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Long non-coding FAM201A accelerates the tumorigenesis and progression of colorectal cancer through miR-3163/MACC1 axis

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ABSTRACT

Colorectal cancer (CRC) is the leading contributor to cancer-relevant deaths worldwide with severe incidence and mortality. An extensive body of evidence has demonstrated that lncRNA plays a critical role in the oncogenicity of CRC. Despite the oncogenic function of FAM201A in esophageal squamous cell cancer and non-small-cell lung cancer, the potential of FAM201A in CRC progression remains unknown. FAM201A expression level was significantly enhanced in CRC cells compared with normal cells. Further, functional experiments illustrated that knockdown of FAM201A restrained cell growth, stemness and promoted chemoresistance of CRC cells. By exploring molecular mechanism of FAM201A, we found that FAM201A acted as a sponge of miR-3163. More importantly, oncogene MACC1 was confirmed to be a direct target of miR-3163 and FAM201A modulated MACC1 expression level via competing for miR-3163. Subsequently, we testified that FAM201A exerted its role in the tumorigenesis and development of CRC through targeting miR-3163/MACC1. Animal assay certified that FAM201A expedited CRC cell growth *in vivo*. In conclusion, our study was the first to unveil that FAM201A promoted cell proliferation and CSC characteristics in CRC via regulation of the miR-3163/MACC1 axis, which provided clues for the clinical treatment of patients with this disease.

Introduction

Colorectal cancer (CRC) is regarded as the third leading cause responsible for deaths ascribed to human malignancy throughout the world [1]. The occurrence and development of CRC are characterized by multiple stages and the involvement of complex genetic and epigenetic variations [2]. On account of economic development and lifestyle changes, the morbidity of CRC trends to rise [3]. Despite tremendous progress in the diagnostic and therapeutic methods, CRC patients succumbed to tumor recurrence and metastasis are over 40% [4]. Thus, deeply understanding the potential mechanism and genetic changes of CRC is necessary to improve the treatment of patients with this disease.

CRC-related mortalities are mostly attributed to tumor relapse, metastasis and chemotherapy resistance [5]. The main obstacle in CRC therapy is acquisition of resistance to a variety of chemotherapy drugs [6]. Multiple lines of evidence has proven that cancer stem cells (CSCs) are of immense importance in the different processes of malignant tumors, such as initiation, development, metastasis as well as chemoresistance [7,8]. CSCs possess natural resistance to chemotherapy resulting from their capacity to repair DNA and regulate the expression of multidrug resistance ABC transporters [9]. As a result, a potent strategy to treat CRC is indispensable to inhibit the CSC properties of CRC cells so that to promote chemotherapy efficacy.

It is extensively accepted that long non-coding RNAs (lncRNAs) are a set of RNA molecules comprising more than 200 nucleotides with little or no protein-coding ability [10]. An increasing number of reports unveil that lncRNAs play a crucial role in modulating the expression of diverse genes participated in the physiological and pathological progression of numerous disorders, including cancer [11,12]. Considering the significant function of abnormally expressed lncRNAs in malignant tumors, mounting researchers pay more attention to lncRNAs and investigate their potential in the development of human cancer [13]. For example, high level of lncRNA PANDAR predicts poor outcomes and facilitates cell proliferation of cervical cancer [14]. LncRNA CCAT1 promotes paclitaxel resistance in nasopharynx cancers cells through targeting miR-181a/CPEB2 pathway [15]. LncRNA SLCO4A1-AS1

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Table 1
Sequences of vectors.

Vector	Sequence
sh-FAM201A	5′-GTACCTCGATCTTTCGTCCATTTACTTCAAGAGAGTAAATGGACGAAAGATCTTTTTGGAAA-3′
sh-MACC1	5′CACCATGGCTTGGTTAAGTCAACCGAAGTTGACTTAACCAAGCCA-3′
sh-NC	5′-UUGCUAAGCGUCGGUCAAUTT-3′

expedites CRC cell growth and metastasis via β-catenin-dependent Wnt pathway [16]. Of note, lncRNA family with sequence similarity 201-member A (FAM201A), located in human chromosome 9p38.62, has been proofed to exert its carcinogenic activity in esophageal squamous cell cancer and non-small-cell lung cancer [17,18]. Unfortunately, the participation of FAM201A in CRC tumorigenesis and progression has not been expounded thoroughly.

In the current study, we planned to shed light on the latent role and regulatory mechanism of FAM201A in CRC. Our experimental results showed that FAM201A functioned as an oncogene in CRC by boosting cell growth and maintaining CSC features. Mechanistically, the oncogenicity of FAM201A was mediated by miR-3163/MACC1 axis.

Materials and methods

Cell culture

Human normal colonic epithelial cell line (HIEC) and four CRC cell lines (HT29, HCT116, SW480 and SW620) were supplied by the Cell Bank of Chinese Academy of Science (Shanghai, China). Cell lines were verified by STR profiles. All cells were maintained in DMEM (Gibco, Rockville, USA) containing 10% FBS (Life Technologie, USA), 100 μg/ml streptomycin as well as 100 U/ml penicillin (Sigma-Aldrich, USA) at 37 °C under 5% CO_2.

Cell transfection and treatment

To silence FAM201A or MACC1, short hairpin RNAs (shRNAs) against FAM201A (sh-FAM201A) or MACC1 (sh-MACC1) were procured from Invitrogen (Carlsbad, USA). Non-specific shRNA (sh-NC) was used as negative control. For overexpression or inhibition of miR-3163, the mimic and inhibitor of miR-3163 and corresponding negative control (NC mimic and NC inhibitor) were designed and synthesized by GenePharma. Cell transfection was conducted with Lipofectamine 2000 kit (Invitrogen) obeying the manufacturer's instructions. 5-fluorouracil (5-FU) and oxaliplatin (Oxa) acquired from Sigma Aldrich were applied for detection of CRC cell drug resistance. At 48 h post transfection, HCT116 and SW480 cells were administrated with different concentrations of 5-FU or Oxa. The oligonucleotides of the indicated plasmids were listed in the following Table 1.

Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA from CRC cells was drawn out by utilization of TRIzol reagent (Invitrogen) based on product manuals. The cDNA synthesis was completed with reverse transcription kit (Takara, Janpan) according to the vendor's instructions. Thereafter, RT-qPCR was implemented on ABI Real-Time PCR System (Applied Biosystems, USA) using SYBR Green Mix Kit (Takara). The expression of all genes was calculated by employment of the $2^{-\Delta\Delta Ct}$ method. U6 and GAPDH were adopted as inherent references. The following sequences of PCR primers were utilized: FAM201A (forward): 5′-TCTCTGATGGGAGCCTCTTTA-3′, FAM201A (reverse): 5′-CAAGCCACAGACGGAGAAA-3′; MACC1 (forward): 5′-AAGGAAGATTGCCACACAGAGAG-3′, MACC1 (reverse): 5′-GCTAGTTCCCTCCAGCCTTTTC-3′; GAPDH (forward): 5′-ACAACTTTGGTATCGTGGAAGG-3′, GAPDH (reverse): 5′-GCCATCACGCCACAGTTTC-3′; U6 (forward): 5′-CCGTATGACCTCCTTCCACAGA-3′, U6 (reverse): 5′-TCTGTCCACCTCTGAAACCAGG-3′

Cell proliferation assay

Cell viability of HCT116 and SW480 cells was examined by the Cell Counting Kit-8 (Dojindo, Japan) in line with directions supplied by the manufacturer. Following transfection, HCT116 and SW480 cells were plated into a 96-well plate (1×10^3 cells per well) and cultured at 37 °C. At the end of matched incubation time points, each well was supplemented with CCK-8 solution and incubated for another 2 h at 37 °C. The absorbance at 450 nm was measured with a Microplate Reader (Bio-Rad, USA).

Colony formation assay

After transfection, 200 HCT116 and SW480 cells were inoculated in 12-well plates and cultivated in DMEM with 10% FBS. The fresh medium was changed every 3 days and the medium was abandoned after two weeks of cultivation. Subsequently, cells were immobilized with 4% paraformaldehyde, treated with 0.5% crystal violet and imaged under a light microscope (Olympus, Japan).

Flow cytometry

In brief, transfected HCT116 and SW480 cells were collected, rinsed with PBS, followed by fixation in 75% ethanol overnight, administrated with RNase for 30 min and dyed with propidium iodide (PI, Sigma-Aldrich). The distribution of cell cycle was analyzed by a FACScan flow cytometry.

Sphere formation assay

In short, cell suspension were seeded into an ultralow attachment 96-well plate complemented with a serum-free conditioned medium containing DMEM/F-12, basic epidermal growth factor, B27 supplement, insulin and 0.4% FBS. The spheres were monitored with an inverted microscope.

Western blot

HCT116 and SW480 cells were lysed with RIPA lysis buffer (Thermo Fisher Scientific, USA). 30 μg protein was loaded for 10% SDS-PAGE, and therewith transferred to PVDF membranes (Sigma-Aldrich). Membranes were blocked in 5% skim milk and went through overnight incubation with primary antibodies for SOX2 (1:1000, ab92494, Abcam, USA), OCT4 (1:1000, ab181557, Abcam, USA), ALDH (1:1000, ab52492, Abcam, USA), MACC1 (1:1000, ab226803, Abcam, USA), and GAPDH (1:1000, ab8245, Abcam, USA) at 4 °C. After treatment with secondary antibodies for 1 h at room temperature, membranes were visualized by the ECL Kit (Millipore, USA). GAPDH served as the internal control.

RNA pull down assay

RNA pull down assays were performed using the Magnetic RNA-Protein Pull Down Kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Briefly, cells were lysed using pierce IP lysis buffer (Thermo Fisher Scientific, Inc.) for 10 min, followed by lysis at 4 °C for 1 h at 13,000 × g for 10 min to pellet cell debris, and the cell supernatant was obtained. The extracts of HCT116 and SW480 cells were treated with the biotin-label miR-3163-WT, miR-3163-Mut or negative control miR-NC, followed by incubation with magnetic beads overnight at 4 °C. The bound RNAs were isolated and purified for RT-qPCR detection.

Table 2
Sequences of luciferase vectors.

Vector	Sequence
FAM201A-WT	5′-auCUGUAUACCUUUUAUUUUAUu-3′
FAM201A-Mut	5′-auGAGAUGACGGGAGUAAAAUAu-3′
MACC1- WT	5′-uugaaggacuugaauUUUUAUa-3′
MACC1-Mut	5′-uugaaggacuugaauAAAAUAa-3′

Subcellular fractionation analysis

The cytoplasmic and nuclear RNAs from HCT116 and SW480 cells were isolated and purified using the Nuclear/Cytosol RNA Purification Kit (Norgen, USA) in line with the vendor's instructions.

Luciferase reporter assay

FAM201A-WT and FAM201A-Mut were constructed through inserting the wild-type and mutant FAM201A into the luciferase vector pGL3 (Promega, USA), respectively. MACC1- WT and MACC1-Mut were generated in the same way. Cells were co-transfected with indicated vectors and miR-3163 mimic or negative control NC mimic via utilization of Lipofectamine 2000 (Invitrogen) following the product instructions. The luciferase activity was tested with Dual-Luciferase Reporter Assay System (Promega). The sequences of the above luciferase vectors were listed in Table 2.

RNA immunoprecipitation (RIP)

The Imprint RNA Immunoprecipitation Kit (Millipore) was adopted for RIP assay complying with the instructions recommended by the vender. The RNAs were immunoprecipitated by Ago2 antibody (Millipore) with IgG as a negative control. Following elution, immunoprecipitated RNAs were determined with RT-qPCR analysis.

Animal experiment

The procedures in the xenograft tumor formation experiment were in conformity with the ordinances set by the Institutional Ethics Committee of Jiangxi Provincial People's Hospital (Ethics approval number: NO.2020042). To conduct *in vivo* assay, 4-week-old BALB/c nude mice were subcutaneously inoculated with 2×10^6 HCT116 cells transfected with adenovirus-mediated sh-NC or sh-FAM201A. The growth of xenografts was recorded every 4 days and nude mice were raised for 4 weeks. Mice were euthanized with intraperitioneal injection of 100 mg/kg pentobarbital. Tumor tissues were harvested after mice were sacrificed.

Immunohistochemistry (IHC) assay

Formalin fixed and paraffin embedded tumor tissue sections were cut into 4.5 μm sections and dried at room temperature for 24 h. Following sections were deparaffinized, washed and rehydrated, slices were antigen retrieved, sealed in 5% FBS-PBS solution and probed by primary antibody for Ki-67 or SOX2 overnight at 4 °C. Afterwards, the sections

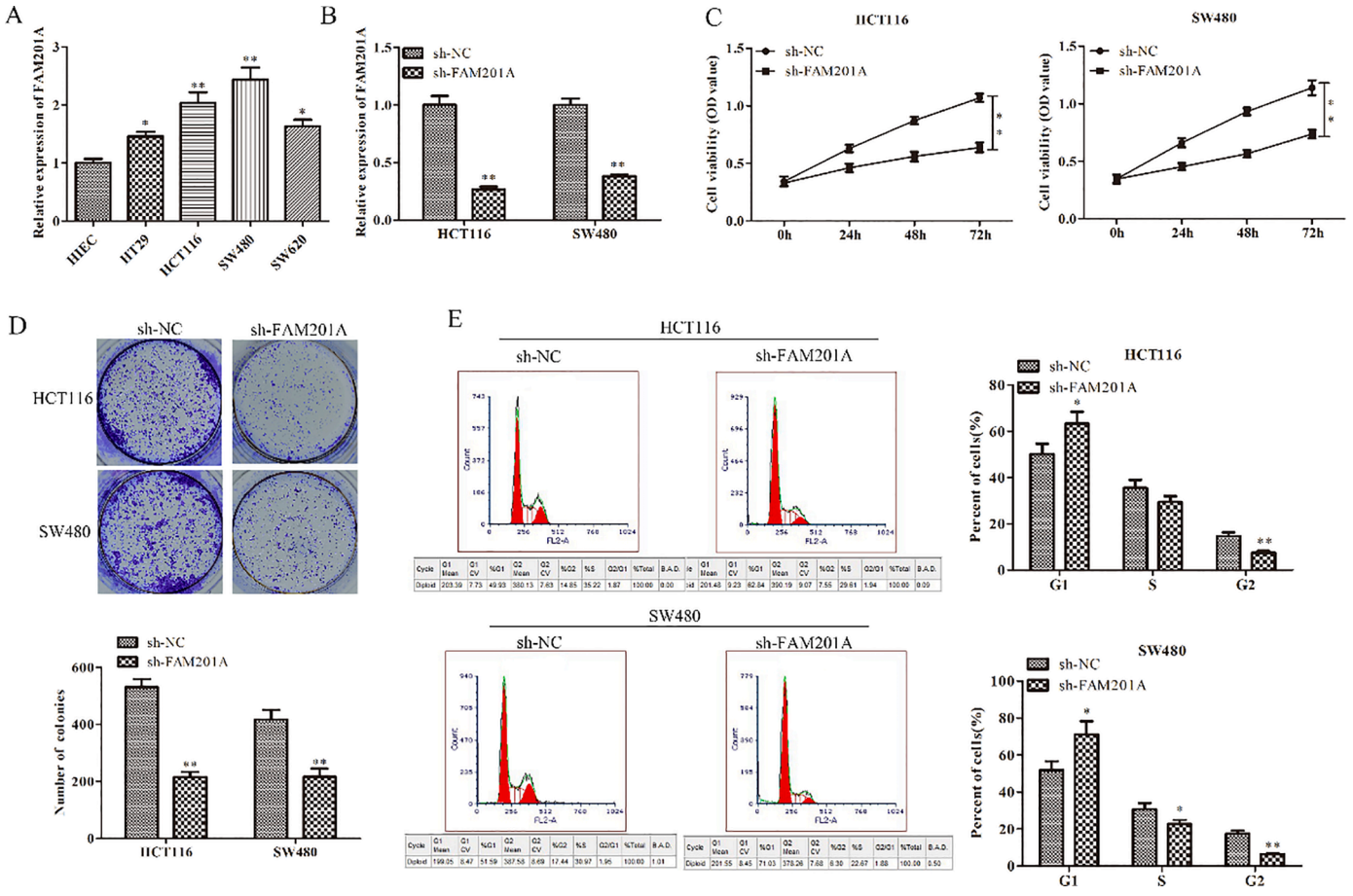

Fig. 1. Silencing FAM201A inhibits CRC cell proliferation. (A) The RT-qPCR results of FAM201A expression in CRC cells (HT29, HCT116, SW480 and SW620) and normal intestinal epitheliums HIEC. (B) The efficacy of FAM201A interference was determined by RT-qPCR. (C) CCK-8 assay and (D) colony formation assay were adopted for estimation of cell proliferation in CRC. (E) Cell cycle distribution was analyzed by flow cytometry. Experimental data were displayed as mean ± SD and all assays were repeated thrice. $^*P < 0.05$, $^{**}P < 0.01$.

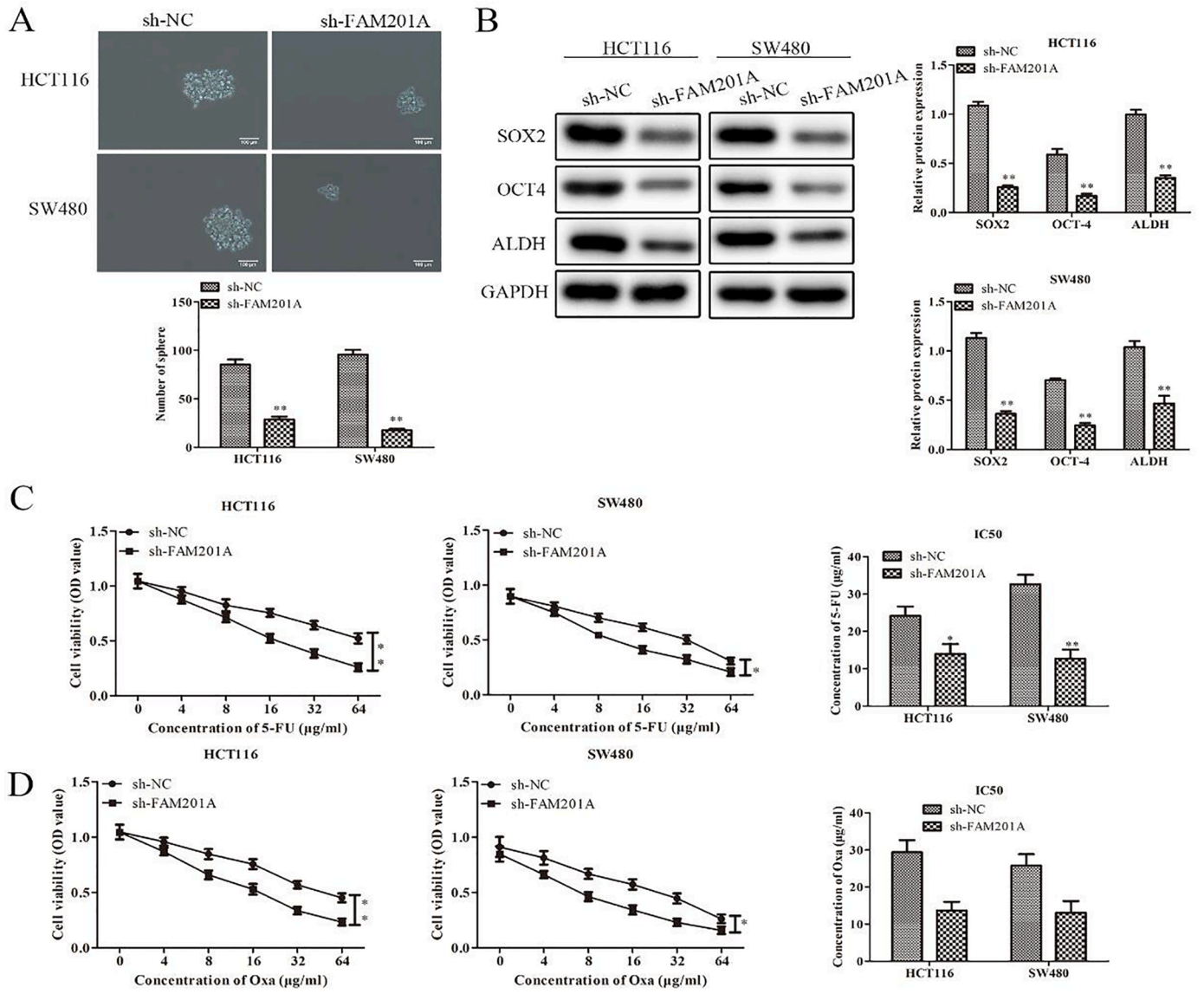

Fig. 2. Knockdown of FAM201A suppresses stemness and chemoresistance. (A) The effects of FAM201A on stemness were determined by sphere formation assay. (B) Western blot was conducted to examine the expression levels of stem markers SOX2, OCT4 and ALDH. GAPDH was adopted as inherent reference. (C,D) The sensitivity of HCT116 and SW480 cells to 5-FU and Oxa was assessed by CCK-8 assay. IC50 value was analyzed. Experimental data were displayed as mean ± SD and all assays were repeated thrice. $^{*}P < 0.05$, $^{**}P < 0.01$.

were incubated with horseradish peroxidase-conjugated secondary antibody for 30 min at room temperature and then treated with diaminobenzidine. Cell nuclei were stained by hematoxylin. Images were photographed by a light microscope (Olympus Microscope).

Statistical analysis

Experimental data were displayed as mean ± SD and all assays were repeated thrice. GraphPad Prism Software 5.0 (GraphPad Software Inc., USA) was applied to carry out statistical analysis. Comparison between the two groups was estimated by Student's t-test. The difference among multiple groups was analyzed by one-way ANOVA or two-way ANOVA followed by Tukey's test. $P < 0.05$ was deemed statistically significant.

Results

Silencing FAM201A inhibits CRC cell proliferation

To explore the involvement of FAM201A in CRC, we originally checked the expression pattern of FAM201A in CRC cell lines. Results of the RT-qPCR assay demonstrated that FAM201A was expressed at a higher level in CRC cells (HT29, HCT116, SW480, and SW620) compared with normal cells HIEC. Among them, the differences were more significant for HCT116 and SW480 (Fig. 1A). Therefore, we chose HCT116 and SW480 for subsequent experiments. Thereafter, we knocked down FAM201A expression in HCT116 and SW480 cells to conduct loss-of-function assays and knockdown efficiency was certified by RT-qPCR. Fig. 1B shows that FAM201A expression levels were significantly reduced in HCT116 and SW480 cells transfected with sh-FAM201A. The CCK-8 assay illustrated that FAM201A silence diminished the viability of HCT116 and SW480 cells (Fig. 1C). The role of FAM201A in CRC cell proliferation was further validated by colony formation assay. Fig. 1D shows that silencing FAM201A significantly inhibited CRC cell proliferation. Likewise, flow cytometry analysis indicated that downregulation of FAM201A led to cell cycle arrest in G0/G1 phase (Fig. 1E). Based on the aforementioned findings, we concluded that the knockdown of FAM201A impeded the growth of CRC cells.

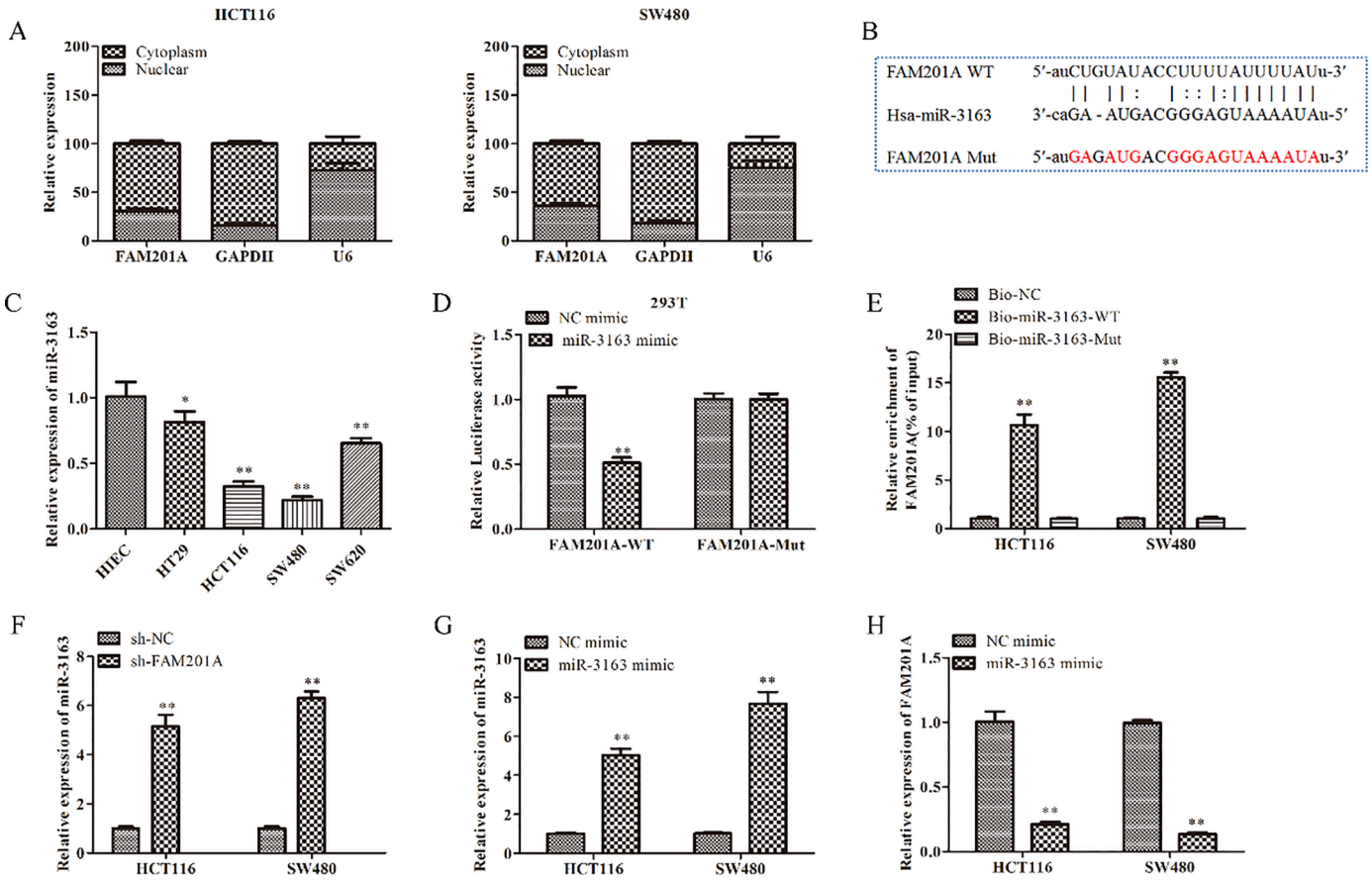

Fig. 3. FAM201A serves as a sponge for miR-3163. (A) The location of FAM201A detected by subcellular fractionation assay. (B) The speculated binding sites between FAM201A and miR-3163 predicted by starBase. (C) The RT-qPCR analysis was applied to measure miR-3163 expression in CRC cells and normal epitheliums. (D,E) The interaction of FAM201A with miR-3163 was confirmed by RNA pull down assay and luciferase reporter assay. (F–H) After different treatments, the levels of FAM201A and miR-3163 expression in HCT116 and SW480 were identified by RT-qPCR. Experimental data were displayed as mean ± SD and all assays were repeated thrice. $*P < 0.05$, $**P < 0.01$.

Knockdown of FAM201A suppresses stemness and chemoresistance

Considering the pivotal role of CSC properties in the tumorigenesis of cancer, we intended to investigate the impacts of FAM201A on stemness and chemoresistance. Our results delineated that suppression of FAM201A contributed to the attenuated sphere formative capability of CRC cells (Fig. 2A). Consistently, western blot suggested that FAM201A silence prominently lessened the expression levels of stem factors SOX2, OCT4, and ALDH (Fig. 2B). Moreover, we observed that knockdown of FAM201A heightened the sensitivity of HCT116 and SW480 cells to 5-FU (Fig. 2C). Similarly, the silencing of FAM201A alleviated the resistance of CRC cells to Oxa (Fig. 2D). Collectively, these data provided strong evidence that silencing FAM201A weakens the CSC characteristics of CRC cells.

FAM201A serves as a sponge for miR-3163

To address the regulatory mechanism underlying FAM201A, we performed subcellular fractionation assay. It was disclosed that FAM201A was principally distributed in the cytosol, indicating that FAM201A might function as a ceRNA to liberate target genes from miRNA-degradation (Fig. 3A). Through the utilization of bioinformatics analysis, we discovered that FAM201A harbored miR-3163 binding sites (Fig. 3B). The RT-qPCR assay illuminated that the level of miR-3163 in CRC cells was lower than that in normal cells (Fig. 3C). In view of the anticancer function of miR-3163 in several cancers, miR-3163 was selected for subsequent study. Our observations depicted that the luciferase activity of FAM201A-WT was only weakened by miR-3163 mimic (Fig. 3D). Besides, RNA pull down assay manifested that FAM201A was abundantly detected in precipitates pulled down by miR-3163-WT, further confirming that FAM201A directly bound to miR-3163 (Fig. 3E). Results showed that suppression of FAM201A resulted in the augment of miR-3163 expression (Fig. 3F). Thereafter, we overexpressed miR-3163 and found that miR-3163 mimic remarkably reduced the level of FAM201A (Fig. 3G,H). Collectively, our findings elucidated that FAM201A interacted with miR-3163 in CRC.

FAM201A regulates the expression of MACC1 via a miRNA-dependent mechanism

Thereafter, miR-3163 binding sites were found in the 3′UTR of oncogene MACC1 (Fig. 4A). In contrast with normal cells HIEC, the expression of MACC1 was markedly elevated in CRC cells (Fig. 4B). Therefore, we further explored the relationship between miR-3163 and MACC1. Luciferase reporter assay presented that the conspicuous decline of luciferase activity was observed in MACC1-WT not in MACC1-Mut, suggesting the interplay of MACC1 with miR-3163 (Fig. 4C). Besides, enhanced expression of miR-3163 overtly lowered the mRNA and protein levels of MACC1 (Fig. 4D,E). Then, RIP experiment expounded that FAM201A, miR-3163 and MACC1 were all enriched by Ago2 antibody (Fig. 4F). Additionally, depletion of FAM201A caused the diminution of MACC1 expression at both mRNA and protein levels (Fig. 4F–H). On the whole, we disclosed that FAM201A modulated MACC1 expression by competing for miR-3163.

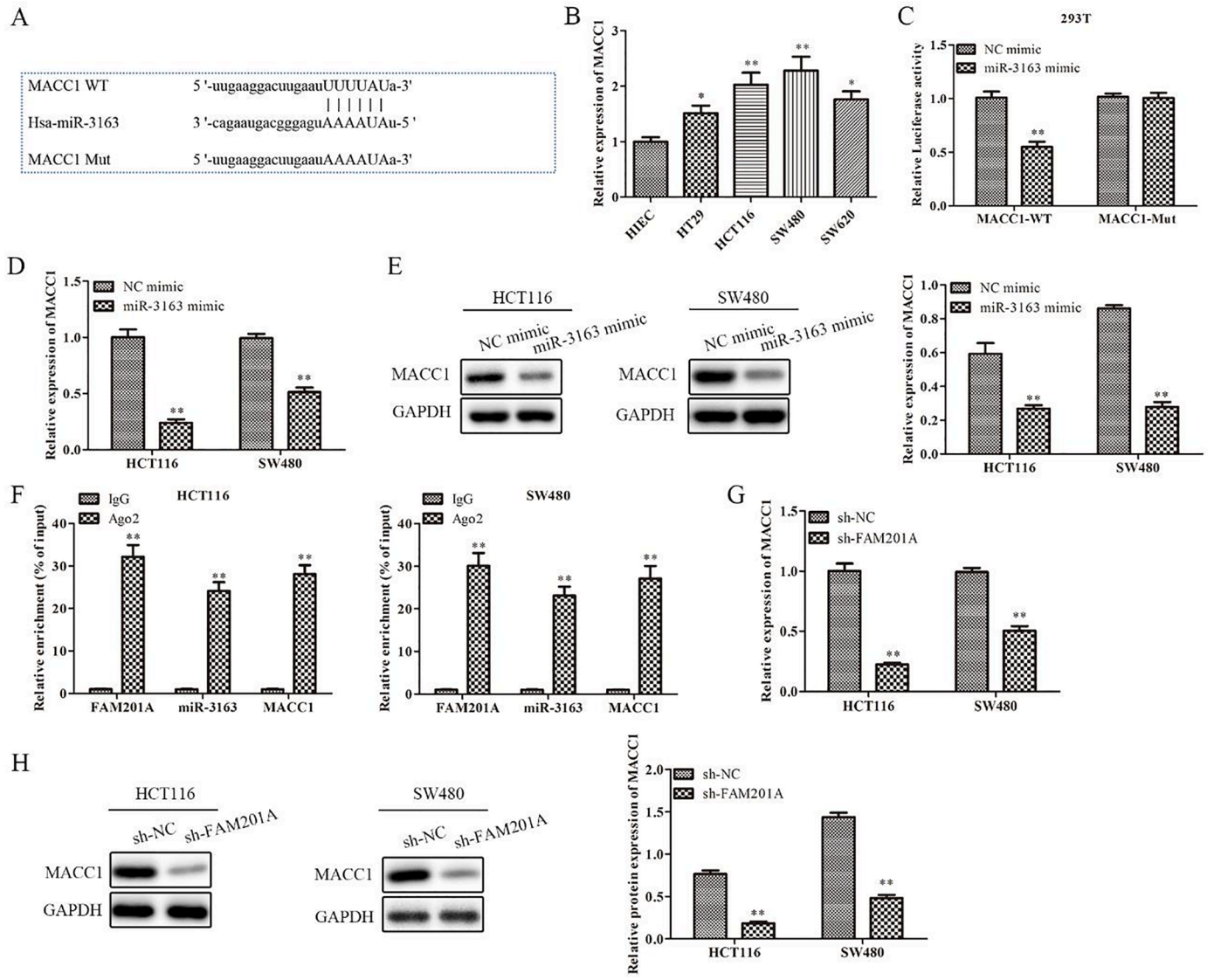

Fig. 4. FAM201A regulates the expression of MACC1 via a miRNA-dependent mechanism. (A) The putative miR-3163 binding sites in the 3′UTR of MACC1. (B) The expression pattern of MACC1 was evaluated by RT-qPCR assay. (C) Luciferase reporter assay validated the interplay of miR-3163 with MACC1. (D,E) The RT-qPCR analysis and western blot were performed to test MACC1 mRNA and protein levels when miR-3163 was overexpressed. (F) The relationship among FAM201A, miR-3163 and MACC1 was validated by RIP experiment. (F) MACC1 expression at mRNA and protein levels determined by RT-qPCR and western blot. Experimental data were displayed as mean ± SD and all assays were repeated thrice. $^{*}P < 0.05$, $^{**}P < 0.01$.

FAM201A facilitates CRC progression by targeting miR-3163/MACC1 axis

Rescue assays were carried out to verify whether the function of FAM201A in CRC was mediated by miR-3163/MACC1 pathway. After transfection, RT-qPCR analysis exhibited that miR-3163 expression was downregulated and MACC1 was silenced in HCT116 cells (Fig. 5A). The CCK-8 assay and colony formation assay indicated that the decreased proliferation of HCT116 cells caused by FAM201A silence was increased by miR-3163 inhibitor and therewith recovered due to knockdown of MACC1 (Fig. 5B,C). Consistently, cell cycle arrest ascribed to down-regulation of FAM201A was abolished by miR-3163 inhibitor and the restoration of cell cycle progression occurred when MACC1 was silenced (Fig. 5D). Furthermore, sphere formation assay illustrated that sphere-forming ability suppressed by FAM201A knockdown was promoted by miR-3163 inhibitor and then renewed by repression of MACC1 (Fig. 5E). In concert with the foregoing, the reduced levels of SOX2, OCT4 and ALDH resulting from FAM201A silence were elevated by miR-3163 inhibitor and knockdown of MACC1 abrogated the impacts of miR-3163 inhibitor on stem factors (Fig. 5F). More importantly, miR-3163 inhibitor heightened the resistance of HCT116 cells sensitized by suppression of FAM201A to 5-FU and Oxa, subsequently MACC1 depletion repaired the sensitization of HCT116 cells to 5-FU and Oxa (Fig. 5G). Taken together, FAM201A induced CRC tumorigenesis and maintained CSC characteristics through sponging miR-3163 to modulate MACC1.

Inhibition of FAM201A restrains the malignant behaviors of CRC cells in vivo

To further confirm the carcinogenic role of FAM201A, tumorigenicity *in vivo* was implemented. Nude mice were subcutaneously inoculated with HCT116 cells transfected with sh-NC or sh-FAM201A. Our observations unraveled that the silencing of FAM201A restrained the tumorigenicity of CRC cells (Fig. 6A). Strikingly, the FAM201A knockdown lowered the size and weight of xenografts (Fig. 6B,C). Furthermore, the expression of FAM201A and MACC1 was obviously declined in neoplasms generated by injection of FAM201A-downregulated HCT116 cells compared with those in the sh-NC group, while the miR-

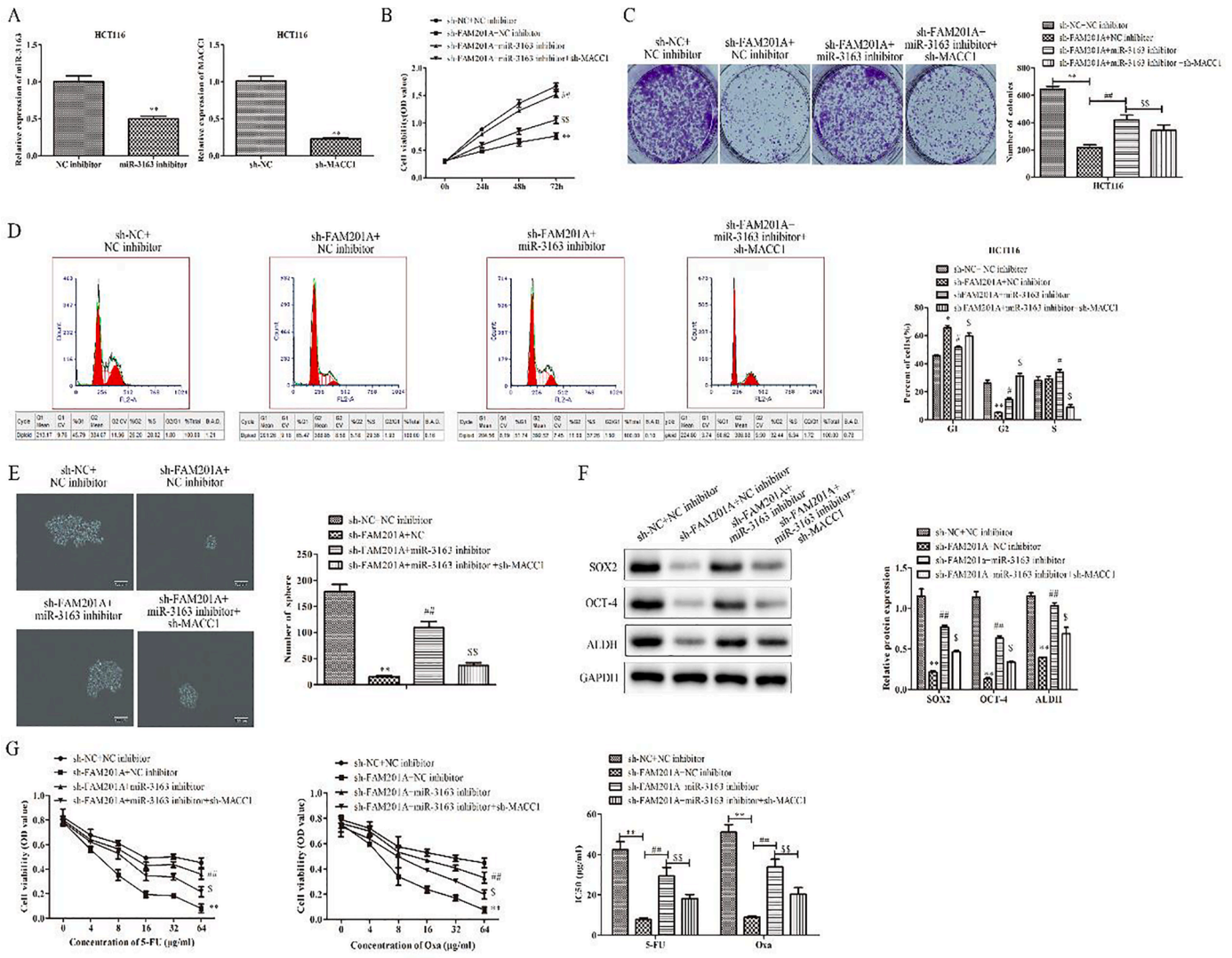

Fig. 5. FAM201A facilitates CRC progression by targeting miR-3163/MACC1 axis. (A) The RT-qPCR assay was implemented to certify knockdown efficiency for miR-3163 and MACC1. (B) CCK-8 assay, (C) colony formation assay and (D) flow cytometry analysis was employed to estimate the role of miR-3163/MACC1 in CRC cell growth. (E–G) The impacts of miR-3163 and MACC1 on FAM201A-regulated CSC properties were evaluated by sphere formation assay (E), western blot (F) and CCK-8 assay (G). Experimental data were displayed as mean ± SD and all assays were repeated thrice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs sh-NC+NC inhibitor group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs sh-FAM201A+NC inhibitor group. $^{\$}P < 0.05$, $^{\$\$}P < 0.01$ vs sh-FAM201A+miR-3163 inhibitor group.

316 level was highly expressed in tumors when FAM201A was silenced (Fig. 6D). IHC assay delineated that inhibition of FAM201A significantly diminished the expression of Ki-67 and SOX2 (Fig. 6E). Namely, FAM201A accelerated the deterioration of CRC *in vivo*.

Discussion

CRC is one of the most prevalent malignancies in digestive tract with third highest morbidity and fourth highest mortality among all cancers [19,20]. Roughly 1.2 million new cases of CRC and 600,000 deaths resulting from CRC annually [21]. In view of its subclinical features and poor prognostic manifestations, CRC is deemed as a severe disease hazard to public health [22]. Despite enormous efforts devoted to develop the treatment regimens for CRC, the prognosis of CRC patients remain dismal and their 5-year relative survival rate is still disappointing [23]. Accordingly, elucidating the underlying mechanism of CRC to discover effective diagnostic and prognostic biomarkers is conducive to the improvement of CRC therapy.

Mounting evidence points out that lncRNAs, as a component of noncoding RNA, are participated in the development and evolution of plenty disorders through serving as molecular scaffolds, inducing transcriptional gene silencing, modulating stem cell pluripotency, maintaining DNA methylation/demethylation and other means [24–27]. A myriad of investigations highlight that deregulated lncRNAs are closely correlated with the tumor progression [28,29]. LncRNA FAM201A has been reported to modulate ATM and mTOR expression to involve in the radiosensitivity of esophageal squamous cell carcinoma by miR-101 [17]. In addition, recent study illustrates that FAM201A reinforces radioresistance in non-small-cell lung cancer through upregulating EGFR expression via miR-370 [18]. Nevertheless, the latent role of FAM201A in CRC tumorigenesis has not been understood. In the current study, we first prospected the expression of FAM201A in CRC cells. In contrast with normal cells, FAM201A was highly expressed in CRC cells. Thereafter, FAM201A was silenced in HCT116 and SW480 cells to carry out loss-of-function experiments. Our results expounded that depletion of FAM201A suppressed cell proliferation and cell cycle progression. As CSC properties play a key role in the carcinogenicity of CRC, we then explored the effects of FAM201A on stemness and chemoresistance and revealed that FAM201A knockdown alleviated sphere formative capacity and enhanced the chemosensitivity of CRC cells. It is well-known that microRNAs (miRNAs) are another type of non-coding RNA transcript with a length of 20–25 nucleotides [30]. Accumulating

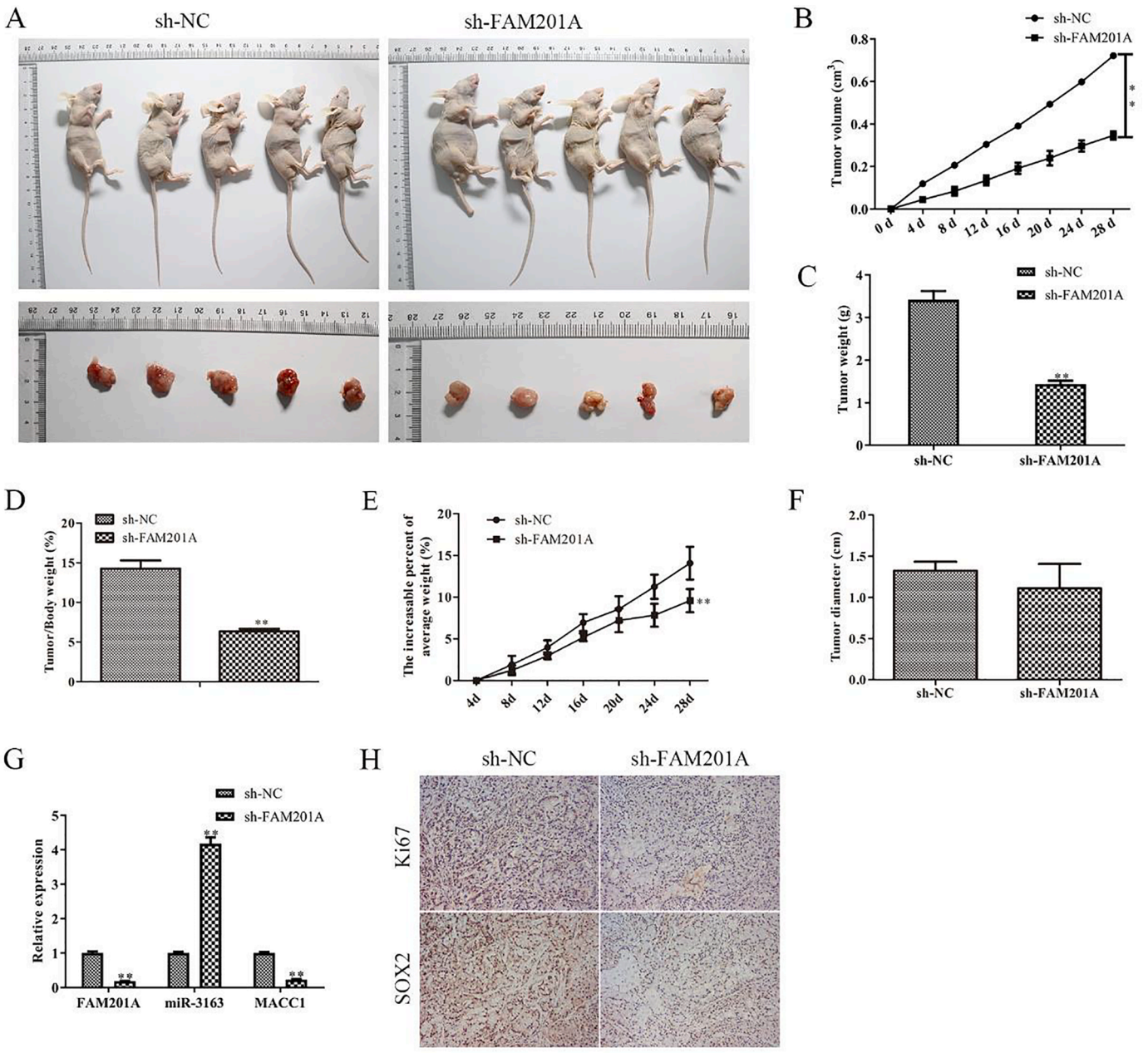

Fig. 6. Inhibition of FAM201A restrains the malignant behaviors of CRC cells *in vivo*. (A) Images of tumors formed by nude mice injected with cells stably transfected with adenovirus-mediated sh-NC or sh-FAM201A. (B,C) The volume and weight of xenografts was shown ($n = 5$). (D) The RT-qPCR detection of FAM201A, miR-3163 and MACC1 expression in neoplasms. (E) IHC assay was conducted to test the levels of Ki-67 and SOX2. Experimental data were displayed as mean ± SD and all assays were repeated thrice. $^{*}P < 0.05$, $^{**}P < 0.01$.

researches emphasize that miRNAs are regarded as core mediators in the oncogenicity of multiple malignant tumors via functioning as oncogenes or tumor suppressors [31,32]. Increasing investigations demonstrate that a wide range of miRNAs are involved in the occurrence and development of CRC [33,34]. A growing body of evidence has proven that lncRNAs are capable of competitively repressing miRNAs to regulate target genes by working as molecular sponges [35,36]. Herein, results of subcellular fractionation assay delineated the dominating expression of FAM201A in the cytoplasm, suggesting that the possibility of FAM201A as a ceRNA [37]. Previous studies illuminated that miR-3163 executed its anti-cancer activities in several cancers, including CRC [38–40]. And miR-3163 was significantly low expressed in CRC tissues and cells [41]. In addition, studies have shown that MACC1 is significantly highly expressed in CRC tissues and cells, and is closely related to CRC cell proliferation, migration and apoptosis [42]. By utilization of bioinformatics tool miR-3163 was found to own binding sites with FAM201A. Thereafter, we validated that miR-3163 was negatively regulated by FAM201A. Further, oncogene MACC1 was testified to be a target of miR-3163. Mechanistically, FAM201A promoted MACC1 expression to act as an oncogene in CRC. Rescue experiments revealed that the function of FAM201A in CRC tumorigenicity was mediated by miR-3163/MACC1 pathway. Moreover, xenograft tumor assay justified that FAM201A expedited the growth of CRC cells *in vivo*. Above results indicated that FAM201A was a pivotal agent in the progression of CRC.

Conclusion

To summarize, we unraveled that FAM201A was a cancer facilitator in CRC both *in vitro* and *in vivo*. Our study corroborated that FAM201A accelerated cell proliferation and maintained CSC features via sponging miR-3163 to elevate MACC1 expression. To our best knowledge, this is the first investigation to shed light on the potential and molecular mechanism of FAM201A in CRC. Our results revealed that FAM201A might be a novel target for the treatment of CRC.

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Data availability

All the data during the current study are included in the article or uploaded as supplementary information.

Ethics approval and consent to participate

The experimental protocols were approved by the Ethics Committee of the Jiangxi Provincial People's Hospital. This paper has not been published elsewhere in whole or in part. All authors have read and approved the content, and agree to submit it for consideration for publication in your journal. There are no ethical/legal conflicts involved in the article. Informed consent was obtained from all individual participants included in the study.

CRediT authorship contribution statement

Lifeng Zeng: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Xiaojiang Luo:** Data curation, Formal analysis, Writing – review & editing. **Zhiyong Zhang:** Data curation, Formal analysis. **Zhengyong Wang:** Data curation, Formal analysis. **Jinrong Qian:** Methodology, Investigation, Data curation, Formal analysis, Visualization, Funding acquisition, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Not applicable.

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