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Original Article

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lncRNA RMST suppresses the progression of colorectal cancer by competitively binding to miR-27a-3p/RXRα axis and inactivating Wnt signaling pathway

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Abstract

Colorectal cancer (CRC) ranks the 3rd in cancer types globally. Long noncoding RNAs (lncRNAs) are related to the initiation and progression of CRC. The current study plans to reveal the action of rhabdomyosarcoma 2-associated transcript (RMST) in CRC. The results show that RMST is downregulated in CRC specimens and cell lines relative to normal specimens and a fetal normal colon cell line (FHC), respectively. Elevation of RMST represses cell proliferation and colony formation and induces cell apoptosis in CRC cells. Bioinformatic analysis reveals a binding site in RMST for miR-27a-3p. The direct association between RMST and miR-27a-3p is confirmed by dual luciferase reporter assay, RNA pull-down assay, and real time-quantitative polymerase chain reaction (RT-qPCR). miR-27a-3p is upregulated in CRC tumor specimens relative to normal specimens, and there is a negative correlation between RMST and miR-27a-3p in CRC tumor specimens. In addition, the effects of RMST overexpression are weakened by the elevation of miR-27a-3p. RMST and retinoid X receptor (RXRα) share the same complementary site with miR-27a-3p. The direct association between RXRα and miR-27a-3p is confirmed by RNA pull-down assay, RT-qPCR and western blot analysis. Overexpression of RMST induces RXRα expression and inactivates the Wnt signaling pathway by decreasing β-catenin levels in CRC cells. Collectively, our findings reveal a pivotal role of RMST in regulating miR-27a-3p/RXRα axis and counteracting Wnt signaling pathway during the progression of CRC.

Key words colorectal cancer, RMST, miR-27a-3p, RXRα, Wnt/β-catenin signaling pathway

Introduction

Colorectal cancer (CRC) remains the 3rd most common cancer type in humans globally [1]. The progression of CRC includes multiple steps with dysregulation of cell growth and programmed cell death [2]. Despite the application of systematic therapeutic approaches in CRC, the prognosis of patients with CRC is unsatisfactory; especially in those with liver metastasis, the 5-year overall survival is less than 10% [3]. Investigations into molecular mechanisms will provide novel targets and biomarkers for patients with CRC.

Long noncoding RNAs (lncRNAs) of > 200 nt in length are a type of transcript with no potential for protein coding [4]. They play pivotal roles in regulating the development and progression of disease [5,6]. Recent reports have suggested that many lncRNAs exert their functions by the competing endogenous RNA (ceRNA) hypothesis [7,8]. According to this model, lncRNAs or mRNAs that share the same sequence with miRNAs are proposed to mutually regulate the expression of each other by competitively interacting with miRNAs [9]. Several lncRNAs are crucial molecules for CRC, including MAFG-AS1 [10], H19 [11], GAS5 [12], and BX357664 [13]. Rhabdomyosarcoma 2-associated transcript (RMST) was initially

identified to be involved in neuron differentiation by a genome-wide approach [14]. In addition, RMST was found to play a role as a tumor suppressor in triple-negative breast cancer (TNBC) by suppressing cell survival and inducing cell apoptosis [15]. However, the function of RMST in the progression of CRC remains unknown.

In this study, we found that RMST is downregulated while miR-27a-3p is upregulated in CRC specimens and cell lines. Elevation of RMST represses cell proliferation and colony formation and induces cell apoptosis in CRC cells. There is a direct association between RMST and miR-27a-3p as well as miR-27a-3p and retinoid X receptor (RXRα). Overexpression of RMST induces RXRα expression and inactivates the Wnt signaling pathway by decreasing β-catenin levels in CRC cells.

Materials and Methods

Samples

Samples were extracted from patients with CRC ($n = 50$) at Tianjin Union Medical Center of Nankai University from July 2017 to January 2019. Patient information was collected, including tumor-node-metastasis (TNM) stage [16]. The protocols were approved by the Ethical Committee of the Tianjin Union Medical Center of Nankai University (approval number: TUMC2017-06). All patients signed informed consent prior to the study. Samples were placed immediately in liquid nitrogen for storage.

Cell culture and transfection

CRC cell lines (HCT116 and SW480) and fetal normal colon cell line (FHC) were obtained from ATCC (Manassas, USA). They were cultured in DMEM (Gibco, Waltham, USA) containing 10% FBS (Gibco) and 1% penicillin/streptomycin (Sigma-Aldrich, St Louis, USA) in a humid incubator with 5% CO_2 at 37°C.

Full-length RMST was synthesized by Sangon Biotech (Shanghai, China) and annealed to pcDNA3. The miR-NC mimic and miR-27a-3p mimic were provided by GenePharma (Suzhou, China). In brief, 2 μg plasmid, 50 nM miR-NC mimic (5′-GUGGAUUUUCCUCUAUGAUUU-3′) or 50 nM miR-27a-3p mimic (5′-UUCACAGUGGCUAAGUUCCGC-3′) was transfected into cells by using Lipofectamine 3000 (Invitrogen, Carlsbad, USA). Forty-eight hours later, the cells were harvested for subsequent experiments.

Bioinformatics analysis

The expression of RMST in 26 tumor types and normal tissues was acquired from TCGA. The CCLE dataset was used to retrieve the expression of RMST in multiple cancer cell lines, including 57 CRC cell lines. Using GEO2R software provided by the GEO database, miR-27a-3p expression in normal colon mucosa, colon adenoma and colon carcinoma based on the GSE115513 dataset from the GEO database (https://www.ncbi.nlm.nih.gov/gds/?term = GSE115513) was analysed. Potential miRNAs for RMST were predicted using ENCORI software (http://starbase.sysu.edu.cn/index.php). ENCORI analysis showed that PAR-CLIP data from a previous study revealed a binding site between miR-27a-3p and RMST [17].

Cell proliferation and cell apoptosis assays

Cell proliferation was measured by using CCK-8 kit (DoJinDo, Tokyo, Japan). Cells were grown in culture medium (containing 10% CCK-8) at 37°C for 1 h. The absorbance (450 nM) was read using a microplate reader (Bio-Rad Laboratories, Hercules. USA). The percentage of apoptotic cells was measured by flow cytometry. Cells were cultured for 48 h at 37°C prior to being harvested by using 0.025% trypsin (Thermo Fisher Scientific, Waltham, USA), then incubated with Annexin V (5 μL) and PI (5 μL) from an Annexin V-FITC-PI Apoptosis Detection Kit (Invitrogen) for 15 min in the dark at 37°C. After that, 400 μL of 1 × Annexin-binding buffer was added and thoroughly mixed. Next, the suspension was loaded for flow cytometric analysis. Annexin V-positive cells were apoptotic. The results were analysed by FlowJo software 10.2 (FlowJo LLC, Ashland, USA).

Colony formation assay

In brief, cells were plated in 6-well plates and cultured for 7 days with the culture medium refreshed every 2 days. Then, the cells were stained with 0.1% crystal violet (Sigma-Aldrich) for 10 min at room temperature. Finally, colonies were observed and counted with a microscope (Nikon, Tokyo, Japan).

RT-qPCR

TRIzol (Invitrogen) was used to isolate RNA from tissues and cell lines. Nuclear and cytoplasmic fractions were isolated with the Nuclear Cytoplasmic Extraction Kit (Pierce, Rockford, USA) following the manufacturer's protocol. cDNA was synthesized from RNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Thereafter, RT-qPCR was carried out with TB Green® Fast qPCR Mix (TaKaRa, Tokyo, Japan) on a CFX96 Real-Time PCR system (Bio-Rad Laboratories). The PCR reaction conditions were as following: predenature at 95°C fo 30 s; 95°C for 5s, 60°C for 10 s, 40 cycles, Relative gene expression was analysed by the $2^{-\Delta\Delta Ct}$ method. *GAPDH* served as a reference for RXRα and RMST, while *U6* served as a reference for miR-27a-3p [18]. The primers are presented in Table 1.

Western blot analysis

Protein lysates of cell lines were obtained by using RIPA buffer (Thermo Fisher Scientific). The concentration of lysates was determined using a BCA Protein Assay kit (Thermo Fisher Scientific). Protein samples (25 μg) were subject to SDS-PAGE (10%) and transferred onto PVDF membranes which were blocked with 5% nonfat milk for 2 h at room temperature and then successively incubated with primary antibodies for 12 h at 4°C and the corresponding secondary antibodies for 1 h at room temperature. Protein bands were developed with an ECL Western Blotting Substrate (Pierce). Band intensities were analysed by ImageJ (version 1.6.1; NIH, Bethesda, USA). GAPDH served as an internal control. The primary antibodies used are as follows: c-Myc (18583, 1:2000; CST, Danvers, USA), β-catenin (8480, 1:2000; CST), GAPDH (97166, 1:5000; CST), APC (2504, 1:2000; CST), RXRα (AB45310, 1:1000; AbSci, College Park, USA), and TERT (AB41486, 1:1000, AbSci). HRP-conjugated secondary antibodies (ab205719, 1:5000; ab6721, 1:5000) were purchased from Abcam (Cambridge, UK).

TOP-flash/FOP-flash assay

Top-flash or Fop-flash plasmid (200 ng), pRL-TK (20 ng), and pcDNA3-RMST (200 ng, 500 ng) or pcDNA3 (200 ng) were cotransfected into cells by using Lipofectamine 3000 (Invitrogen). Twenty-four hours after transfection, harvested cells were lysed. Firefly luciferase activities were evaluated by using a Dual Luciferase Reporter System kit (Promega, Madison, USA), which was referred to as Renilla luciferase.

Table 1. Sequences of primers used for RT-qPCR

Primer	Sequence (5′→3′)
RMST-F	GCTGCGGGGAAATATAATCA
RMST-R	AGGAACACCATCTGCCTTTG
U6-F	CTCGCTTCGGCAGCACATATAC
U6-R	GGAACGCTTCACGAATTTGC
GAPDH-F	TGCACCACCAACTGCTTAGC
GAPDH-R	GGCATGGACTGTGGTCATGAG
MYC-F	CCTGGTGCTCCATGAGGAGAC
MYC-R	CAGACTCTGACCTTTTGCCAGG
TERT-F	AAATGCGGCCCCTGTTTCT
TERT-R	CAGTGCGTCTTGAGGAGCA
TCF1-F	AACACCTCAACAAGGGCACTC
TCF1-R	CCCCACTTGAAACGGTTCCT
TWIST2-F	GCAAGATCCAGACGCTCAAGCT
TWIST2-R	ACACGGAGAAGGCGTAGCTGAG
SNAIL1-F	TCGGAAGCCTAACTACAGCGA
SNAIL1-R	AGATGAGCATTGGCAGCGAG
VEGFA-F	AGGGCAGAATCATCACGAAGT
VEGFA-R	AGGGTCTCGATTGGATGGCA
miR-27a-3p stem loop	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGT TGAGGCGGAA
miR-27a-3p-F	GCCGAGTTCACAGTGGCTAA
miR-27a-3p-R	CTCAACTGGTGTCGTGGA
miR-27b-3p stem loop	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGT TGAGGCAGAA
miR-27b-3p-F	ACACTCCAGCTGGGTTCACAGTGGCTA
miR-27b-3p-R	CTCAACTGGTGTCGTGGA
RXRα-F	ATGGACACCAAACATTTCCTGC
RXRα-R	GGGAGCTGATGACCGAGAAAG

Dual luciferase reporter assay

The RMST sequence was cloned into pmirGLO (Promega). pmirGLO-RMST-WT or the mutant construct pmirGLO-RMST-MUT was cotransfected with miR-NC mimic or miR-27a-3p mimic into cells by using Lipofectamine 3000 (Invitrogen). Forty-eight hours following transfection, harvested cells were lysed. Firefly luciferase activities were evaluated with a Dual Luciferase Reporter System kit (Promega) on a Synergy H1 microplate reader (BioTek Instruments, Winooski, USA), which were referred to as Renilla luciferase.

Pulldown assay with biotinylated miRNA

Cells were transfected with biotinylated miR-27a-3p (20 nM) or biotinylated miR-NC (20 nM) by using Lipofectamine 3000 (Invitrogen). Forty-eight hours after transfection, cell lysates were collected and incubated with streptavidin magnetic beads (Invitrogen) as described previously [19]. The bound RNAs were purified and reverse-transcribed into cDNA for RT-qPCR analysis as mentioned above.

Statistical analyses

All experiments were repeated three times. Data were analysed by GraphPad Prism 6.0 and are shown as the mean ± SD. Two groups were analysed by Student's *t*-test. More than two groups were analysed by one-way ANOVA followed by Tukey's analysis. The associations between RMST and clinicopathologic parameters were analysed by the *chi*-square test. The association between RMST and miR-27a-3p was analysed by Pearson correlation analysis. $P < 0.05$ indicates statistical significance.

Results

RMST was downregulated in CRC tissues and cell lines

To unveil the role of RMST in cancer, the expression of RMST was analysed from TCGA datasets, which revealed the downregulation of RMST in half of the selected cancer types (13 out of 26), including COAD and COADREAD (Figure 1A). Meanwhile, in the collected samples in the current study, RMST was validated to be significantly decreased in CRC tumor samples relative to normal samples (Figure 1B), RMST was significantly decreased in moderate-low differentiated tumors compared to that in well differentiated tumors (Figure 1C), and lower RMST expression was observed in metastatic tumors than in nonmetastatic tumors (Figure 1D). The *chi*-square test showed that in patients with CRC, RMST was related to patients' histological stage and metastasis status but not to patients' sex, age, tumor size, T stage status or N stage status (Table 2). Furthermore, RMST was significantly decreased in HCT116 and SW480 cells compared to that in FHC cells (Figure 1E), and by analysing CCLE data, we found that RMST was barely detected in most CRC cell lines (32 out of 57), including HCT116 and SW480 cells (Figure 1F). These data collectively indicated that RMST expression was downregulated in CRC.

Overexpression of RMST induced cell apoptosis and inhibited cell proliferation and colony formation in CRC cells

To further unveil the action of RMST in CRC, RMST was overexpressed in HCT116 cells (Figure 2A). In HCT116 cells, RMST overexpression significantly enhanced cell apoptosis (Figure 2B) and restrained cell proliferation (Figure 2C) and colony formation (Figure 2D). Consistently, the findings in SW480 cells were similar to those in HCT116 cells (Figure 2E–H). The data together indicated a pivotal function of RMST in CRC cell growth and survival.

RMST sponged miR-27a-3p in CRC cells

According to the prediction of ENCORI software, miR-27a-3p and miR-27b-3p harbored complementary binding sites for RMST (Figure 3A and Supplementary Figure S1A). In HCT116 cells, RMST overexpression significantly reduced miR-27a-3p expression (Figure 3B). miR-27a-3p expression was confirmed to be elevated by the miR-27a-3p mimic, while RMST expression was reduced by the miR-27a-3p mimic (Figure 3C,D); miR-27a-3p elevation significantly depressed the luciferase activity of HCT116 cells transfected with RMST-WT but not RMST-Mut (Figure 3E); RMST was pulled down by biotin-labelled miR-27a-3p oligos (Figure 3F). The results in SW480 cells were similar to those in HCT116 cells, as mentioned above (Figure 3G–K). However, miR-27b-3p expression was not altered upon RMST overexpression in HCT116 or SW480 cells (Supplementary Figure S1B). We next detected the subcellular localization of RMST and miR-27a-3p in HCT116 cells. As expected, miR-27a-3p was mainly located in the cytoplasmic fraction, and RMST was also detected in the cytoplasm (approximately 30%),

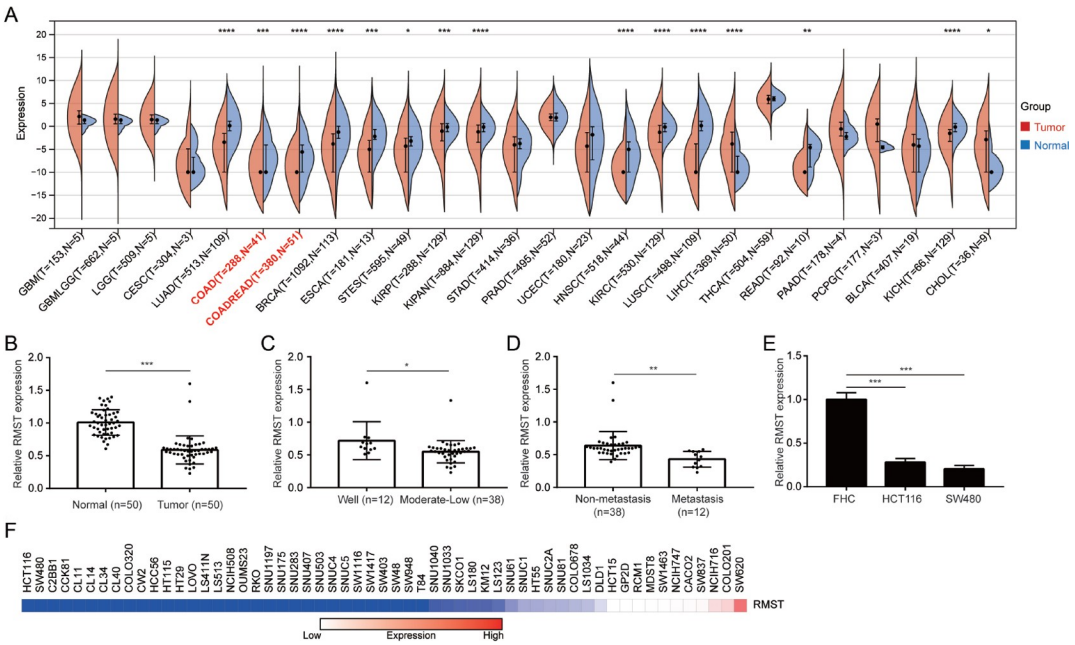

Figure 1. RMST expression profile in CRC cells and tumor tissues (A) RMST expression in 26 cancer types and normal samples was analysed in the TCGA dataset. (B) RMST expression was compared in 50 pairs of CRC tumor samples to normal samples. (C) RMST expression was compared in CRC tumor samples of moderate-low differentiation to those of well differentiation. (D) RMST expression was compared between nonmetastatic and metastatic CRC tumor samples. (E) RMST expression was compared in HCT116 and SW480 cells to that in FHCs. (F) RMST expression in 57 CRC cell lines was retrieved from the CCLE dataset. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$.

Table 2. Association between RMST expression and clinicopathological features of colorectal cancer patients

Characteristics	RMST expression		*P* value
	Low	High	
Sex			0.095
Male	17	18	
Female	8	7	
Age (years)			0.136
> 55	20	21	
≤ 55	5	4	
Tumor size (cm)			0.802
> 5	18	15	
≤ 5	7	10	
Histological differentiation			0.023
Well	7	15	
Moderately-Poorly	18	10	
T stage			0.081
1	3	8	
2	6	9	
3	6	5	
4	10	3	
N stage			0.571
0	13	10	
1–2	12	15	
M stage			0.018
0	15	23	
1	10	2	

although most of the transcript was located in the nuclear fraction (Figure 3L). The above findings indicated a direct interaction between RMST and miR-27a-3p.

RMST was inversely correlated with miR-27a-3p in CRC tumor tissues

Additionally, the present bioinformatics analysis indicated that miR-27a-3p is an upregulated miRNA in colon carcinoma relative to colon adenoma and colon mucosa (Figure 4A). Meanwhile, in the collected samples in the current study, we also observed an elevation of miR-27a-3p in CRC tumor samples relative to normal samples (Figure 4B). Furthermore, miR-27a-3p was negatively correlated with RMST in CRC tumor samples (Figure 4C).

Overexpression of RMST induced CRC cell apoptosis while inhibiting cell proliferation and colony formation by sponging miR-27a-3p

In HCT116 cells, RMST overexpression triggered significant cell apoptosis (Figure 5A) and inhibited cell growth and colony formation (Figure 5B,C), which were significantly attenuated by miR-27a-3p overexpression. Consistently, the findings in SW480 cells were similar to those in HCT116 cells (Figure 5D–F). Altogether, the above data demonstrated that RMST exerted its role as a tumor suppressor by sponging miR-27a-3p in CRC.

RXRα was regulated by RMST/miR-27a-3p in CRC cells

As reported in a previous study, by direct interaction, miR-27a-3p controls several mRNAs, including RXRα [20]. Interestingly, the RXRα 3′UTR and RMST shared the same binding site with miR-27a-3p (Figure 6A). Since the activity of β-catenin can be suppressed by the RXRα-mediated signaling pathway [21] and β-catenin is negatively associated with RXRα [22], the effects of RMST and

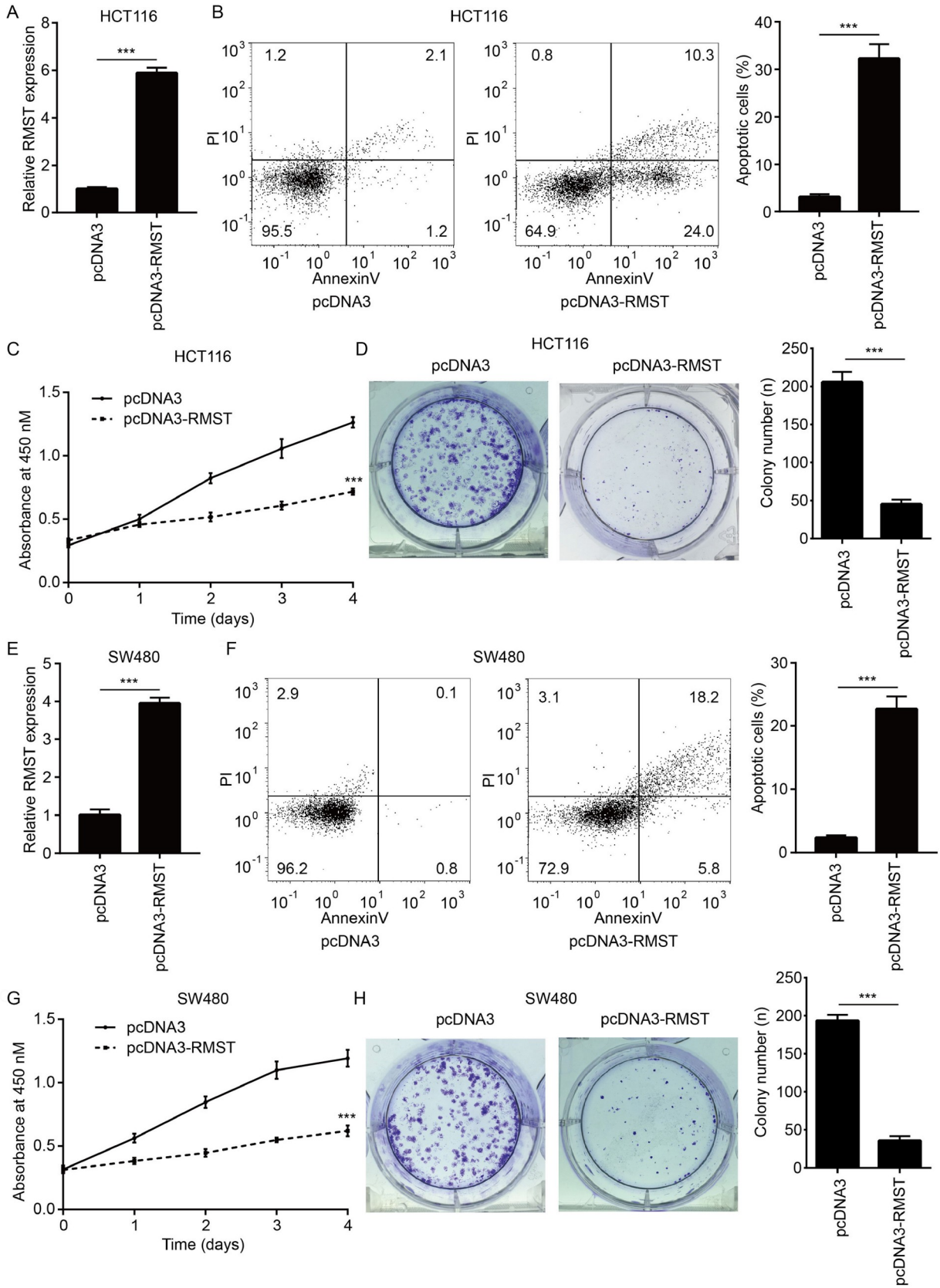

Figure 2. Effect of RMST on cell proliferation, colony formation and apoptosis of CRC cells (A–D) Overexpression of RMST increased RMST level and cell apoptosis while repressing cell proliferation and colony formation in HCT116 cells. (E–H) In SW480 cells, the results were similar to those in HCT116 cells. ***$P < 0.001$.

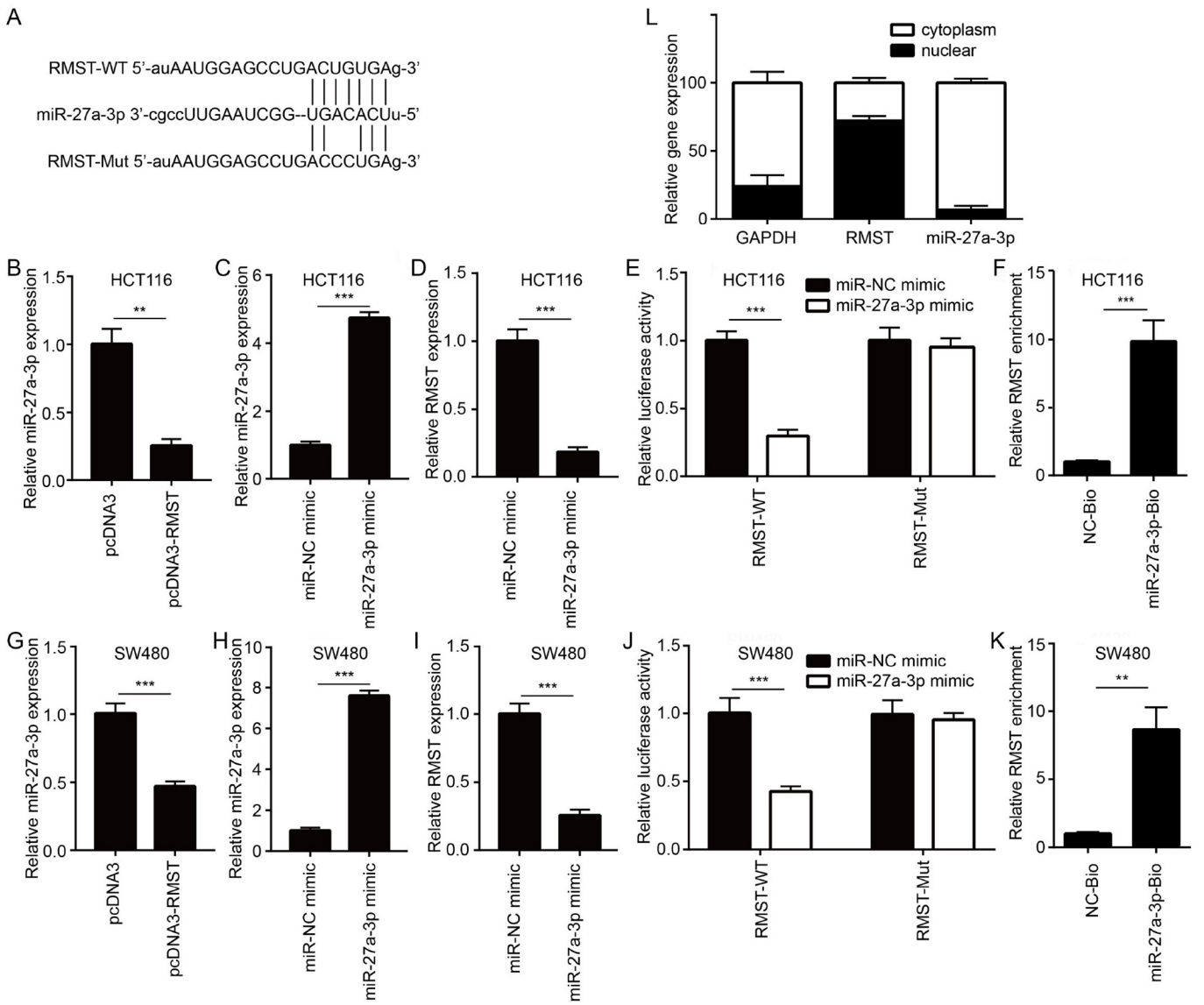

Figure 3. RMST sponged miR-27a-3p in CRC (A) The binding between RMST and miR-27a-3p was predicted by the ENCORI database. In HCT116 cells, (B) overexpression of RMST reduced miR-27a-3p expression; (C–E) miR-27a-3p overexpression increased miR-27a-3p expression and decreased RMST expression and the luciferase activity of RMST-WT. (F) RMST was pulled down by biotin-labelled miR-27a-3p oligos. (G–K) In SW480 cells, the results were similar to those in HCT116 cells. (L) miR-27a-3p was mainly located in the cytoplasmic fraction, and RMST was mainly located in the nuclear fraction. $*P<0.05$, $**P<0.01$, $***P<0.001$.

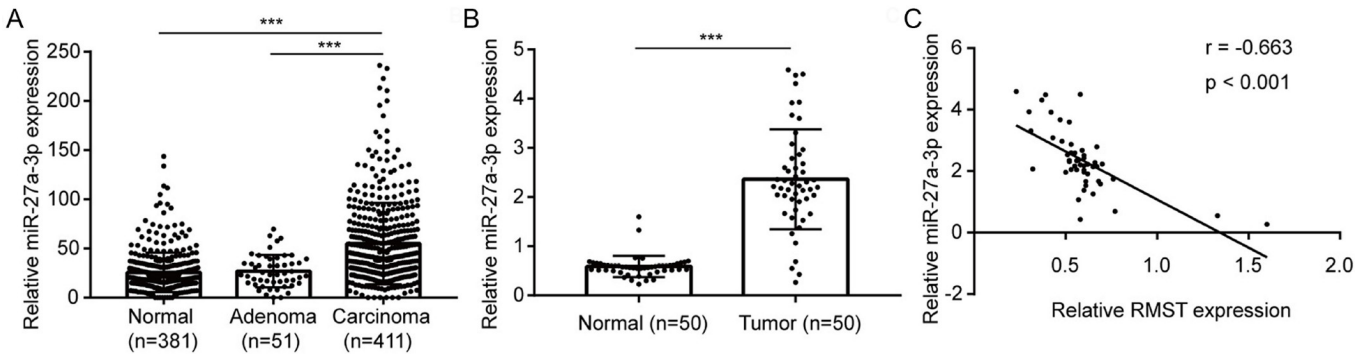

Figure 4. RMST negatively regulated miR-27a-3p in CRC (A) miR-27a-3p was upregulated in colon carcinoma relative to colon adenoma and colon mucosa according to GSE115513. (B) miR-27a-3p was elevated in CRC tumor samples compared with normal samples. (C) RMST was inversely related to miR-27a-3p in CRC tumor samples. $***P<0.001$.

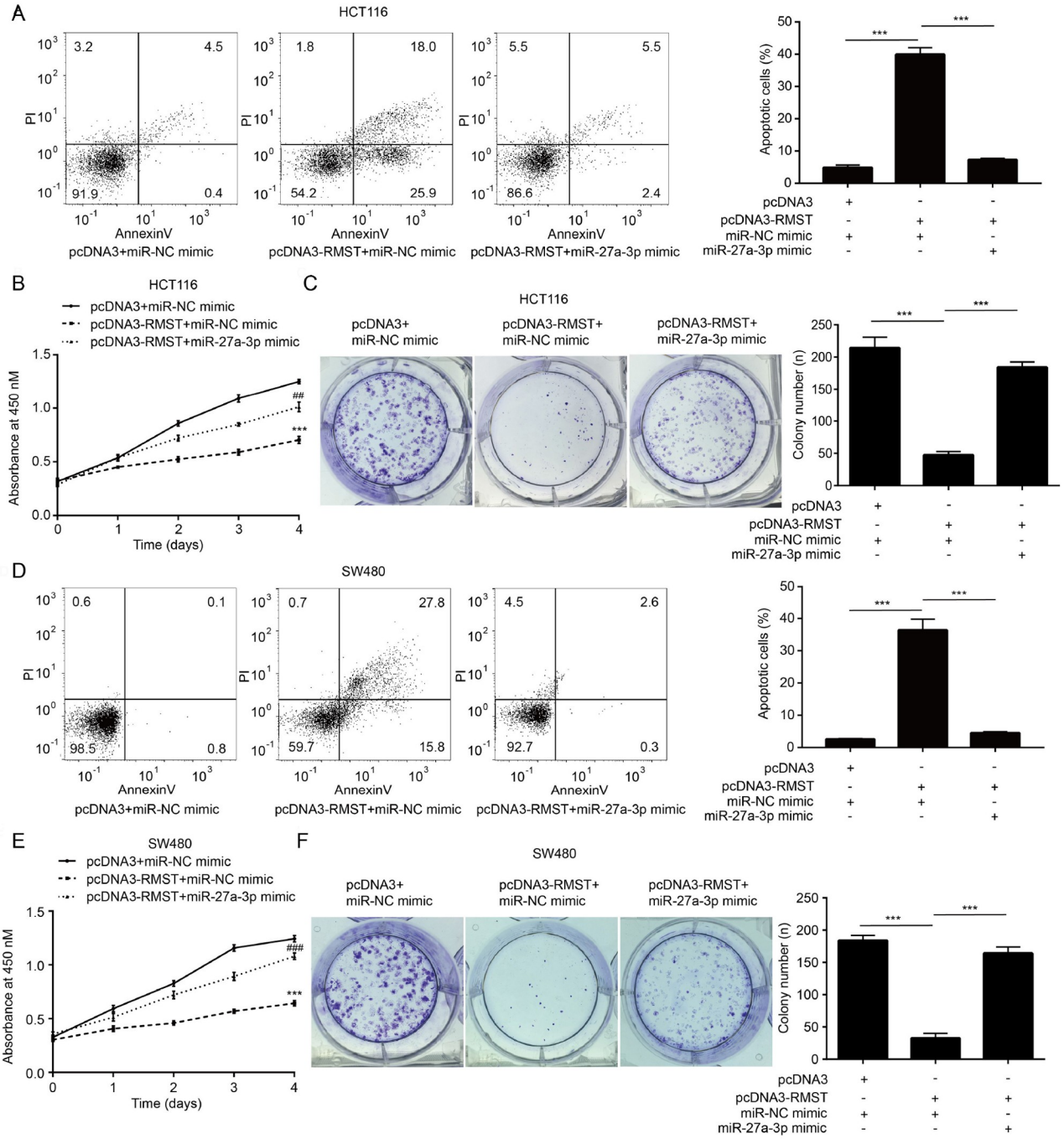

Figure 5. RMST controlled cell proliferation, colony formation and apoptosis by sponging miR-27a-3p in CRC (A–C) In HCT116 cells, miR-27a-3p overexpression partially reversed RMST-induced cell apoptosis and growth inhibition. (D–F) In SW480 cells, the results were similar to those in HCT116 cells. $^{***}P < 0.001$ vs pcDNA3 + miR-NC mimic; $^{###}P < 0.001$ vs pcDNA3-RMST + miR-NC mimic.

miR-27a-3p on β-catenin were also explored. In HCT116 cells, RMST elevation increased RXRα mRNA level, which was weakened after miR-27a-3p overexpression (Figure 6B). Meanwhile, RMST elevation increased RXRα protein level and reduced β-catenin protein level, which were weakened after miR-27a-3p overexpression (Figure 6C). Furthermore, RXRα was pulled down by biotin-labelled miR-27a-3p oligos (Figure 6D). Consistently, the findings in SW480 cells were similar to those in HCT116 cells (Figure 6E–G).

RMST repressed the activity of the Wnt signaling pathway in CRC cells

The Wnt signaling pathway is one of the most well-known oncogenic signaling pathways in many cancer types, including CRC [23,24]. Upon activation of the Wnt signaling pathway, the ligands (Wnt1a, *etc.*) can stabilize β-catenin [25]. To investigate the association between RMST and the Wnt signaling pathway, the activity of the Wnt signaling pathway in RMST-overexpressing HCT116 and SW480 cells was detected by a β-catenin/TCF4

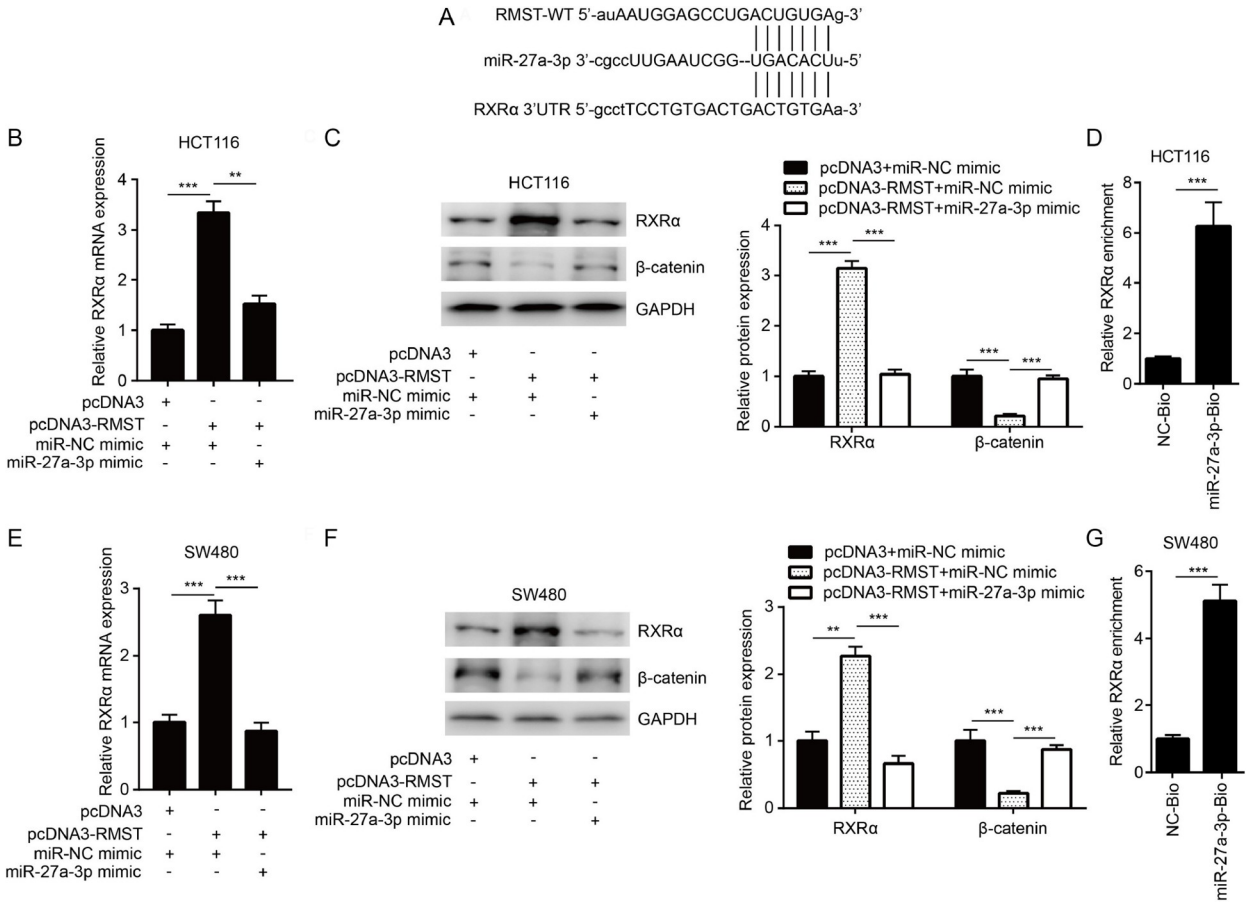

Figure 6. RXRα was regulated by RMST/miR-27a-3p in CRC (A) Complementary sites exist among RXRα, RMST and miR-27a-3p. (B,C) In HCT116 cells, overexpression of RMST enhanced RXRα mRNA and protein levels and reduced β-catenin protein level, which were weakened upon miR-27a-3p elevation. (D) RXRα was pulled down by biotin-labelled miR-27a-3p oligos. (E–G) In SW480 cells, the results were similar to those in HCT116 cells. $^{**}P < 0.01$, $^{***}P < 0.001$.

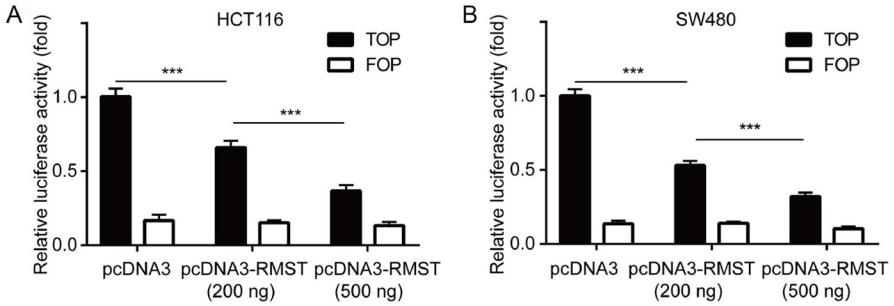

Figure 7. Effect of RMST on the Wnt signaling pathway in CRC cells (A,B) Overexpression of RMST inactivated the Wnt signaling pathway in a dose-dependent manner in HCT116 and SW480 cells. $^{***}P < 0.001$.

luciferase reporter (TOP-Flash/FOP-Flash) assay. Overexpression of RMST significantly inhibited TOP-Flash luciferase in a dose-dependent manner in HCT116 and SW480 cells (Figure 7A,B).

Discussion

Accumulating evidence has suggested that lncRNAs are implicated in the initiation, metastasis, drug resistance and progression of CRC [4,7,26,27]. Recently, RMST was identified as a lowly expressed lncRNA in breast cancer by microarray [15]. Consistently, the current study also revealed that RMST expression is downregulated in CRC tumor samples as well as in cell lines compared with that in normal tissue samples and cells, respectively. In addition, overexpression of RMST induced cell apoptosis and inhibited cell proliferation in CRC cell lines, indicating the tumor suppressive role of RMST in CRC. However, the underlying molecular mechanisms of RMST in CRC have not yet been investigated.

Unlike the subcellular location in neuron cells, RMST is dominantly localized in the cytoplasm of cells in breast cancer [15,28], indicating that RMST might be involved in the progression of cancer by a ceRNA mechanism [29]. By bioinformatics analysis, a putative binding site was predicted between miR-27a-3p and RMST; by RT-qPCR, dual luciferase reporter assay and RNA pull-down

assay, a mutual regulatory relationship and a direct interaction between RMST and miR-27a-3p were uncovered. However, the interaction between RMST and miR-27a-3p in CRC has not yet been reported.

Identified by microarray, miR-27a-3p was found to be a dysregulated miRNA in CRC [30]. We further explored the miR-27a-3p level in a large cohort (GSE115513); consistently, it was upregulated in colon carcinoma relative to colon mucosa. One miRNA can be sponged by different lncRNAs, and miR-27a-3p is also sponged by several different lncRNAs in different cell types [31,32]. For example, in clear cell renal cell carcinoma, miR-27a-3p is sponged by ADAMTS9-AS2 [31], and in periodontal ligament stem cells, miR-27a-3p is sponged by MEG3 [32]. Herein, we revealed an inverse relationship between RMST and miR-27a-3p in CRC tumor specimens. Additionally, the effects of RMST overexpression on CRC cell apoptosis and proliferation were partially reversed by miR-27a-3p overexpression. Thus, the present study demonstrated that RMST could function as a ceRNA for miR-27a-3p to suppress the progression of CRC.

As previously reported, by direct interaction, miR-27a-3p controls several mRNAs, including RXRα [20]. Interestingly, the current study predicted that the RXRα 3′UTR and RMST shared the same binding site with miR-27a-3p. Afterwards, the current study confirmed for the first time that RMST upregulated RXRα by sponging miR-27a-3p in CRC. RXRα is a negative regulator of the Wnt signaling pathway, and its downregulation facilitates the progression of CRC [33]. The Wnt/β-catenin signaling pathway remains a well-documented oncogenic pathway in CRC [34], and its uncontrolled activation mainly is resulted from the aberrant elevation of β-catenin due to the loss of negative regulators [35]. Dysregulation of several lncRNAs is responsible for the hyperactivation of the Wnt/β-catenin signaling pathway in CRC [36,37]; for instance, PART1 sponges miR-150-5p to activate the Wnt/β-catenin signaling pathway [36], and ASB16-AS1 activates the Wnt/β-catenin signaling pathway by sponging miR-1305 [37]. Current data further identified RMST as a new negative regulator of the Wnt signaling pathway in CRC.

In conclusion, the current study demonstrated that RMST inhibits the progression of CRC by sponging the miR-27a-3p/RXRα axis and inactivates the Wnt/β-catenin signaling pathway. Herein, we proposed a novel RMST/miR-27a-3p/RXRα axis in CRC. Our results indicated that RMST serves as a novel biomarker for patients with CRC. However, there was a limitation in the current study, *i.e.*, RMST is downregulated in CRC tumor tissues compared to normal tissues. The current study mainly investigated whether overexpression of RMST can attenuate cell proliferation and colony formation while promoting cell apoptosis; therefore, there was a lack of lncRNA knockdown experiments. To prove the real function of RMST, we will overexpress RMST first, followed by knockdown of *RMST* to observe whether there are rescues of the phenotype in our future work.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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