a bridge of the Wnt signaling pathway with epithelial-mesenchymal transformation (EMT) (13). Studies have testified that EMT is a primary cause of the recurrence and metastasis of cancer because it can increase the migration and invasion of cancer cells and plays a critical role in the modulation of CRC diagnosis and prognosis (25,26). The trigger of Wnt/β-catenin signaling has been demonstrated to be a vital player in EMT required for CRC metastasis (27). In addition, *NCAPG* has been shown to activate Wnt/β-catenin signaling in many diseases. For example, *NCAPG* overexpression suppressed apoptosis and promoted EMT in cardia adenocarcinoma via triggering Wnt/β-catenin signaling (28). Elsewhere, *NCAPG* facilitated the progression of CRC and EMT process via activation of Wnt/β-catenin signaling (13).

This study was undertaken to investigate the functional role of *NCAPG* in CRC and to explore its mechanism, aiming to provide a theoretical foundation for further studies. We present the following article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-63/rc).

Highlight box

Key findings

- *NCAPG* might help to impede the progression of CRC.

What is known and what is new?

- *NCAPG* transcriptionally regulated by *CBX3* activated the Wnt/β-catenin signaling pathway to promote proliferation and cell cycle in HCT116 cells.
- *NCAPG* transcriptionally regulated by *CBX3* activated the Wnt/β-catenin signaling pathway to inhibit apoptosis in HCT116 cells.

What is the implication, and what should change now?

- *NCAPG* might be a therapeutic target for the amelioration of CRC.

Methods

Cell culture and treatment

Normal colon mucosal epithelial cell line NCM460 was supplied from Biovector (Beijing, China) while CRC cell lines (HCT116, SW480, and HT-29) were provided by Procell Life Science & Technology Co., Ltd. (Wuhan, China). All cells were cultivated in Dulbecco's modified Eagle's medium (DMEM, Biosharp Life Sciences, Hefei, China), supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific Inc., Waltham, MA, USA) and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA), at 37 ℃ with 5% CO_2.

Cell transfection

HCT116 cells were harvested in the logarithmic growth phase and then injected into 6-well plates at a density of 1×10^5 cells/mL. Plasmids carrying *CBX3* (Ov-CBX3), pcDNA3.1 empty vector (Ov-NC), short hairpin specific to *CBX3* (sh-CBX3) and *NCAPG* (sh-NCAPG), and the corresponding scrambled sequence as a negative control (sh-NC) were synthesized by Obio Technology Co., Ltd. (Shanghai, China). The transfection of 100 nM of recombinants into HCT116 cells was performed at 37 ℃ for 48 hours using Lipofectamine 2000 reagent (Thermo Fisher Scientific Inc.) After 48 hours, the cells were collected for subsequent experiments.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

RNA was extracted from HCT116 cells with TRIzol reagent (Biosharp) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using the PrimeScript RT Reagent Kit with genomic DNA (gDNA) Eraser (Takara Bio Inc., Kusatsu, Shiga, Japan). Subsequently, PCR amplification was conducted with TB Green Premix Ex Taq II on the ABI PRISM 7900 Sequence Detection System (Applied Biosystems) in line with the suggested recommendations. The following thermocycling conditions were used: initial denaturation at 95 ℃ for 5 minutes, followed by 40 cycles of denaturation at 95 ℃ for 10 seconds and annealing at 60 ℃ for 30 seconds, and then extension for 10 minutes at 72 ℃. The primer sequences were as follows: *NCAPG* forward (F), 5'-GAGGCTGCTGTCGATTAAGGA-3' and reverse (R), 5'-AACTGTCTTATCATCCATCGTGC-3', *CBX3* F, 5'-TAGATCGACGTGTAGTGAATGGG-3' and R, 5'-TGTCTGTGGCACCAATTATTCTT-3' and β-actin F, 5'-AGCGAGCATCCCCCAAAGTT-3' and R, 5'-GGGCACGAAGGCTCATCATT-3'. The relative gene expression was determined with $2^{-\Delta\Delta CT}$ (29).

Western blot

HCT116 cells were harvested in the logarithmic growth phase and then injected into 6-well plates at a density of 1×10^5 cells/mL. Total proteins were extracted from HCT116 cells with radioimmunoprecipitation assay (RIPA) lysis buffer (Biosharp) and then quantified with the application of a bicinchoninic acid (BCA) protein assay kit (Biosharp). After separation with 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), equal amounts of protein (30 μg/lane) were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Burlington, MA, USA). Membranes were blocked with 5% bovine serum albumin (BSA, BioFroxx) at room temperature for 1 hour. The membranes were then cultivated with primary antibodies targeting NCAPG (24563-1-AP; 1:5,000; Proteintech, Wuhan, China), cyclin D1 (60186-1-AP; 1:5,000; Proteintech), cyclin-dependent kinase 4 (CDK4) (66950-1-Ig; 1:5,000; Proteintech), Bcl2 (12789-1-AP; 1:2,000; Proteintech), Bax (50599-2-Ig; 1:2,000; Proteintech), CBX3 (ab217999; 1:2,000; Abcam), Wnt3a (26744-1-AP; 1:500; Proteintech), β-catenin (66379-1-Ig; 1:5,000; Proteintech) or β-actin (20536-1-AP; 1:2,000; Proteintech) overnight at 4 ℃. Next, the membranes were rinsed with tris-buffered saline with Tween (TBST) and then incubated with horseradish peroxidase (HRP)-conjugated affinipure goat anti-mouse IgG (SA00001-1; 1:2,000; Proteintech) or HRP-conjugated affinipure goat anti-rabbit IgG (SA00001-2; 1:2,000; Proteintech) at 37 ℃ for 2 h. The antibody-labeled proteins were visualized using enhanced chemiluminescence (ECL) detection reagent (Shanghai Yeasen Biotechnology Co., Ltd., Shanghai, China) and then analyzed using ImageJ, version 1.49 (National Institutes of Health, USA).

Cell Counting Kit-8 (CCK-8) assay

HCT116 cells harvested in the logarithmic growth phase were injected into 96-well plates at a density of 3×10^4 cells/mL and then cultivated at room temperature for 24 hours. Afterwards, 15 μL CCK-8 solution was added into each well to further incubate HCT116 cells in the dark

at 37 ℃ with 5% CO_2 for 2 hours. The optical density was determined utilizing a microplate reader (Thermo Fisher Scientific Inc.) at 450 nm.

Flow cytometry

HCT116 cells harvested in the logarithmic growth phase were injected into 6-well plates at a density of 1×10^5 cells/mL and incubated at room temperature. After centrifugation at 1,500 r/min for 5 minutes, the cell precipitate was collected and the supernatant was discarded. Subsequently, the collected precipitate was rinsed with pre-chilled PBS and subjected to 75% ethanol fixation at 4 ℃ overnight. The cells were then exposed to 100 µL RNase A (Sigma-Aldrich) at 37 ℃ for 30 minutes and stained with 400 µL propidium iodide (Sigma-Aldrich) at 4 ℃ away from light for 30 minutes. Finally, the cells were analyzed using a flow cytometer (Beckman Coulter, Brea, CA, USA).

Terminal-deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay

The apoptosis of HCT116 cells was determined using a TUNEL kit (Beyotime Institute of Biotechnology, Shanghai, China). HCT116 cells harvested in the logarithmic growth phase were injected into 48-well plates at a density of 1×10^4 cells/mL and then treated as aforementioned. Subsequently, HCT116 cells were subjected to 4% paraformaldehyde (Beyotime Institute of Biotechnology) fixation at room temperature for 30 minutes and 0.25% Triton X-100 (Beyotime Institute of Biotechnology) permeation at room temperature for 5 minutes. Next, HCT116 cells were rinsed with phosphate-buffered saline (PBS) twice and then incubated with 50 µL reaction solution for 1 hour as per the standard protocol, after which 0.1 µg/mL 4',6-diamidino-2-phenylindole (DAPI) was applied for the staining of cell nuclei at room temperature for 10 minutes. Finally, apoptotic cells in 5 randomly selected fields were photographed under a florescent microscope (Olympus, Tokyo, Japan).

Colorimetric caspase activity assay

HCT116 cells harvested in the logarithmic growth phase were injected into 6-well plates at a density of 1×10^5 cells/mL for incubation. According to manufacturer's instructions, the activity of caspase3 and caspase9 was assessed with caspase3 assay kit and caspase9 assay kit (Elabscience, Houston, TX, USA), respectively. The optical density was determined at 405 nm with a microplate reader (Thermo Fisher Scientific Inc.).

Luciferase report assay

HumanTFDB predicted the binding sites of *CBX3* and *NCAPG* promoters. Luciferase assay was implemented for the verification of their interaction on the Luciferase Reporter System (Promega, Madison, WI, USA). *NCAPG* wild-type (WT) as well as mutant (MUT) reporter plasmids containing *CBX3* mimic or mimic NC binding sites were constructed (GenePharma, Shanghai, China) to be transfected into HCT116 cells with Lipofectamine 2000 reagent (Thermo Fisher Scientific Inc.) as per standard protocol. Luciferase activity was normalized to that of *Renilla*.

Statistical analysis

All experiments were repeated at least 3 times. The collected experimental data are presented in the form of mean ± standard deviation (SD) and were analyzed with GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) with Tukey's post-hoc test was applied for comparisons among multiple groups. P less than 0.05 indicated statistical significance.

Results

NCAPG silence inhibited the proliferation and cell cycle of HCT116 cells

The messenger RNA (mRNA) and protein expressions of *NCAPG* in normal colon mucosal epithelial cell line NCM460 and CRC cell lines (HCT116, SW480, and HT-29) were first assessed using RT-qPCR and western blot. Compared with the NCM460 group, the mRNA and protein expressions of *NCAPG* were greatly elevated in CRC cell lines (*Figure 1A,1B*). It was noted that *NCAPG* had higher expression in HCT116 cells, and thus they were chosen for subsequent experiments. To reduce *NCAPG* expression, sh-NCAPG was transfected into HCT116 cells, and RT-qPCR as well as western blot was utilized to examine transfection efficacy. Compared with the sh-NC group, the expression of *NCAPG* was the lowest in the sh-NCAPG#3 group, and thus sh-NCAPG#3 was selected for following studies (*Figure 1C,1D*). CCK-8 was conducted to determine

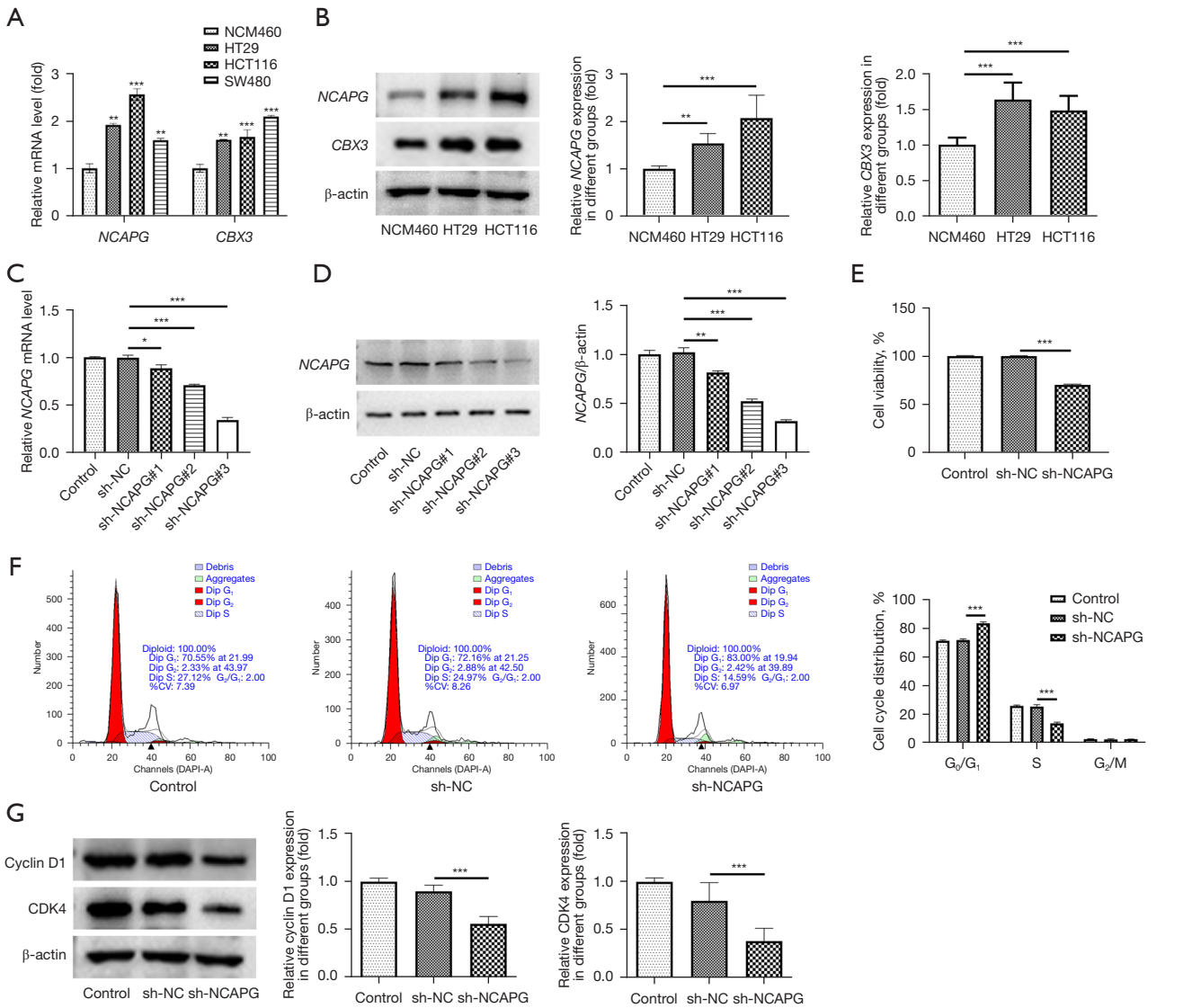


Figure 1 *NCAPG* silence inhibited the proliferation and cell cycle of HCT116 cells. (A,B) The mRNA and protein expressions of *NCAPG* and *CBX3* were detected using RT-qPCR and western blot. (C,D) The transfection efficacy of sh-NCAPG was detected using RT-qPCR and western blot. (E) The viability of transfected HCT116 cells was detected using CCK-8. (F) The cell cycle of transfected HCT116 cells was detected using flow cytometry. (G) The expression of cyclin D1 and CDK4 was detected using western blot. Data are expressed as mean ± SD. *P<0.05; **P<0.01; ***P<0.001. NCAPG, non-SMC condensin I complex subunit G; SMC, structural maintenance of chromosomes; CBX3, chromobox protein homolog 3; mRNA, messenger RNA; sh-NC, short hairpin specific to negative control; sh-NCAPG, short hairpin specific to NCAPG; CV, coefficient of variation; CDK4, cyclin-dependent kinase 4; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; CCK-8, Cell Counting Kit-8; SD, standard deviation.

the effects of *NCAPG* knockdown on the proliferation of HCT116 cells. As depicted in *Figure 1E*, the proliferation of HCT116 cells was conspicuously reduced after depleting *NCAPG* expression when compared with the sh-NC group. In addition, results obtained from flow cytometry showed that *NCAPG* deficiency markedly decreased the number of HCT116 cells in the S phase of the cell cycle in comparison with the sh-NC group (*Figure 1F*). Moreover, the expressions of cell cycle-related proteins were analyzed using western blot, which showed that the expressions of

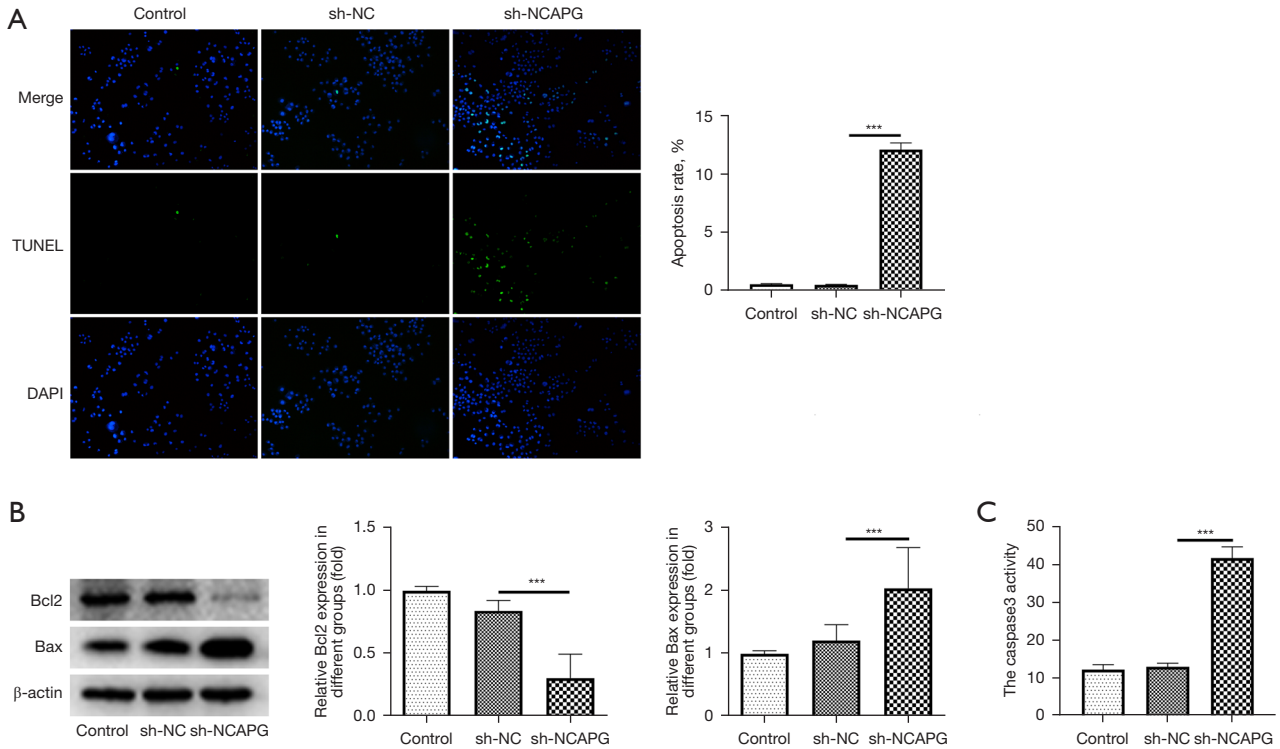


Figure 2 *NCAPG* silence induced the apoptosis of HCT116 cells. (A) The apoptosis of transfected HCT116 cells was detected using TUNEL. DAPI was used for staining. Magnification, ×200. (B) The expression of Bax and Bcl2 was detected using western blot. (C) The activity of cleaved caspase3 was detected using colorimetric caspase activity assay. Data are expressed as mean ± SD. ***P<0.001. TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; DAPI, 4',6-diamidino-2-phenylindole, sh-NC, short hairpin specific to negative control; sh-NCAPG, short hairpin specific to NCAPG; NCAPG, non-SMC condensin I complex subunit G; SMC, structural maintenance of chromosomes; SD, standard deviation.

cyclin D1 and CDK4 were markedly decreased in NCAPG-silenced HCT116 cells (*Figure 1G*). The above results indicated that *NCAPG* depletion exhibited inhibitory effects on the proliferation and cell cycle of HCT116 cells.

NCAPG silence induced the apoptosis of HCT116 cells

The effects of *NCAPG* silence on the apoptosis of HCT116 cells were evaluated with TUNEL. As demonstrated in *Figure 2A*, the apoptosis level of HCT116 cells was conspicuously enhanced after silencing *NCAPG* expression in contrast with the sh-NC group. The contents of apoptosis-related proteins were determined with western blot, which revealed that *NCAPG* knockdown elevated Bax expression and decreased Bcl2 expression compared with those in the sh-NC group (*Figure 2B*). In comparison with the sh-NC group, the activity of cleaved caspase3 in HCT116 cells was markedly enhanced after cell transfection with sh-NCAPG (*Figure 2C*). To conclude, the apoptosis of HCT116 cells could be induced by silencing *NCAPG*.

NCAPG is transcriptionally regulated by CBX3

HumanTFDB predicted the binding sites of *CBX3* and *NCAPG* promoters (*Table 1* and *Figure 3A*). According to the ENCORI database, *CBX3* was positively correlated with *NCAPG* (*Figure 3B*). The mRNA and protein expressions of *CBX3* were assessed by RT-qPCR and western blot. The results showed that the mRNA and protein expressions of *CBX3* were markedly increased in CRC cell lines (HCT116, SW480, and HT-29) when compared to NCM460 cells (*Figure 1A*,*1B*). To overexpress *CBX3*, Ov-CBX3 was transfected into HCT116 cells, which led to a significant elevation of the mRNA and protein expressions of *CBX3* in HCT116 cells. Sh-CBX3 was also transfected into HCT116 cells, and the results showed that the expression of *CBX3* in

Table 1 The binding sites of *CBX3* and *NCAPG* promoter region predicted by HumanTFDB

TF	Site	Start	Stop	Score	P value	Matched sequence
CBX3	Site 3	581	592	12.9605	1.77E-05	CAGAAAATCCAT
CBX3	Site 4	797	818	11.6282	2.85E-05	ACGCAGAGGAGTCCCACCATGG
CBX3	Site 2	234	249	11.2632	2.94E-05	TACAATGGAGTACTAT
CBX3	Site 5	1,738	1,746	11.3947	3.02E-05	CAGCCAGGC
CBX3	Site 1	122	133	10.6184	6.16E-05	CACAAAGCAAAA

CBX3, chromobox protein homolog 3; NCAPG, non-SMC condensin I complex subunit G; SMC, structural maintenance of chromosomes; HumanTFDB, Human Transcription Factor Database; TF, transcription factor.

HCT116 cells was conspicuously decreased compared with the sh-NC group (*Figure 3C,3D*). Evidently, sh-CBX3#2 had better transfection efficacy, as shown by the lower expression of *CBX3* in the sh-CBX3#2 group. In view of this, sh-CBX3#2 was selected for subsequent experiments. Results obtained from luciferase report assay revealed that conspicuously stronger luciferase activity was observed in HCT116 cells transfected with Ov-CBX3 and *NCAPG* WT than that in HCT116 cells transfected with Ov-NC and *NCAPG* WT (*Figure 3E*). Compared with Ov-NC, the expression of *NCAPG* in *CBX3*-overexpressed HCT116 cells was greatly increased, while the expression of *NCAPG* in *CBX3*-silenced HCT116 cells was reduced compared with the sh-NC group (*Figure 3F,3G*). To sum up, *NCAPG* was transcriptionally regulated by *CBX3*.

NCAPG transcriptionally regulated by CBX3 activated the Wnt/β-catenin signaling pathway to regulate proliferation, the cell cycle, and apoptosis in HCT116 cells

To determine the relationship between *CBX3* and the Wnt/β-catenin signaling pathway, western blot was performed to evaluate the contents of Wnt/β-catenin signaling pathway-related proteins. Compared with the Ov-NC group, *CBX3* overexpression elevated the expressions of Wnt3a and β-catenin in HCT116 cells, indicating that *CBX3* could activate the Wnt/β-catenin signaling pathway (*Figure 4A*). Results obtained from CCK-8 demonstrated that the reduced viability in HCT116 cells due to *NCAPG* depletion was partially revived after overexpressing *CBX3* (*Figure 4B*). Compared with the sh-NC group, *NCAPG* deficiency increased the number of cells in the G_0/G_1 phase and decreased that in the S phase, which was then reversed by *CBX3* overexpression (*Figure 4C*). Additionally, the reduced protein expressions of cyclin D1 and CDK4 in NCAPG-silenced HCT116 cells were elevated after cell transfection with Ov-CBX3 compared with the sh-NCAPG + Ov-NC group (*Figure 4D*). Cell apoptosis was determined with TUNEL, and the results showed that *NCAPG* knockdown induced apoptosis in HCT116 cells compared with the sh-NC, while *CBX3* overexpression exhibited the opposite effect, as indicated by diminished apoptosis in the sh-NCAPG + Ov-CBX3 group (*Figure 4E*). Compared with the sh-NC group, *NCAPG* interference increased Bax expression and reduced Bcl2 expression, which were subsequently reversed by *CBX3* overexpression (*Figure 4F*). Further, *CBX3* overexpression also suppressed the enhanced activity of cleaved caspase3 and cleaved caspase9 in NCAPG-depleted HCT116 cells in comparison with the sh-NCAPG + Ov-NC group (*Figure 4G*). Collectively, our results showed that *NCAPG* was transcriptionally regulated by *CBX3* and activated the Wnt/β-catenin signaling pathway to promote proliferation and the cell cycle and inhibit apoptosis in HCT116 cells.

Discussion

CRC is one of the most common cancers worldwide (30). Due to tumor heterogeneity, significant differences in phenotypic features such as proliferation and apoptosis are often exhibited during the onset and in the aggressiveness of CRC (31). As is commonly known, abnormal proliferation is one of the characteristics of cancer cells (32). As a critical regulator of cancer progression, the cell cycle has become an emerging target for treatment in breast cancer (33). Further, dysregulated apoptotic cell death is a hallmark for cancer because the changes in apoptosis are closely related with tumor development (34). In view of this, it can be concluded that the interruption of the above processes is an effective way to protect against cancer development.

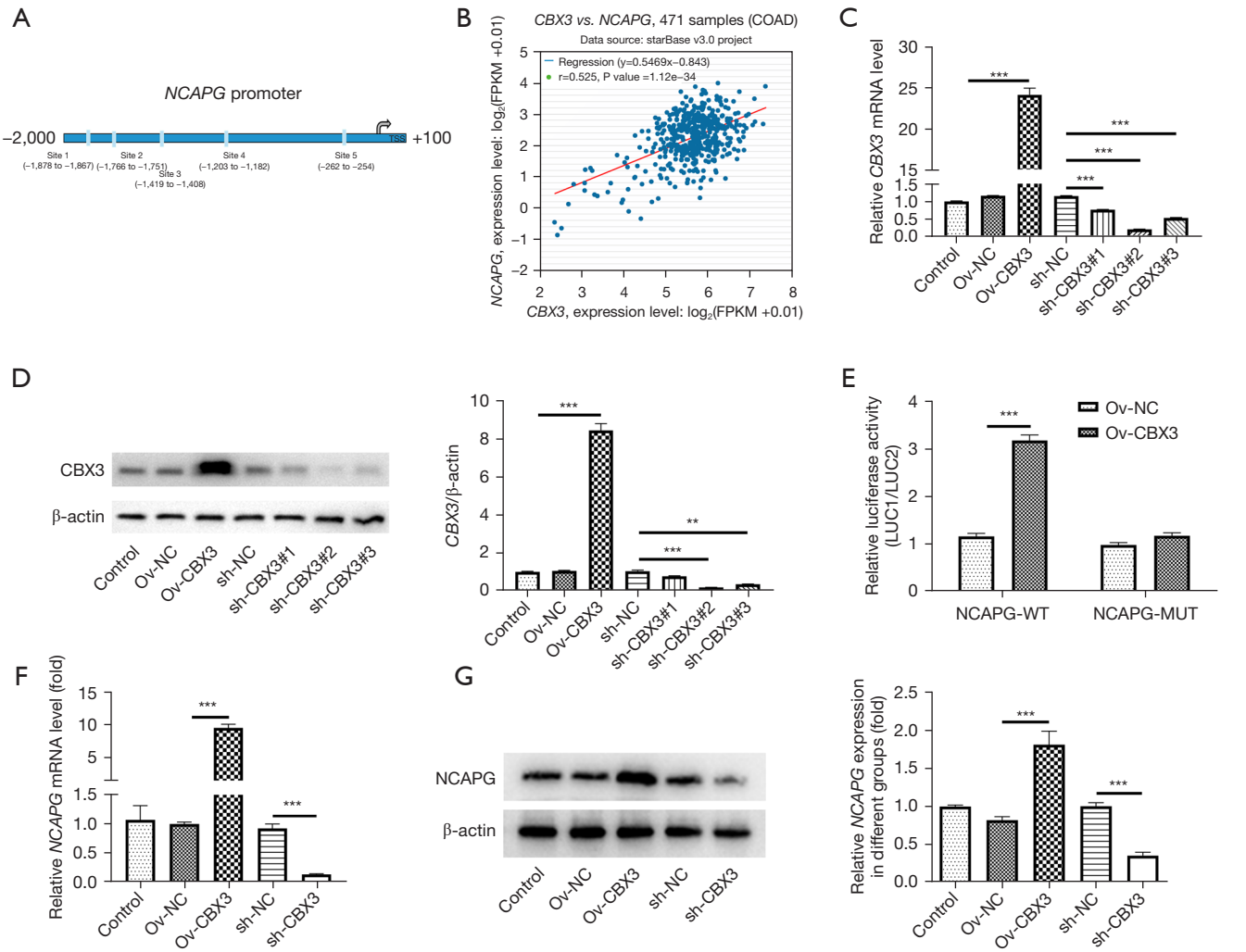
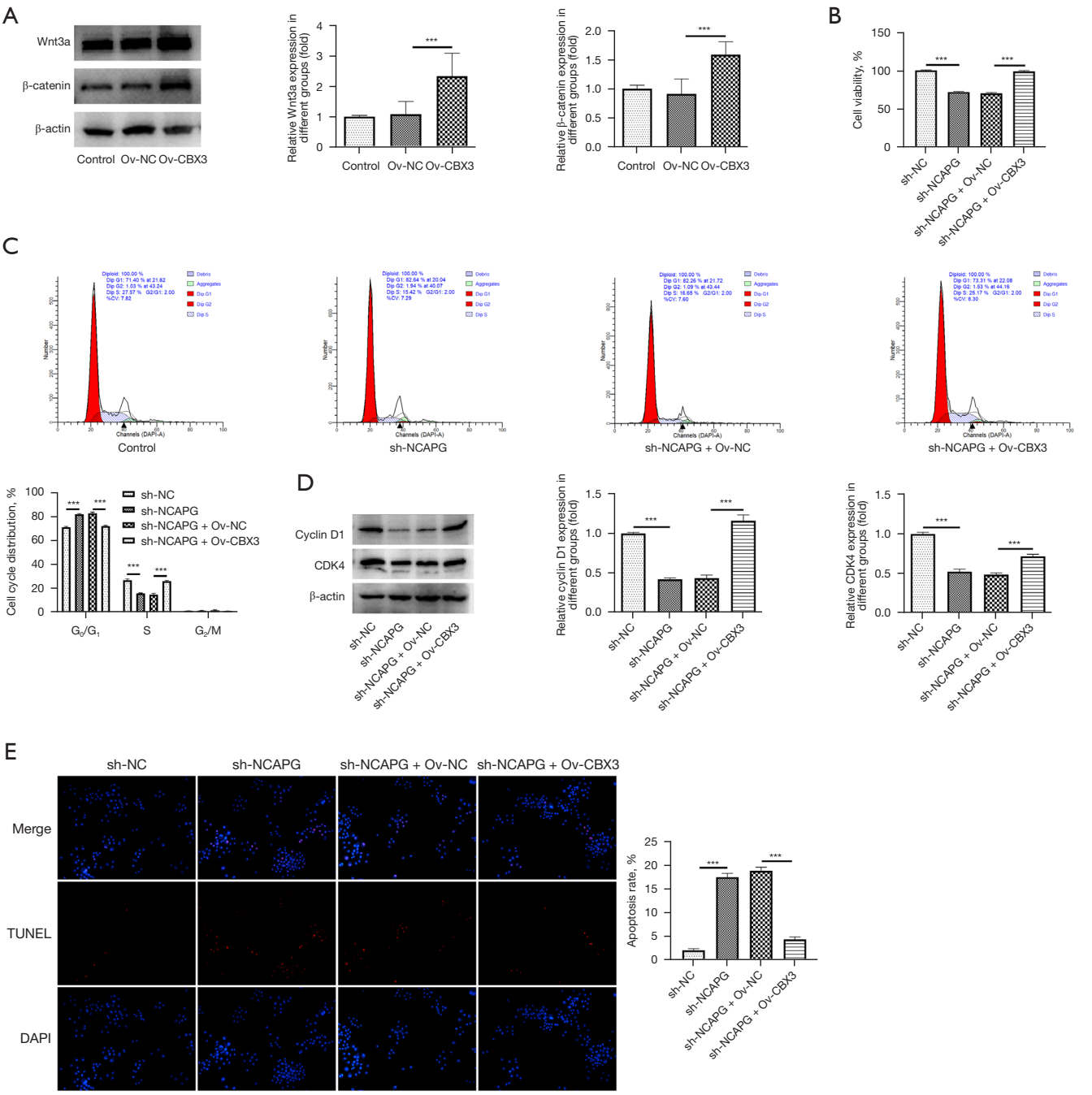


Figure 3 *NCAPG* is transcriptionally regulated by *CBX3*. (A) HumanTFDB predicted the binding sites of *CBX3* and *NCAPG* promoters. (B) According to the ENCORI database, *CBX3* was positively correlated with *NCAPG*. (C,D) The transfection efficacy of Ov-CBX3 and sh-CBX3 were detected using RT-qPCR and western blot. (E) The activity of *NCAPG* promoters was detected using luciferase report assay. (F,G) The mRNA and protein expressions of *NCAPG* were detected using RT-qPCR and western blot. Data are expressed as mean ± SD. **P<0.01; ***P<0.001. NCAPG, non-SMC condensin I complex subunit G; SMC, structural maintenance of chromosomes; FPKM, fragments per kilobase million; CBX3, chromobox protein homolog 3; COAD, colon adenocarcinoma; mRNA, messenger RNA; Ov-NC, pcDNA3.1 empty vector; Ov-CBX3, plasmids carrying CBX3; sh-NC, short hairpin specific to negative control; sh-CBX3, short hairpin specific to CBX3; WT, wild type; MUT, mutant; HumanTFDB, Human Transcription Factor Database; ENCORI, Encyclopedia of RNA Interactomes; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; SD, standard deviation.

Besides, previous studies have found many important biomarkers in the progression of CRC and claimed that molecular targeted-therapy is critical for the treatment of CRC (35,36). For instance, long noncoding RNA SNHG14 was confirmed to facilitate CRC progression (37). Additionally, IMPDH2 was also showed to promote the malignant development of CRC cells (38). *NCAPG* is a condensin complex subunit encoded by the *NY-MEL-3* gene and is upregulated in CRC (13). In addition, *NCAPG* has been found to promote proliferation and suppress apoptosis in CRC cells (39), and NCAPG is involved in cell cycle progression (40). In the present study, RT-qPCR



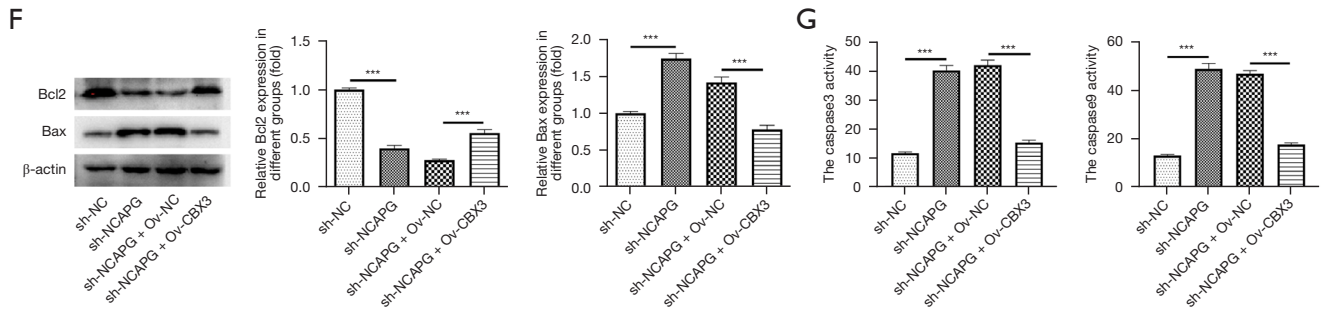


Figure 4 *NCAPG* transcriptionally regulated by *CBX3* activated the Wnt/β-catenin signaling pathway to regulate proliferation, cell cycle, and apoptosis in HCT116 cells. (A) The protein expression of Wnt3a and β-catenin was detected using western blot. (B) The viability of HCT116 cells cotransfected with sh-NCAPG and Ov-CBX3 was detected using CCK-8. (C) The cell cycle of HCT116 cells cotransfected with sh-NCAPG and Ov-CBX3 was detected using flow cytometry. (D) The expression of cyclin D1 and CDK4 in HCT116 cells cotransfected with sh-NCAPG and Ov-CBX3 was detected using western blot. (E) The apoptosis of HCT116 cells cotransfected with sh-NCAPG and Ov-CBX3 was detected using TUNEL. DAPI was used for staining. Magnification, ×200. (F) The expression of Bax and Bcl2 in HCT116 cells cotransfected with sh-NCAPG and Ov-CBX3 were detected using western blot. (G) The activity of cleaved caspase3 and cleaved caspase9 in HCT116 cells cotransfected with sh-NCAPG and Ov-CBX3 was detected using colorimetric caspase activity assay. Data are expressed as mean ± SD. ***P<0.001. Ov-NC, pcDNA3.1 empty vector; Ov-CBX3, plasmids carrying CBX3; CBX3, chromobox protein homolog 3; sh-NC, short hairpin specific to negative control; sh-NCAPG, short hairpin specific to NCAPG; NCAPG, non-SMC condensin I complex subunit G; SMC, structural maintenance of chromosomes; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; DAPI, 4',6-diamidino-2-phenylindole, CCK-8, Cell Counting Kit-8; SD, standard deviation.

and western blot showed that the mRNA and protein expressions of *NCAPG* were upregulated in CRC cells (HCT116, HT-29, and SW480). After transfection with sh-NCAPG, the expression of *NCAPG* was greatly reduced. Moreover, *NCAPG* knockdown was found to repress the proliferation. The cell cycle in CRC cells was also repressed by *NCAPG* interference, accompanied by decreased protein expressions of cyclin D1 and CDK4. However, *NCAPG* deficiency induced apoptosis in CRC cells, together with increased Bax expression and reduced Bcl2 expression. The above findings implied that NCAPG knockdown helped to fight against CRC.

CBX3 has a close association with transcriptional activation or repression, cell differentiation and growth, as well as epigenetic modification (19,41). Previous study has reported that *CBX3* is elevated in many cancers and is crucial for the advancement of tumors and the modulation of growth-related genes (42). For instance, *CBX3* was highly expressed in osteosarcoma and predictive of an unfavorable prognosis for osteosarcoma patients (43). Peng *et al.* reported that *CBX3* expression was enhanced in glioblastoma (GBM) and promoted the proliferation, invasion, and tumorigenesis of GBM cells (44). Moreover, *CBX3* was shown to be positively expressed in CRC tissues (45). In this study, we found that the mRNA and protein expressions of *CBX3* were conspicuously elevated in CRC cells, which was consistent with the findings of a previous study (45). After transfection with Ov-CBX3, the expression of *CBX3* in HCT116 cells was highly expressed, while sh-CBX3 reduced *CBX3* expression. HumanTFDB predicted the binding sites of *CBX3* and *NCAPG* promoters, and according to the ENCORI database, *CBX3* was positively correlated with *NCAPG*. Here, results obtained from luciferase report assay demonstrated that the luciferase activity in HCT116 cells transfected with Ov-CBX3 and *NCAPG* WT was higher than that in HCT116 cells transfected with Ov-NC and *NCAPG* WT. Additionally, Ov-CBX3 elevated the expression of *NCAPG* while sh-CBX3 decreased that in HCT116 cells, indicating that *NCAPG* could be regulated by *CBX3*.

The aberrant Wnt/β-catenin signaling pathway has been shown to promote cancer stem cell renewal as well as cell proliferation, thereby acting as a critical player in tumorigenesis and therapy response (46). A number of studies have highlighted the therapeutic potential of agents targeting Wnt/β-catenin signaling in cancer (47), including colon cancer (48). Mukherjee *et al.* reported that targeting Wnt/β-catenin signaling was an effective method in the

treatment of breast cancer (21). Interestingly, Disoma and co-workers put forward that Wnt/β-catenin signaling should be a pivotal therapeutic target for CRC (49). In the present study, the expressions of Wnt/β-catenin signaling-related proteins (Wnt3a and β-catenin) were determined, and the results showed that the contents of Wnt3a and β-catenin were enhanced in CBX3-overexpressed HCT116 cells, implying that *CBX3* could activate Wnt/β-catenin signaling in CRC. To investigate the mechanism of Wnt/β-catenin signaling, further experiments were conducted, with the results showing that the effects of *NCAPG* knockdown on the proliferation, cell cycle, and apoptosis on HCT116 cells were reversed by *CBX3* overexpression.

Conclusions

To sum up, this study investigated the functional role of *NCAPG* in HCT116 cells and identified that *NCAPG* could be transcriptionally regulated by *CBX3*, revealing the mechanism through which the progression of CRC is facilitated by *NCAPG*. To the best our knowledge, this study was the first to explore the mechanism of *NCAPG* and *CBX3* in CRC, which shed novel insights into the targeted treatment for CRC and laid foundation for further researches.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-63/rc

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