MiR-23b-3p Targeting NCAPG Promotes Cell Proliferation and Anti-Apoptosis By Regulating PI3K/AKT Signaling Pathway in Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is one of the major malignancies with poor prognosis. NCAPG is reportedly upregulated in human malignant tumors. However, its clinicopathological and biological significance in CRC are still indistinct. In this investigation, NCAPG was upregulated in human CRC specimens and CRC cells. NCAPG overexpression was positively correlated with clinicopathological characteristics, such as differentiation and tumor size, and was independently associated with poor survival. Consistent with clinical observations, NCAPG significantly promoted tumorigenesis and inhibited apoptosis of CRC cells in vitro and in vivo. Moreover, we found knockdown of NCAPG inhibited proliferation through regulating PI3K/AKT pathway. Furthermore, we showed that NCAPG was a potential target whose expression is negatively modulated by miR-23b-3p. Taken together, the miR-23b-3p/NCAPG/PI3K/AKT axis plays tumorigenic roles in colorectal carcinogenesis and acts as a therapeutic or diagnostic target.

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, and its morbidity and mortality are increasing yearly in China [1]. In recent years, despite great advancements in diagnosis measures and therapeutic methods, the overall outcome of CRC patients is still poor [2]. Therefore, it is of huge necessary to identify emerging predictive biomarkers and underlying mechanisms to promote the development of more individualized treatment for CRC patients.

Non-structural maintenance of chromosomes condensin I complex subunit G (NCAPG) is a chromosomal condensed protein associated with mitosis [3]. Human NCAPG actives condensin via regulating ATPase activity to exert is effects [4]. For the past few years, more and more studies have reported that NCAPG was related to the pathogenesis of various cancers, including breast cancer [5], gastric cancer [6-8], prostate cancer [9], hepatocellular carcinoma [10] and ovarian cancer [11]. In our previous study, we discovered that NCAPG is overly expressed in CRC specimens via gene microarrays [12]. However, as far as we can ascertain, the biologic functions and clinical implication of NCAPG in CRC have never been defined.

MicroRNAs (miRNAs), highly conserved small non-coding RNA molecules, primarily act on preventing mRNA translation via binding to the 3’ untranslated region (UTR) of downstream target mRNAs at the post-transcriptional level [13]. Growing evidence has increasingly suggested that miRNAs might participate in regulating the biological behaviors in variety of cancer [14]. Latest studies have disclosed that miR-23b-3p is lowly expressed in several cancers [15]. In CRC, down regulation of miR-23b-3p has previously been reported is related with 5-fluorouracil resistance [16]. However, the exact mechanisms of NCAPG and its relationship with miR-23b-3p in CRC are barely understood.

In our current study, NCAPG expression was detected in samples and cells of CRC. After that, we evaluated the clinical significance of NCAPG expression in CRC, and explored the influence of NCAPG on
the proliferation and apoptosis of CRC cells. Moreover, we further discussed the mechanisms of NCAPG by recognizing its potential regulated pathway.

**Materials And Methods**

**Patients and tissue samples**

The CRC samples and corresponding non-carcinoma samples were acquired from 88 CRC patients receiving surgical resection at The Affiliated Hospital of North Sichuan Medical College between March 2012 and May 2014, and all patients signed the informed consents. Among the 88 patients, 5 patients were excluded from our study for the reason that the diagnosis of liver or lung metastasis which number was too small to make statistical analysis meaningful. No patients in this study had ever treated with preoperative chemotherapy, radiotherapy or other therapy modalities. After the excision, these specimens were stored at liquid nitrogen at once and then transmitted into a refrigerator at −80°C. The Ethics Committee of The Affiliated Hospital of North Sichuan Medical College approved this research. (Approval number 2021ER(A)005).

**Cell culture**

The CRC cells (HCT116, HCT15, SW480, SW620, LoVo), FHC cells (human normal colon epithelium cell line) and human embryonic kidney 293T cells were bought from American Type Culture Collection (ATCC, Manassas, USA). The CRC cell lines were cultured in DMEM (Chinese Academy of Sciences, Shanghai, China) supplemented with 10% FBS (fetal bovine serum, Chinese Academy of Sciences), while FHC cells and 293 T cells were cultured in DMEM/F12 supplied with 10% FBS. All of the cells were kept in a circumstance at 37°C and with 5% CO₂ humidified incubator.

**Quantitative real time PCR (qRT-PCR)**

Total RNAs were purified from CRC tissues or cell lines using TRIZol Reagent (Invitrogen, Carlsbad, CA) as per the instruction of manufacture. For NACPG mRNA detection, reverse transcription was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Quantitative PCR was performed using SYBR Premix Ex Taq (Takara, Dalian, China) on the ABI 7500 real-time PCR System (Applied Biosystems). For detection of miRNAs, qPCR was exerted using the QuantMir RT Kit (System Biosciences, Mountain View, CA, USA) with miR-23b-3p specific primers (Applied Biosystems). GAPDH and U6 were supposed to the internal control respectively. 2^DDCT method was applied to calculate the relative expression. The primer sequences are as follow: NCAPG, forward: 5’-AAGTTAGACGGCGACGTGTTATC-3’, reverse: 5’-CAGCTTTCTGACAGCCTCTT-3’; MiR-23b-3p, forward: 5’-ATCACATTGCCAGGGATTACCTTTTT-3’; Reverse: 5’-AATTAAAAAGGTAAATCCCTGGCAATGTGATGGCC-3’; GAPDH, forward: TGACTTCAACAGCGACACCCA, reverse: CA CCCTGTTGCTGATGAGCCAAA; U6, forward: CTCGGCTT CGGCAGCACA, reverse: AACGCTTCAG AATTTGCGT.
Complete cell lysis (Roche, Applied Science, USA) containing protease/phosphatase inhibitor Cocktail (Cell Signaling Technology, Beverly, MA, USA) was used to isolate total proteins from cultured cell samples. After being separating via SDS-PAGE gel, the proteins were transferred to PVDF membrane which was then blocked with 5% fit-free milk. Next, the blocked membrane was probed with primary antibodies against PI3K (1:1000), p-PI3K (1:1000), AKT (1:1000), p-AKT (1:1000), Bcl-2 (1:1000), Bax (1:1000), GAPDH (1:500) (all, Cell Signaling Technology) and NCAPG (1:750; Abcam) at 4°C for a whole night and further treated with horseradish-conjugated secondary antibody (Abcam, Cambridge, MA, USA). ECL regents (Millipore, MA, USA) were employed to visualize signals and GAPDH was regarded as internal reference.

**Cell transfection**

To construct NCAPG knockdown CRC cells, lentiviral short hairpin RNAs targeting NCAPG were designed and provided by Genechem (Shanghai, China). The sequences of the NCAPG short hairpin RNAs (shRNAs) were used were as follows, shNCAPG1, forward: 5’-GATCCCGGGCAGCATGTATCTGTTATCA TGTATTCTGAGGCTACGATAACACTGCCCCGTTTTTTTTTTT-3’, reverse: 5’- CTTAAAAAAACGG GCAGTGTATCCTCGAGAATACATGATAACACTCGCCGCC; shNCAPG2, forward: 5’-GAT CCGCCTTAACGTAACATGACAATCTCGAGATTTGTACGACTGTATGTAAAGCTTTTTTTTTTTT-3’, reverse: 5’- CTTAAAAAAAGCGCTTAACAGTACATGACAATCTCGAGATTTGTACGACTGTATGTAAAGC. Lipofectamine® 2000 reagent was used for lentivirus packaging in the 293T cells. Lentiviruses were generated by transfecting the shRNA containing vectors and the corresponding packaging vectors into 293T cells. Viral titers were measured using standardized protocols. The recombinant lentivirus-transfected units were used to infect cells with polybrene (8 mg/ml; Sigma-Aldrich; Merck KGaA). Stable knockdown CRC cells were filtered out using puromycin and successful transfection was confirmed using RT-qPCR and western blotting. For miR-23b-3p transfection, miR-23b-3p inhibitor (anti-miR-23b-3p) or mimics and their negative controls were obtained from RiboBio (Guangzhou, China). Before transfection, 2 x 10^6 cells were cultured in 6-well plate for 24hours. Lipofectamine (Invitrogen) was applied to transfect.

**Cell proliferation analysis**

In this study, analysis of CRC cell proliferation was monitored via colony formation assays and CCK-8 assays. In brief, for CCK-8, after the transfection, approximately 3 x 10^3 target cells were cultured at 96-well culture plate and sustained at 37°C for one night. At the time 0, 24, 48, 72 and 96 hours, cells were dealt with cell counting kit-8 (Beyotime, Shanghai, China) and incubated for 1 hour. Then, the spectrophotometer was utilized to observe OD (optical density) value at 450 nm in every wells. For colony formation assay, cells were seeded into 6-well plates and cultivated for about 2 weeks. The appearance of cell colonies was the mark. Paraformaldehyde was used to fix proliferating colonies, and then crystal violet was employed to stain the colonies. Finally, the stained colonies which were more than 50 cells were counted for further statistical analysis.

**Cell apoptosis assay**
Flow cytometry (FCM) was performed to assess cell apoptosis. Trypsin-EDTA was used to harvest transfected cells. After washing with PBS for two times, cells were suspended in binding buffer. Then, prepared cells were stained with an Annexin V-FITC Cell Apoptosis Detection Kit (Beyotime, Jiangsu, China) and propidium iodide solution (PI) on the basis of the manufacturer’s directions. The apoptosis of CRC cells was observed via FACscan flow cytometry (Becton Dickinson, Heidelberg, Germany).

**Xenograft tumor assay**

All 12 female BALB/C nude mice (1-2 months of age, Shanghai Laboratory Animal Center, Shanghai, China) were randomized into two groups (six mice per group) and then were subcutaneously injected with shNCAPG or shCtrl CRC cell suspension (1x10⁶ cells/ml) in the right flanks. After 4 weeks of injection, all mice were killed and resected the tumors to weigh. The Experimental Animal Ethics Committee of North Sichuan Medical College approved all animal experiments in this study.

**Dual-luciferase reporter assay**

Dual-Luci Reporter assay system (Promega, Madison, USA) was employed to perform luciferase assay. Wild-type or mutant 3′-UTR of NCAPG were subcloned into the XhoI and NotI site of the psicheck-2 vector (Promega, Madison, WI, USA). And the vectors were respectively named into psicheck-2-NCAPG-WT and psicheck-2-NCAPG-MUT. The following primers were used to amplify specific fragments. NCAPG-wt, forward: 5’-CAACTCGAGGTGAGGAAGACCAGTATAACG-3’, reverse: 5’-CACAAACACAGCGCCGCTTTCTGTGGTTTGCAGAATACGTGTTGTTTGCA -3’. NCAPG-mut, forward: 5’-CACAACTCGAGTGTGGATAAATTATCTAGACCAGATATTACACTCAGGAACCTTCTC, reverse: CACAACTCGAGTGTGGATAAATTATCTAGACCAGATATTACACTCAGGAACCTTCTC. HEK 293T cells were plated onto 24-well plates at 2x10⁴ cells/well and transfected with 200 ng of psicheck-2-NCAPG-wt or psicheck-2-NCAPG-mut and 40 nM miR-23b-3p inhibitor using Lipofectamine 2000 (Invitrogen). Firefly luciferase was used to normalize the Renilla luciferase. After transfection for 48 h, cells were harvested and calculated with Dual Luciferase Reporter Assay System (Promega Corporation, Madison, WI, USA) based on the manufacturer’s protocols.

**Bioinformatic analysis**

To verify the expression of NCAPG and miR-23b-2p in CRC and normal tissues, Oncomine (https://www.oncomine.org/), GEPIA (http://gepia.ancer-pku.cn/index.html) and dbDEMC 2.0 (https://www.picb.ac.cn/ dbDEMC/index.html) were used. In addition, The Human Protein Atlas (THPA, https://www.proteinatlas.org/) was used to get the images of immunohistochemistry about NCAPG expression in CRC. To validate the survival analysis result, the survival curve of CRC was obtained from GenomicsScape (http://www.genomicscape.com). The correlation curve between NCAPG and PI3K or AKT gene family was downloaded from GEPIA, and the relation curve between NCAPG and miR-23b-3p was acquired from Starbase (http://starbase.sysu.edu.cn). Moreover, Targetscan (http://www.targetscan.org), Starbase and MiRcode (http://www.mircode. org/index.php) were utilized to predict the targeting relationship of miR-23b-3p and NCAPG.
**Statistical analysis**

In this study, all experiments have been repeatedly performed at least three times. Continuous variables were expressed as mean ± standard deviation (SD). Differences between groups were analyzed using Student's t-test or variance analysis, while chi-square test was employed to analyze categorical data. Colorectal cancer patients’ postoperative survival rate was analyzed by Kaplan-Meier method, the differences of survival rates were valued by performing log-rank test. Univariate and multivariate Cox proportional hazards model analysis was applied to measure the independent prognostic factors of NCAPG. The correlation between NCAPG and miR-23b-3p was determined by Spearman's correlation analysis. All assays or clinical data were processed by the SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA), and when the *p* value less than 0.05, the differences were regarded statistically significant.

**Results**

**NCAPG expression in CRC tissues and cell lines**

To investigate the expression of NCAPG in CRC specimens and tumor-adjacent normal tissues, the database Oncomine and GEPIA were used to check NCAPG expression in CRC. The results revealed that NCAPG was significantly overexpressed in CRC tissues (Fig. 1a, b). In addition, the database THPA also identified its high expression in CRC tissues at immunohistochemical level (Fig. 1c). Furthermore, we confirmed the high NCAPG expression in the collected primary CRC tissues compared to the corresponding normal tissues (Fig. 1d). Similar outcomes were observed in the CRC cell lines (Fig. 1e). The results show that the level of NCAPG is high in CRC tissues and cell lines.

**Correlation between NCAPG expression and clinicopathological characteristics of CRC patients**

To assess the correlation between NCAPG expression and clinicopathological features, the CRC patients were divided into two groups base on the median value of NCAPG expression. As illustrated in Table 1, the high expression of NCAPG in the cohort was closely related with poor differentiation (*p* = 0.006) and large tumor size (*p* = 0.026). Survival curve plotted according to the survival status and follow-up time showed that high NCAPG expression correlated with shorter OS (overall survival) (Fig. 1f). This above result was also verified by the Kaplan-Meier survival analysis obtained from the GenomicsScape (Fig. 1g). Furthermore, we performed Cox proportional hazards regression analysis to ascertain independent prognostic markers for OS. Since T stage and lymph node metastasis determine TNM stage in CRC, TNM stage was not further included into the Cox analysis in our study. The results affirmed that T stage, lymph node metastasis and NCAPG expression were independent prognostic factors (Table 2), demonstrating that NCAPG may be involved in the progression of CRC and predicting OS in CRC.

**Effect of NCAPG on CRC cell proliferation in vitro and in vivo**

To verify whether NCAPG expression influence CRC cell growth, we executed proliferation assays. Inhibition of NCAPG was confirmed by western blotting and RT-qPCR (Fig. 2a, b). As shown in Fig. 2c and
2d, NCAPG silencing caused a prominent reduction in proliferation in HCT15 and HCT116 cells.

Subsequently, to further determine the effect on tumor growth in CRC, xenograft models in BALB/C nude mice were constructed. The results showed that the average weight of xenograft tumors in shNCAPG group was prominently smaller than that of shCtrl group (Fig. 2e, f). Hence, these assay outcomes display the inhibition of NCAPG restrains CRC cell proliferation both in vitro and in vivo.

Effect of NCAPG on CRC cell apoptosis

To investigate the role of NCAPG on CRC cell apoptosis, Bcl-2 and Bax, apoptosis-related proteins, were detected in CRC cells. The results displayed that Bcl-2 was downregulated, while Bax was upregulated when CRC cell lines were dealt with shNCAPG (Fig. 3g). Besides, flow cytometry assay showed that silenced NCPAG exhibited a higher percentage of apoptosis compared with the control group (Fig.2h). The above data suggest NCAPG suppresses apoptosis of CRC cells.

NCAPG regulates PI3K/AKT pathway in CRC cells

The PI3K/AKT signaling pathway has crucial effects on the biological progression of various cancers [17]. Some previous researches had revealed that PI3K/AKT pathway was modulated by NCAPG in cardia adenocarcinoma [8] and hepatocellular carcinoma [18]. Thus, we hypothesized that NCAPG might regulate PI3K/AKT pathway in CRC.

Firstly, the GEPIA database disclosed that NCAPG expression was positively correlated with PI3K/AKT gene family, PIK3CA, PIK3CB, AKT1 and AKT2 (Fig. 3a). Moreover, western blotting results demonstrated that phosphorylation of both PI3K (p-PI3K), AKT (p-AKT) decreased significantly after NCAPG silenced in CRC cells, while 740Y-P (an activator of PI3K) could release the suppression of shNCAPG for p-PI3K and p-AKT (Fig. 3b). Additionally, 740Y-P rescued cell proliferation that caused by NCAPG silencing (Fig. 3c, 3d). Therefore, these conclusions highlight that NCAPG might regulate CRC cells proliferation through AKT/PI3K pathway.

MiR-23b-3p directly targets NCAPG in CRC cells

Previous studies in hepatocellular carcinoma [19] and prostate cancer [19] have demonstrated regulation of NCAPG expression by microRNAs. The publicly available databases Starbase, Targetscan and MiRcode were used to predict that NCAPG is a theoretically possible target of miR-23b-3p (Fig. S1). Moreover, the heatmap downloaded from the database dbDEMC 2.0 suggested miR-23b-3p was down-regulated in CRC (Fig. 4a). Strikingly, a negative relationship between the level of NCAPG and miR-23b-3p was observed in Starbase (Fig. 4b), which was consistent with our cohort (Fig. 4c).

To confirm whether miR-23b-3p can directly target NCAPG in CRC, we performed luciferase report assays. The results showed miR-23b-3p overexpression induced relative luciferase activity significantly decrease, whereas miR-23b-3p inhibition increased the activity (Fig. 4f). Additionally, miR-23b-3p binding site mutation in the 3'UTR of NCAPG abrogated both the function of miR-23b-3p and anti-miR-23b-3p,
suggesting that miR-23b-3p can bind to the NCAPG 3'UTR. Moreover, RT-qPCR and western blotting analysis indicated that NCAPG were obviously reduced in CRC cells overexpressing miR-23b-3p as compared with those transfected with anti-miR-23b-3p (Fig. 6g, h). To explore whether miR-23b-3p exerted its function through NCAPG, we accomplished a rescue experiment in HCT116 cells. As can be seen shown in Fig. 6i, we found that HCT116 cells transfected with anti-miR-23b-3p had a stronger growth ability comparing with non-treated CRC cells, which means reintroduction of anti-miR-23b-3p could partly reverse the inhibiting effects attracted by shNCAPG in CRC cells. In brief, the results demonstrated that miR-23b-3p may affect CRC cells growth of by directly targeting NCAPG.

Discussion

Mounting evidence manifested ectopic NCAPG expression was related with several types of malignant tumors, including gastric cancer [21, 22], hepatocellular carcinoma [23], and ovarian cancer [11]. In current study, we found high NCAPG expression in CRC tissues and cell lines, consistent with our previous study [24]. Elevated NCAPG expression was found to be relevant with TNM stage, distant metastasis, nodal status and vascular invasion in gastric cancer [7]. In this study, it is demonstrated that high NACPG level was related with poor differentiation, large tumor size, and shorter OS of the CRC patients, which indicated that NCAPG could be involved in the occurrence and development and may be regarded as a potentially possible prognostic biomarker in CRC. NCAPG served as an oncogene to promote tumor growth and metastasis in other types of cancer [20, 25, 26, 29, 27]. Similar with these studies, we also explored that NCAPG significantly promoted CRC cells tumorigenesis and inhibited apoptosis in vitro and in vivo. NCAPG might thus play a carcinogenic role in CRC.

To investigate the molecular mechanisms of the changes mediated by NCAPG in CRC, the phosphoinositide 3-kinase (PI3K)-AKT pathway was selected because it was reported be regulated by NCAPG in several cancers [28, 29]. PI3K/AKT pathway performs crucial effects in a plurality of cellular processes [30] and is included in the genesis and progression of various tumors. The procedure of CRC evolution remains a multi-step manner, which was mediated by the abnormal regulation of several distinct pathways including PI3K/AKT signaling pathway [31]. The products of activating PI3K bind to the AKT PH domain can not only mediate the AKT translocation of membrane from the cytoplasm, but also accelerate its conformational change [32]. The activated AKT can further affect invasion, migration, proliferation and apoptosis in cancer cells [33]. In this regard, we reported that knockdown of NCAPG markedly impede the phosphorylation levels of PI3K and AKT. However, PI3K signaling activator (740 Y-P) rescued the roles of shNCAPG in the above effects. Moreover, activation of PI3K/AKT pathway impaired the proliferation reduction in CRC cells induced by NCAPG knockdown. Hence, we concluded that the suppressed proliferation of CRC cells was induced by NCAPG inhibiting the PI3K/Akt pathway.

MicroRNAs regulate gene expression at post-transcriptional levels, and increasing researches have focalized on the role of microRNAs in the carcinogenesis and development of cancers [34]. It has been shown that NCAPG was negatively regulated by miR-181c in hepatocellular carcinoma [34] and miR-99a-3p in prostate cancer [20]. Whereas, there was no evidence that miRNAs could regulate NCAPG in CRC.
cells. In our evaluation of the upstream miRNAs of NCAPG, miR-23b-3p was selected as a candidate miRNA via bioinformatic analysis. MiR-23b-3p has been largely described as an important modulator of cellular physiology and is strongly associated with cancer development [35]. The lowly-expressed miR-23b-3p was reported in cervical cancer [36], lung cancer [37] and prostate cancer [38]. Consistent with these studies, we observed the down-regulation of miR-23b-3p in CRC tissues. However, some scholars have been reported that up-regulation of miR-23b-3p in several types of malignant tumors, including pancreatic cancer [39], renal cancer [40], lung cancer [41] and pancreatic cancer [42]. The discrepant results may be due to the different tissues or diseases. Moreover, we also found a statistically significant inverse correlation between NCAPG and miR-23b-3p. Luciferase assay, western blotting and cell growth assay further confirmed those targeting and modulating relationship. Altogether, these findings provide sufficient evidences that NCAPG could be directly targeted by miR-23b-3p in CRC cells.

Our study still has limitation that the precise mechanism for NCAPG regulation in CRC is unclear. Meanwhile, other limitations are that this is a single institute study and the number of cases is small.

**Conclusion**

In summary, this study suggests that NCAPG can promote growth and inhibit apoptosis in CRC via regulating the PI3K/AKT pathway, and these effects were modulated by miR-23b-3p. These results reveal that NCAPG act as an oncogene role and represents a potential molecular target of therapy in CRC.

**Declarations**

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**Availability of data and materials**

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

**Authors' contributions**
PL and JW designed the study, analyzed and interpreted the data, and wrote the manuscript. XR, ZY, YZ, HT and SL performed the data analysis and interpretation. XT and GZ make data analysis and interpretation, and wrote the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The present study was approved by the Medical Ethics Committee of North Sichuan Medical College (Approval number 2021ER(A)005). All patients provided written informed for participation in the present study. The animal experiments were approved by the Experimental Animal Ethics Committee of North Sichuan Medical College (Approval number 20190917).

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Tables

Table 1. Relationship between NCAPG and clinicopathological features of 88 CRC patients.
| Variable                      | NCAPG expression | p-value |
|-------------------------------|------------------|---------|
|                               | Low(n=41)        | High (n=42) |
| Age                           |                  |         |
| < 60                          | 14               | 10      | 0.340 |
| ≥60                           | 27               | 32      |       |
| Gender                        |                  |         |
| male                          | 21               | 20      | 0.827 |
| female                        | 20               | 22      |       |
| T stage                       |                  | 0.826   |
| T1 and T2                     | 23               | 25      |       |
| T3 and T4                     | 18               | 17      |       |
| Lymph node metastasis         |                  | 0.383   |
| Absent                        | 24               | 22      |       |
| present                       | 17               | 20      |       |
| Differentiation               |                  | 0.006*  |
| Well                          | 18               | 10      |       |
| Moderate                      | 16               | 11      |       |
| Poor                          | 7                | 21      |       |
| Tumor size                    |                  | 0.026*  |
| < 5cm                         | 29               | 19      |       |
| > 5cm                         | 12               | 23      |       |
| Tumor location                |                  | 0.625   |
| Colon                         | 12               | 10      |       |
| Rectum                        | 29               | 32      |       |

**Abbreviation** NACPG: Non-structural maintenance of chromosomes condensin I complex subunit G; CRC: colorectal cancer. * P<0.05

**Table 2.** Univariate and Multivariate Cox regression analysis of clinicopathological factors for overall survival on the basis of clinical information of collected CRC patients.
| Univariate analysis | Multivariate analysis |
|---------------------|-----------------------|
|                      | HR  | 95% CI      |   p  | HR  | 95% CI      |   p  |
| Age                 | 2.326 | 0.798—6.780 | 0.122 |      |                |     |
| (<60 years Vs >60 years) |     |            |       |      |                |     |
| Gender              | 0.705 | 0.320—1.555 | 0.387 |      |                |     |
| (Male Vs Female)    |     |            |       |      |                |     |
| T stage             | 2.295 | 1.030—5.111 | 0.042* | 2.548 | 1.130—5.747 | 0.024* |
| (T1 + T2 Vs T3+T4)  |     |            |       |      |                |     |
| Lymphatic invasion  | 3.514 | 1.467—8.415 | 0.005* | 3.225 | 1.143—9.100 | 0.027* |
| (Absent Vs Present) |     |            |       |      |                |     |
| Differentiation     | 1.758 | 1.054—2.930 | 0.031* | 1.068 | 0.575—1.984 | 0.834 |
| (Well Vs Moderate Vs Poor) |     |            |       |      |                |     |
| Tumor size          | 2.217 | 0.995—4.939 | 0.051 |      |                |     |
| (< 5cm Vs > 5cm)    |     |            |       |      |                |     |
| Tumor location      | 1.340 | 0.502—3.576 | 0.558 |      |                |     |
| (Colon Vs Rectum)   |     |            |       |      |                |     |
| NCAPG expression    | 3.357 | 1.340—8.412 | 0.010* | 3.095 | 1.095—8.747 | 0.033* |
| (Low Vs High)       |     |            |       |      |                |     |

**Abbreviation** HR, Hazard Ratio; CI, confidence interval; well, well differentiated; moderate, moderately differentiated; poor, poorly differentiated; NACPG: Non-structural maintenance of chromosomes condensin I complex subunit G. * P<0.05.

**Figures**
Figure 1

NCAPG was overexpressed in CRC samples and cells which was related poor differentiation, large tumor size and shorter survival times in CRC. a NCAPG expression in all cancer cases obtained from the Oncomine database. b NCAPG expression in CRC tissues and normal tissues downloaded from GEPIA database. c The results of immunohistochemistry of NCAPG in normal colorectal tissues and CRC tissues acquired from THPA. d The relative expression of NCAPG in collected tumor tissues and adjacent tissues. e The relative expression of NCAPG in normal colon cell line FHC and CRC cell lines. f Kaplan–Meier survival curve based on NCAPG expression status of collected CRC patients. g Kaplan–Meier survival curve of CRC patients based on NCAPG from the database GenomicScape. **P < 0.01
Figure 2

Effect of NCAPG on CRC cell proliferation and apoptosis. a,b Western blotting and RT-qPCR was used to detect the expression of NCAPG in CRC cells transfected with negative control lentivirus (shCtrl) or shRNA lentivirus (shNCAPG). c,d Clone formation assay and CCK-8 assay was performed to determine the proliferation capacity of CRC cells and transduced cells. e,f Representative images of xenograft tumor nude mice and the corresponding excised tumors in shCtrl group and shNCAPG group. g Western blotting was used to detect the effect of NCAPG knockdown on apoptosis-related proteins, Bcl and Bax. h Influence of NCAPG inhibition on CRC cells apoptosis measured by flow cytometry. **P<0.01
Figure 3

NCAPG regulates PI3K/AKT pathway in CRC cells. a The correlation between NCAPG and PI3K/AKT gene family at mRNA expression level from GEPIA. b western blotting was used to show the relative expression of PI3K-p-PI3K, AKT and p-AKT at protein level in transduced and 740Y-P treated CRC cells. c,d CCK-8 and colony formation assays were performed to confirm the proliferation ability of transduced CRC cells. * P<0.05, **P<0.01.
Figure 4

MiR-23b-3p directly targets NCAPG in CRC cells. a The heatmap about expression of miR-23b-3p in digestive system cancers from dbDEMC 2.0 database. B Correlation between miR-23b-3p and NCAPG at expression level in 450 CRC patients based on the Starbase. c The relationship between NCAPG and miR-23b-3p at expression level plotted on the basis of detection in collected CRC samples. d The predicted binding sites of miR-23b-3p and NCAPG and wild-type and mutant of putative miR-23b-3p target sequences of NCAPG 3'UTR. e RT-qPCR was applied to measure the relative expression of miR-23b-3p in CRC cells transfected with anti-miR-23b-3p or miR-23b-3p mimics; f Analysis of the luciferase activity of psicheck-2-NCAPG 3'UTR WT and MUT vector in HCT116 cells by miR-23b-3p or anti-miR-23b-3p. g,h RT-qPCR and western blotting were utilized to detect NCAPG levels in the indicated HCT15 or HCT116 cells. i CCK-8 assay was applied to measure the growth ability of indicated HCT 116 cells in rescue experiment. *P<0.05, **P<0.01.
Supplementary Files

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