LncRNA RP11-708J19.2 promotes colorectal cancer progression by binding to SIRT7 *via* regulating H3K18ac

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ABSTRACT

Colorectal cancer (CRC) is a prevalent malignancy with a complex genetic basis. Recent genome-wide association studies (GWAS) have identified a susceptibility locus at 3p21.31, however, the functional SNP(s) underlying the association between the 3p21.31 region and CRC remain to be elucidated. In this study, we identified rs2101247 as the potential functional SNP and further demonstrated that rs2101247 is significantly associated with the expression of the nearby long non-coding RNA (lncRNA) RP11-708J19.2 (ENSG00000271161.1). Functional experiments showed that RP11-708J19.2 is upregulated in CRC tumor tissues, and its knockdown reduces cell viability while promoting apoptosis in SW1116 and HCT116 cell lines. Mechanistically, RP11-708J19.2 interacts directly with the deacetylase SIRT7, modulating histone H3K18 acetylation (H3K18ac). Specifically, RP11-708J19.2 knockdown leads to a significant upregulation of H3K18ac levels, implicating a SIRT7-mediated epigenetic pathway in CRC progression. Our findings elucidate a novel functional SNP-lncRNA axis that contributes to CRC pathogenesis, providing potential biomarkers for early detection and therapeutic targets for intervention.

Key words: colorectal cancer; SNP variant rs2101247; LncRNA RP11-708J19.2.

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Introduction

Colorectal cancer (CRC) is a prevalent malignancy and the second leading cause of cancer-related mortality worldwide.[1] Despite significant progress in neoadjuvant chemotherapy and targeted therapy,[2-5] the prognosis remains poor, particularly for advanced-stage patients, with a 5-year survival rate of only 12.5% in AJCC stage IV and nearly 50% diagnosed at an advanced stage.[6] Therefore, elucidating the molecular mechanisms underlying CRC development and identifying early biomarkers are essential for improving clinical outcomes.

Epidemiological and molecular studies suggest that CRC arises from a complex interplay of genetic and environmental factors.[7,8] Genome-wide association studies (GWAS) have identified over 60 susceptibility single nucleotide polymorphisms (SNPs) for CRC, highlighting the significant role of genetic variation in disease risk.[9]

However, the functional mechanisms linking these SNPs to tumorigenesis remain largely unexplored. Notably, many disease-associated SNPs reside within non-coding RNA (ncRNA) regions, suggesting that they may influence disease susceptibility by modulating ncRNA expression.[10] Among the various ncRNA classes, long non-coding RNAs (lncRNAs) have garnered considerable attention due to their ability to regulate tumor stemness, proliferation, metastasis, and prognosis through epigenetic mechanisms.[11-14] In particular, SNP loci located in non-coding regions can promote CRC occurrence by influencing the expression of multiple lncRNAs, providing a more direct and simplified approach for disease prediction and drug sensitivity assessment.[15-17]

Materials and Methods

In silico analysis

HaploReg v4.1[18] and UCSC Genome Browser (https://genome.ucsc.edu) were used to analyze the histone modification status of the loci that were in linkage disequilibrium with the tag SNP rs8180040 (with r2 >0.6).

Luciferase reporter gene assay

Luciferase reporter assay was conducted using the Luciferase Reporter Gene Assay Kit (Yeasen, Shanghai, China) per the manufacturer's suggestions. Briefly, SW1116 cells were co-transfected withvrs1201247 locus either carrying the G allele or A allele using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 48 h. Then, the Firefly luciferase activity of each sample was normalized to the Renilla luciferase activity.

Fluorescence *in situ* hybridization (FISH)

The RNA FISH assay was carried out to detect lncRNA RP11-708J19.2 (RP11-708J19.2) in tissues of 7 CRC patients as well as on CRC tissue microarrays. A probe that targeted RP11-708J19.2 sequence was designed by and purchased from GenePharma (Shanghai, China). The sequence of probe was 5'-FAM-CTGCAGCTTCTCCTGGAGTTATTTGGTGCT-GAGTTCTTTTCTATA-3'. RNA FISH assay was performed with a Ribo FISH kit (RiboBio Biotechnology Co., Ltd., Guangzhou, China) per the manufacturer's recommended procedures. In brief, paraffin-embedded sections were deparaffinized, dehydrated with ethanol and rehydrated in 50% formamide. Then the specimens were fixed with 4% polyoxymethylene and permeabilized with 0.5% Triton X-100 at 4°C for 5 min. Hybridization with the probe was performed at 37°C by overnight incubation in the dark. DAPI was used for counterstaining the nuclei in the next day. Following staining, the samples were observed with a 20x objective lens using a confocal microscopy (LSM710, Zeiss, Oberkochen, Germany).

Patient and sample collection

Cancer tissue and adjacent cancer formalin-fixed paraffin-embedded specimens from 7 CRC patients were commercially available and was obtained from Shanghai Outdo Biotech Co., Ltd. (OD-CT-RpUtr-03; Shanghai, China).

Tissue microarrays

In addition to the samples we collected from 7 CRC patients, a human CRC tissues microarray (TMA) was also used as a supplement to enrich number of samples of this study. The TMA was commercially available and was obtained from Shanghai Outdo Biotech Co., Ltd. (OD-CT-RpUtr-03). Our TMA was composed of 166 samples CRC tissues and corresponding adjacent tissues from 83 CRC patients. The composition of both normal and cancer TMAs is described in detail in the Results section.

Cell culture

Human colorectal cancer cell lines SW1116 (Cat. CTCC-400-0285) and HCT116 (Cat. CTCC-001-0002) was acquired from Meisen Cell (Hangzhou, China). SW1116 cells were cultured in RPMI-1640 medium (Gibco Laboratories, Grand Island, NY, USA) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. HCT116 cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco Laboratories) supplemented with 10% fetal bovine serum (FBS) (Gibco Laboratories). All cells were cultured in humidified incubator at 37°C with 5% CO_2 supplied.

Design and synthesis of siRNA

Small interfering RNA (siRNA) was used to knockdown the expression of lncRNA RP11-708J19.2 to validate its role in SW1116 and HCT116 cells. Two pairs of siRNA and random negative control were synthesized by RiboBio Biotechnology Co., Ltd. The siRNAs (siRNA-1 and 3; 10 nM) were transfected into SW1116 or HCT116 cells (2×10^5 cells/well) using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) for 48 h at 37°C

Reverse transcription-quantitative PCR (RT-qPCR)

Total cellular RNAs were extracted from SW1116 and HCT116 cell lines using the Trizol reagent (Invitrogen). cDNA was reversely transcribed using EntiLink™ 1st Strand cDNA Synthesis Kit (ELK Biotechnology, Wuhan, China) following the manufacturer's recommended protocol. Quantitative PCR was performed using the EnTurbo™ SYBR Green PCR SuperMix kit (ELK Biotechnology) following the manufacturer's instructions to determine the RNA expression of lncRNA RP11-708J19.2 (ENSG00000271161.1) on a StepOne™ Real-Time PCR (Thermo Fisher Scientific, Inc). The primers used were provided by RiboBio Biotechnology Co., Ltd. Primers sequences for RP11-708J19.2 were sense 5′-GAACTCAGCACCAAATAACTCCA-3′ and antisense 5′-GCCTCCAGCTAGCCACATATTTA-3′. GAPDH was used as endogenous control to normalize the differences in the amount of total RNA in each sample for the purpose of quantification. Primers sequences for GAPDH were sense 5′-CATCATCC-CTGCCTCTACTGG-3′ and antisense 5′-GTGGGTGTCGCT-GTTGAAGTC-3′. The relative expression levels of lncRNA RP11-708J19.2 were calculated using the 2-ΔΔCt method.

Cell viability assay by CCK-8

Cell viability was determined using the CCK-8 assay; 1×10^4 cells were seeded into 96-well plates overnight at 37°C followed by the transfection of siRNAs. Then, the cells were incubated with 10 µL of CCK-8 reagent (Beyotime Biotech, Shanghai, China) for 3 h at 37°C. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Richmond, CA, USA). Each measurement was performed in triplicate.

TUNEL staining

Cell apoptosis was detected by using the One Step TUNEL Apoptosis Assay kit (Keygen Biotech Co., Ltd., Nanjing, China) according to the manufacturer's instructions. Cells were fixed with 4 % paraformaldehyde for 30 min at room temperature, followed by rinse with PBS, and then permeabilized in a 0.1% Triton X-100 solution for 2 min on ice. The TUNEL reaction mixture solution was added to the cells and then incubated for 1 h in a humidified chamber at 37°C in the dark. The cells were then washed twice in PBS for 5 min, and counterstained with the fluorescent dye 4′-6-diamidino-2-phenylindole (DAPI) for visualization of the nuclei. All images were observed under a fluorescence microscope (LSM710, Zeiss). The ratio of TUNEL-positive stained cells to total cells in three random fields were calculated.

RNA pull-down and mass spectrometry

RNA pull-down followed by mass spectrometry and Western blot was used to identify the protein factors which bind to RP11-708J19.2. The biotin-labeled probes targeting RP11-708J19.2 were synthesized using MAXIscript™ Kit (Thermo Fisher Scientific, Inc) according to manufacturer's instruction. The sequences of sense and antisense probes were (RP11-708J19.2: 5'-GAGGGTTTTCTGCTCAAAAGCATGG-3). RNA pull-down assay was performed with Pierce™ Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific, Inc) according to the manufacturer's recommended protocol. For each reaction, 500 µL of cell lysate was added, and input RNA was simultaneously collected for normalization. Following a 30-minute incubation at room temperature with probe-conjugated beads, the complexes were washed three times using the wash buffer. The retrieved proteins were detected by western blot or mass spectrometry analysis at SpecAlly Life Technology Co., Ltd. (Wuhan, China).

Western blot analysis

SW1116 cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors for 10 min on ice. Total proteins were extracted and the protein concentrations were determined using the Bicinchoninic Acid Protein Assay Kit (Thermo Fisher Scientific, Inc). The aliquots of lysates were subjected to SDS-PAGE, then transferred onto PVDF membranes (Millipore, MA, USA). After blocking with 5% non-fat milk at room temperature for 1h, the membranes were incubated with primary antibodies overnight at 4°C. The membranes were then incubated with the secondary antibodies at room temperature for 2 h. The protein bands were visualized using ECL chemiluminescent reagent kit (Beyotime, Beijing, China). The primary antibodies used in the current study were listed below: anti-SIRT7 (1:1000; Cat. 12994-1-AP; Proteintech, Wuhan, China), anti-KAT2A (1:1000; Cat. 66575-1-Ig; Proteintech), anti-Histone H3 (1:1000; Cat. #9717; Cell Signaling Technology, Danvers, MA, USA), anti-H3K18ac (1:1000; Cat. PTM-114RM, PTM Biotechnology, Inc., Hangzhou, China), anti-H3K9ac (1:1000; Cat. PTM-112RM; PTM Biotechnology, In.c), anti-H3K27ac (1:1000; Cat. PTM-160; PTM Biotechnology, Inc.), anti-H3K4m1 (1:1000; Cat. PTM-611RM; PTM Biotechnology, Inc.), anti-H3K4m3 (1:1000; Cat. PTM-613; PTM Biotechnology, Inc.), anti-H3K9m3 (1:1000; Cat. PTM-616RM; PTM Biotechnology, Inc.), anti-H3K36m3 (1:1000; Cat. PTM-653RM; PTM Biotechnology, Inc.) and anti-β-actin (1:1000; Cat. 66009-1-Ig; Proteintech).

Immunofluorescence analysis

Immunofluorescence staining was performed to investigate the expressions of H3K9ac, H3K18ac and H3K27ac in SW1116 cells. The cells were fixed with 4% paraformaldehyde for 10 min at room temperature, washed with PBS, and then permeabilized with 0.2% Triton-X-100 at room temperature for 15 min. After that, the cells were blocked in goat serum for 1 h at room temperature. The cells were then incubated with the primary antibodies overnight at 4°C, followed by FITC-conjugated secondary antibody for 1 h. DAPI was used for counterstaining the nuclei for 2 min. The stained cells were visualized and images were acquired using a LSM710 confocal microscope. H3K9ac (Cat. PTM-112RM), H3K18ac (Cat. PTM-114RM) and H3K27ac (Cat. PTM-160) were purchased from PTM Biotechnology, Inc. (1:200). The negative control samples were prepared by omitting the primary antibody and substituting it with PBS.

Statistical analysis

The data are presented as the mean ±SD. One-way analysis of variance and Turkey *post-hoc* test were used to evaluate the differences among the groups (>2 groups). Results are manifested as the mean ± SE. GraphPad Prism 10.0 (La Jolla, CA, USA) was used for statistical analysis. A p-value <0.05 was considered statistically significant. All experiments described above were performed in triplicate.

Results

rs2101247 is identified as the functional SNP located in the colorectal cancer correlated 3p21.31 region

A recent case-control study conducted in Spain identified a susceptibility locus for CRC within the 3p21.31 region of the human genome,[19] where rs8180040 acts as the tag single nucleotide polymorphism (tag SNP) of this region [odds ratio (OR) =0.695]. Ke *et al*. validated the 3p21.31 region in the Chinese population and also found a significant association between rs8180040 and CRC in this cohort.[20] Given that rs8180040, the tag SNP of this region, is not located in gene transcription or regulatory sequence regions, it is unlikely to be a functional SNP.[21-23] Thus, the functional SNP(s) underlying the association between the 3p21.31 region and CRC remain to be elucidated. Notably, gene regulatory elements are typically characterized by specific histone modifications: for instance, enhancer regions show enriched trimethylation of histone H3 at lysine 4 (H3K4m1), while promoter regions exhibit abundant trimethylation of histone H3 at lysine 4 (H3K4m3) and acetylation of histone H3 at lysine 27 (H3K27ac).[24-26] We analyzed histone modifications of SNPs in linkage disequilibrium (LD) with the tag SNP rs8180040 (r^2>0.6) using bioinformatic tools including HaploReg v4.1 and the UCSC Genome Browser. Our analysis revealed that the genomic region harboring rs2101247 potentially contains an enhancer element, leading us to hypothesize that rs2101247 is the functional SNP in the 3p21.31 region associated with CRC (Figure 1 A,B). The supporting evidence is as follows: i) rs2101247 is mapped to an intronic region and does not affect protein coding, and it is in

strong LD with the tag SNP rs8180040 (D' = 0.88); ii) the genomic region encompassing rs2101247 displays enhancer-associated epigenetic markers across eight distinct tissue types; iii) H3K4m1 and H3K27ac are well-recognized epigenetic signatures for enhancer activation and promoter activation, respectively. Analysis *via* the UCSC Genome Browser demonstrated significant enrichment of H3K4me1 around rs2101247, whereas H3K27ac enrichment was not evident at this locus (Figure 1 A,B). To further investigate whether rs2101247 is the functional SNP locus, the luciferase assay method was used. The results showed that in SW1116 cells, the rs1201247 locus carrying the G allele had significantly higher luciferase activity compared to the A allele (p<0.01), suggesting that rs1201247[G] has stronger transcriptional activity (Figure 1C). Thereby, we hypothesized that the potential functional locus in the 3p21.31 region is rs2101247.

LncRNA RP11-708J19.2 (ENSG00000271161.1) is associated with SNP rs2101247

Based on prior analyses, the region surrounding the rs2101247 locus was predicted to contain an enhancer. Consistent with this hypothesis, subsequent experiments revealed that distinct genotypes at rs2101247 exhibited divergent luciferase activities. On this basis, we infer that the rs2101247 locus modulates the activity of this enhancer, which exerts its regulatory function by controlling the expression of a nearby transcript. Considering the potential correlation between disease-associated SNPs and the modulation of ncRNA expression, we speculated that rs2101247 may exert its effect by regulating non-coding RNA.[10] Using GTEx analysis to examine the association between the SNP locus and the adjacent lncRNAs, a significant association was observed between rs2101247 and the expression levels of lncRNA RP11-708J19.2 (ENSG00000271161.1), a transcript located 46 kb downstream (p<0.01) (Figure 2A).

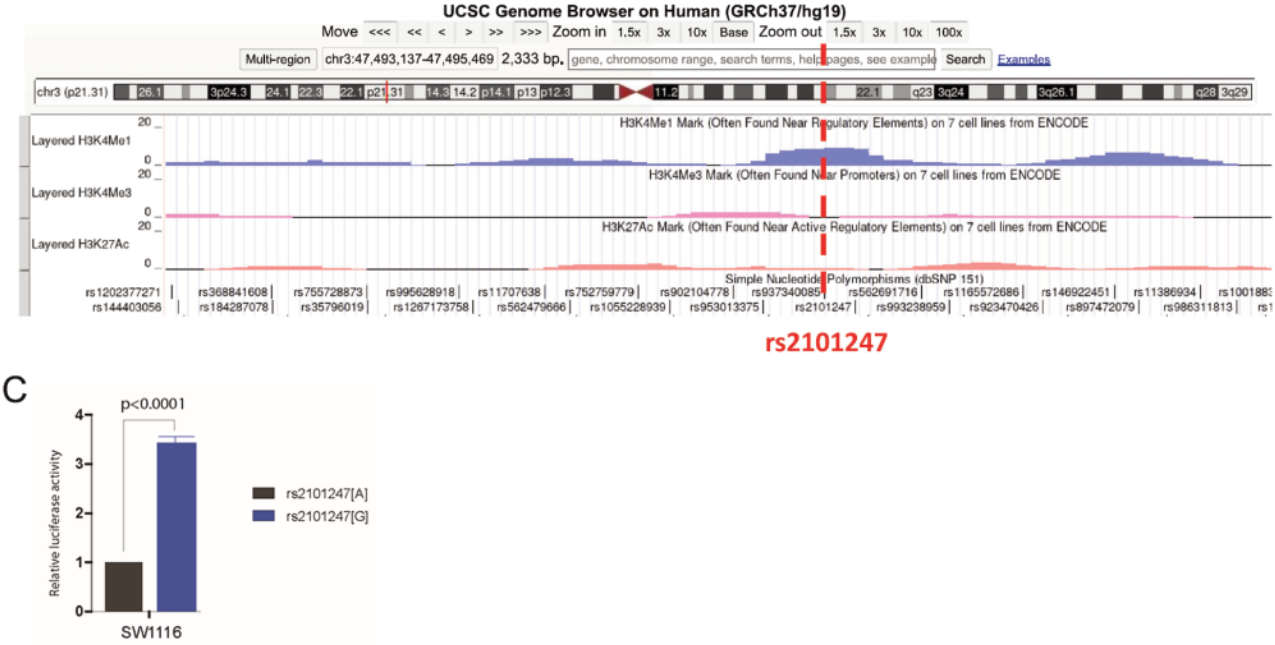
LncRNA RP11-708J19.2 is upregulated in tumor tissues of colorectal cancer patients

To explore the association between RP11-708J19.2 and colorectal cancer, FISH was employed to detect the expression of RP11-708J19.2 in tumor tissues and adjacent tissues from colorectal cancer patients. The results revealed that RP11-708J19.2 was highly expressed in colorectal cancer tumor tissues (Figure 2B). In addition, FISH analysis of the tissue microarray revealed that RP11-708J19.2 gene expression was significantly elevated in colorectal cancer tissues compared with normal and adjacent non-

A

variant	Ref	Alt	AFR freq	AMR freq	ASN freq	EUR freq	SiPhy cons	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	NHGRI/EBI GWAS hits	GRASP QTL hits	Selected eQTL hits
rs2101247	G	A	0.25	0.42	0.54	0.40			8 tissues			HNF1			20 hits
rs7649234	G	C	0.57	0.55	0.46	0.60			8 tissues	LNG		GCNF,NF-kappaB			17 hits
rs878659	G	A	0.29	0.52	0.46	0.60			6 tissues	ESDR,IPSC		Foxj2			26 hits
rs6792488	C	T	0.25	0.43	0.54	0.40			8 tissues			4 altered motifs			21 hits
rs12492433	G	A	0.32	0.52	0.46	0.60		4 tissues	14 tissues			ZID			27 hits
rs62248599	G	A	0.25	0.42	0.54	0.41		24 tissues		30 tissues		GATA			22 hits

B



C

Figure 1. rs2101247 is identified as the functional SNP located in the colorectal cancer correlated 3p21.31 region. **A)** Analysis using HaploReg v4.1. **B**) Analysis using UCSC Genome Browser. **C**) In SW1116 cells, the rs1201247 locus carrying the G allele showed a significantly higher luciferase activity compared to the A allele. n=3.

tumor tissues (Figure 2C). Taken together, we observed significantly elevated expression of RP11-708J19.2 in colorectal cancer patients.

Knockdown of RP11-708J19.2 decreased the viability and increased the apoptosis of SW1116 and HCT116 cells

To explore the function of RP11-708J19.2 in colorectal cancer cells, we investigated its effects on proliferation and apoptosis in the SW1116 and HCT116 cell lines. RP11-708J19.2 expression was knocked down in SW1116 and HCT116 cells using siRNA1 and siRNA2, and the reduction in RP11-708J19.2 levels was confirmed via RT-qPCR. Subsequently, we employed CCK-8 to assess cell proliferation and TUNEL staining for apoptosis analysis. The results indicated that siRNAs successfully reduced the elevated expression of RP11-708J19.2 in SW1116 and HCT116 cells (Figure 3 A,B). CCK-8 assays demonstrated that the proliferative capacity of SW1116 and HCT116 cells was significantly inhibited by RP11-708J19.2 siRNAs (Figure 3 C,D). The results of TUNEL staining revealed that the knockdown of RP11-708J19.2 led to a significantly increase of apoptosis in both SW1116 and HCT116 cells (Figure 3 E,F).

RP11-708J19.2 interacts with SIRT7 through SIRT7-mediated histone epigenetic modifications (mainly H3K18ac)

Our previous experiments confirmed that RP11-708J19.2 primarily localizes within the nucleus. Studies have shown that nuclear lncRNAs can exert regulatory functions through interactions with transcription factors, epigenetic modifiers, and transcriptional regulators.[11,27,28] To further elucidate the functional mechanism mediated by RP11-708J19.2, we employed an unbiased screening approach to identify proteins interacting with RP11-708J19.2. Biotin-labeled RP11-708J19.2 and antisense-RP11-708J19.2 were incubated with SW1116 cell protein extracts for RNA pull-down analysis. Subsequent SDS-PAGE electrophoresis and silver staining revealed distinct protein bands (Figure 4 A,B). Differential bands were excised for mass spectrometry analysis, identifying 92 potential RP11-708J19.2-binding proteins (*Supplementary Table S1*). Eight proteins including EIF3I, MBD3, SIRT7, ZRANB2, EIF3G, WDR5, AP1G1 and KAT2A (Figure 4B), which most likely involved in transcriptional regulation were selected for the following validation.[29-31] The results of western blot suggested that only SIRT7 directly interacted with RP11-708J19.2 (Figure 4C). SIRT7 functions as a deacetylase was identified as a highly selective enzyme for histone H3K18ac, with reduced H3K18 acetylation closely associated with tumorigenesis.[32] In addition, aberrant epigenetic modifications are common hallmarks of cancer.[33-35]

LncRNAs have been shown to participate in tumor development through epigenetic regulation.[36] We thus hypothesized that RP11-708J19.2 may exert its effects through SIRT7-mediated histone epigenetic modifications. Consequently, we analyzed histone modification changes (including H3K9ac, H3K18ac, H3K27ac, H3K4m1, H3K4m3, H3K9m3, H3K36m3) in SW1116 cells following RP11-708J19.2 knockdown *via* Western blot. The results indicated that knockdown of RP11-708J19.2 resulted in a significant upregulation of global histone acetylation levels, with a marked increase observed in H3K18ac specifically (Figure 4D). Other histone modifications showed no significant alterations, consistent with our hypothesis. Collectively, these findings suggest that RP11-708J19.2 exerts its regulatory effects primarily through SIRT7-mediated histone epigenetic modifications, particularly H3K18ac.

H3K18ac was significantly downregulated in SW1116 cells after the knockdown of RP11-708J19.2

To further characterize the relationship between RP11-708J19.2 knockdown and histone acetylation levels in SW116 cells, immunofluorescence assay was used to detect alterations in histone modifications, including H3K9ac, H3K18ac and

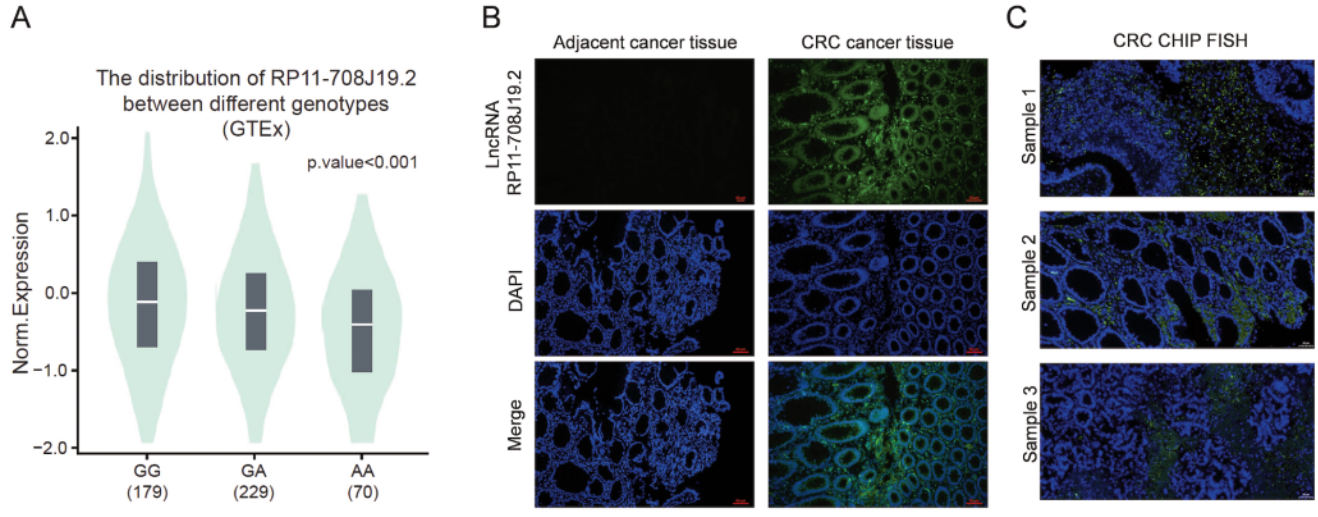


Figure 2. LncRNA RP11-708J19.2 is upregulated in tumor tissues of colorectal cancer patients. **A**) lncRNA RP11-708J19.2 (ENSG00000271161.1) is associated with SNP rs2101247. **B**) Expression levels of RP11-708J19.2 detected by FISH in cancerous and adjacent non-cancerous tissues from 7 colorectal cancer patients. **C**) Expression levels of RP11-708J19.2 detected by FISH in a tissue microarray of colorectal cancer specimens (3 samples). n=3.

H3K27ac. The results demonstrated knockdown of RP11-708J19.2 resulted in a significant reduction in H3K18ac level in SW1116 cells, with no corresponding changes observed in H3K9ac or H3K27ac levels (Figure 5 A-C). These findings aligned with the aforementioned Western blot results.

Collectively, our findings reveal that RP11-708J19.2 is upreg-

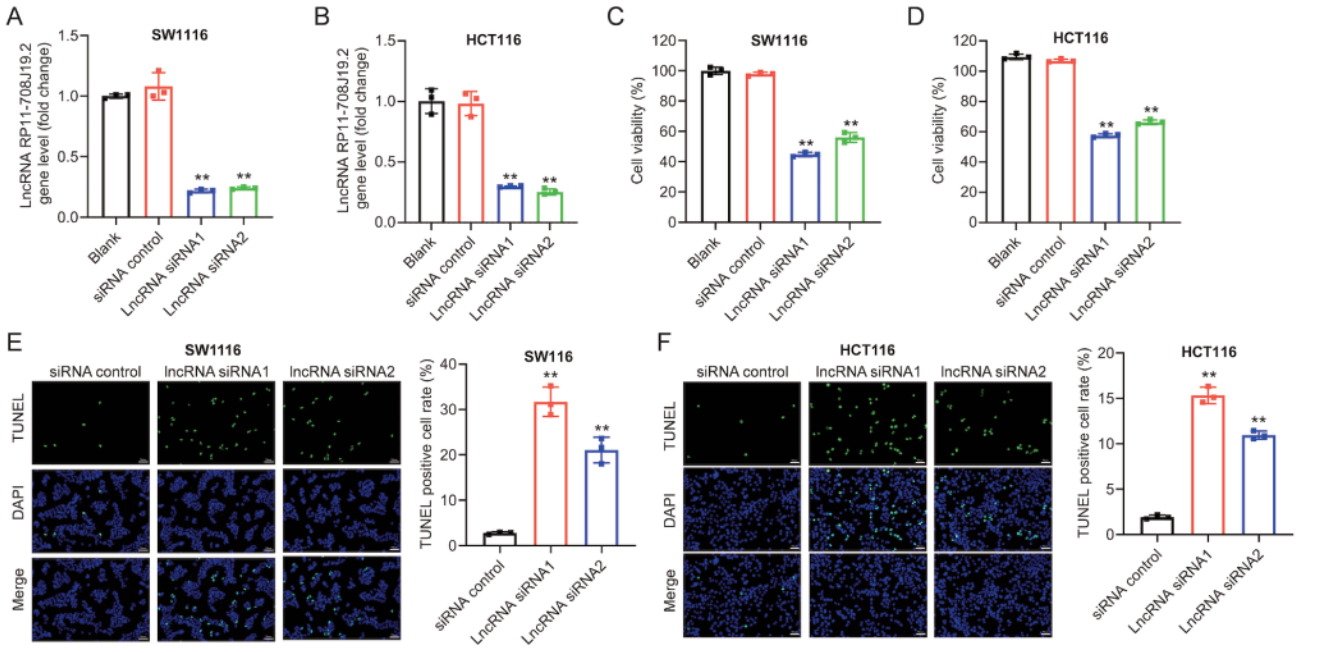


Figure 3. Knockdown of RP11-708J19.2 decreased the viability and increased the apoptosis of SW1116 and HCT116 cells. SW1116 and HCT116 cells were transfected with RP11-708J19.2 -specific siRNA1 or siRNA2 for 48 h. **A,B**) The gene level of RP11-708J19.2 in SW1116 cells and in HCT116 cells were detected with RT-qPCR. **C,D**) The proliferation of SW1116 and HCT116 cells were detected with CCK8 assay. **E**) The apoptosis situation after knockdown of RP11-708J19.2 in in SW1116 cells and in HCT116 cells were detected TUNEL staining. **F**) Quantitative analysis of TUNEL positive cell rate. $^{**}p<0.01$ comparing with siRNA control group, n=3.

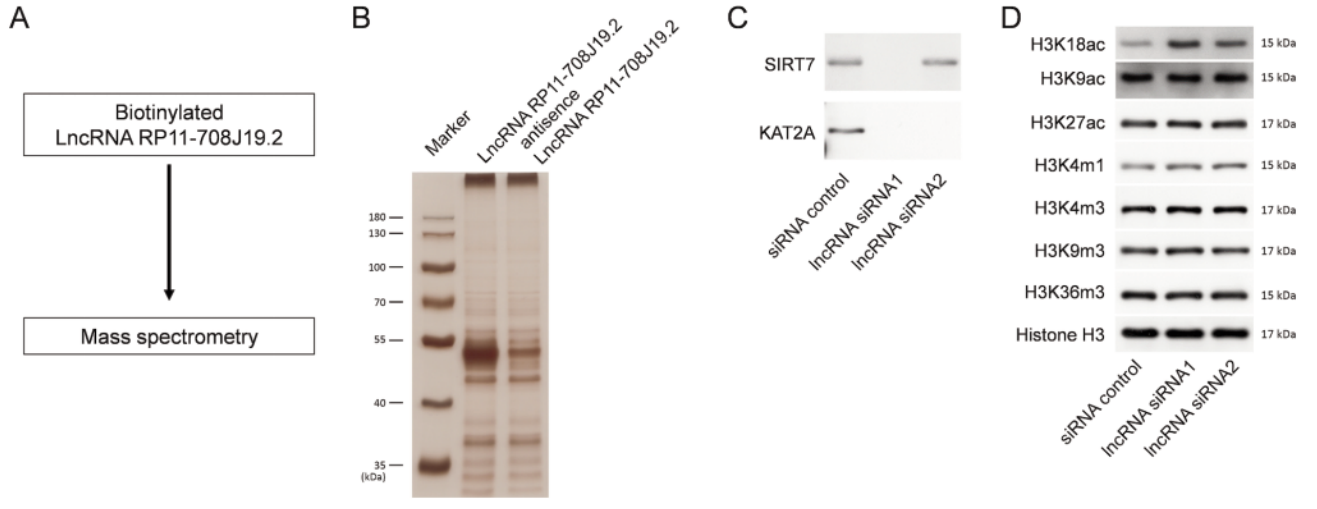


Figure 4. RP11-708J19.2 interacts with SIRT7 and promotes SIRT7-mediated histone epigenetic modifications (mainly H3K18ac) in SW1116 cells. **A**) RNA pull-down and Mass spectrometry of biotinylated RP11-708J19.2. **B**) SDS-PAGE electrophoresis of EIF3I, MBD3, SIRT7, ZRANB2, EIF3G, WDR5, AP1G1, and KAT2A. **C**) After RNA pull-down assay, Western blot results demonstrated that only SIRT7 directly interacted with RP11-708J19.2. **D**) The histone acetylation proteins including H3K18ac, H3K9ac, H3K27ac, H3K4m1, H3K4m3, H3K9m3 and H3K36m3 in SW1116 cells were detected with Western blot. n=3

ulated in colorectal cancer and correlates with poor prognosis. Functional analyses confirm that RP11-708J19.2 promotes colorectal cancer cell proliferation and maintains stem cell properties. Mechanistically, RP11-708J19.2 regulates histone acetylation, predominantly H3K18ac, *via* its interaction with SIRT7, which ultimately promotes colorectal cancer development and progression.

Discussion

CRC has been one of the most common malignant tumors.[1] The research of GWAS has identified over 60 susceptibility SNPs for CRC, which has a profound impact on understanding the mechanism of colorectal cancer occurrence.[9] For example, one previous study indicated that SNP rs17042479(G) change the expression of

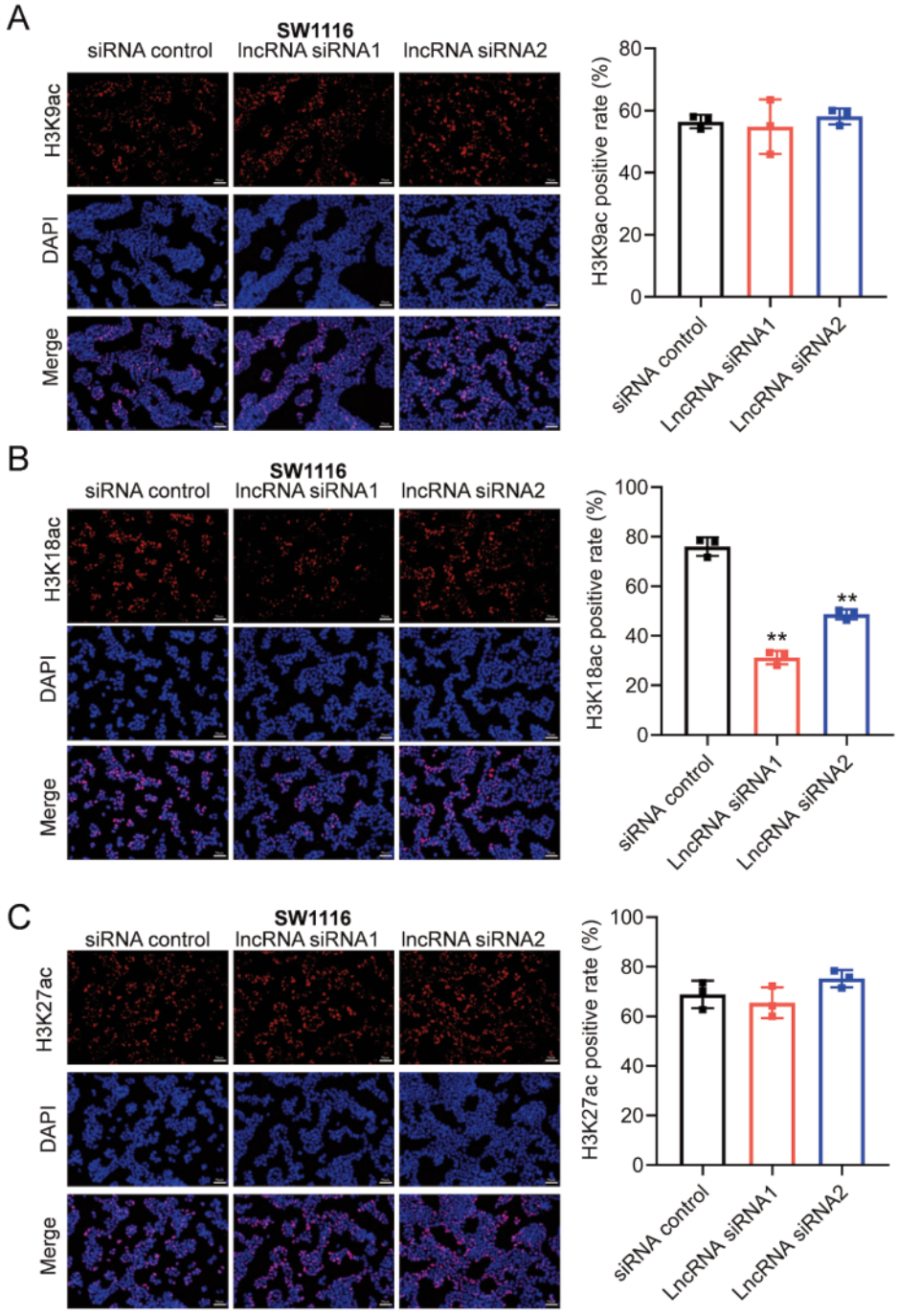


Figure 5. Knockdown of RP11-708J19.2 significantly reduced H3K18ac levels in SW1116 cells without affecting H3K9ac or H3K27ac. **A**) Immunofluorescence images and quantification of H3K9ac. **B**) Image and quantification of H3K18ac. **C**) Immunofluorescence images and quantification of H3K27ac in SW1116 cells. $^{**}p<0.01$ comparing with siRNA control group, n=3.

NAF1 and thereby increases the risk of developing CRC.[37] However, there was little research on SNP locus rs2101247, as well as the association between rs2101247 and CRC. Our study indicated that SNP locus rs2101247 is closely associated with CRC, which contribute the research on SNP locus rs2101247 and may inspire more research on SNP locus rs2101247 in CRC as well as in other disease.

LncRNAs have attracted intensive attention because many lncRNAs have been found participate in the process of tumorigenesis of cancers including CRC.[38] For example, lncRNA SPRY4-IT1 was reported to promote proliferation and invasion in CRC through acting as a ceRNA of miR-101-3p.[38] In this study, we first identified lncRNA RP11-708J19.2 (RP11-708J19.2) is associated with the expression of rs2101247 locus. RP11-708J19.2 is a newly identified that plays an important role in CRC, which may shed light on extensive studies of this lncRNA in other diseases. This study capitalizes on the complementary advantages of lncRNAs and SNPs, extending our prior work to advance the field of molecular research. We discovered that RP11-708J19.2 could regulate SIRT7 expression by modification of histone acetylation (mainly H3K18ac), thereby influencing the occurrence and development of colorectal cancer. This mechanism provides new insights into colorectal cancer pathogenesis and advances our understanding of the role played by genetic variations in tumorigenesis. At the same time, this study identified RP11-708J19.2 as a key molecular target and elucidated its underlying mechanism, thus advancing our understanding of the interplay among genetic variations, lncRNAs, and colorectal carcinoma. These findings may help identify high-risk colorectal cancer populations, uncover new tumor biomarkers, and guide the development of intervention targets and prevention strategies.

The regulation of H3K18ac by lncRNAs were also revealed in other previously published studies. One previous study reported that the elevated lncRNA BCAR4 expression correlates with advanced breast cancers. lncRNA BCAR4 direct binds with protein complex containing Smad nuclear-interacting protein 1 (SNIP1) and Serine/threonine-protein phosphatase 1 regulatory subunit 10 (PNUTS). Mechanistically, the BCAR4-SNIP1 binding releases the inhibitory role of SNIP1 on p300 histone acetyltransferase (HAT) activity, resulting in the acetylation of histones including H3K18ac.[39] Additionally, it was reported in another study that lncRNA DIGIT correlates with liver diseases by interacting with protein BRD3 at sites of H3K18ac, regulating gene expression and promoting endoderm differentiation.[40] These studies are in line with our findings and together will inspire extensive research on the interaction between lncRNAs and H3K18ac in different diseases. However, the mechanisms underlying the rs2101247-RP11-708J19.2-SIRT7 axis, its relationship with established CRC pathways, and how this SNP regulates lncRNA expression remain to be fully elucidated and warrant further investigation. Taken together, this study elucidated a novel rs2101247-RP11-708J19.2-SIRT7 axis that contributes to CRC pathogenesis, providing potential biomarkers for early detection and therapeutic targets for intervention.

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Online Supplementary Material
Table S1. Potential binding proteins.

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