COPS3 Promotes Proliferation, Invasion, and EMT of Colorectal Cancer Cells by MEK/ERK Pathway

Yanchao Xie, Zhijiang Wei, and Chi Cheng

Department of Gastroenterology, Cangzhou Central Hospital, Cangzhou, Hebei 061000, China

Correspondence should be addressed to Yanchao Xie; xyc0810_dr@163.com

Received 12 May 2022; Accepted 1 July 2022; Published 19 July 2022

Academic Editor: Chia-Jung Li

Copyright © 2022 Yanchao Xie et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Colorectal cancer (CRC) is one of the most aggressive cancers with poor prognosis and high mortality. The study of the pathogenesis of CRC is a top priority in providing effective diagnostic and prognostic strategies for CRC. COPS3 protein is a subunit of the COP9 signaling body (CSN), which is closely associated with the development of multiple types of tumors. However, there are few studies on the role of COPS3 in colon adenocarcinoma (COAD). This study investigated the effects of COPS3 on proliferation, motility, and EMT of colorectal cancer cells and related mechanisms. COPS3 was highly expressed in COAD. The depletion of COPS3 suppressed the viability and stimulated the apoptosis of COAD cells. Depletion of COPS3 suppressed the motility and EMT process of COAD cells. Mechanically, we found that COPS3 could mediate MEK/ERK pathway and therefore affected the process of COAD cells. We thought that COPS3 could serve as a promising COAD target.

1. Introduction

Colorectal cancer (CRC), as one of the most aggressive cancers with poor prognosis, causes a large number of deaths worldwide and affects millions of people every year [1, 2]. CRC mainly affects the distal rectum, sigmoid colon, and descending colon [3]. More and more CRC risk factors have been reported recently, such as ageing, unhealthy diet, smoking, obesity, physical inactivity, inflammatory bowel disease, and genetic factors [4]. Treatment for CRC includes surgery, chemotherapy, and radiotherapy [5, 6]. However, because the detailed mechanism of CRC development is not fully understood, the 5-year survival rate for CRC is low, especially in the later stages [7]. Therefore, a better understanding of the pathogenesis of CRC is a top priority in providing effective diagnostic and prognostic strategies for patients with CRC.

COPS3 protein is a subunit of the COP9 signaling body [8], located in chromosome region 17p11.2 and plays a role in deubiquitination and protein kinase activity in a variety of processes [9]. COPS3 is closely associated with tumor development [10, 11]. Knockdown of COPS3 significantly reduced lung metastasis of osteosarcoma cells in mouse models, downregulated MEK and ERK signaling, and inhibited EMT by 90 kDa ribosomal S6 kinase (RSK), reducing metastasis of osteosarcoma cells [12]. In addition, COPS3 depletion inhibited tumor growth in nude mice by blocking cell cycle progression [13]. However, there are few studies on the role of COPS3 in colorectal cancer, particularly colon adenocarcinoma (COAD).

MEK/ERK cell signaling pathway plays an important role in various human tumors and is involved in cell proliferation, survival, metabolism, and cell migration [14]. For example, sophorine inhibits tumorigenesis in colorectal cancer by downregulating the MEK/ERK/VEGF pathway [15]. Epithelial mesenchymal transformation (EMT) is a biological process in which cancer cells lose their epithelial features and acquire mesenchymal markers, which make tumor cells more mobile and invasive [16]. EMT is marked by decreased E-cadherin expression and increased N-cadherin or Vimentin expression [17]. The process of EMT is controlled by transcription factors and certain pathways.

This study investigated the effects of COPS3 on proliferation, migration, invasion, and EMT of colorectal cancer cells and related mechanisms. Our data revealed that COPS3 was highly expressed in human COAD cells and affected the
viability, motility, and EMT of COAD cells via MEK/ERK pathway. We thought that COPS3 could serve as a promising COAD target.

2. Materials and Methods

2.1. Antibodies, Primers, and Plasmids. The antibodies used were anti-COPS3 (1:500 dilution, ab231344, Abcam), anti-E-cadherin (1:1000 dilution, ab76055, Abcam), anti-N-cadherin (1:1000 dilution, ab76011, Abcam), anti-Vimentin (1:500 dilution, ab8978, Abcam), anti-MEK (1:1000 dilution, 178876, Abcam), anti-p-MEK (1:1000 dilution, ab278564, Abcam), anti-ERK (1:1000 dilution, ab184699, Abcam), anti-p-ERK (1:500 dilution, ab201015, Abcam), and anti-β-actin (1:2000 dilution, 60008-1-Ig, Proteintech).

The quantitative PCR primer sequences of COPS3 are forward, 5′-GCGAGGAAUUGCAUCCUUTT-3′ and reverse, 5′-AAGGAUGCAUUCUCCGCTT-3′. The quantitative PCR primer sequences of GAPDH are 5′-TCCGCCGTGTG TACGTCATT-3′ and 5′-TCCGCCGTGTG TACGTCATT-3′.

siRNA of COPS3 and control siRNA was bought from Riobio (China).

2.2. Cell Culture. The normal cell line NCM460 and 4 COAD cell lines, including SW480, HCT116, LoVo, and DLD-1, were all purchased from ATCC. Both of the cells were maintained in DMEM, supplemented with 10% of fetal bovine serum, and incubated at 37°C in a 5% CO₂ incubator.

2.3. Immunoblot Assay. The samples were lysed with the lysis buffer (RIPA, Beyotime, China) and then separated by 10% SDS-PAGE experiment; sequentially, the total proteins were transferred onto PVDF membranes (Millipore, USA). Then, the PVDF membranes were blocked with the use of 5% dry milk in TBST buffer and antibodies. After washing with TBST for 3 times, the membranes were treated with the secondary antibodies for 45 min. Each blot was then visualized using the ECL kit (GE, SA).

2.4. Cell Viability Assays. For CCK-8, COAD cells were plated into the 96-well plates (1000 cell per well) and maintained in complete growth media for 24 h at 37°C. The cells were exposed to CCK-8 reagent at 37°C for 1.5 h. The relative cell viability was assessed with microplate spectrophotometer at 450 nm (Bio-Rad, U.S.A.).

For colony formation assay, COAD cells were plated into 24-well plates (1000 cell per well) and maintained in complete growth media for 14 d at 37°C. Subsequently, the cells were incubated with 0.2% crystal violet and washed, and then, the cells were photographed by a fluorescence microscope (Zeiss, Germany).

2.5. Cell Apoptosis Assay. The cells after transfection for 48 h were washed with PBS. Subsequently, the cells were fixed with precool 70% ethanol at -20°C for 1 h. Subsequently, the cells were stained with propidium iodide (PI) and FITC-labelled Annexin V at 4°C for 10 min, and the apoptosis levels were measured by BD FACS caliber.

Figure 1: COPS3 was highly expressed in COAD tissues and cell lines. (a) TCGA database showed the levels of transcript per million (TPM) in 286 tumor tissues compared to the 41 normal tissues. (b) qPCR assays showed the mRNA levels of COPS3 in normal cell line NCM460 and 4 COAD cell lines, including SW480, HCT116, LoVo, and DLD-1. (c and d) Immunoblot assays showed the protein levels of COPS3 in normal cell line NCM460 and 4 COAD cell lines, including SW480, HCT116, LoVo, and DLD-1. Data are presented as mean ± SD. **p < 0.01 and ***p < 0.001.
Disease Markers

Figure 2: Continued.
2.6. Tumor Growth In Vivo Assay. All experimental procedures were according to the criteria outlined in the Regulations of Experimental Animal Administration (http://www.most.gov.cn). Female BALB/c nude mice (8-week-old; weight, ~20 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. None of the mice died during the study. A total of 10 athymic nude mice were randomly divided into control (n = 5) and transfection (n = 5) groups. HCT116 cells which were stably transfected with shRNA plasmids were injected into the right flank of female nude mice. After 2 weeks, the volume of tumors was estimated every week, and the tumor growth curves of 7 consecutive weeks were calculated. The final tumor volume was calculated according to the equation: Tumor volume (mm³) = tumor length (mm) × tumor width (mm)/2/2.

2.7. Statistics. Data were represented as mean ± SD. The statistical significance of the difference was evaluated by Student’s t test, and p < 0.05 was considered significant.

3. Results

3.1. COPS3 Was Highly Expressed in COAD. We first detected the expression levels of COPS3 in COAD tissues through the analysis in TCGA database. We noticed that the transcript per million of COPS3 in primary tumor tissues (n = 286) was higher than normal (n = 41), suggesting the high expression in COAD (Figure 1(a)). COPS3 mRNA level increased, indicating that COPS3 high expression may be transcriptional. We then detected the expression of COPS3 in normal cell line NCM460 and 4 COAD cell lines, including SW480, HCT116, LoVo, and DLD-1, through qPCR and immunoblot assays. We found that COPS3 was highly expressed in COAD cell lines at mRNA and protein levels (Figures 1(b) and 1(d)). We therefore thought that COPS3 was highly expressed in COAD.

3.2. COPS3 Depletion Suppressed the Viability of COAD Cells and Stimulated Apoptosis. Then, the effects of COPS3 on the viability and apoptosis of COAD cells were evaluated by the transfection of its siRNA in COAD cells including SW480 and HCT116. qPCR and immunoblot confirmed that the transfection of its siRNA decreased the expression of COPS3, compared to the control and NC-siRNA groups in these cells at mRNA and protein levels (Figures 2(a) and 2(b)). Through CCK-8 assays, we found that COPS3 ablation decreased the OD value at 450 nm wavelength, suggesting the inhibition of cell viability (Figure 2(c)). Further through colony formation, we found that the knockdown of COPS3 also decreased colony number in SW480 and HCT116 cells (Figures 2(d) and 2(e)). In addition, FCM assays showed that the depletion of COPS3 contributed to the apoptosis of SW480 and HCT116 cells, with the increased percentage of apoptosis cells (Figures 2(f) and 2(g)). We further detected the expression of cleaved caspase-3 and Bcl-2 in control and COPS3 siRNA cells and further confirmed the previous conclusion (Figure 2(h)). Therefore, COPS3 depletion suppressed the viability of COAD cells and stimulated apoptosis.

3.3. The Knockdown of COPS3 Suppressed the Motility of COAD Cells. We then detected the effects of COPS3 on the motility of COAD cells. We found that its ablation increased the wound width at 24th hour time point, in SW480 and
HCT116 cells (Figures 3(a) and 3(b)). We therefore thought depletion of COPS3 suppressed COAD cell migration. Further, we found its knockdown suppressed the invasion of SW480 and HCT116 cells, with the decreased number of invasive cells (Figures 3(c) and 3(d)). Therefore, COAD3 knockdown inhibited the motility of COAD cells.

3.4. Knockdown of COPS3 Suppressed the EMT in COAD Cells. Since previously we showed the effects of COPS3 on COAD3 cell viability and migration, we then investigated its role in the COAD cell EMT process. We detected the expression of several EMT markers. Through immunoblot assays, we found that COPS3 knockdown increased the protein levels of E-cadherin and downregulation of N-cadherin and Vimentin, in both SW480 and HCT116 cells (Figure 4). Therefore, depletion of COPS3 suppressed the EMT process in COAD cells.

3.5. COPS3 Mediated the MEK/ERK Pathway in COAD Cells. Then, we investigated the possible mechanism underlying...
Figure 4: Depletion of COPS3 suppressed the EMT process in COAD cells. Immunoblot assays showed the expression of E-cadherin, N-cadherin, and Vimentin in SW480 and HCT116 cells upon the transfection of control or COPS3 siRNAs or without transfection (control). Data are presented as mean ± SD. **p < 0.01 and ***p < 0.001, siCOPS3 vs. siControl.

Figure 5: COPS3 mediated the MEK/ERK pathway in COAD cells. Immunoblot assays showed the expression of phosphorylated MEK and ERK and expression of these proteins in SW480 and HCT116 cells upon the transfection of control or COPS3 siRNAs or without transfection (control). Data are presented as mean ± SD. **p < 0.01 and ***p < 0.001, siCOPS3 vs. siControl.
COPS3 affecting COAD progression. The previous study indicated the effects of COPS3 on the MEK/ERK pathway, which could mediate the proliferation, motility, and EMT in several types of tumor cells [12]. We then detected whether COPS3 could mediate this pathway in COAD cells. Through immunoblot assays, the knockdown of COPS3 decreased the phosphorylation levels of MEK and ERK in both SW480 and HCT116 cells (Figure 5). Therefore, we thought COPS3 could mediate the MEK/ERK in COAD cells.

3.6. COPS3 Depletion Suppressed Tumor Growth In Vivo. To further confirm whether COPS3 deficiency was able to repress tumor growth, the in vivo assays were constructed. Through injection of COPS3 deficiency HCT116 cells into nude mice, we measured and calculated the growth curves of tumors. Consistent with our hypothesis, the volumes of tumors in COPS3-depleted groups were markedly smaller than the negative control groups (Figure 6(a)). We then confirmed the silencing efficiency of COPS3 siRNA, we detected the expression of COPS3 in tumor tissues of mice via IHC and immunoblot assays, and the data revealed that compared with the negative groups, the protein levels of COPS3 were efficiently restrained by COPS3 siRNA in the COPS3 depletion groups (Figures 6(b) and 6(c)). We further detected the expression of E-cadherin, Erk, p-Erk, Mek, and p-Mek through immunoblot, and the data further confirmed our previous conclusion (Figure 6(d)). Therefore, COPS3 depletion suppressed tumor growth in vivo.

4. Discussion

CRC is a common gastrointestinal malignancy occurring in the colon [18]. CRC inchoate symptom is more not apparent and often already was in progress period when seeing a doctor, right now commonly used remedial measure [2]. To improve the resection rate, reduce the recurrence rate, and improve the survival rate, the treatment of intermediate and advanced CRC is based on surgery, supplemented by chemotherapy, immunotherapy, traditional Chinese medicine, and other supportive therapies [18]. Recently, targeted therapy has made a series of positive progress and has great potential to improve the survival rate of patients with advanced colorectal cancer [19]. However, there are new and more therapeutic targets for the CRC treatment. Here, we noticed that COPS3 was highly expressed in COAD. It affected the viability, motility, and EMT of COAD cells. We thought it could act as a target of COAD.

Through a series of in vitro assays, we concluded that COPS3 was highly expressed in human COAD cells. We further confirmed its effects on the viability, motility, and the process of EMT in COAD cells. COPS3 is an important oncogene involved in metastasis of osteosarcoma [9]. COPS3 depletion could inhibit the growth of lung cancer...
and liver cancer cells and induce apoptosis [13, 20]. A previous study also revealed that COPS3 played a vital role in linking Raf-1/MEK/ERK pathway and autophagic regulation in osteosarcoma [12]. Depletion of COPS3 could suppress the progression of prostate cancer through reducing phosphorylated p38 MAPK and impairs the EMT [21]. In addition, the overexpression of COPS3 could contribute to the progression of clear cell renal cell carcinoma (ccRCC) via regulation of phospho-AKT, Cyclin D1, and Caspase-3 [22]. The ablation of COPS3 suppressed the proliferation of lung cancer cells via induction of cell cycle arrest and stimulation of apoptosis [13]. These studies with our findings confirmed that COPS3 could serve as a promising target of cancers.

The multiple biological functions of COPS3 have been widely revealed [10]. COP9S3 played a role in regulating mouse oocytes meiosis by regulating MPF activity and securing degradation [23]. The COPS3 is necessary for early embryo survival by way of a stable protein deposit in mouse oocytes [24]. COPS3 is also poised to facilitate communication between the extracellular matrix and the nucleus [25]. Therefore, we guess that COPS3 could induce the deubiquitination of the downstream proteins or the protein kinase activity and therefore mediate the progression of COAD. However, the precise mechanism needs further study.

MEK/ERK signaling pathway can promote the progression of multiple types of cancers, including COAD [26]. The MEK/ERK pathway has been revealed to affect the proliferation, apoptosis, and motility of tumors and affect the EMT progression [12]. Multiple proteins or drugs affected COAD progression via this pathway. For example, Verticillin A could increase the BIM/MCL-1 ratio to overcome ABT-737 resistance in COAD cells by this pathway [27]. These studies all confirmed that MEK/ERK pathway could serve as a promising target of COAD.

In summary, we noticed the high expression of COPS3 in COAD cells. COPS3 contributed to the viability, motility, and EMT of COAD cells via MEK/ERK pathway. We therefore thought COPS3 could serve as a target of COAD.

Data Availability

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

Conflicts of Interest

The authors state that there are no conflicts of interest to disclose.

Authors’ Contributions

Yanchao Xie designed the study and supervised the data collection, Zhijiang Wei analyzed the data and interpreted the data, and Chi Cheng prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

References

[1] Y. Jin, L. Meng, H. Yang et al., “The IL-22 gene rs2227478 polymorphism significantly decreases the risk of colorectal cancer in a Han Chinese population,” *Pathology, research and practice*, vol. 228, article 153690, 2021.
[2] D. Lucchetti, I. V. Zurlo, F. Colella et al., “Mutational status of plasma exosomal KRAS predicts outcome in patients with metastatic colorectal cancer,” *Scientific Reports*, vol. 11, no. 1, p. 22868, 2021.
[3] A. Zuhan, I. Riwanto, D. E. Listiana, F. Djannah, and R. M. Rosyidi, “The extent of distal intramural spread of colorectal cancer cell study of it's relationship with histological grading, stage of disease and CEA level,” *Annals of medicine and surgery*, vol. 64, p. 102227, 2021.
[4] A. Ottaiano, L. Circelli, M. Santorsola et al., “Metastatic colorectal cancer and type 2 diabetes: prognostic and genetic interactions,” *Molecular oncology*, vol. 16, no. 2, pp. 319–332, 2022.
[5] V. Lago, L. Sala Climent, B. Segarra-Vidal, M. Frasson, B. Flor, and S. Domingo, “Ghost ileostomy: prevention, diagnosis, and early treatment of coloanastomosis leakage in advanced ovarian cancer,” *International journal of gynecological cancer*, vol. 32, no. 1, pp. 109–110, 2022.
[6] Y. Luan, M. Li, Y. Zhao et al., “Centrosomal-associated proteins: potential therapeutic targets for solid tumors?,” *Biomedicine & pharmacotherapy*, vol. 144, article 112292, 2021.
[7] J. Nian, L. Tao, and W. Zhou, “Prior endoscopic resection does not affect the outcome of secondary surgery for T1 colorectal cancer, a systematic review and meta-analysis,” *International journal of colorectal disease*, vol. 37, no. 2, pp. 273–281, 2022.
[8] E. Oron, M. Mannervik, S. Rencus et al., “COP9 signalosome subunits 4 and 5 regulate multiple pleiotropic pathways in Drosophila melanogaster,” *Development*, vol. 129, no. 19, pp. 4399–4409, 2002.
[9] T. Yan, J. S. Wunder, N. Gokgoz et al., “COPS3 amplification and clinical outcome in osteosarcoma,” *Cancer*, vol. 109, no. 9, pp. 1870–1876, 2007.
[10] M. van Dartel and T. J. Hulsebos, “Amplification and overexpression of genes in 17p11.2 ~ p12 in osteosarcoma,” *Cancer genetics and cytogeneics*, vol. 153, no. 1, pp. 77–80, 2004.
[11] J. Both, T. Wu, A. L. Ten Asbroek, F. Baas, and T. J. Hulsebos, “Oncogenic properties of candidate oncogenes in chromosome region 17p11.2p12 in human osteosarcoma,” *Cytogenetic and genome research*, vol. 150, no. 1, pp. 52–59, 2017.
[12] F. Zhang, T. Yan, W. Guo et al., “Novel oncogene COPS3 interacts with Beclin1 and Raf-1 to regulate metastasis of osteosarcoma through autophagy,” *Journal of experimental & clinical cancer research : CR.*, vol. 37, no. 1, p. 135, 2018.
[13] J. Pang, X. Yan, H. Cao et al., “Knockdown of COPS3 inhibits lung cancer tumor growth in nude mice by blocking cell cycle progression,” *Journal of Cancer*, vol. 8, no. 7, pp. 1129–1136, 2017.
[14] J. Xue, Y. Li, J. Yi, and H. Jiang, “HAVCR1 affects the MEK/ERK pathway in gastric adenocarcinomas and influences tumor progression and patient outcome,” *Gastroenterology research and practice*, vol. 2019, Article ID 6746970, 10 pages, 2019.
[15] P. Zhang, H. Kawakami, W. Liu et al., “Targeting CDK1 and MEK/ERK overcomes apoptotic resistance in BRAF-mutant human colorectal cancer,” *Molecular cancer research : MCR*, vol. 16, no. 3, pp. 378–389, 2018.
[16] N. L. Tran, R. B. Nagle, A. E. Cress, and R. L. Heimark, “N-cadherin expression in human prostate carcinoma cell lines. An epithelial-mesenchymal transformation mediating adhesion with stromal cells,” *The American journal of pathology*, vol. 155, no. 3, pp. 787–798, 1999.

[17] X. Zhang, G. Liu, Y. Kang, Z. Dong, Q. Qian, and X. Ma, “N-cadherin expression is associated with acquisition of EMT phenotype and with enhanced invasion in erlotinib-resistant lung cancer cell lines,” *PLoS One*, vol. 8, no. 3, article e57692, 2013.

[18] W. Song, J. Ren, R. Xiang, C. Kong, and T. Fu, “Identification of pyroptosis-related subtypes, the development of a prognosis model, and characterization of tumor microenvironment infiltration in colorectal cancer,” *Oncoimmunology*, vol. 10, no. 1, p. 1987636, 2021.

[19] H. H. Hashbullah and M. Musa, “Gene therapy targeting p53 and KRAS for colorectal cancer treatment: a myth or the way forward?,” *International journal of molecular sciences*, vol. 22, no. 21, article 11941, 2021.

[20] Y. S. Yu, Z. H. Tang, Q. C. Pan, X. H. Chen, X. N. Liu, and G. Q. Zang, “Inhibition of Csn3 expression induces growth arrest and apoptosis of hepatocellular carcinoma cells,” *Cancer Chemotherapy and Pharmacology*, vol. 69, no. 5, pp. 1173–1180, 2012.

[21] Z. Zhu, Y. Hong, F. Zhang et al., “Knockdown of COPS3 inhibits the progress of prostate cancer through reducing phosphorylated p38 MAPK expression and impairs the epithelial-mesenchymal transition process,” *The Prostate*, vol. 79, no. 16, pp. 1823–1831, 2019.

[22] Y. Hong, X. Huang, L. An et al., “Overexpression of COPS3 promotes clear cell renal cell carcinoma progression via regulation of phospho-AKT(Thr308), cyclin D1 and caspase-3,” *Experimental cell research*, vol. 365, no. 2, pp. 163–170, 2018.

[23] E. Kim, S. J. Yoon, E. Y. Kim et al., “Function of COP9 signalosome in regulation of mouse oocytes meiosis by regulating MPF activity and securing degradation,” *PLoS One*, vol. 6, no. 10, article e25870, 2011.

[24] S. Israel, H. C. A. Drexler, G. Fuellen, and M. Boiani, “The COP9 signalosome subunit 3 is necessary for early embryo survival by way of a stable protein deposit in mouse oocytes,” *Molecular human reproduction*, vol. 27, no. 8, 2021.

[25] C. Hunter, J. Evans, and M. L. Valencik, “Subunit 3 of the COP9 signalosome is poised to facilitate communication between the extracellular matrix and the nucleus through the muscle-specific beta1D integrin,” *Cell communication & adhesion*, vol. 15, no. 3, pp. 247–260, 2008.

[26] N. Cui, L. Li, Q. Feng, H. M. Ma, D. Lei, and P. S. Zheng, “Hexokinase 2 promotes cell growth and tumor formation through the Raf/MEK/ERK signaling pathway in cervical cancer,” *Frontiers in oncology*, vol. 10, p. 581208, 2020.

[27] Y. Guan, K. Tu, Q. Huang, and F. Liu, “Verticillin A increases the BIMEL/MCL-1 ratio to overcome ABT-737-resistance in human colon cancer cells by targeting the MEK/ERK pathway,” *Biochemical and biophysical research communications*, vol. 567, pp. 22–28, 2021.