Neoplasma 2021; 68(4): 702–710

doi:10.4149/neo_2021_201031N1158

SPOCK1 promotes the proliferation and migration of colon cancer cells by regulating the NF-κB pathway and inducing EMT

Han-Xiong LIU[1], Yuan-Yuan CAO[2], Jia-Yao QU[3,*]

[1]Department of Gastroenterology, The First People's Hospital of Chenzhou, Chenzhou, China; [2]Department of Critical Care Medicine, The First People's Hospital of Chenzhou, Chenzhou, China; [3]Department of Clinical Laboratory, The Third People's Hospital of Shenzhen, Southern University of Science and Technology, National Clinical Research Center for Infectious Diseases, Shenzhen, China

**Correspondence: qujiayao17@163.com*

Received October 31, 2020 / Accepted February 11, 2021

Sparc/osteonectin, cwcv, and kazal-like domains proteoglycan 1 (SPOCK1) has been shown to promote various tumors, but its role in colon cancer (CRC) has not been clearly illuminated. The aim of this study was to investigate the effects of SPOCK1 interference on the proliferation, migration, and EMT of CRC cells. First, we analyzed the expression of SPOCK1 in various CRC datasets. Then, we investigated the correlation between SPOCK1 and prognosis in CRC patients. We overexpressed SPOCK1 and knocked down SPOCK1 expression in HCT-116 and SW480 cells, respectively. Then, cell proliferation was assayed with a CCK-8 assay, and cell migration was evaluated with a Transwell migration assay. NF-κB and EMT-related proteins were studied by western blotting. The results indicated that the mRNA levels of SPOCK1 were relatively high in CRC tissues and that high expression of SPOCK1 was negatively correlated with patient prognosis. With SPOCK1 overexpression in HCT-116 cells, cell proliferation and migration were increased, while SPOCK1 knockdown had the opposite effects. With SPOCK1 overexpression in HCT-116 cells, the expression levels of NF-κB and EMT-related proteins were elevated, while SPOCK1 knockdown produced the opposite results. In conclusion, our study demonstrates that SPOCK1 may activate the NF-κB/Snail signaling cascade to promote the proliferation and migration of CRC cells. SPOCK1 may serve as a new prognostic indicator and potential therapeutic target in CRC.

Key words: SPOCK1, colon cancer, cell proliferation, NF-κB, EMT

Colon cancer (CRC) is one of the most common cancer types and has the third-highest morbidity rate of all tumors following prostate cancer and lung cancer [1]. Despite advances in early detection, surgical techniques, and systemic treatment, the 5-year survival rate of CRC patients is relatively low [2, 3]. Most patients with CRC are diagnosed in an intermediate- or late-stage and have a poor prognosis [3]. Thus, investigating the mechanisms underlying the occurrence and development of CRC and identifying molecular markers for early diagnosis of CRC are of great significance for early diagnosis and clinical treatment.

Sparc/osteonectin, cwcv, and kazal-like domains proteoglycan 1 (SPOCK1), also known as testtican-1, was initially identified in the human testes [4]. As a member of the cysteine-rich acidic secreted protein (SPARC) family, SPOCK1 encodes a calcium-binding matricellular glycoprotein [4]. As a kind of adhesion protein, SPOCK1 participates in cell-cell and cell-matrix interactions [5], cell proliferation, and migration [6] and regulates a variety of physiological and pathological processes [5]. In recent studies, the mRNA levels of SPOCK1 were analyzed in several types of tumors [6–8]. In prostate cancer patients, high SPOCK1 expression is associated with an advanced stage, a high T value, and a high Gleason grade [9]. In patients with pancreatic cancer or gastric cancer, high expression of SPOCK1 is positively correlated with a poor prognosis [10, 11]. However, little is known about the detailed function and underlying molecular mechanism of SPOCK1 in the pathology of CRC.

In our previous bioinformatics analysis, we found that the expression of SPOCK1 might be associated with proliferation and migration in CRC. In the current study, we found that SPOCK1 expression tended to be upregulated in CRC tissues and was closely associated with patient prognosis. Moreover, we confirmed that SPOCK1 might promote CRC proliferation and migration by inducing epithelial-mesenchymal transition (EMT) via the NF-κB/Snail signaling pathway. Our study may provide a novel foundation for future CRC diagnostics and treatment.

Materials and methods

Reagents. Fetal bovine serum (FBS), OptiMEM, and RPMI-1640 medium were purchased from Gibco (Thermo, USA). A penicillin-streptomycin solution and trypsin-EDTA (0.25%) were purchased from HyClone (Thermo, USA). TRIzol and SYBR Green kit were acquired from Invitrogen (Thermo, USA). CCK-8 kit, a nuclear protein extraction kit, RIPA lysis buffer, and bicinchoninic acid (BCA) protein quantitation kits were purchased from Biyuntian Biotechnology (Biyuntian, China). Antibodies against SPOCK1 (ab229935), E-cadherin (ab1416), Vimentin (ab92547), GAPDH (ab9485), and Snail (ab53519) were purchased from Abcam (Abcam, USA). Antibodies against IKBα (4814S), p-IKBα (2859S), NF-κB p65 (8242S), p-NF-κB p65 (3033S), and H3 (4499S) were purchased from CST (CST, USA).

Cell culture. The human CRC cell lines LOVO, HT29, HCT-116, SW620, SW480, and SW116 were obtained from the Committee of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI-1640 medium supplemented with 10% FBS and 1% streptomycin/penicillin. The cell lines were propagated in a 5% CO_2, 37 °C humidified incubator.

Cell transfection. Cells were grown in six-well plates until reaching 80–90% confluence. Then, SPOCK1-siRNA or pcDNA-SPOCK1 was transfected into the cells with Lipofectamine™ 3000 (Invitrogen, USA) according to the manufacturer's protocol. A vector group or si-NC group was included in the experiment. Then, RT-PCR and western blotting were performed to confirm the transfection efficiency. After 48 h of transfection, cells were collected for subsequent experiments. siRNAs and pcDNA plasmids were designed by and purchased from Guangzhou Fullen Biotechnology (Fullen, China).

Cell proliferation and migration assays. To detect cell proliferation, CCK-8 assays were performed according to the manufacturer's instructions. Briefly, a total of 3×10^3 cancer cells were seeded in each well of a 96-well plate, and a CCK-8 kit was used every 24 h. The absorbance was measured 24 h later at 450 nm, and cell growth curves were generated. To detect cell migration, Transwell migration assays were used. A total of 5×10^4 cells were seeded in the upper chamber for 24 h for the migration assay. Medium containing 10% FBS was placed in the lower chamber. Twenty-four hours later, the cells were fixed with 4% paraformaldehyde for 20 min and then stained with 0.1% crystal violet for 10 min. The cells in the upper chamber were removed with a cotton swab and dried. Images were captured with an inverted microscope.

Real-time polymerase chain reaction (RT-PCR). According to the manufacturer's instructions, total RNA was isolated by using TRIzol reagent. Reverse transcription was performed using the GoScript Reverse Transcription System (Promega, USA) following the manufacturer's protocols. RT-PCR was performed using the SYBR Green kit on the Light Cycle480 Real-Time PCR Detection System (Roche, Germany) following the manufacturer's instructions. Relative mRNA levels were normalized against GAPDH levels using the $2^{-\Delta\Delta}$Ct formula. The primer sequences were as follows: GAPDH forward, 5'-ACCACAGTCCATGCCATCAC-3' and reverse, 5'-TCCACCCTGTTGCTGTA-3'; and SPOCK1 forward, 5'-CCCGTTACTGCCGGTGATTA-3' and reverse, 5'-CCAGGTCTGGACAAGCTGAG-3'.

Western blot analysis. Cells were lysed in cold lysis buffer with PMSF and protease and phosphatase inhibitors. Proteins were extracted according to the reagent instructions and denatured with loading buffer for 10 min at 99 °C. Nuclear proteins were extracted following the protocol of a nuclear protein extraction kit. BCA assays were used to detect total protein concentrations. Equal amounts of proteins were loaded, separated by 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and electrotransferred to polyvinylidene fluoride (PVDF) membranes. Each membrane was incubated with different antibodies (anti-SPOCK1, 1:500; anti-E-cadherin, 1:500; anti-Vimentin, 1:500; anti-Snail, 1:500; anti-IKBα, 1:1,000; anti-p-IKBα, 1:1,000; anti-NF-κB p65, 1:1,000; anti-p-NF-κB p65, 1:1,000; anti-GAPDH, 1:5,000; anti-H3, 1:1,000). Immunoreactive bands were visualized using an ECL detection reagent (Biyuntian Biotechnology, China).

Statistical analysis. Statistical Package for the Social Sciences (SPSS) V.20 was used to run statistical analyses. Quantitative data are expressed as the mean ± SD. A p-value <0.05 was considered to indicate a significant difference.

Results

SPOCK1 expression is upregulated in CRC tissues. We first investigated the expression of SPOCK1 in human CRC tumors. We analyzed the expression of SPOCK1 in TCGA CRC datasets using the UALCAN website (http://ualcan.path.uab.edu/index.html). SPOCK1 expression was significantly higher in CRC tissues than in normal colon tissues (Figure 1A). The Kaiser colon and Skrzypczak colorectal2 dataset data were obtained from the Oncomine database. SPOCK1 mRNA levels were significantly upregulated in CRC tissues compared to normal tissues (Figures 1B, 1C). Furthermore, these results were supported by analysis of the individual dataset GSE35834 in the NCBI GEO database (Figure 1D). These results suggest that SPOCK1 expression is upregulated in CRC.

SPOCK1 upregulation is associated with lymphatic metastasis and a poor patient prognosis. To explore the significance of SPOCK1 in CRC, we next analyzed the effects of SPOCK1 expression on the prognosis and tumor stage of CRC patients. As shown in Figures 2A and 2B, CRC patients with high expression of SPOCK1 had markedly lower overall survival (OS) and disease-free survival (DFS) rates than those with low expression of SPOCK1. Similar results were obtained with the GSE17536 dataset (Figures 2C, 2D). We also observed that SPOCK1 expression was significantly

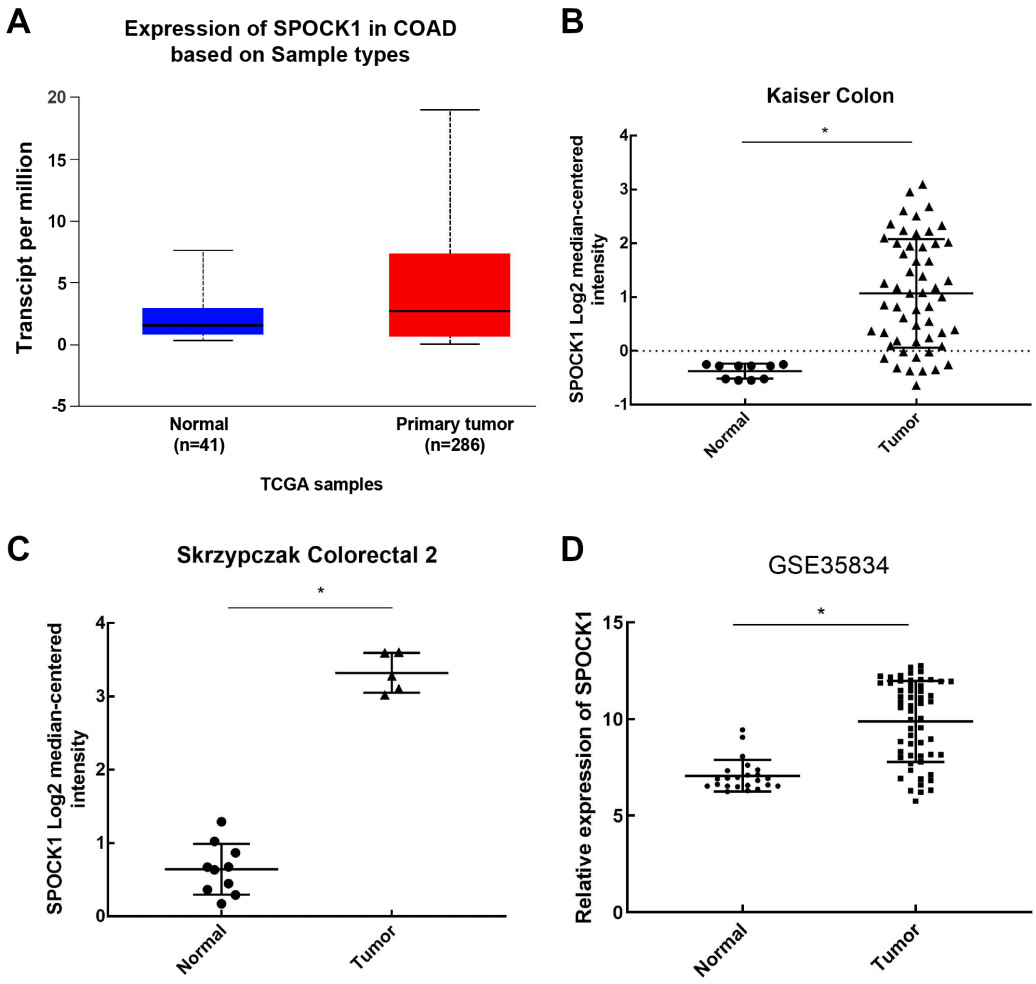


Figure 1. SPOCK1 was highly expressed in CRC patients. A) Relative expression levels of SPOCK1 in colon tissues and CRC tissues in the TCGA COAD dataset. B, C) Relative expression levels of SPOCK1 in colon tissues and CRC tissues in the Kaiser colon and Skrzypczak colorectal2 datasets, respectively. D) Relative expression levels of SPOCK1 in colon tissues and CRC tissues in the GSE35834 dataset. *p<0.05, compared with normal colon tissue.

associated with tumor stage and the lymphatic metastasis status (Figures 2E, 2F). Taken together, these results suggest that SPOCK1 may play an important role in CRC progression.

SPOCK1 promotes the proliferation and migration of CRC cells. To further determine the biological function of SPOCK1 in CRC, SPOCK1 expression was analyzed in six human CRC cell lines (LOVO, HT29, HCT-116, SW620, SW480, and SW116) by RT-qPCR and western blotting. The results showed that SPOCK1 was expressed at lower levels in the HCT-116 cell line and highly expressed in the SW480 cell line (Figures 3A, 3B). Then, we established an HCT-116 cell line overexpressing SPOCK1 and knocked down SPOCK1 expression in SW480 cells. Overexpression and knockdown were confirmed using RT-PCR and western blotting (Figures 3C, 3D). Next, to further study the role of SPOCK1 in proliferation, we used CCK-8 assays. As shown in Figures 3E and 3F, SPOCK1 overexpression significantly increased cell proliferation, whereas SPOCK1 knockdown significantly suppressed cell proliferation. Furthermore, Transwell migration assays showed that SPOCK1 overexpression promoted cell migration compared with vector transfection. Opposite results were obtained after silencing SPOCK1 (Figures 3G, 3H). Thus, these findings suggested that overexpression of SPOCK1 promoted cell proliferation and migration in CRC cells.

High expression of SPOCK1 upregulates the expression of NF-κB pathway components. Then, we wanted to study the signaling pathways responsible for regulating the SPOCK1-promoted proliferation and migration of CRC cells. For this purpose, SPOCK1 in the TCGA COAD dataset was analyzed by bioinformatics methods. Interestingly, we found that the expression of SPOCK1 in CRC was associated with the NF-κB signaling pathways in the TCGA COAD dataset (Supplementary Figure S1). Previous studies have shown that the NF-κB signaling plays a critical role in tumor cell

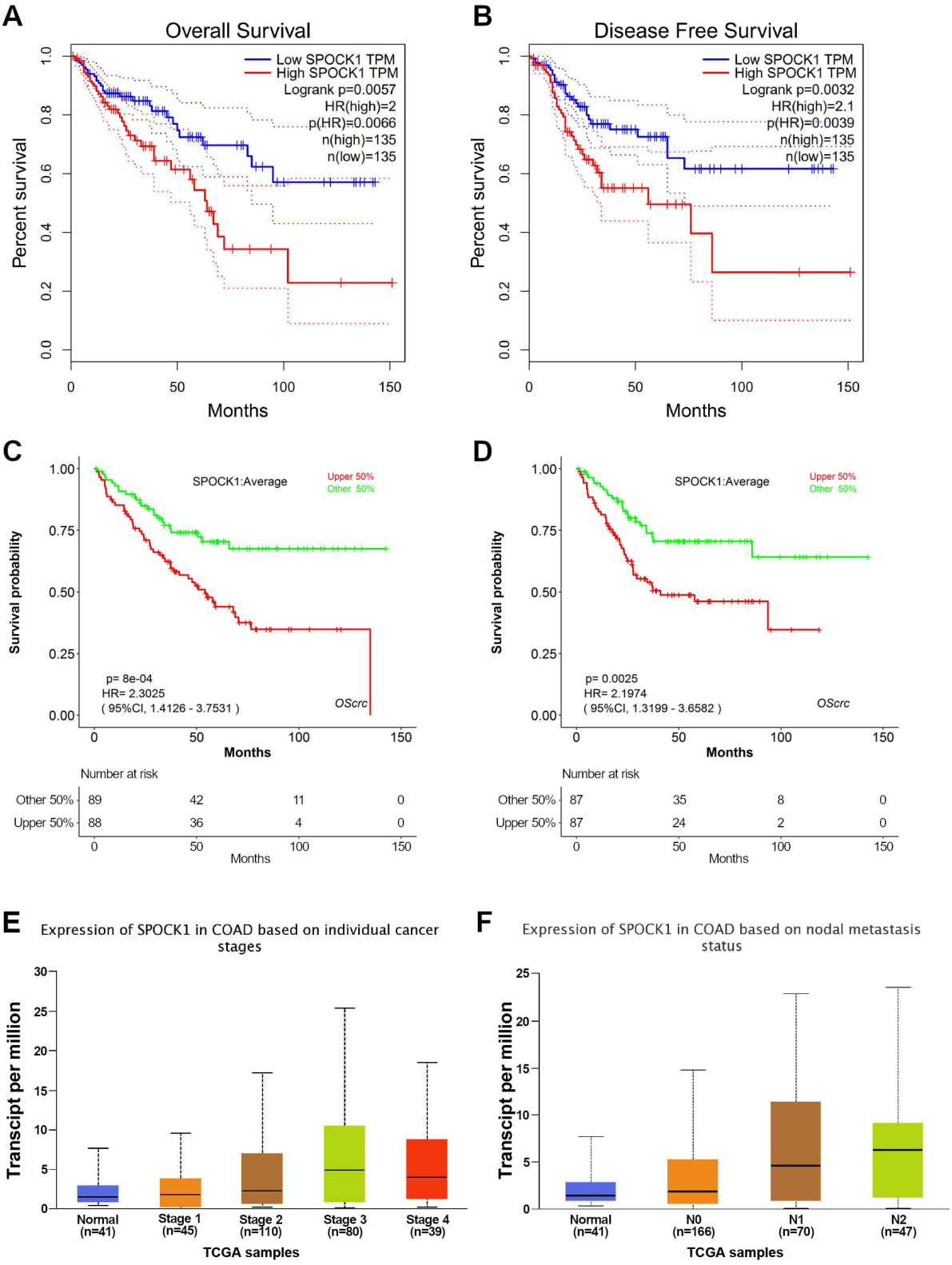


Figure 2. High expression of SPOCK1 is negatively correlated with patient prognosis. A, B) Overall survival and disease-free survival of patients with CRC, in relation to low or high expression of SPOCK1 in the TCGA COAD dataset. C, D) Overall survival and disease-free survival of patients with CRC, in relation to low or high expression of SPOCK1 in GSE17536. E) Expression levels of SPOCK1 in CRC patients with different tumor stages. F) Expression levels of SPOCK1 in CRC patients with different lymphatic metastasis statuses. *p<0.05, compared with normal colon tissue.

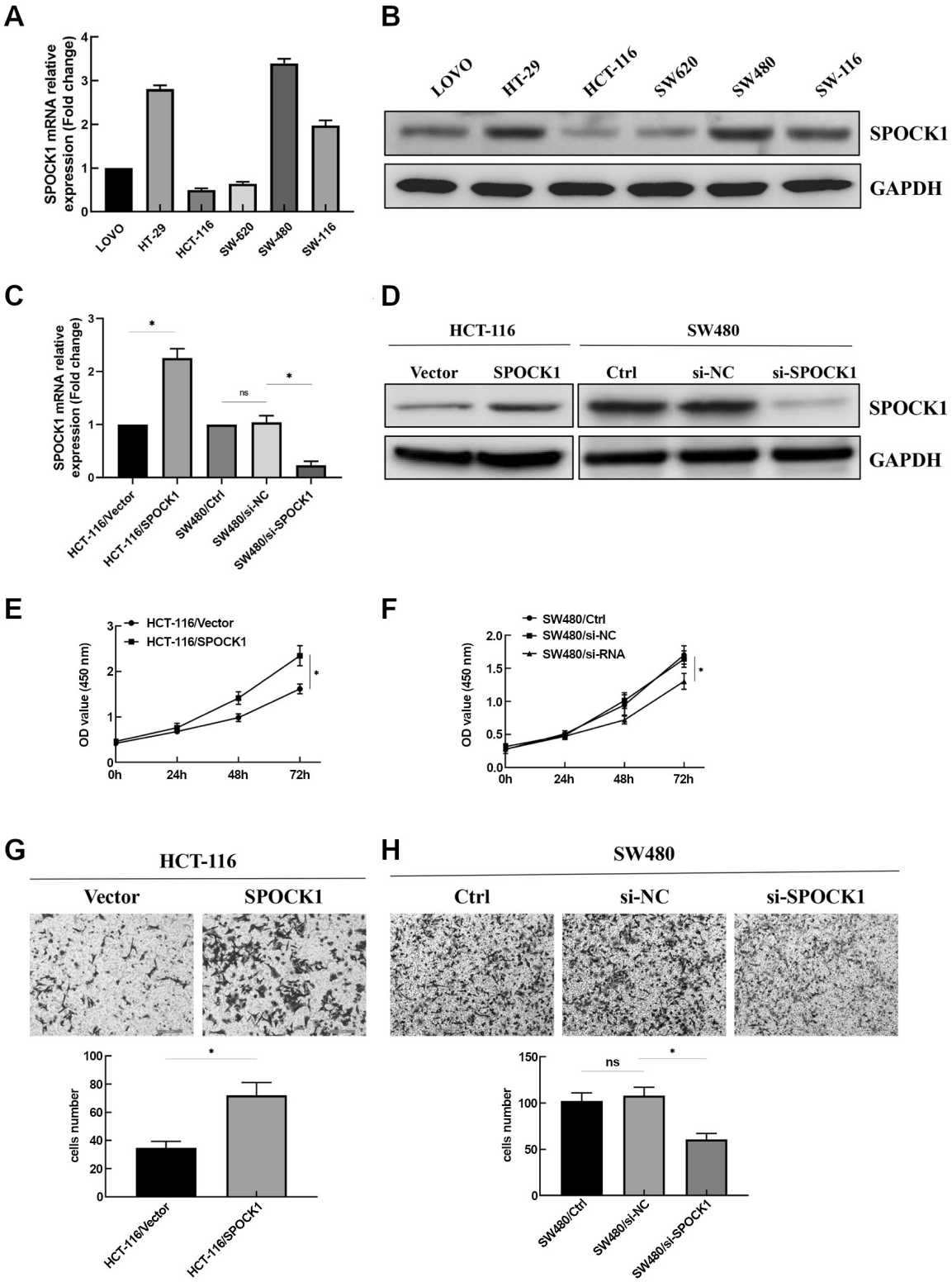


Figure 3. High expression of SPOCK1 promotes CRC cell proliferation and migration. A, B) The expression levels of SPOCK1 in the LOVO, HT29, HCT-116, SW620, SW480, and SW116 cell lines were detected by RT-qPCR and western blot analysis, respectively. C, D) The expression levels of SPOCK1 in HCT-116/Vector, HCT-116/SPOCK1, SW480/Ctrl, SW480/si-NC, SW480/si-SPOCK1 cells were detected by RT-qPCR and western blot analysis, respectively. E, F) The effect of SPOCK1 on cell proliferation was analyzed by a CCK-8 assay. G, H) The effect of SPOCK1 on cell migration was analyzed by a Transwell migration assay. *p<0.05, compared with control cells.

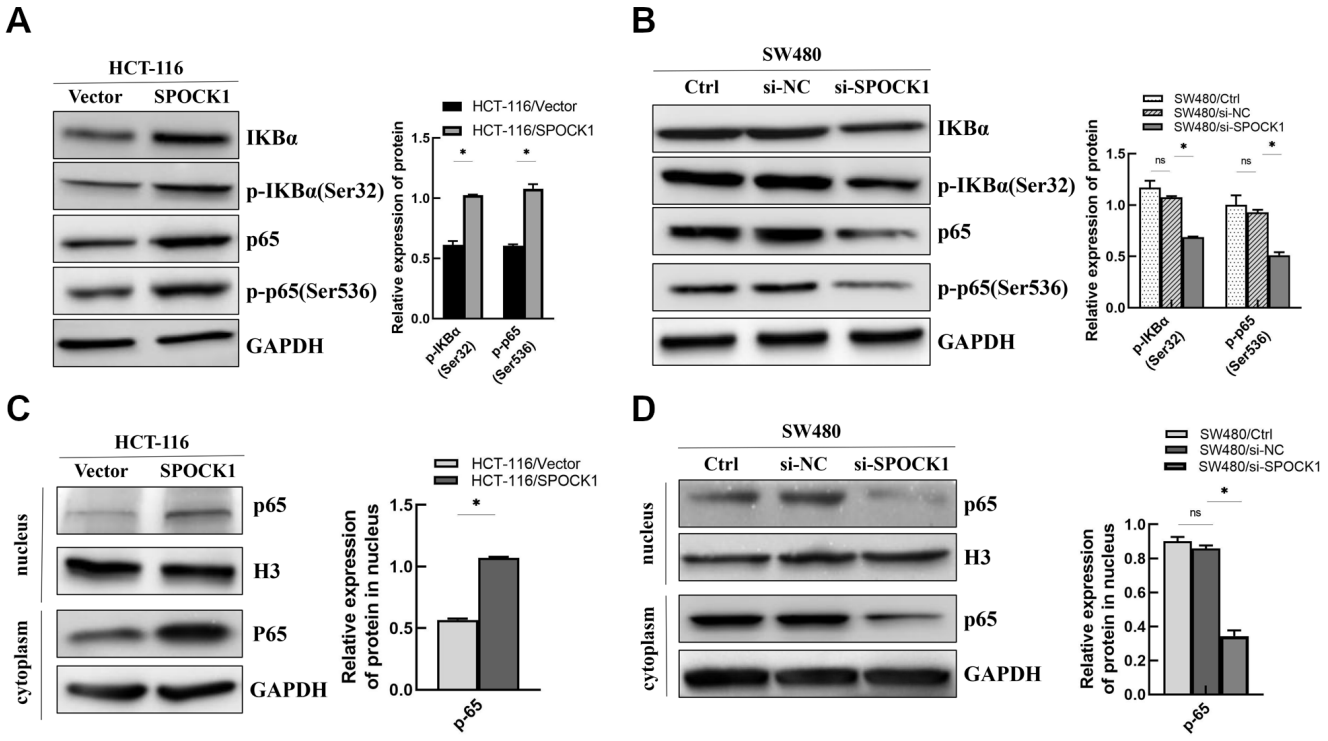


Figure 4. Effect of SPOCK1 on NF-κB-related protein expression. A, B) The expression levels of IKBα, p-IKBα (Ser32), NF-κB p65, and p-NF-κB p65 (Ser536) were detected by western blotting. C, D) The expression levels of nuclear NF-κB p65 were detected by western blotting. *p<0.05, compared with control cells.

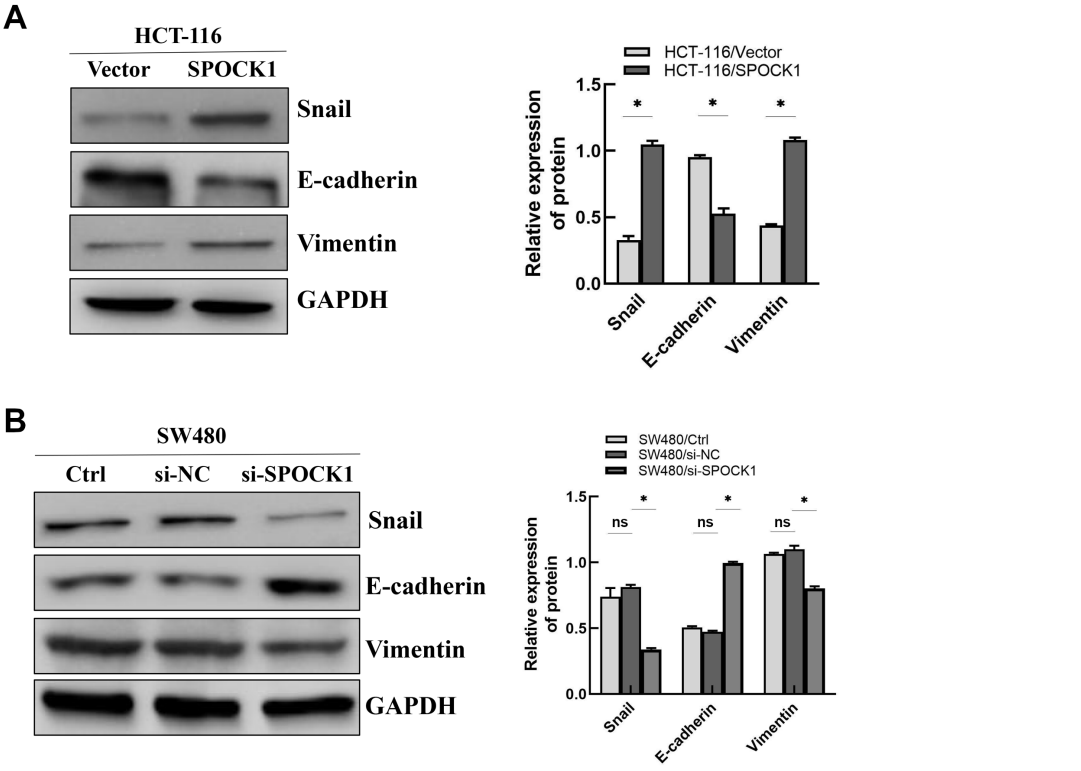


Figure 5. Effect of SPOCK1 on EMT-related protein expression. A, B) The expression levels of E-cadherin, Vimentin, and Snail were detected by western blotting. *p<0.05, compared with control cells.

progression [12]. Hence, we examined the phosphorylation levels of both IKBα and NF-κB p65 in SPOCK1-knockdown and SPOCK1-overexpressing CRC cells by western blotting. The western blotting results showed that the expression of phosphorylated IKBα and phosphorylated NF-κB p65 was increased in SPOCK1-overexpressing cells and decreased in SPOCK1-knockdown cells (Figures 4A, 4B). NF-κB is a known nuclear transcription factor. Activation of the NF-κB pathway results in translocation of NF-κB subunits, including p65, to the nucleus. Therefore, we examined the expression of NF-κB p65 in the nucleus. As shown in Figure 4C, the expression levels of the p65 subunit of NF-κB in the nucleus were increased in HCT-116-overexpressing SPOCK1 cells. A significant reduction in p65 protein expression in the nucleus was detected after SPOCK1 knockdown (Figure 4D). Taken together, these results suggest that increased SPOCK1 expression may activate the NF-κB signaling pathway.

SPOCK1 promotes EMT via NF-κB signaling. In our previous work, we found that the expression of SPOCK1 was associated with extracellular matrix (ECM) regulation in the TCGA COAD dataset (Supplementary Figure S2A). EMT has been tightly associated with ECM degradation, particularly in metastasis. Therefore, western blotting was performed to detect the expression of E-cadherin and Vimentin in CRC cell lines with SPOCK1 overexpression or knockdown. As expected, the level of E-cadherin, a hallmark of EMT, was significantly decreased in HCT-116 cells overexpressing SPOCK1 (Figure 5A). Additionally, overexpression of SPOCK1 in HCT-116 cells increased the expression levels of Vimentin (Figure 5A). Conversely, after SPOCK1 knockdown in SW480 cells, the opposite result was observed (Figure 5B). These findings suggest that active SPOCK1 promotes EMT in CRC cells. Previous studies have implicated the NF-κB pathway in the regulation of EMT in many cancer cells [13]. EMT-related transcription factors, such as Snail, Slug, and Twist1, can be regulated by NF-κB signaling to induce EMT [13]. In our bioinformatics analysis, we found that SPOCK1 expression in the TCGA COAD dataset was significantly positively correlated with the expression of SNAI1 (Supplementary Figure S2B). Snail is an important EMT mediator and transcription factor in tumor cells. In our results, overexpression of SPOCK1 significantly increased the expression of Snail compared to control expression, while knockdown of SPOCK1 expression markedly inhibited the expression of Snail (Figures 5A, 5B). In summary, these results indicate that SPOCK1 may mediate EMT and metastasis in CRC cells via the NF-κB/Snail signaling cascade.

Discussion

Despite recent progress in the clinical diagnosis and treatment of CRC, the long-term survival rates of patients with advanced disease remain disappointing. Studying the occurrence and developmental mechanism of colorectal cancer at the molecular level and identifying potential diagnostic and treatment targets remain of great significance.

As a member of the SPARC family, SPOCK1 was previously studied in the nervous system [14]. A few studies have shown that SPOCK1 is closely correlated with the progression of cancer [5]. In breast cancer [15], non-small cell lung cancer [8], and gastric cancer [11], high SPOCK1 expression levels in patients are correlated with a poor prognosis. A study on glioblastoma multiforme (GBM) showed that SPOCK1 might mediate temozolomide resistance in GBM [16]. Furthermore, SPOCK1 plays an important role in tumor cell matrix regulation and EMT [17]. In our study, we found that SPOCK1 was highly expressed in CRC patients. Elevated expression of SPOCK1 in CRC patients was negatively correlated with overall survival and disease-free survival rates. Similar findings were also reported in another similar study [18, 19]. More importantly, upregulated SPOCK1 expression was related to tumor stage and the lymphatic metastasis status. With further tumor progression, SPOCK1 expression increased, which further supports the critical role of SPOCK1 in CRC.

EMT was first discovered during embryonic development, in which cells lose their epithelial phenotype and acquire mesenchymal-like properties that enable them to survive and form metastases in distant sites [20]. EMT can be divided into three types, with type III being the cancer type [21]. Prior studies have indicated that loss of the E-cadherin transcript, a hallmark of EMT, plays a central role in the EMT process and promotes the migration of tumor cells [20, 22]. Chen *et al.* [11] showed that SPOCK1 promoted the invasion and metastasis of gastric cancer through Slug-mediated EMT. In our study, we showed that overexpression of SPOCK1 enhanced the migration of CRC cells, while knockdown of SPOCK1 expression decreased migration. The increased migratory potential could be attributed to a decrease in E-cadherin expression upon SPOCK1 overexpression in HCT-116 cells. Furthermore, we showed that SPOCK1 regulated the expression of Snail, which is an EMT-related transcription factor [22]. Unlike previously reported studies, we found that SPOCK1 promoted EMT in CRC cells by regulating Snail signaling.

NF-κB, discovered in 1986, binds to the enhancer region of the κB chain of immunoglobulin as a nuclear factor in B cells [23]. NF-κB transcriptional activity is important in regulating intracellular signaling, the stress response, proliferation, survival, differentiation, and inflammation [12]. Many studies have indicated that NF-κB signaling is frequently activated in CRC and critical for CRC cell growth [24]. It was reported that NF-κB activation could drive EMT via EMT-related transcription factors [13]. In this study, we examined the effect of SPOCK1 on the phosphorylation of IKBα and NF-κB in CRC cells and found that the overexpression of SPOCK1 obviously increased the levels of phosphorylated IKBα and NF-κB in HCT-116 cells. Further-

more, overexpression of SPOCK1 upregulated NF-κB p65 protein expression in the nucleus. Opposing results were obtained after SPOCK1 knockdown in SW480 cells. Interestingly, NF-κB is also one of the upstream signals of Snail [25]. NF-κB pathway regulates Snail expression through transcriptional and post-translational mechanisms, and NF-κB increases the transcription of Snail [26, 27]. Thus, these data suggest that SPOCK1 promotes proliferation and migration via the NF-κB/Snail signaling pathway cascade. Another study showed that SPOCK1 could promote the proliferation and migration/invasion of CRC cells via the PI3K/AKT signaling pathway [19]. SPOCK1 may affect several signaling pathways in CRC.

In summary, the present study demonstrates that elevated expression levels of SPOCK1 in CRC patients correlate with a poor prognosis. SPOCK1, as a critical oncoprotein, may activate the NF-κB/Snail signaling cascade to promote proliferation and migration in CRC cells. SPOCK1 may serve as a new prognostic indicator and a potential therapeutic target in CRC.

Supplementary information is available in the online version of the paper.

Acknowledgments: This study was supported by a grant from the Scientific Research Project of Health Commission of Hunan Province, China (No. 202103030567).

References

[1] SIEGEL RL, MILLER KD, JEMAL A. Cancer statistics, 2020. CA Cancer J Clin 2020; 70: 7–30. https://doi.org/10.3322/caac.21590

[2] CHAKRABARTI S, PETERSON CY, SRIRAM D, MAHIPAL A. Early stage colon cancer: Current treatment standards, evolving paradigms, and future directions. World J Gastrointest Oncol 2020; 12: 808–832. https://doi.org/10.4251/wjgo.v12.i8.808

[3] SIEGEL RL, MILLER KD, GODING SAUER A, FEDEWA SA, BUTTERLY LF et al. Colorectal cancer statistics, 2020. CA Cancer J Clin 2020; 70: 145–164. https://doi.org/10.3322/caac.21601

[4] BRADSHAW AD. Diverse biological functions of the SPARC family of proteins. Int J Biochem Cell Biol 2012; 44: 480–488. https://doi.org/10.1016/j.biocel.2011.12.021

[5] SUN LR, LI SY, GUO QS, ZHOU W, ZHANG HM. SPOCK1 Involvement in Epithelial-to-Mesenchymal Transition: A New Target in Cancer Therapy. Cancer Manag Res 2020; 12: 3561–3569. https://doi.org/10.2147/CMAR.S249754

[6] LI J, KE J, FANG J, CHEN JP. A potential prognostic marker and therapeutic target: SPOCK1 promotes the proliferation, metastasis, and apoptosis of pancreatic ductal adenocarcinoma cells. J Cell Biochem 2020; 121: 743–754. https://doi.org/10.1002/jcb.29320

[7] SHU YJ, WENG H, YE YY, HU YP, BAO RF et al. SPOCK1 as a potential cancer prognostic marker promotes the proliferation and metastasis of gallbladder cancer cells by activating the PI3K/AKT pathway. Mol Cancer 2015; 14: 12. https://doi.org/10.1186/s12943-014-0276-y

[8] WANG T, LIU X, TIAN Q, LIANG T, CHANG P. Reduced SPOCK1 expression inhibits non-small cell lung cancer cell proliferation and migration through Wnt/β-catenin signaling. Eur Rev Med Pharmacol Sci 2018; 22: 637–644. https://doi.org/10.26355/eurrev_201802_14288

[9] CHEN ML, HO CJ, YEH CM, CHEN SL, SUNG WW et al. High SPOCK1 Expression is Associated with Advanced Stage, T Value, and Gleason Grade in Prostate Cancer. Medicina (Kaunas) 2019; 55: 343. https://doi.org/10.3390/medicina55070343

[10] VEENSTRA VL, DAMHOFER H, WAASDORP C, STEINS A, KOCHER HM et al. Stromal SPOCK1 supports invasive pancreatic cancer growth. Mol Oncol 2017; 11: 1050–1064. https://doi.org/10.1002/1878-0261.12073

[11] CHEN D, ZHOU H, LIU G, ZHAO Y, CAO G et al. SPOCK1 promotes the invasion and metastasis of gastric cancer through Slug-induced epithelial-mesenchymal transition. J Cell Mol Med 2018; 22: 797–807. https://doi.org/10.1111/jcmm.13357

[12] TANIGUCHI K, KARIN M. NF-κB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol 2018; 18: 309–324. https://doi.org/10.1038/nri.2017.142

[13] LEE YJ, PARK JH, OH SM. Activation of NF-κB by TOPK upregulates Snail/Slug expression in TGF-β1 signaling to induce epithelial-mesenchymal transition and invasion of breast cancer cells. Biochem Biophys Res Commun 2020; 530: 122–129. https://doi.org/10.1016/j.bbrc.2020.07.015

[14] CHARBONNIER F, PÉRIN JP, ROUSSEL G, NUSSBAUM JL, ALLIEL PM. [Cloning of testican/SPOCK in man and mouse. Neuromuscular expression perspectives in pathology]. C R Seances Soc Biol Fil 1997; 191: 127–133.

[15] FAN LC, JENG YM, LU YT, LIEN HC. SPOCK1 Is a Novel Transforming Growth Factor-β-Induced Myoepithelial Marker That Enhances Invasion and Correlates with Poor Prognosis in Breast Cancer. PLoS One 2016; 11: e0162933. https://doi.org/10.1371/journal.pone.0162933

[16] YU F, LI G, GAO J, SUN Y, LIU P et al. SPOCK1 is upregulated in recurrent glioblastoma and contributes to metastasis and Temozolomide resistance. Cell Prolif 2016; 49: 195–206. https://doi.org/10.1111/cpr.12241

[17] ALSHARGABI R, SANO T, YAMASHITA A, TAKANO A, SANADA T et al. SPOCK1 is a novel inducer of epithelial to mesenchymal transition in drug-induced gingival overgrowth. Sci Rep 2020; 10: 9785. https://doi.org/10.1038/s41598-020-66660-z

[18] ZHAO P, GUAN HT, DAI ZJ, MA YG, LIU XX et al. Knockdown of SPOCK1 Inhibits the Proliferation and Invasion in Colorectal Cancer Cells by Suppressing the PI3K/Akt Pathway. Oncol Res 2016; 24: 437–445. https://doi.org/10.3727/096504016X14685034103554

[19] ZHANG J, ZHI X, SHI S, TAO R, CHEN P et al. SPOCK1 is up-regulated and promotes tumor growth via the PI3K/AKT signaling pathway in colorectal cancer. Biochem Biophys Res Commun 2017; 482: 870–876. https://doi.org/10.1016/j.bbrc.2016.11.126

[20] DONGRE A, WEINBERG RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol 2019; 20: 69–84. https://doi.org/10.1038/s41580-018-0080-4

[21] CUMMING GR, CARR W. Hemodynamic response to exercise after beta-adrenergic and parasympathetic blockade. Can J Physiol Pharmacol 1967; 45: 813–819. https://doi.org/10.1139/y67-096

[22] PEINADO H, OLMEDA D, CANO A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype. Nat Rev Cancer 2007; 7: 415–428. https://doi.org/10.1038/nrc2131

[23] SEN R, BALTIMORE D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell 1986. 46: 705–716. J Immunol 2006; 177: 7485–7496.

[24] CHEN J, YANG X, LIU R, WEN C, WANG H et al. Circular RNA GLIS2 promotes colorectal cancer cell motility via activation of the NF-κB pathway. Cell Death Dis 2020; 11: 788. https://doi.org/10.1038/s41419-020-02989-7

[25] LIU J, WU Z, HAN D, WEI C, LIANG Y et al. Mesencephalic Astrocyte-Derived Neurotrophic Factor Inhibits Liver Cancer Through Small Ubiquitin-Related Modifier (SUMO) ylation-Related Suppression of NF-κB/Snail Signaling Pathway and Epithelial-Mesenchymal Transition. Hepatology 2020; 71: 1262–1278. https://doi.org/10.1002/hep.30917

[26] WU Y, ZHOU BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. Br J Cancer 2010; 102: 639–644. https://doi.org/10.1038/sj.bjc.6605530

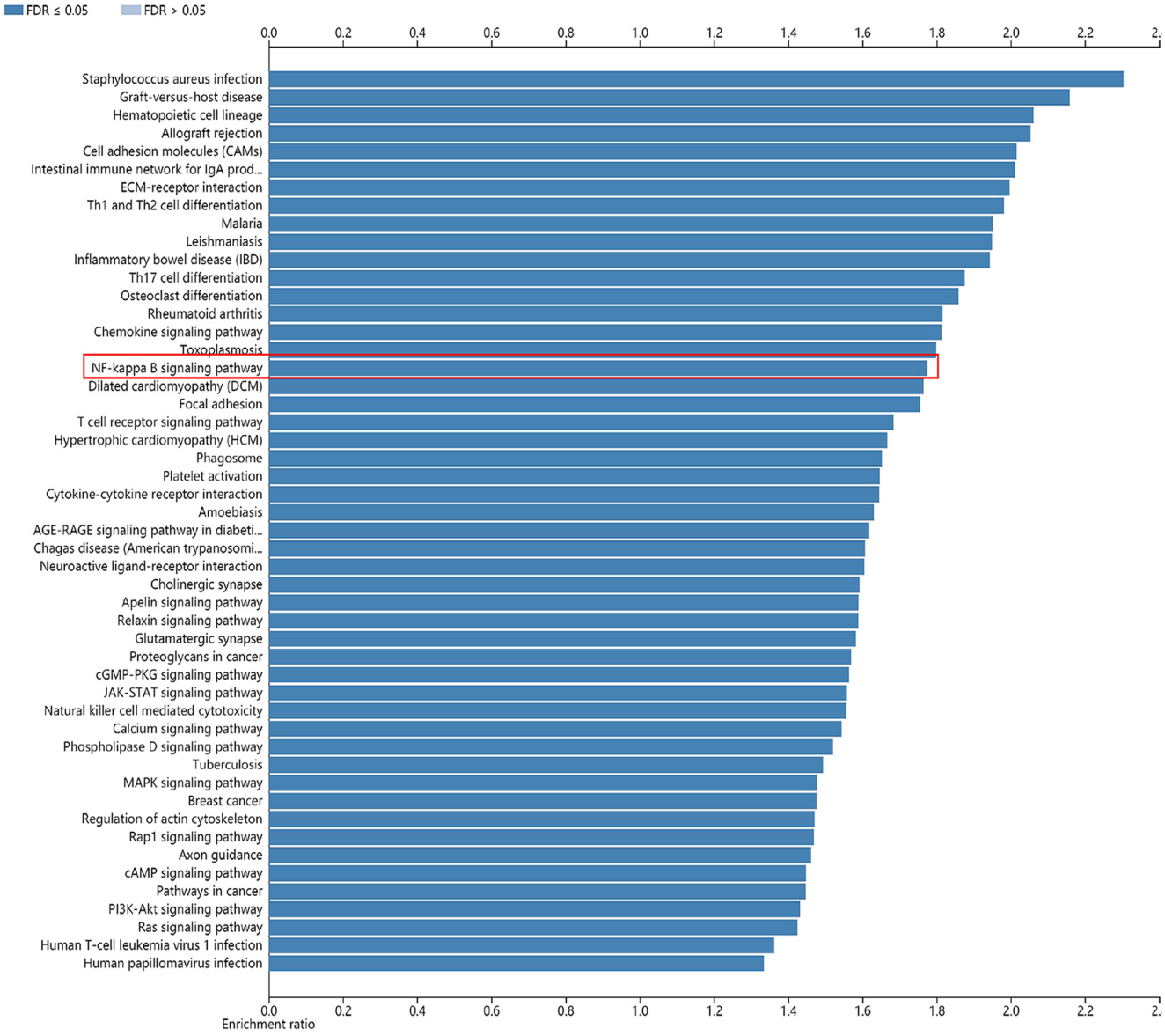
[27] WU D, KE Y, XIAO R, LIU J, LI Q et al. Long non-coding RNA GClnc1 knockdown suppresses progression of epithelial ovarian cancer by recruiting FOXC2 to disrupt the NOTCH1/NF-κB/Snail pathway. Exp Cell Res 2021; 399: 112422. https://doi.org/10.1016/j.yexcr.2020.112422

https://doi.org/10.4149/neo_2021_201031N1158

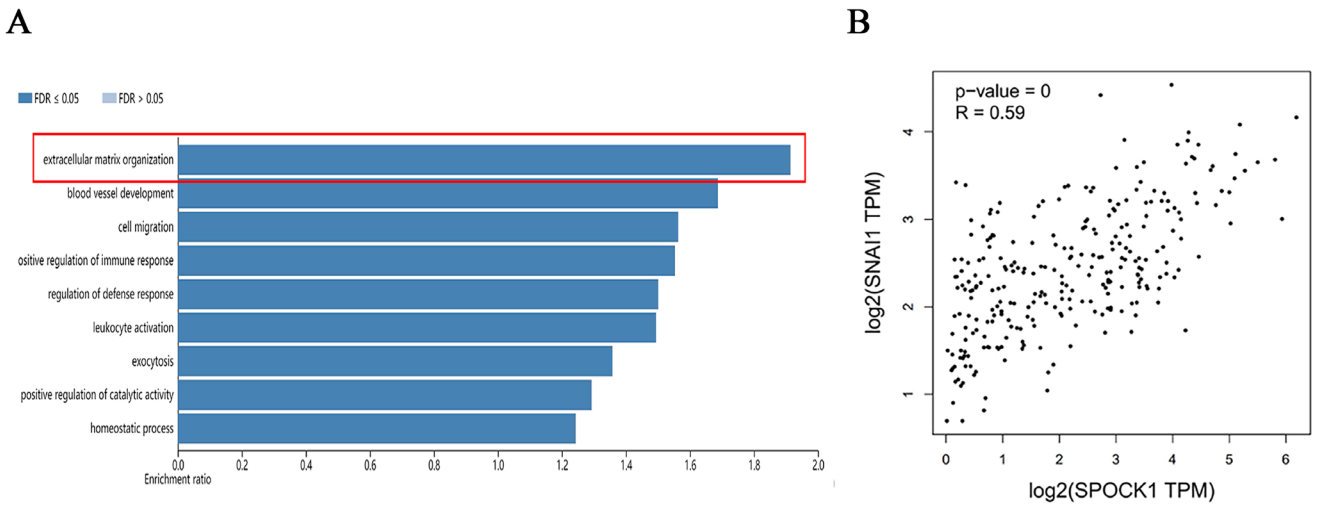
SPOCK1 promotes the proliferation and migration of colon cancer cells by regulating the NF-κB pathway and inducing EMT

Han-Xiong LIU[1], Yuan-Yuan CAO[2], Jia-Yao QU[3,*]

Supplementary Information



Supplementary Figure S1. Expression of SPOCK1 in CRC is associated with NF-κB signaling pathway.



Supplementary Figure S2. SPOCK1 is involved in the regulation of EMT. A) Expression of SPOCK1 is associated with ECM regulation; B) The expression of SPOCK1 is positively correlated SNAI1, *p<0.05