ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 3/2023
517–523
https://doi.org/10.18388/abp.2020_6457

Regular paper

Circ-POLA2-mediated miR-138-5p/SEMA4C axis affects colon cancer cell activities

YanDong Huang[1#], QingYang Bai[1#], HongBo Yu[2], YanRu Li[1], Hao Lu[1], HuiMin Kang[1], XueWei Shi[1] and Kai Feng[1]✉

[1]Department of Oncology, The First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou City, Inner Mongolia Autonomous Region, 014017, China; [2]Practical Teaching Skills Center, Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou City, Inner Mongolia Autonomous Region, 014017, China

This study aimed to investigate the mechanism of circ-POLA2 in colon cancer (CC). Circ-POLA2, miR-138-5p, and SEMA4C levels in CC tissues and cells were recorded. The influences mediated by circ-POLA2, miR-138-5p or SEMA4C on cell proliferation, migration, invasion, and apoptosis were determined. The feedback loop of circ-POLA2/miR-138-5p/SEMA4C was surveyed. As measured, circ-POLA2 and SEMA4C were highly expressed, while miR-138-5p was poorly expressed. Meanwhile, circ-POLA2 could mediate SEMA4C through miR-138-5p targeting. Circ-POLA2 knockdown caused the blockade for cell activities, but this effect was alleviated by miR-138-5p inhibition or SEMA4C overexpression. Overall, circ-POLA2 is tumorigenic for CC through miR-138-5p/SEMA4C axis, which may provide a promising molecular target for CC therapy.

Keywords: Circ-POLA2, miR-138-5p, SEMA4C, colon cancer, proliferation, migration, invasion, apoptosis

Received: 09 August, 2022; revised: 11 March, 2023; accepted: 21 March, 2023; available on-line: 17 August, 2023

✉e-mail: fengkai8018266@hotmail.com
#These authors contributed equally to this work.
Abbreviations: CC, colon cancer; CRC, colorectal cancer

INTRODUCTION

More than 1,090,000 new cases of colon cancer (CC) are diagnosed annually, of which approximately 50% die due to the high rate of metastasis (Siegel *et al.,* 2017; Bray *et al.,* 2018). With changes in people's lifestyle and diet, new cases of CC and young patients are increasing year by year (Zheng *et al.,* 2017). Today, the mainstay of treatment for CC is segmental or total colectomy followed by anastomosis, possibly with the introduction of adjuvant chemotherapy if necessary (Benson *et al.,* 2017; Sun *et al.*, 2020). However, high recurrence/metastasis rates after surgery have not been effectively solved, which is a future direction for CC management.

Circular RNAs (circRNAs) take part in the occurrence, development, metastasis and invasion of CC, and confer diagnostic and prognostic values (Zhao *et al.*, 2019; Han *et al.,* 2021). Indeed, circRNAs are newly discovered in a variety of cells (Wang *et al.,* 2022). Currently, the interaction between circRNAs and miRNAs has been a study focus (Lux & Bullinger, 2018). miRNAs primarily act on target mRNA 3'UTR and are key regulatory roles in CC (Wang *et al.,* 2022; Siciliano *et al.,* 2013; Hollis *et al.,* 2015). Circ-POLA2, a cancer-associated circRNA, has been clarified to express abnormally in endometrial cancer (Fang *et al.,* 2021), acute myeloid leukemia (Li *et al.,* 2021), cervical squamous cell carcinoma (Cao *et al.,* 2020) and lung cancer (Fan *et al.,* 2020). But its relationship with CC has not been reported. Through multiple bioinformatics tools, miRNA (miR-138-5p) and mRNA (SEMA4C) of circ-POLA2 were predicted. miR-138-5p has been confirmed to have decreased expression in colorectal cancer (CRC) and have anti-tumor effects (Wei *et al.,* 2021) and SEMA4C has been illustrated to promote CC progression (Hung *et al.,* 2022). Based on this, circ-POLA2 is likely to competitively upregulate SEMA4C by sponging miR-138-5p, thereby participating CC. Here, circ-POLA2/miR-138-5p/SEMA4C axis-related function and mechanisms were explored, finding new insights into CC treatment.

METHODS

Clinical samples

Following Declaration of Helsinki, the study procedures proceeded with approval of the Ethics Committee of The First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology. All patients has informed of the study and signed written informed consent. CC tumor tissues and adjacent tissues were operationally collected from 40 patients (19 males and 21 females) and immediately frozen in liquid nitrogen. None of patients received preoperative chemotherapy or radiotherapy. Patients with other intestinal diseases, primary cancers, or organ failure were excluded.

Cell lines and culture

SW480, SW620, HT29, and HCT116 were human CC cell lines purchased from ATCC, and NCM460 was normal human colonic mucosal epithelial cell line provided by INCELL Corporation LLC. In a Dulbecco's modified Eagle's medium made of 10% fetal bovine serum (Thermo Fisher Scientific, USA), 100 IU/mL penicillin (SigmaAldrich, USA), and 100 μg/mL streptomycin (Sigma-Aldrich), the cell lines were maintained.

Cell transfection

Short hairpin RNA (shRNA) stably against circ-POLA2 (sh-POLA2), circ-POLA2 overexpression (oe-POLA2), miR-138-5p mimic, miR-138-5p inhibitor, pcDNA-SEMA4C, and corresponding controls were obtained from RiboBio (Guangzhou, China). After Lipo-

fectamine 2000 (Invitrogen)-guided transient transfection, cells were amasses after 24 h.

CCK-8

On the 96-well plates, each well was covered with 5×10^3 SW480 cells and added with 10 μL of CCK-8 solution (Beyotime) after 72 h. The absorbance at 450 nm was recorded on a microplate reader (Tecan Sunrise, Mannedorf, Switzerland) after 4 h.

Clone formation

On the 6-well plates, each well was filled with 1×10^3 SW480 cells for 14 d until staining with 1% crystal violet (Corning). The stained colonies were counted using a microplate reader (Tecan Sunrise).

Transwell

SW480 cells (2×10^5) were seeded into upper chambers (Corning) containing serum-free medium. Matrigel (BD Biosciences, USA) was only coated for testing invasion. Fresh medium containing 10% FBS (Thermo Fisher Scientific) in the lower chamber aimed to attract cell movement. The transmembrane cells during 24 h were conditioned to crystal violet dyeing (0.1%) and microscopical counting.

Flow cytometry

On 1×10^6 SW480 cells, apoptosis was detected by the AnnexinV-fluorescein isothiocyanate/propidium iodide kit (BD Pharmingen, USA) and the apoptosis rate was measured by a BD FACS Aria (BD Biosciences).

RT-qPCR

All tissues and cells were processed for extracting total RNA using Trizol® reagent (Thermo Fisher Scientific). After determination of RNA quality and concentration by NanoDrop 2000 (Thermo Fisher Scientific), reverse transcription was done with SuperScript IV VILO kit (Invitrogen) and All-in-One First-Strand cDNA Synthesis kit (GeneCopoeia, Guangzhou, China). With ABI SYBR Green Master Mix (Invitrogen), PCR was carried out to measure gene expression according to the calculation method 2-ΔΔCT. The primer sequences are shown in Table 1.

Immunoblotting

Sample lysates collected by RIPA lysis buffer (Pierce, USA) were measured by BCA assay kit (Beyotime, China). Proteins were separated by 10-12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (Beyotime), loaded onto a polyvinylidene fluoride membrane (Millipore), after which 5% nonfat dry milk was added, and primary antibodies SEMA4C (1:10000; sc-136445; Santa Cruz Biotechnology) and GAPDH (1:1000; ab8245; Abcam), and horseradish peroxidase-conjugated secondary antibody (1:5000; sc-2054; Santa Cruz Biotechnology) were supplemented. Protein levels were detected by Bio-Rad Gel Imaging System using enhanced chemiluminescence (Thermo Fisher Scientific).

Dual-luciferase reporter gene assay

pmiR-GLO (Promega) was inserted with the amplified wild-type or mutant sequences (POLA2-WT, SEMA4C-WT, POLA2-MUT, SEMA4C-MUT) and co-transfected with miR-138-5p or miR-NC into SW480 cells with Lipofectamine 2000 (Thermo Fisher Scientific), and the luciferase activity was detected using a dual-luciferase reporter gene detection system (Genomeditech, Shanghai, China).

Table 1. Sequences used for PCR

Genes	Sequences (5′–3′)
circ-POLA2	Forward; TGAGCTTGTGAGTGAGTGGT
	Reverse; GCAAGGAGAATGGCGAGATG
miR-138-5p	Forward; CGAGCTGGTGTTGTGAATC
	Reverse; GCAGGGTCCGAGGTATTC
SEMA4C	Forward; ACCTTGTGCCGCGTAAGACAG
	Reverse; CGTCAGCGTCAGTGTCAGGAA
U6	Forward; CTCGCTTCGGCAGCACA
	Reverse; AACGCTTCACGAATTTGCGT
GAPDH	Forward; CACCCACTCCTCCACCTTTG
	Reverse; CCACCACCCTGTTGCTGTAG

Note: circ-POLA2, circular RNA POLA2; miR-138-5p, microRNA-138-5p; SEMA4C, Semaphorin 4C; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

RNA pull-down

Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific) was used. SW480 cells (1×10^7) were lysed in RIP lysis buffer (Thermo Fisher Scientific), followed by interaction with circ-POLA2 probe-coated C-1 magnetic beads. The final results were obtained by RT-qPCR.

Statistical analysis

SPSS 21.0 software and GraphPad Primer 6.0 were employed to perform statistical analysis. All data presented as mean ± standard deviation were analyzed by Student's *t*-test and one-way ANOVA. The Kaplan-Meier method was utilized to assess survival and analyzed by the Log rank test while Pearson analysis was to analyze the gene correlation. $P<0.05$ was considered statistically significant.

RESULTS

circ-POLA2 is associated with CC progression

High circ-POLA2 was expressed in CC patients' tumor tissues (Fig. 1A), and circ-POLA2 expression was further increased in CC patients' tumor tissues in TNM stage III and with lymph node metastasis (Fig. 1B, C). CC patients were divided into high or low circ-POLA2 expression groups defined by circ-POLA2 median value, and Kaplan-Meier analysis evaluated that patients with high circ-POLA2 expression had lower overall survival (Fig. 1D). These findings suggest that circ-POLA2 is upregulated in tumor tissues of patients with CC and is associated with poor prognosis.

Circ-POLA2 knockdown inhibits activities of CC cells

Circ-POLA2 in CC cells and normal colon epithelial cells was detected by RT-qPCR. The results showed that circ-POLA2 expression was increased in CC cell lines (SW480, SW620, HT29 and HCT116), and circ-

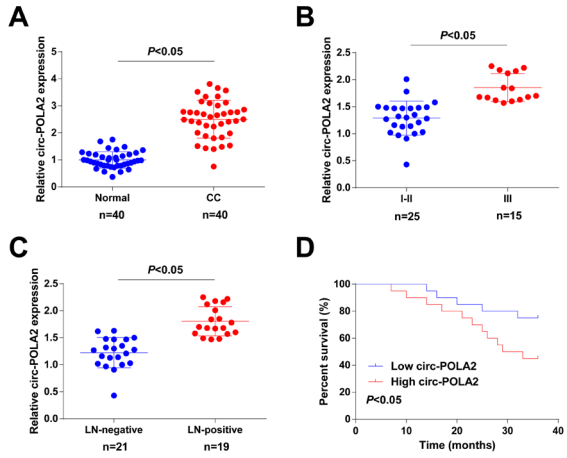

Figure 1. Upregulation of circ-POLA2 is associated with CC progression
(**A**) circ-POLA2 in tumor tissues of CC patients was detected by RT-qPCR. (**B**) circ-POLA2 in tumor tissues of CC patients with TNM stage III was detected by RT-qPCR. (**C**) circ-POLA2 in tumor tissues of CC patients with lymph node metastasis was detected by RT-qPCR. (**D**) Kaplan-Meier analysis of overall survival of CC patients; values are expressed as mean ± standard deviation.

POLA2 expression was most significantly increased in SW480 cells (Fig. 2A). Therefore, SW480 cells were analyzed for subsequent experiments. After transfection of sh-POLA2 or oe-POLA2, circ-POLA2 in SW480 cells decreased or increased accordingly (Fig. 2B), indicating that the cells were successfully transfected. CCK-8 assay, colony formation assay, Transwell assay, and flow cytometry were then performed to explore the role of circ-POLA2 in CC cells. CCK-8 results showed that circ-POLA2 knockdown inhibited SW480 cell activity (Fig. 2C). Meanwhile, colony formation experiments determined that circ-POLA2 knockdown inhibited the proliferation of SW480 cells (Fig. 2D, E). Transwell analysis presented that circ-POLA2 knockdown inhibited SW480 cell migration and invasion (Fig. 2F, G). In addition, flow cytometry suggested that circ-POLA2 knockdown promoted apoptosis of SW480 cells (Fig. 2H, I). However, the effect of circ-POLA2 overexpression on CC cells was opposite to that of circ-POLA2 knockdown (Fig. 2C–I). In conclusion, circ-POLA2 plays a carcinogenic role by promoting proliferation, migration, and invasion of CC cells and inhibiting cell apoptosis.

Circ-POLA2 confers mediation of miR-138-5p

miR-138-5p was cross-screened on bioinformatics websites (CircBank, starBase, miRanda, and RNAhybrid) to be the potential miRNA of circ-POLA2 (Fig. 3A). Measurements of luciferase activity demonstrated that miR-138-5p inhibited the luciferase activity of POLA2-WT (Fig. 3B) and RNA pull-down test discovered that circ-POLA2 significantly increased miR-138-5p enrichment (Fig. 3C). Actually, miR-138-5p expression kept lowly in CCpatients' tumor tissues (Fig. 3D), which was in an inverse correlation with circ-POLA2 expression (Fig. 3E). Likewise, miR-138-

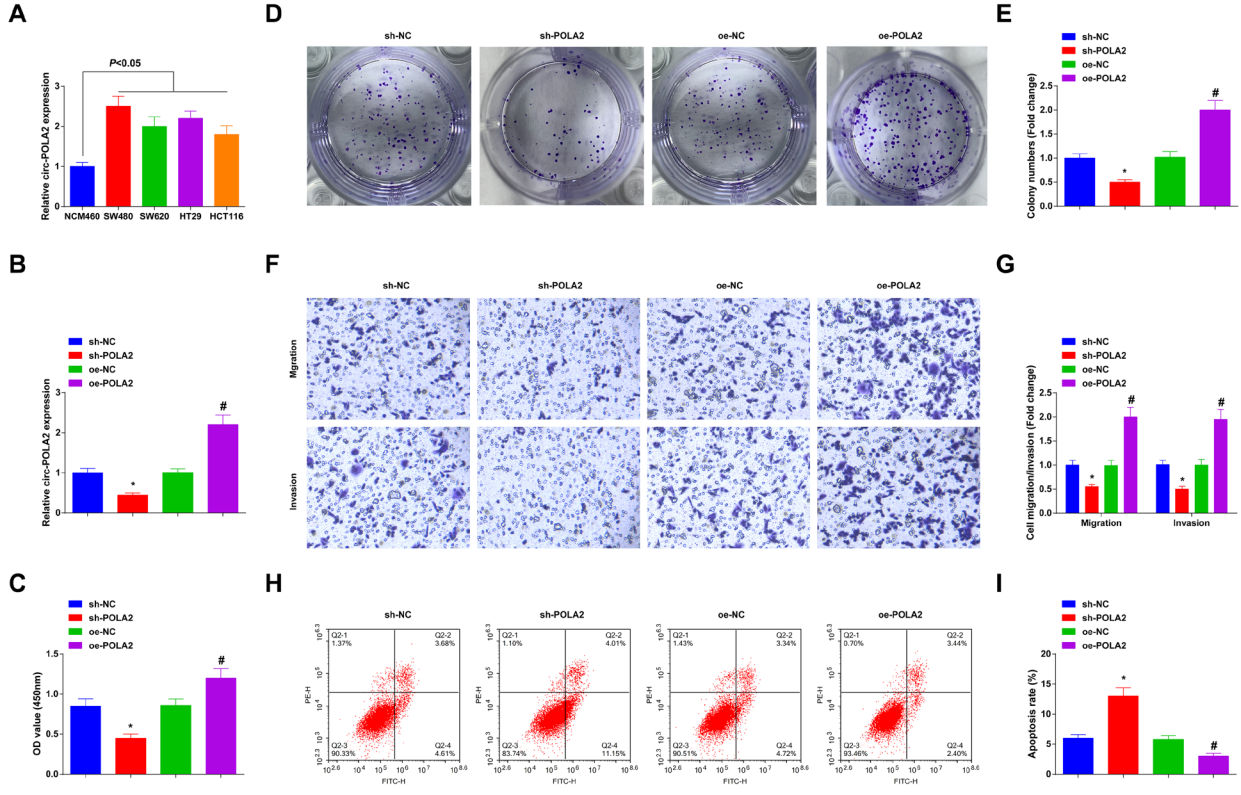

Figure 2. Circ-POLA2 knockdown inhibits activities of CC cells
(**A**) circ-POLA2 in CC cell lines was detected by RT-qPCR. (**B**) The transfection efficacy of sh-POLA2, sh-NC, oe-POLA2 or oe-NC was verified by RT-qPCR. (**C**) Cell viability was detected by CCK-8 after circ-POLA2 expression intervention. (**D–E**) Cell proliferation was detected by colony formation assay after circ-POLA2 expression intervention. (**F–G**) Cell migration and invasion were analyzed by Transwell after circ-POLA2 expression intervention. (**H–I**) Apoptosis was analyzed by flow cytometry after circ-POLA2 expression intervention; values are expressed as mean ± standard deviation. **P*<0.05 *vs*. sh-NC; #*P*<0.05 *vs*. oe-NC.

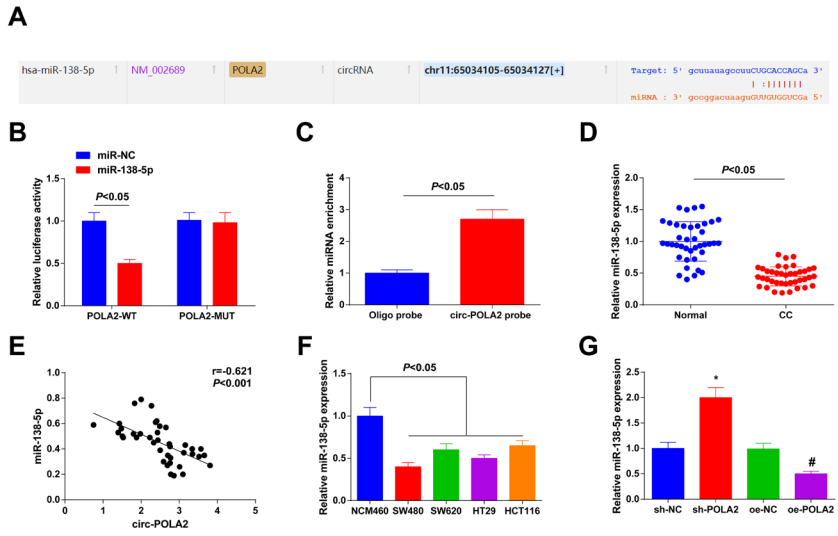

Figure 3. circ-POLA2 confers mediation of miR-138-5p
(**A**) Bioinformation sites predicted the binding sites of circ-POLA2 and miR-138-5p. (**B–C**) The binding relationship between circ-POLA2 and miR-138-5p was verified by dual luciferase reporter assay and RNA Pull-Down assay. (**D**) miR-138-5p in tumor tissues of CC patients was detected by RT-qPCR. (**E**) The correlation between circ-POLA2 and miR-138-5p expression in CC tissues was evaluated by Pearson correlation coefficient. (**F**) miR-138-5p in CC cell lines was detected by RT-qPCR. G: miR-138-5p was detected by RT-qPCR after circ-POLA2 expression intervention; values are expressed as mean ± standard deviation. **P*<0.05 *vs*. sh-NC group; #*P*<0.05 *vs*. oe-NC.

5p expression was downregulated in CC cells lines (SW480, SW620, HT29, and HCT116) (Fig. 3F). While miR-138-5p was up-regulated in SW480 cells intervened with sh-POLA2 and down-regulated in those transfected with oe-POLA2 (Fig. 3G). In conclusion, circ-POLA2 acts as a molecular sponge for miR-138-5p to inhibit miR-138-5p expression.

miR-138-5p prevents the aggressive activities of CC cells

After transfection with miR-138-5p mimic or inhibitor, miR-138-5p in SW480 cells increased or decreased accordingly (Fig. 4A). CCK-8 detection results showed that upregulating miR-138-5p inhibited the activity of SW480 cells (Fig. 4B). Colony formation assay found that upregulating miR-138-5p inhibited the proliferation

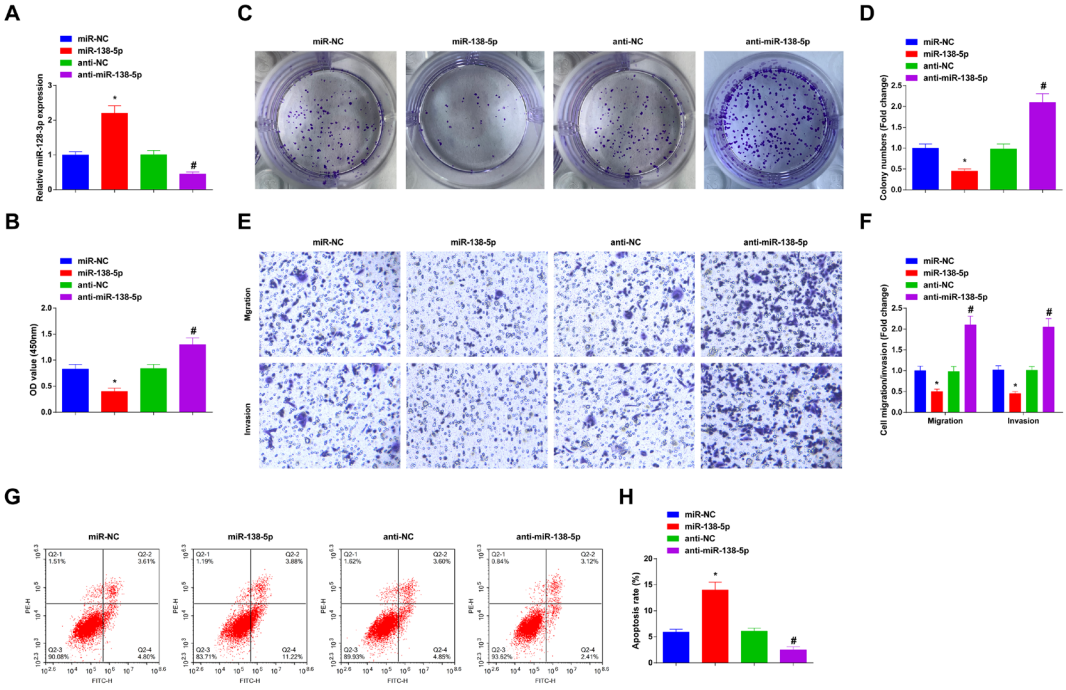

Figure 4. miR-138-5p prevents the malignant activities of CC cells
(**A**) The transfection efficacy of miR-138-5p, miR-NC, anti-miR-138-5p, or anti-NC was verified by RT-qPCR. (**B**) Cell viability was detected by CCK-8 after miR-138-5p expression intervention. (**C–D**) Cell proliferation was detected by colony formation assay after miR-138-5p expression intervention. (**E–F**) Cell migration and invasion were analyzed by Transwell after miR-138-5p expression intervention. (**G–H**) Apoptosis was analyzed by flow cytometry after miR-138-5p expression intervention; values are expressed as mean ± standard deviation. **P*<0.05 *vs*. miR-NC; #*P*<0.05 *vs*. anti-NC.

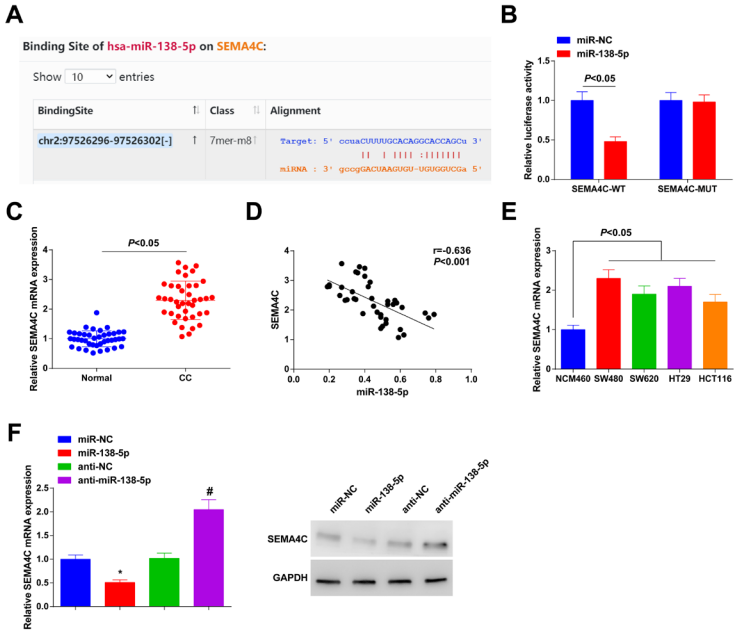

Figure 5. miR-138-5p has a modificatory action on SEMA4C expression
(**A**) Bioinformation sites predicted the binding sites of miR-138-5p and SEMA4C. (**B**) The binding relationship between miR-138-5p and SEMA4C was verified by dual luciferase reporter assay. (**C**) SEMA4C in tumor tissues of CC patients was detected by RT-qPCR. (**D**) The correlation between miR-138-5p and SEMA4C expression in CC tissues was evaluated by Pearson correlation coefficient. (**E**) SEMA4C in CC cell lines was detected by RT-qPCR. (**F**) SEMA4C was detected by RT-qPCR and Western blot after miR-138-5p expression intervention; values are expressed as mean ± standard deviation. **P*<0.05 *vs*. sh-NC group; #*P*<0.05 *vs*. oe-NC.

of SW480 cells (Fig. 4C, D). Transwell analysis showed that overexpressing miR-138-5p inhibited the migration and invasion of SW480 cells (Fig. 4E, F). Flow cytometry showed that upregulating miR-138-5p promoted apoptosis of SW480 cells (Fig. 4G, H). Down-regulation of miR-138-5p had opposite effects on CC cells compared with upregulation of miR-138-5p (Fig. 4B–H). These data indicate that miR-138-5p plays an antitumor function by inhibiting cell proliferation, migration, and invasion and promoting cell apoptosis.

miR-138-5p has a modificatory action on SEMA4C expression

On the bioinformatics website starBase, miR-138-5p had a targeted binding site with SEMA4C (Fig. 5A). Further detection results revealed that the luciferase activity of the SEMA4C-WT was inhibited by miR-138-5p transfection (Fig. 5B). High SEMA4C expression was measured in CC patients' tumor tissues (Fig. 5C) in a negative correlation with miR-138-5p expression (Fig. 5D). The upregulation of SEMA4C was also seen in CC cells lines (SW480, SW620, HT29, and HCT116) (Fig. 5E) and cellular SEMA4C levels could be negatively modified by miR-138-5p (Fig. 5F). These results suggest that SEMA4C expression is negatively regulated by miR-138-5p.

miR-138-5p/SEMA4C axis can intervene the influences of circ-POLA2 on CC

miR-138-5p or SEMA4C expression was intervened in sh-POLA2-modified SW480 cells and the successful transfection was verified, as evidenced by miR-138-5p inhibitor-induced decline of miR-138-5p and SEMA4C-induced elevation of SEMA4C expression (Fig. 6A). CCK-8 assay showed that the sh-POLA2-mediated decrease in SW480 cell viability was reversed by anti-miR-138-5p or SEMA4C (Fig. 6B). Meanwhile, colony formation assay showed that anti-miR-138-5p or SEMA4C could attenuate sh-POLA2-induced proliferation inhibition in SW480 cells (Fig. 6C, D). Furthermore, anti-miR-138-5p or SEMA4C mitigated Sh-PolA2-mediated decrease in SW480 cell migration and invasion (Fig. 6E, F). In addition, anti-miR-138-5p or SEMA4C mitigated an increase in sh-POLA2-mediated apoptosis in SW480 cells (Fig. 6G, H). In conclusion, circ-POLA2 can promote proliferation, migration, and invasion of CC cells and inhibit cell apoptosis by regulating miR-138-5p/SEMA4C axis.

DISCUSSION

Accumulating evidence suggests that circRNAs can act as tumor biomarkers and regulate CC progression (Chen and Shen 2020). Ju and others (Ju *et al.,* 2019) analyzed 667 cases of stage II/III CC with R0 resection and found that circRNA could predict postoperative recurrence of stage II/III CC. Abnormal expression of circRNAs is indicative of patients' prognosis in CC (Hsiao *et al.,* 2017; Zhou *et al.,* 2020; Zheng *et al.,* 2019). In CC patients' tumor, circ-POLA2 was upregulated, having an association with TNM stage III, lymph node metastasis, as well as low overall survival rate. Furthermore, our findings suggest that circ-POLA2 exerts oncogenic effects via miR-138-5p/SEMA4C axis.

The up-regulated expression and stability of circ-POLA2 make it a potential biomarker and diagnostic and therapeutic target for human cancer (Fang *et al.,* 2021; Li *et al.,* 2021; Cao *et al.,* 2020; Fan *et al.,* 2020). Furthermore, circ-POLA2 can sponge different miRNAs, including miR-31, miR-34a, miR-326 and miR-34a, to intercede tumor progression. Similarly, this study clarified a competitive binding of circ-POLA2 to miR-138-5p.

miR-138-5p is a cancer-related miRNA in gastric cancer (Zhang *et al.*, 2020), prostate cancer (Huang *et al.,*

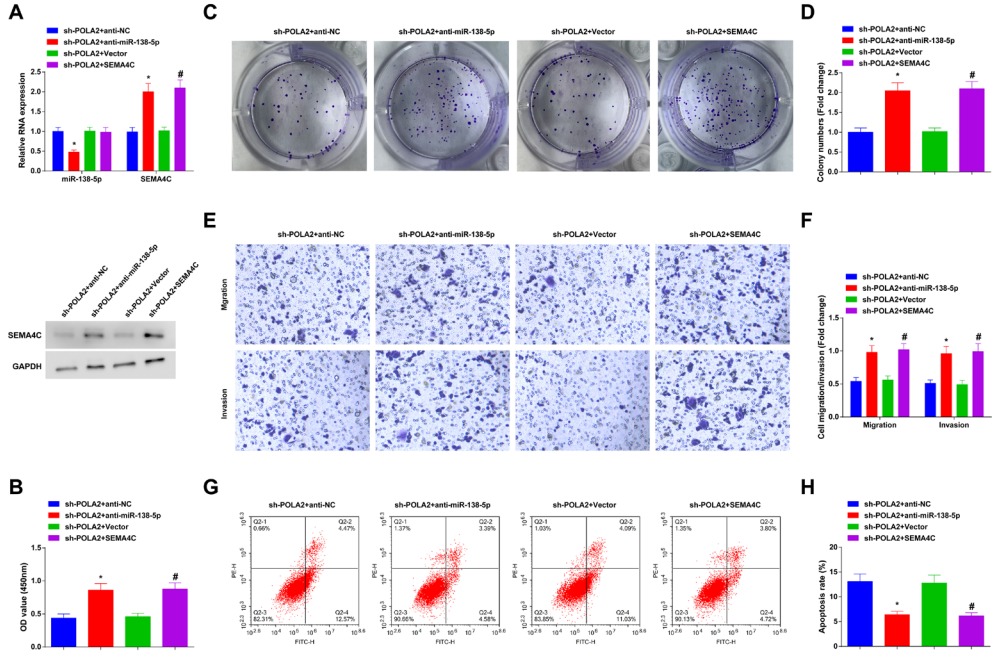

Figure 6. miR-138-5p/SEMA4C axis can intervene the influences of circ-POLA2 on CC
(**A**) The transfection efficacy of sh-POLA2 + anti-miR-138-5p, sh-POLA2 + anti-NC, sh-POLA2 + SEMA4C, or sh-POLA2 + Vector was verified by RT-qPCR and Western blot. (**B**) Cell viability was detected by CCK-8. (**C–D**) Cell proliferation was detected by colony formation assay. (**E–F**) Cell migration and invasion were analyzed by Transwell. (**G–H**) Apoptosis was analyzed by flow cytometry; values are expressed as mean ± standard deviation. **P*<0.05 *vs*. sh-POLA2+ anti-NC; #*P*<0.05 *vs*. sh-POLA2 + Vector.

2020), and cervical cancer. Notably, it is known that miR-138-5p is decreased in CRC and that miR-138-5p serves to disrupt the aggressive activities of CRC cells (Wei *et al.,* 2021). Here, miR-138-5p expression was reduced in CC and was anti-tumorigenic achieved by impeding cell malignancy.

SEMA4C, a member of the semaphorin family originally named M-SemaF, can mediate directional growth of axons and the development of myotubes (Yang *et al.,* 2020). As miRNAs target, SEMA4C is associated with various tumors. For example, SEMA4C mediates chemoresistance in cervical cancer (Jing *et al.,* 2019), induces tumor progression in pancreatic cancer (Fei *et al.,* 2020), and is considered an epigenetic regulator involved in CC progression (Hung *et al.,* 2022). Here, SEMA4C was upregulated in CC, and involved in circ-POLA2/miR-138-5p axis-regulated progression of CC. In detail, miR-138-5p downregulation or SEMA4C upregulation could attenuate the reduction of cell activities mediated by circ-POLA2 knockdown.

Although this study found that circ-POLA2/miR-138-5p/SEMA4C axis promotes CC progression in vitro, it has not been further validated *in vivo*. Circ-POLA2 expression in serum of CC patients should also be detected in the future to verify whether circ-POLA2 is of feasibility for blood biopsy of CC patients. Furthermore, circRNAs may encode proteins or peptides to participate in tumor progression (Qian *et al.,* 2018; Peng *et al.,* 2021; Wang *et al.,* 2021; Wu *et al.,* 2021). However, this research did not investigate the coding ability of circ-POLA2.

CONCLUSION

Circ-POLA2 has potent oncogenic activity in CC and an association with patients' poor prognosis. Furthermore, circ-POLA2 upregulates SEMA4C expression by miR-138-5p, thereby promoting cancer development. This study extends the understanding of circRNA function in CC pathogenesis and proposes a novel circRNA as a therapeutic target for CC.

Declarations

Acknowledgments: Not applicable.
Funding Statements: Not applicable.
Conflicts of Interest: The authors declare no conflicts of interest.
Data available: Data is available from the corresponding author on request.

REFERENCES

Benson AB, 3rd, Venook AP, Cederquist L, Chan E, Chen YJ, Cooper HS, Deming D, Engstrom PF, Enzinger PC, Fichera A, Grem JL, Grothey A, Hochster HS, Hoffe S, Hunt S, Kamel A, Kirilcuk N, Krishnamurthi S, Messersmith WA, Mulcahy MF, Murphy JD, Nurkin S, Saltz L, Sharma S, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Wu CS, Gregory KM, Freedman-Cass D (2017) Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* **15**: 370–398. https://doi.org/10.6004/jnccn.2017.0036

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **68**: 394–424. https://doi.org/10.3322/caac.21492

Cao Y, Li J, Jia Y, Zhang R, Shi H (2020) CircRNA circ_POLA2 promotes cervical squamous cell carcinoma progression *via* regulating miR-326/GNB1. *Front Oncol* **10**: 959. https://doi.org/10.3389/fonc.2020.00959

Chen S, Shen X (2020) Long noncoding RNAs: functions and mechanisms in colon cancer. *Mol Cancer* **19**: 167. https://doi.org/10.1186/s12943-020-01287-2

Fan Z, Bai Y, Zhang Q, Qian P (2020) CircRNA circ_POLA2 promotes lung cancer cell stemness via regulating the miR-326/GNB1 axis. *Environ Toxicol* **35**: 1146–1156. https://doi.org/10.1002/tox.22980

Fang X, Wang J, Chen L, Zhang X (2021) circRNA circ_POLA2 increases microRNA-31 methylation to promote endometrial cancer cell proliferation. *Oncol Lett* **22**: 762. https://doi.org/10.3892/ol.2021.13023

Fei X, Jin HY, Gao Y, Kong LM, Tan XD (2020) Hsa-miR-10a-5p promotes pancreatic cancer growth by BDNF/SEMA4C pathway. *J Biol Regul Homeost Agents* **34**: 927–934. https://doi.org/10.23812/20-61-a-47

Han Z, Chen H, Guo Z, Zhu J, Xie X, Li Y, He J (2021) Bioinformatics analysis: the regulatory network of hsa_circ_0007843 and hsa_circ_0007331 in colon cancer. *Biomed Res Int* **2021**: 6662897. https://doi.org/10.1155/2021/6662897

Hollis M, Nair K, Vyas A, Chaturvedi LS, Gambhir S, Vyas D (2015) MicroRNAs potential utility in colon cancer: Early detection, prognosis, and chemosensitivity. *World J Gastroenterol* **21**: 8284–8292. https://doi.org/10.3748/wjg.v21.i27.8284

Hsiao KY, Lin YC, Gupta SK, Chang N, Yen L, Sun HS, Tsai SJ (2017) Noncoding effects of circular RNA CCDC66 promote colon cancer growth and metastasis. *Cancer Res* **77**: 2339–2350. https://doi.org/10.1158/0008-5472.Can-16-1883

Huang H, Xiong Y, Wu Z, He Y, Gao X, Zhou Z, Wang T (2020) MIR-138-5P inhibits the progression of prostate cancer by targeting FOXC1. *Mol Genet Genomic Med* **8**: e1193. https://doi.org/10.1002/mgg3.1193

Hung YH, Lai MD, Hung WC, Chen LT (2022) Semaphorin 4C promotes motility and immunosuppressive activity of cancer cells via CRMP3 and PD-L1. *Am J Cancer Res* **12**: 713–728

Jing L, Bo W, Yourong F, Tian W, Shixuan W, Mingfu W (2019) Sema4C mediates EMT inducing chemotherapeutic resistance of miR-31-3p in cervical cancer cells. *Sci Rep* **9**: 17727. https://doi.org/10.1038/s41598-019-54177-z

Ju HQ, Zhao Q, Wang F, Lan P, Wang Z, Zuo ZX, Wu QN, Fan XJ, Mo HY, Chen L, Li T, Ren C, Wan XB, Chen G, Li YH, Jia WH, Xu RH (2019) A circRNA signature predicts postoperative recurrence in stage II/III colon cancer. *EMBO Mol Med* **11**: e10168. https://doi.org/10.15252/emmm.201810168

Li H, Bi K, Feng S, Wang Y, Zhu C (2021) CircRNA circ_POLA2 is Upregulated in acute myeloid leukemia (AML) and promotes cell proliferation by suppressing the production of mature miR-34a. *Cancer Manag Res* **13**: 3629–3637. https://doi.org/10.2147/cmar.S281690

Lux S, Bullinger L (2018) Circular RNAs in cancer. *Adv Exp Med Biol* **1087**: 215–230. https://doi.org/10.1007/978-981-13-1426-1_17

Peng Y, Xu Y, Zhang X, Deng S, Yuan Y, Luo X, Hossain MT, Zhu X, Du K, Hu F, Chen Y, Chang S, Feng X, Fan X, Ashktorab H, Smoot D, Meltzer SJ, Hou G, Wei Y, Li S, Qin Y, Jin Z (2021) A novel protein AXIN1-295aa encoded by circAXIN1 activates the Wnt/β-catenin signaling pathway to promote gastric cancer progression. *Mol Cancer* **20**: 158. https://doi.org/10.1186/s12943-021-01457-w

Qian L, Yu S, Chen Z, Meng Z, Huang S, Wang P (2018) The emerging role of circRNAs and their clinical significance in human cancers. *Biochim Biophys Acta Rev Cancer* **1870**: 247–260. https://doi.org/10.1016/j.bbcan.2018.06.002

Siciliano V, Garzilli I, Fracassi C, Criscuolo S, Ventre S, di Bernardo D (2013) MiRNAs confer phenotypic robustness to gene networks by suppressing biological noise. *Nat Commun* **4**: 2364. https://doi.org/10.1038/ncomms3364

Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, Jemal A (2017) Colorectal cancer statistics, 2017. *CA Cancer J Clin* **67**: 177–193. https://doi.org/10.3322/caac.21395

Sun Y, Cao B, Zhou J (2020) Roles of DANCR/microRNA-518a-3p/MDMA ceRNA network in the growth and malignant behaviors of colon cancer cells. *BMC Cancer* **20**: 434. https://doi.org/10.1186/s12885-020-06856-8

Wang J, Zhou L, Chen B, Yu Z, Zhang J, Zhang Z, Hu C, Bai Y, Ruan X, Wang S, Ouyang J, Wu A, Zhao X (2022) Circular RNA circCSPP1 promotes the occurrence and development of colon cancer by sponging miR-431 and regulating ROCK1 and ZEB1. *J Transl Med* **20**: 58. https://doi.org/10.1186/s12967-022-03240-x

Wang L, Zhou J, Zhang C, Chen R, Sun Q, Yang P, Peng C, Tan Y, Jin C, Wang T, Ji J, Sun Y (2021) A novel tumour suppressor protein encoded by circMAPK14 inhibits progression and metastasis of colorectal cancer by competitively binding to MKK6. *Clin Transl Med* **11**: e613. https://doi.org/10.1002/ctm2.613

Wei J, Lin Y, Wang Z, Liu Y, Guo W (2021) Circ_0006174 accelerates colorectal cancer progression through regulating miR-138-5p/MACC1 axis. *Cancer Manag Res* **13**: 1673–1686. https://doi.org/10.2147/cmar.S295833

Wu P, Fang X, Liu Y, Tang Y, Wang W, Li X, Fan Y (2021) N6-methyladenosine modification of circCUX1 confers radioresistance of hypopharyngeal squamous cell carcinoma through caspase1 pathway. *Cell Death Dis* **12**: 298. https://doi.org/10.1038/s41419-021-03558-2

Yang L, Yu Y, Xiong Z, Chen H, Tan B, Hu H (2020) Downregulation of SEMA4C inhibit epithelial-mesenchymal transition (EMT) and the invasion and metastasis of cervical cancer cells *via* inhibiting transforming growth factor-beta 1 (TGF-β1)-induced hela cells p38 mitogen-activated protein kinase (MAPK) activation. *Med Sci Monit* **26**: e918123. https://doi.org/10.12659/msm.918123

Zhang W, Liao K, Liu D (2020) MiR-138-5p inhibits the proliferation of gastric cancer cells by targeting DEK. *Cancer Manag Res* **12**: 8137–8147. https://doi.org/10.2147/cmar.S253777

Zhao X, Cai Y, Xu J (2019) Circular RNAs: biogenesis, mechanism, and function in human cancers. *Int J Mol Sci* **20**. https://doi.org/10.3390/ijms20163926

Zheng R, Zeng H, Zhang S, Chen W (2017) Estimates of cancer incidence and mortality in China, 2013. *Chin J Cancer* **36**: 66. https://doi.org/10.1186/s40880-017-0234-3

Zheng X, Chen L, Zhou Y, Wang Q, Zheng Z, Xu B, Wu C, Zhou Q, Hu W, Wu C, Jiang J (2019) A novel protein encoded by a circular RNA circPPP1R12A promotes tumor pathogenesis and metastasis of colon cancer *via* Hippo-YAP signaling. *Mol Cancer* **18**: 47. https://doi.org/10.1186/s12943-019-1010-6

Zhou P, Xie W, Huang HL, Huang RQ, Tian C, Zhu HB, Dai YH, Li ZY (2020) circRNA_100859 functions as an oncogene in colon cancer by sponging the miR-217-HIF-1α pathway. *Aging (Albany NY)* **12**: 13338–13353. https://doi.org/10.18632/aging.103438