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RESEARCH ARTICLE

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Hsa_circ_0005100 regulates tumorigenicity of colorectal carcinoma via miR-145-5p/*MACC1* axis

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Abstract

Background: Circular RNAs (circRNAs) are a kind of RNA molecules involved in the regulation of cancer progression, including colorectal carcinoma (CRC); nevertheless, their regulation mode is blurry. In the present work, we attempted to reveal the characteristics of hsa_hsa_circ_0005100 in CRC.

Methods: Differential expressions of hsa_circ_0005100, *FMN2* mRNA, microRNA-145-5p (miR-145-5p), and MACC1 were indicated by qRT-PCR and Western blot. The capacities of cell growth and motility were validated by the MTT assay, flow cytometry assay, EdU assay, colony formation assay, and transwell assay. Moreover, the targeted relationship of miR-145-5p and hsa_circ_0005100 or *MACC1* was distinguished by dual-luciferase reporter assay. The animal experiment was implemented to confirm the influence of hsa_circ_0005100 on tumorigenesis in vivo.

Results: Hsa_circ_0005100 and *MACC1* expression levels were increased, but miR-145-5p expression level was diminished in CRC. Hsa_circ_0005100 knockdown repressed cell proliferation, cell cycle, migration, and invasion, while expedited cell apoptosis in CRC cells. Furthermore, miR-145-5p was disclosed to block CRC via overturning *MACC1*. Hsa_circ_0005100 targeted miR-145-5p to modulate *MACC1*. Additionally, hsa_circ_0005100 knockdown also attenuated tumorigenesis in vivo.

Conclusion: Hsa_circ_0005100 was a vital regulator in the development of CRC by miR-145-5p/*MACC1* axis, which deepened the understanding of CRC pathogenesis from circRNA insights.

KEYWORDS
colorectal carcinoma, hsa_circ_0005100, *MACC1*, miR-145-5p

1 | INTRODUCTION

Colorectal carcinoma (CRC) is strictly associated with people's chronic inflammation, diet habits, genetic factors, and so on.[1,2] Clinically, the 5-year survival rate for local CRC was about 90%, and it could drop sharply to 10% if distant metastasis occurred.[3] At present, the effective measures to prevent CRC are mainly colonoscopy.[4] Therefore, novel treatment strategies are impending.

Circular RNAs (circRNAs) are classified into non-coding RNAs, but evidence reveals that certain circRNAs have potential

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protein-coding ability.[5–8] CircRNAs are imperative participators in various cancers, including CRC.[9,10] For instance, circLONP2 could enhance CRC invasion and metastasis.[11] CircRNA_103809 could participate in the progression of CRC.[12] Hsa_circ_0005100 might function as potential diagnostic and prognostic indicators for CRC detection.[13] Hsa_circ_0005100 could trigger the malignant phenotypes of prostate cancer cells,[14] and it also promoted cancer cell proliferation in CRC.[15] Hsa_circ_0005100 is generated by back-splicing from formin 2 gene (FMN2), also known as circFMN2. Nevertheless, the detailed monitoring mode of hsa_circ_0005100 in CRC was not legible.

MicroRNAs (miRNAs) are small RNAs that regulate various cellular biological functions.[5,6] For instance, miR-145-5p regulated stemness in glioma.[16] In addition, miR-145-5p prevented the evolution of breast cancer.[17,18] Besides, miR-210, miR-21, and miR-126 could act as diagnostic markers in CRC.[19] Moreover, miR-212 could suppress the progression of CRC.[20] Yet, the definite upshot of miR-145-5p in CRC was vague.

Metastasis-associated in colon cancer-1 (*MACC1*) situated on human chromosome 7 (7p21.1) was an important biomarker for predicting distant metastasis of colon cancer.[21] Meanwhile, the level of *MACC1* was faithfully correlated to the recurrence of many cancers.[21–24] *MACC1* promoted the proliferation of cancer cells.[25] In addition, *MACC1* could also affect the angiogenesis of gastric cancer,[26] but the functional effects of *MACC1* in CRC were still uncertain.

Herein, we revealed the functions of hsa_circ_0005100 in CRC cells. Hsa_circ_0005100 might expedite CRC progression via miR-145-5p/*MACC1*. Our conclusions will afford original perceptions into the monitoring mode of hsa_circ_0005100 in CRC evolution.

2 | MATERIALS AND METHODS

2.1 | Clinical samples

The experiment was sustained by the Third Hospital Affiliated to Hebei Medical University. Forty-three pairs of CRC tissues and paracarcinoma tissue were collected from the Third Hospital Affiliated to Hebei Medical University, and written informed consent was attained from all subjects. Then, all samples were frozen. CRC patients who had never received any therapies against CRC (such as chemotherapy and radiotherapy) and had no other types of cancers or severe systemic diseases were included in our study. The characteristics of included CRC patients are listed in Table 1.

2.2 | Cell lines and transfection

We chose CRC cell lines HCT116 and SW480, using the NCM460 as a non-cancer control. All cells were acquired from Chuan Qiu Biotechnology and cultured in 37°C incubators with 5% CO_2.

TABLE 1 Correlation between circ_FMN2 expression and clinical clinicopathological parameters of CRC patients ($n = 43$)

Parameters	No. of cases	Circ_FMN2 expression (n)		p-value
		High ($n = 22$)	Low ($n = 21$)	
Age (years)				
≤60	26	15	11	0.2895
>60	17	7	10	
Gender				
Female	23	14	9	0.1721
Male	20	8	12	
Lymph node metastasis				
Yes	23	16	7	0.0096*
No	20	6	14	
TNM stage				
I–II	20	7	13	0.0480*
III	23	15	8	
Tumor size (cm)				
≤5	18	6	12	0.0472*
>5	25	16	9	
Tumor location				
Colon	18	10	8	0.6249
Rectum	25	12	13	

[a]Chi-square test.

*$P < 0.05$ indicates statistical difference.

The si-hsa_circ_0005100, sh-hsa_circ_0005100, the control (si-NC and sh-NC), miR-145-5p mimics, miR-145-5p inhibitors and controls, MACC1 overexpression (MACC1), and control (pcDNA) were acquired from Sangon Biotech. CRC cells were transfected with oligonucleotides or vectors using Lipofectamine 2000 reagent (Invitrogen).

2.3 | RNA extraction and qRT-PCR

TRIzol Reagent (Invitrogen) was enforced to isolate total RNA. The qRT-PCR was implemented by SYBR Green kit (Takara). GAPDH or RNU6 (U6) was applied as an endogenous control. The $2^{-\Delta\Delta Ct}$ method was enforced to analyze relative expression. The primers were listed in Table 2.

2.4 | Western blot

The process of Western blot was performed as recounted previously.[27] The antibodies were listed as follows: anti-MACC1 (ab226803; 1:1000; Abcam), anti-PCNA (ab92552; 1:1000; Abcam), and anti-GAPDH (ab9485; 1:2500; Abcam).

2.5 | RNase R digestion assay

Total RNA from the experimental cells was dealt with RNase R (Sigma-Aldrich). Finally, the hsa_circ_0005100 and *FMN2* mRNA contents were revealed by qRT-PCR.

2.6 | MTT assay

After post-transfection, CRC cells (2×10^3 per well) were seeded in 96-well plates. The 20 μl of MTT (Sigma) solution was supplemented and incubated. Then, the absorbance values were measured.

2.7 | Flow Cytometry assay

CRC cells (1×10^6 per well) were planted in 6-well plates. Annexin V-FITC/PI kit (Sigma) and PI Flow Cytometry Kit (Abcam) were implemented to distinguish the cell apoptotic and cycle in line with the instructions. Finally, the cells were examined by a flow cytometer (Beckman Coulter, Miami, FL, USA).

2.8 | Cell proliferation assay

CRC cells were cultured in 96-well plates (2×10^4/well). Next, the EdU Apollo In Vitro Imaging Kit (Sigma) was employed in line with the guide. In brief, cells in 96-well plates were cultured for 48 hours and then co-cultured with EdU for another 8 h. After EdU labeling, cell fixing was performed using the fixative solution from Kit, and then, cell nucleus was stained with DAPI. The number of EdU-positive cells was observed by light microscopy (Nikon).

2.9 | Colony formation assay

CRC cells were plated in 6-well plates (300 cells/well). The cell culture medium was changed every 3 days for 2 weeks. Subsequently, the colonies were washed with PBS, fixed by 4% paraformaldehyde, and then stained with 0.5% crystal violet for 15 min. A light microscope was used to observe the colonies.

2.10 | Transwell assay

After post-transfection, CRC cells were assessed by a transwell with 8-μm pore polycarbonate membrane (BD Biosciences). In brief, 4×10^5 transfected CRC cells, resuspended in 100 μl DMEM without serum, were planted into the top chamber. Then, the lower chamber of the transwell contained 500 μl of DMEM and 10% FBS. Next, the cells on the inferior surface of the membrane were stained. The same method was enforced to detect the invasion ability with the chamber was precoated with matrigel (BD Biosciences). Eventually, a light microscope was performed to validate the count of cells.

TABLE 2 Primers used for qRT-PCR

Name	Primers for PCR (5′–3′)
circ_FMN2	
Forward	AGAACCCCAGGACCTTTTTCA
Reverse	GAGAGCTTGAAGGGTCTCCAG
MACC1	
Forward	GTCATGTGGCTGTGGGAGAA
Reverse	TTTCCAACAACGGGCTCACA
miR-145-5p	
Forward	AGGGGGTCCAGTTTTCCCAGG
Reverse	GTGCGTGTCGTGGAGTCG
GAPDH	
Forward	TCCCATCACCATCTTCCAGG
Reverse	GATGACCCTTTTGGCTCCC
U6	
Forward	CTCGCTTCGGCAGCACATATACT
Reverse	ACGCTTCACGAATTTGCGTGTC
FMN2	
Forward	GCGAACGCTGTTGGAGAAG
Reverse	CTGATTACACGGTTCCCTGAAG

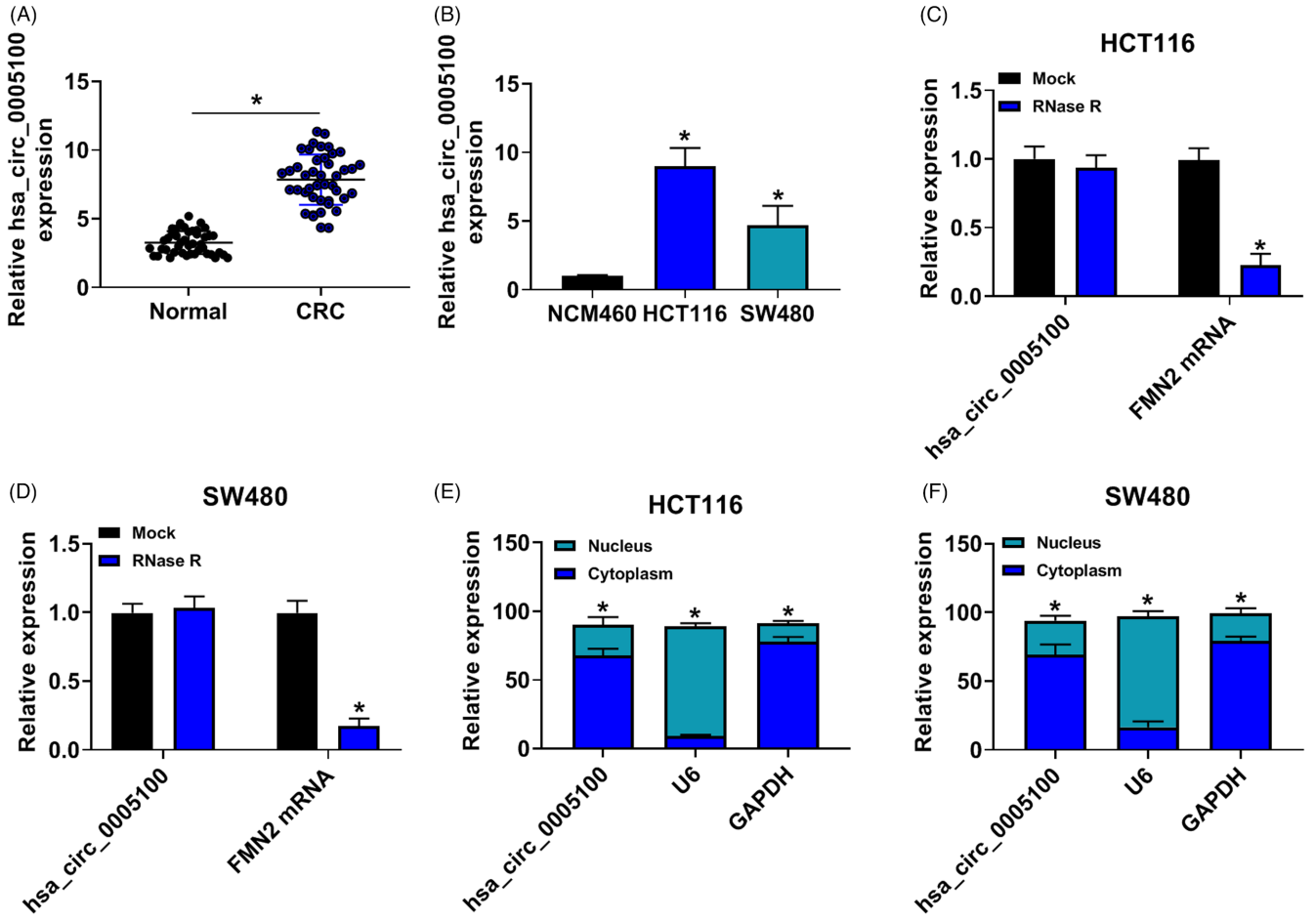

FIGURE 1 Hsa_circ_0005100 was enhanced in CRC. (A) The hsa_circ_0005100 level in CRC tumor tissues was examined. (B) The hsa_circ_0005100 level in CRC cells was assessed. (C, D) The contents of hsa_circ_0005100 and *FMN2* mRNA was quantified. (E, F) The expression of hsa_circ_0005100 in CRC cells was assessed. $^*P < 0.05$

2.11 | Dual-luciferase reporter assay

The targeted relationship of miR-145-5p and hsa_circ_0005100 or *MACC1* was anticipated by circbank and starbase. Then, the pmirGLO vector was used to construct luciferase reporter vectors, and the hsa_circ_0005100 or *MACC1* wild-type (WT) and mutant-type (MUT) reporter vectors were produced by Sangon Biotech (hsa_circ_0005100-WT, *MACC1*-WT or hsa_circ_0005100-MUT, *MACC1*-MUT). Luciferase activity was quantified using dual-luciferase assay kit (Sigma) along with the directives.

2.12 | In vivo tumorigenicity

The Animal Care and Use Committee of the Third Hospital Affiliated to Hebei Medical University supervised the whole test process. These nude mice (female; 6-week-old; 18–22 g) were gotten from Shanghai Laboratory Animal Company (SLAC, Shanghai, China). HCT116 cells (5×10^6 cells per mouse) infected with the sh-hsa_circ_0005100 or the sh-NC were vaccinated in mice ($n = 6$/group). The volume (mm^3) = length × width^2 × 0.5. Next, the tissues were used for examination.

2.13 | IHC assay

The cleaved caspase-3 (ab90437; 1:1000; Abcam) and Ki67 (ab92742; 1:1000; Abcam) contents in tumor were distinguished by IHC assay. The detailed procedures were conducted as reported by Zou et al.[28] Briefly, tumor tissues from mice were cut into 4-μm thickness slides, followed by dewaxing, rehydration, and antigen retrieval. Then, the slides were challenged with the antibodies targeting cleaved caspase-3 and Ki67. After incubation of matched secondary antibody, the slides were stained using the DAB kit from Abcam. The positive staining was observed by light microscopy.

2.14 | Statistical assay

The data were investigated by GraphPad Prism 7. The experiment was reiterated at least three times. Pearson's correlation analysis was enforced to quantity the correlation between two groups. Student's *t* test and ANOVA were implemented to detect the difference. $p < 0.05$ was significant.

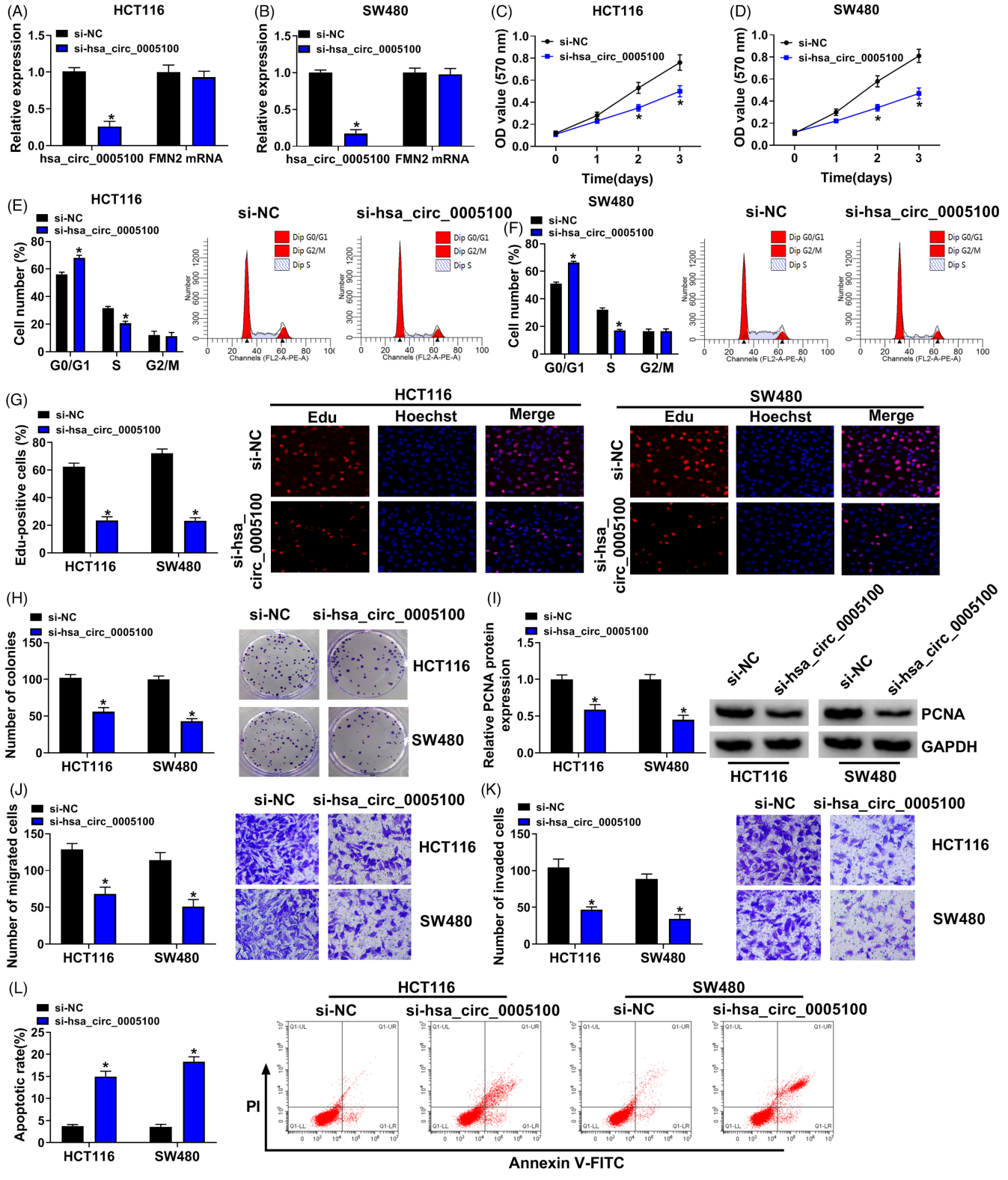

FIGURE 2 Hsa_circ_0005100 downregulation inhibits CRC. (A, B) The hsa_circ_0005100 contents were assessed. (C, D) The cell viability was assessed. (E, F) The cell mitotic cycle was assessed. (G, H) The EdU-positive cell was detected. (I) The PCNA level was detected. (J, K) The cell migration and invasion were validated. (L) The apoptosis of CRC cells was observed. $^*P < 0.05$

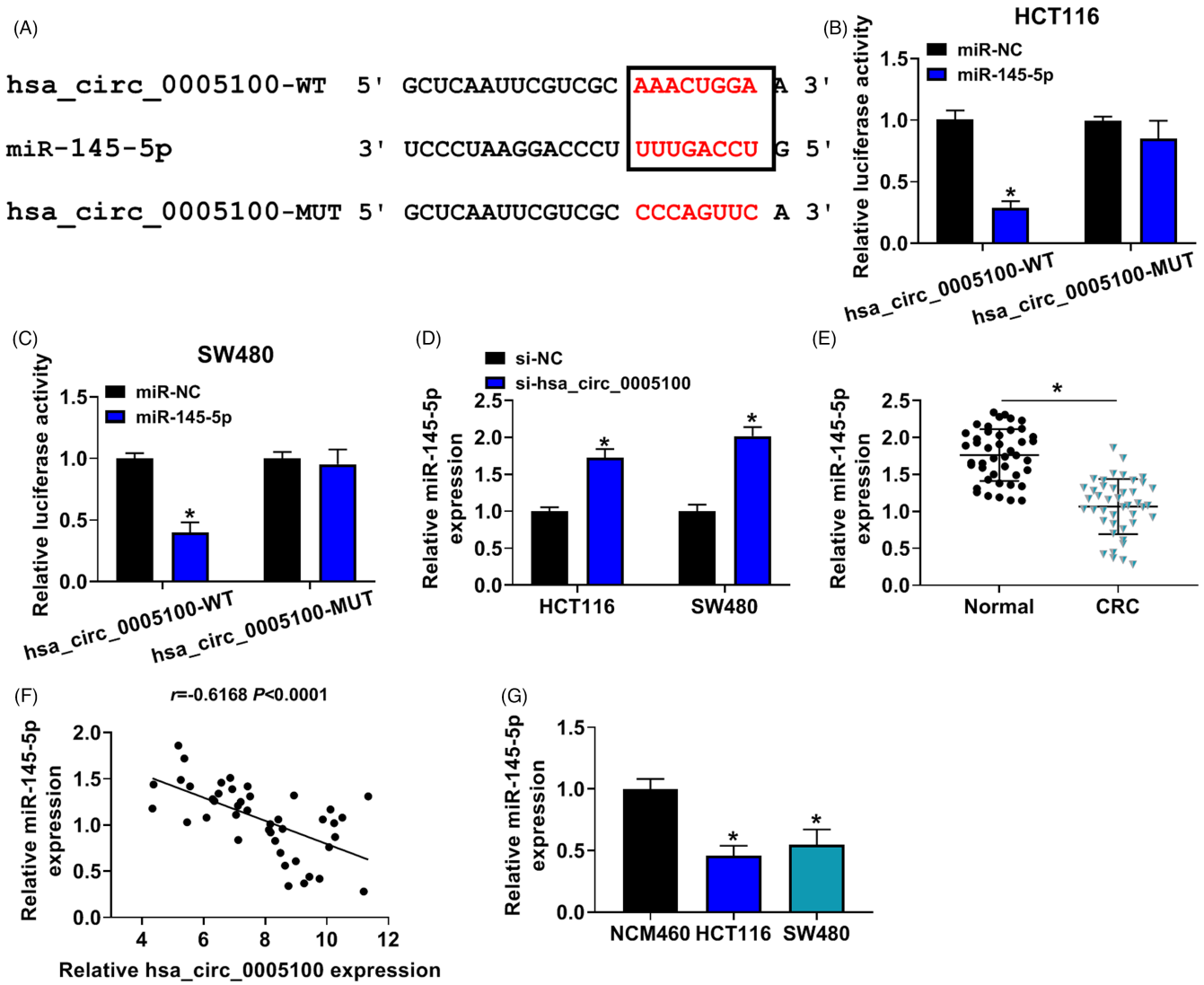

FIGURE 3 Hsa_circ_0005100 targeted miR-145-5p. (A) The bound miRNAs of hsa_circ_0005100 were forecasted by circbank. (B, C) The connection between hsa_circ_0005100 and miR-145-5p. (D, E) The miR-145-5p content was detected. (F) Hsa_circ_0005100 was negatively linked with miR-145-5p ($R = -0.6168$) in CRC. (G) The level of miR-145-5p in CRC cells was assessed. $^*P < 0.05$

3 | RESULTS

3.1 | Hsa_circ_0005100 abundance was elevated in CRC

To scrutinize the possible role of hsa_circ_0005100 in CRC, its abundance was confirmed in CRC by qRT-PCR. First of all, we reconnoitered that hsa_circ_0005100 level was elevated in CRC (Figure 1A,B). As exposed in Figure 1C,D, the *FMN2* mRNA was significantly reduced after RNase R treatment, while hsa_circ_0005100 was basically not altered, verifying the cyclic structure of hsa_circ_0005100. Moreover, hsa_circ_0005100 was mainly distributed in the cytoplasm relative to the nucleus. (Figure 1E,F). These outcomes exposed that hsa_circ_0005100 was elevated in CRC. Additionally, hsa_circ_0005100 was substantiated to have a circular structure.

3.2 | Downregulation of hsa_circ_0005100 restrained CRC development

To reconnoiter the effect of hsa_circ_0005100 in CRC cells, HCT116 and SW480 cells were transfected with si-NC or si-hsa_circ_0005100. Hsa_circ_0005100 content was elevated in CRC cells after si-hsa_circ_0005100 transfection (Figure 2A,B). Moreover, hsa_circ_0005100 downregulation lessened the cell vitality (Figure 2C,D). Besides, si-hsa_circ_0005100 transfection considerably hindered CRC cells in the G0/G1 phase (Figure 2E,F). Hsa_circ_0005100 deficiency diminished the cell proliferation of CRC cells (Figure 2G). Furthermore, the consequences presented the downregulated hsa_circ_0005100 reduced the amount of colonies (Figure 2H). PCNA was demonstrated to be connected with cell proliferation. Herein, we substantiated that si-hsa_circ_0005100 abridged PCNA level in CRC cells (Figure 2I). Silencing of hsa_circ_0005100 reserved migration and invasion of CRC cells

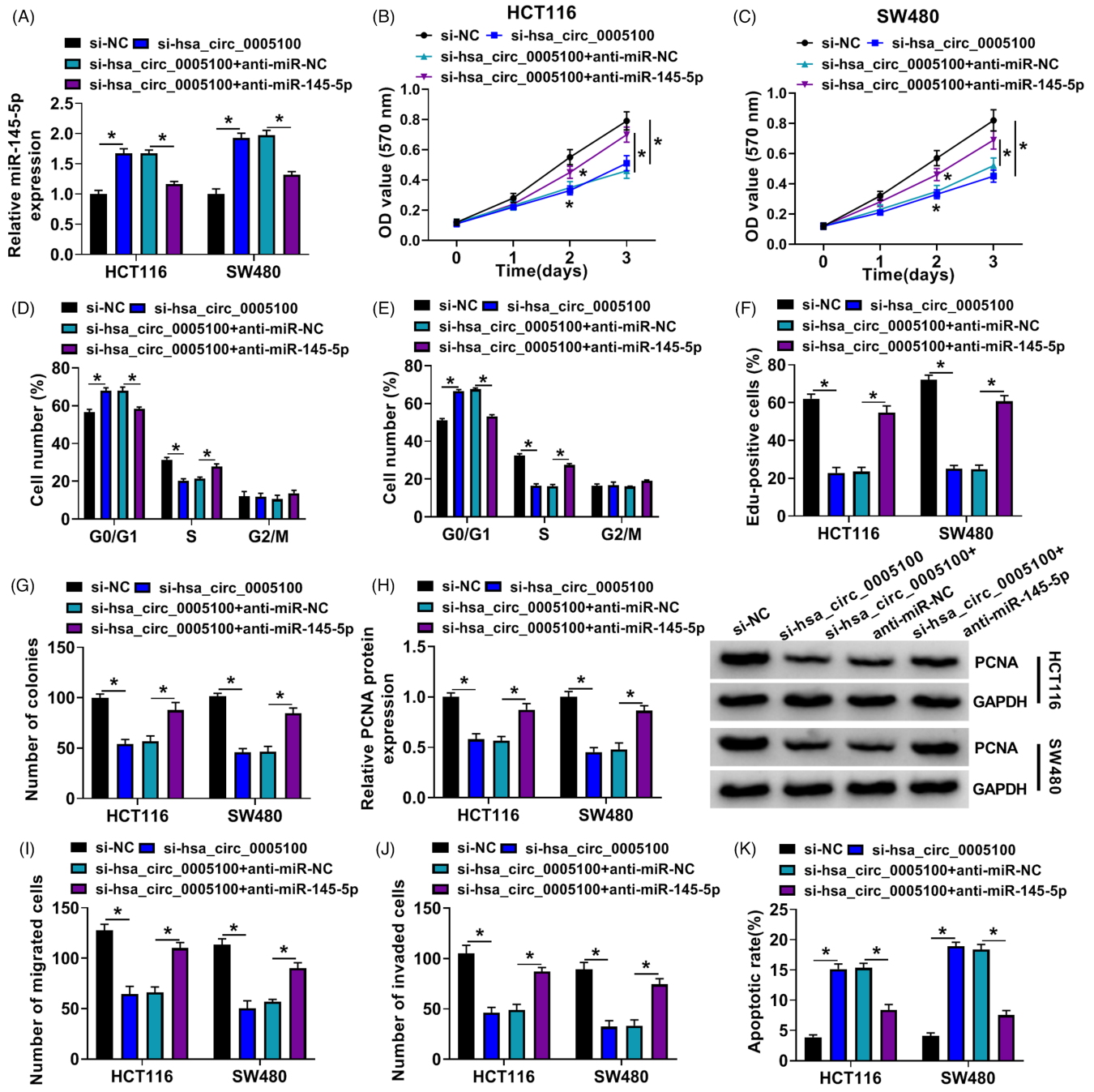

FIGURE 4 Hsa_circ_0005100 expedited CRC via miR-145-5p. (A) The miR-145-5p content was inspected. (B, C) The cell viability, (D, E) the cell mitotic cycle, (F) the rate of EdU-positive cells, (G) the number of colonies, (H) the PCNA content, (I, J) the migration and invasion, and (K) the cell apoptosis were examined. $^{*}P < 0.05$

(Figure 2J,K). Besides, hsa_circ_0005100 deficiency tempted cell apoptosis in CRC cells (Figure 2L). Our consequences directed that downregulated hsa_circ_0005100 prevented CRC.

3.3 | MiR-145-5p targeted hsa_circ_0005100

To confirm whether hsa_circ_0005100 served as a miRNA sponge, the possible target of hsa_circ_0005100 was predicted and assessed. Circbank was employed to envisage that miR-145-5p bound to hsa_circ_0005100 (Figure 3A). Besides, the luciferase activity was diminished in hsa_circ_0005100-WT with miR-145-5p transfection, but there was no alteration in the hsa_circ_0005100-MUT group (Figure 3B,C). Additionally, we exposed that miR-145-5p was amplified by silencing hsa_circ_0005100 (Figure 3D). The miR-145-5p content was lesser in CRC (Figure 3E,G). In addition, Pearson's correlation analysis indorsed that miR-145-5p level was negatively linked with hsa_circ_0005100 in CRC (Figure 3F).

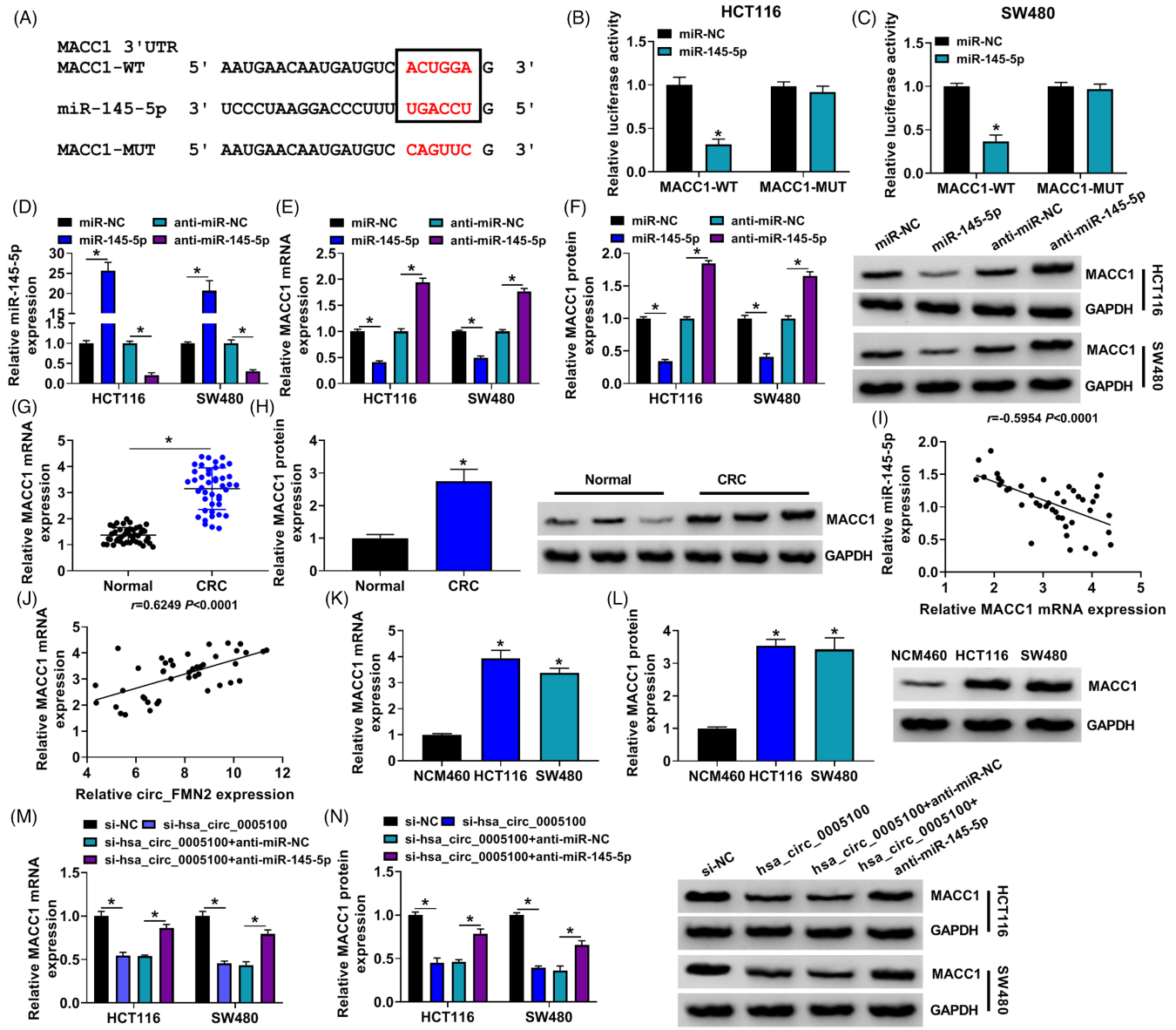

FIGURE 5 MiR-145-5p targets *MACC1*. (A) The connection of miR-145-5p and *MACC1* was inspected. (B, C) The targeted relationship of miR-145-5p and *MACC1*. (D–F) The miR-145-5p and *MACC1* contents were identified. (G, H) The content of MACC1 was detected. (I) The *MACC1* was negatively related to miR-145-5p ($R = -0.5954$) in CRC. (K) The *MACC1* was positively linked with hsa_circ_0005100 ($R = 0.6249$) in CRC. (K–N) The MACC1 level was measured. $^*P < 0.05$

3.4 | Hsa_circ_0005100 expedited CRC by targeting miR-145-5p

In consideration that miR-145-5p was a target of hsa_circ_0005100, we hypothesized that hsa_circ_0005100 regulated the development of GRC cells via miR-145-5p. Primarily, miR-145-5p content was increased by si-hsa_circ_0005100, whereas declined by anti-miR-145-5p in CRC cells (Figure 4A). Besides, hsa_circ_0005100 downregulation reduced the cell vitality; nevertheless, the inhibitory cell viability was recovered by anti-miR-145-5p (Figure 4B,C). Moreover, silence of hsa_circ_0005100 could considerably hinder CRC cell cycle in the G0/G1 phase, whereas this outcome was damaged by downregulated miR-145-5p (Figure 4D,E). In the meantime, the results exposed that hsa_circ_0005100 knockdown weakened the cell proliferation, while this upshot was reduced by anti-miR-145-5p (Figure 4F,G). Furthermore, miR-145-5p inhibitors reserved the influences of hsa_circ_0005100 knockdown on diminished PCNA level in CRC cells (Figure 4H). Transwell assay revealed that si-hsa_circ_0005100 transfection diminished the cell migration and invasion, yet this consequence was decreased by miR-145-5p deficiency (Figure 4I,J). Furthermore, downregulation of hsa_circ_0005100 prompted cell apoptosis, while this influence was debilitated by anti-miR-145-5p (Figure 4K). Our discoveries verified that si-hsa_circ_0005100 repressed CRC cell behaviors by targeting miR-145-5p.

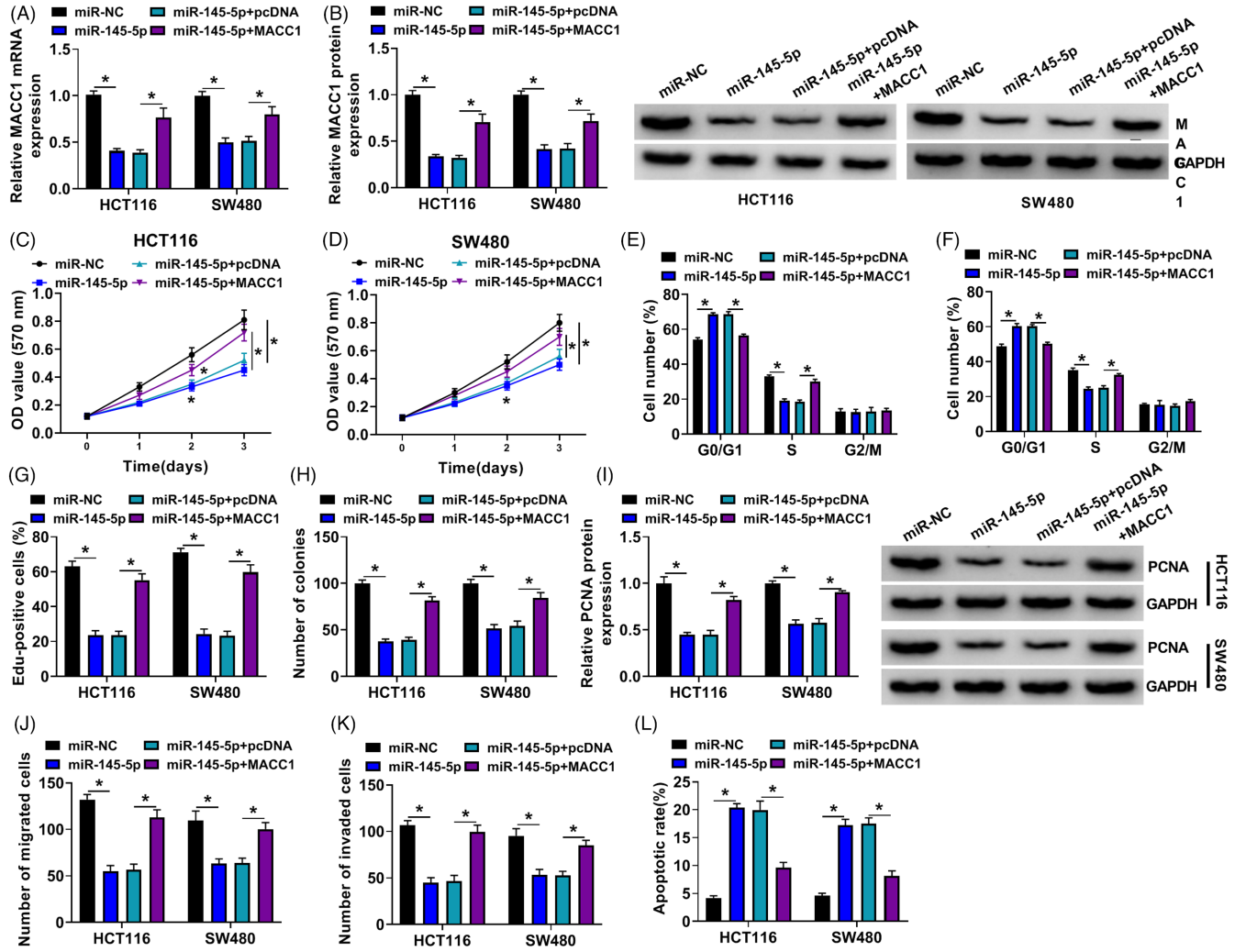

FIGURE 6 MiR-145-5p regulated CRC via *MACC1*. (A, B) The MACC1 level was measured. (C, D) The cell viability, (E, F) the cell mitotic cycle, (G, H) the cell proliferation, (I) the protein level of PCNA, and (J and K) the cell migration and invasion (L) the rate of apoptosis were examined. $^*P < 0.05$

3.5 | MiR-145-5p targeted *MACC1* in CRC cells

MiRNAs could regulate tumor progression by targeting mRNAs. Consequently, the imaginable target genes of miR-145-5p were foretold. The targeted sites of miR-145-5p in *MACC1* 3'UTR were displayed in Figure 5A. The luciferase activity of *MACC1* 3'UTR-WT was retarded after miR-145-5p treatment. Yet, the *MACC1* 3'UTR-MUT group was not reformed (Figure 5B,C). Additionally, miR-145-5p content was increased by miR-145-5p mimics and reduced by anti-miR-145-5p (Figure 5D). However, the expression of *MACC1* was lessened by miR-145-5p mimics and augmented by anti-miR-145-5p (Figure 5E,F). Additionally, the *MACC1* content was upregulated in CRC (Figure 5G,H). As well, Pearson's correlation analysis corroborated that miR-145-5p level was negatively linked with *MACC1* mRNA content (Figure 5I). Nevertheless, the *MACC1* content was positively linked with hsa_circ_0005100 (Figure 5J). Figure 5K,L exhibited that *MACC1* was upregulated in CRC cells (HCT116 and SW480) when compared to NCM460 cells. Furthermore, miR-145-5p inhibitors alleviated the influences of hsa_circ_0005100 silencing on reduced content of *MACC1* in CRC cells (Figure 5M,N). As a group, these sightings advocated that miR-145-5p bound to *MACC1*.

3.6 | MiR-145-5p blocked CRC by regulating *MACC1*

To uncover whether miR-145-5p affected CRC progression via *MACC1*, recovery tests were implemented. Firstly, the *MACC1* content was declined by transfection with miR-145-5p, but this influence was partly impaired by *MACC1* overexpression in CRC cells (Figure 6A,B). Afterward, miR-145-5p confined the cell viability; nevertheless, this influence was weakened by *MACC1* (Figure 6C,D). The miR-145-5p impeded CRC cell cycle in the G0/G1 phase, while this consequence was diminished by *MACC1* (Figure 6E,F). Besides, miR-145-5p diminishes the cell proliferation of CRC cells, while *MACC1* overexpression recovered miR-145-5p-depleted cell proliferation

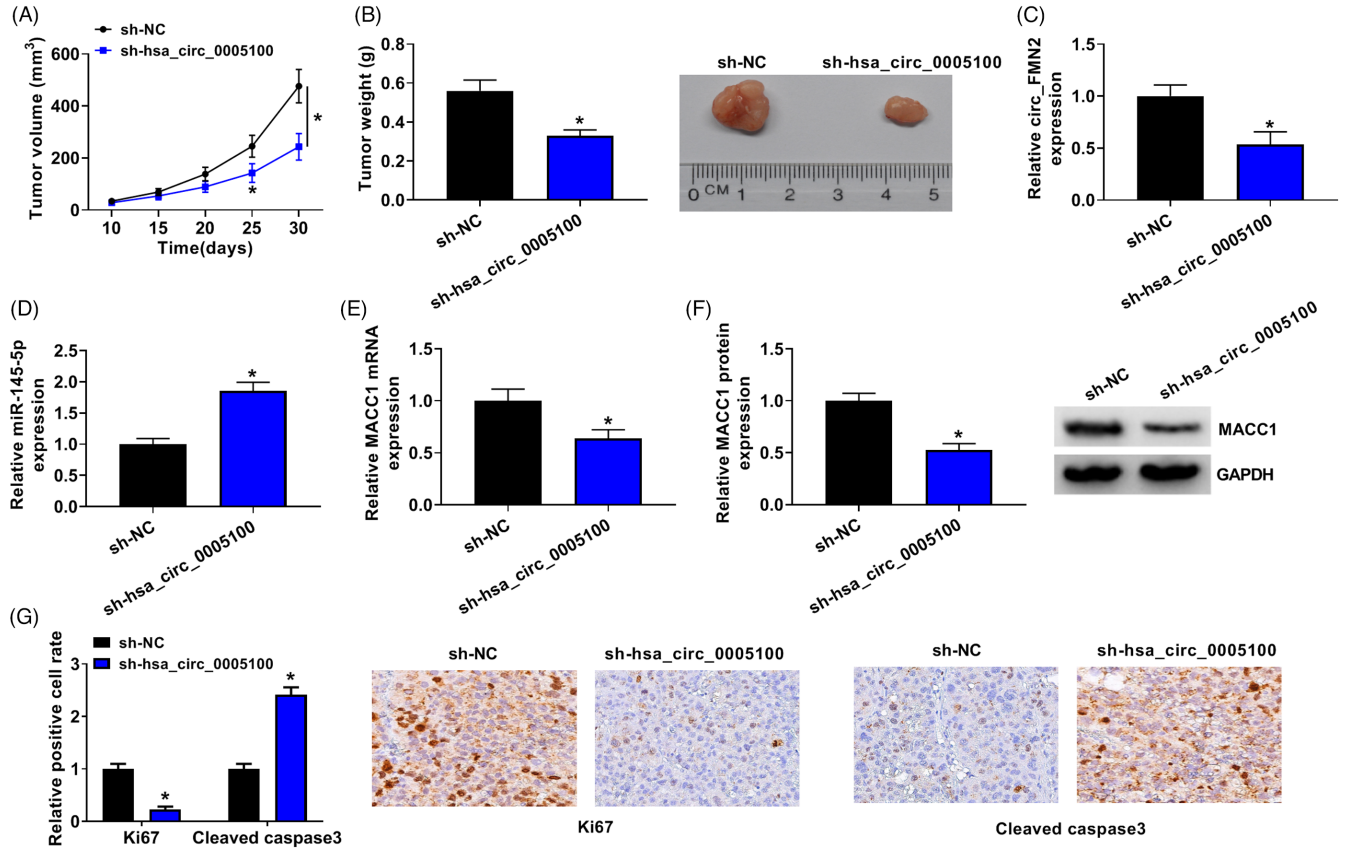

FIGURE 7 Hsa_circ_0005100 downregulated diminished tumorigenesis. (A, B) Tumorigenesis was distinguished. (C, D) The hsa_circ_0005100 and miR-145-5p levels were quantified. (E, F) The levels of MACC1 mRNA and protein were quantified. (G) The Ki67 and cleaved caspase-3 levels were examined by IHC. $^{*}P < 0.05$

(Figure 6G,H). Figure 6I established that *MACC1* reversed the influence of miR-145-5p mimics on dwindled PCNA level in CRC cells. Moreover, miR-145-5p weakened the cell migration and invasion; conversely, this consequence was weakened by *MACC1* (Figure 6J,K). Successively, we observed that miR-145-5p expedited cell apoptosis in CRC cells, and the effect was limited by *MACC1* (Figure 6L). In summary, all facts demonstrated that miR-145-5p controlled CRC development via *MACC1*.

3.7 | Silencing hsa_circ_0005100 constrained tumorigenesis in vivo

To reconnoiter the clinical application of hsa_circ_0005100 on CRC in vivo, the xenograft model was implemented. The sh-hsa_circ_0005100 constrained tumor volume and weight (Figure 7A,B). Furthermore, hsa_circ_0005100, MACC1, and Ki67 were apparently abridged, but miR-145-5p and cleaved caspase-3 were exceptionally amplified in sh-hsa_circ_0005100 group (Figure 7C–G). These consequences pointed out that silencing hsa_circ_0005100 repressed tumorigenesis via miR-145-5p/MACC1 axis.

4 | DISCUSSION

Studies have found the mortality of CRC was snowballing.[29,30] The growth of CRC is associated with lifestyle factors, such as smoking, lack of exercise, obesity, red meat consumption, and excessive alcohol consumption.[31] CRC has become a challenge for people. Meanwhile, there were many circRNAs that were singularly expressed in CRC. Nevertheless, the characteristics of these circRNAs in CRC were still uncertain. Hence, our study inspected the part of hsa_circ_0005100.

Preceding readings have discovered that many circRNAs are vital for CRC. For example, hsa_circ_102958 encouraged tumorigenesis of CRC.[32] Besides, circCAMSAP1 promoted tumor growth in CRC.[33] In our study, our outcomes designated that downregulation of hsa_circ_0005100 prevented CRC. In addition, our in vivo study further discovered that knockdown hsa_circ_0005100 impaired tumor growth. The circRNAs could control targeted genes and bound to miRNAs. For example, hsa_circ_102958 bound to miR-585 and circCAMSAP1 could sponge miR-328-5p in CRC.[32,33] Herein, hsa_circ_0005100 impelled CRC via miR-145-5p, which was comparable to former discoveries.

MiR-145-5p was an imperative participator to suppress the progress of hepatocellular carcinoma, malignant melanoma, and breast

cancer, and studies declaimed that miR-145-5p exerted the inhibitory effects on cancer cell proliferation, migration, and invasion, and the stimulative effects on cell apoptosis and cell cycle via controlling its target genes.[34–36] Here, we demonstrated that miR-145-5p played a curbed part in CRC by binding *MACC1*. *MACC1* was reported to be highly upregulated in gastric cancer and acts as a vital role in gastric cancer by enhancing immune killing.[37] MACC1 was also overexpressed in osteosarcoma, and its silencing arrested cell cycle in G0/G1 phase and induced cell apoptosis of osteosarcoma cells.[38] Moreover, MACC1 deficiency blocked cervical cancer cell proliferation through interfering cell mitosis and cell cycle progression.[39] In this study, *MACC1* expression was enhanced in CRC. We witnessed that miR-145-5p retarded CRC cell growth, arrested cell cycle, blocked cell migration/invasion, and induced cell apoptosis; however, these anticancer effects caused by miR-145-5p were considerably diminished by *MACC1*. miR-145-5p knockdown attenuated the restrained outcome of downregulated hsa_circ_0005100 on *MACC1* expression in CRC cells. These consequences sustained the monitoring mode of the hsa_circ_0005100/miR-145-5p/*MACC1* in CRC.

There are still some limitations in the present study. For example, the amount of clinical samples is not enough, and more samples should be used to identify hsa_circ_0005100 expression in future work. Besides, we only focus on the miR-145-5p/*MACC1* axis downstream of hsa_circ_0005100, and other miRNA/mRNA signals targeted by hsa_circ_0005100 should be further identified.

In summary, hsa_circ_0005100 and *MACC1* levels were enhanced and miR-145-5p level was lessened in CRC. Additionally, our study firstly demonstrated that hsa_circ_0005100 deficiency suppressed CRC malignant progression via miR-145-5p/*MACC1* axis. We provided perception into the monitoring network in CRC and presented new thinking for curing CRC patients.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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