Article

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LncRNA *ZNF667-AS1*: A Promising Therapeutic Target for Colorectal Cancer by Regulating *PNRC2*-Mediated Cell Proliferation, Invasion and Apoptosis

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Background: Long non-coding RNA (lncRNA) zinc finger protein 667-antisense RNA 1 (*ZNF667-AS1*) is closely related to the advancement of a variety of cancers, but its functional role in colorectal cancer remains unclear. This study was designed to explore the function and molecular mechanisms of lncRNA *ZNF667-AS1* in colorectal cancer.
Methods: Reverse transcriptase real-time quantitative polymerase chain reaction (RT-qPCR) was used for the detection of *ZNF667-AS1* and proline-rich nuclear receptor co-activator protein 2 (*PNRC2*) expression level. Cell counting kit-8 (CCK-8), 5-Ethynyl-2′- deoxyuridine (EdU), and colony formation assays were conducted to assess cell proliferation; flow cytometry and transwell invasion assay were performed separately to measure cell apoptosis and invasion. RNA immunoprecipitation (RIP) assay was utilized to analyze the relationship between *ZNF667-AS1* and *PNRC2*. Western blot was to test the PNRC2 protein expression. The *in vivo* role of *ZNF667-AS1* in the advancement of colorectal cancer was evaluated by tumor xenograft assay.
Results: LncRNA *ZNF667-AS1* and *PNRC2* were both decreased in colorectal cancer tissue samples and cells ($p < 0.05$). *ZNF667-AS1* overexpression remarkably restrained proliferation and invasion in HCT-116 and LOVO cells, but enhanced cell apoptosis ($p < 0.0001$). Moreover, *ZNF667-AS1* directly targeted *PNRC2*, and positively regulated its expression. The influence of *ZNF667-AS1* overexpression on invasion, apoptosis, and proliferation was suppressed by *PNRC2* knockdown in HCT-116 and LOVO cells. Additionally, *ZNF667-AS1* overexpression markedly inhibited tumor growth via upregulation of *PNRC2* in mice *in vivo* ($p < 0.05$).
Conclusion: LncRNA *ZNF667-AS1* expressed low in colorectal cancer. LncRNA *ZNF667-AS1* repressed proliferation and invasion, and enhanced apoptosis of colorectal cancer cells by targeting *PNRC2*.

Keywords: lncRNA *ZNF667-AS1*; colorectal cancer; PNRC2

Introduction

Colorectal cancer occurs in the digestive tract, and is a common malignancy [1]. Based on the 2020 global cancer statistics, the mortality of colorectal cancer patients ranks second among all tumor-related deaths worldwide [2]. As a heterogeneous disease, cellular environment and genetic aberrations affect tumor occurrence, development, and metastasis in colorectal cancer [3]. The comprehensive therapeutic treatments for colorectal cancer include surgery in combination with postoperative adjuvant therapy, neoadjuvant therapy, and other therapies [4]. Despite improvements in early diagnosis, most colorectal cancer patients at Stages 2 and 3 still suffer an unsatisfactory survival rate because of the high rate of recurrence and metastasis [5–7]. An understanding of molecular mechanisms underlying colorectal cancer is still lacking, which limits the search for a more effective treatment for patients. As such, it is critical to explore the mechanisms of colorectal cancer development, including apoptosis, metastasis, and proliferation.

Long non-coding RNAs (lncRNAs) are RNAs with no protein-coded function with a length of more than 200 nucleotides. LncRNAs have been elucidated to exert crucial effects on numerous diseases via a variety of pathways, especially in cancers, including lung adenocarcinoma, malignant pleural mesothelioma, and pancreatic cancer [8–10]. LncRNAs serve to monitor malignant metastasis and as a therapeutic target for restraining malignancy development in colorectal cancer [11]. Comprehensive bioinformatics analysis also revealed that lncRNA zinc finger protein 667-antisense RNA 1 (*ZNF667-AS1*) is involved in the disease progression of colorectal cancer [12]. LncRNA *ZNF667-AS1* has been reported to be involved in the pathological progression of various diseases including tumors [13–15]. LncRNA *ZNF667-AS1* has been shown to restrain angiogenesis, invasion, and propagation by modulating N-Cadherin and vascular endothelial growth factor A (VEGFA) expression in gastric cancer [16]. However, whether *ZNF667-AS1* affects colorectal cancer development, and the underlying molecular mechanisms remain to

be explored.

Proline-rich nuclear receptor co-activator protein 2 (PNRC2) belongs to the PNRC family and is a 16 kDa co-activator that interacts with many nuclear receptors via the SH3-binding motif [17]. PNRC2 was first observed in breast cancer [17]. Since then, PNRC2 has been found in human tissues and mammalian cells, with the strongest expression identified in the skeletal muscle, heart, kidney, brain, and placenta [18]. Moreover, a previous study reported a low level of PNRC2 in malignant meningioma patients, and PNRC2 was linked to the malignant progression in meningioma [19]. Nevertheless, the mechanisms of PNRC2 in the development of colorectal cancer still require further investigation.

Our study examined *ZNF667-AS1* and PNRC2 levels in tissues and cells of colorectal cancer. In addition, the relationship between *ZNF667-AS1* and PNRC2 was also explored. Furthermore, we investigated in depth whether the *ZNF667-AS1*/PNRC2 axis affects the cellular phenotype as well as tumor growth of colorectal cancer, which may provide potential therapeutic targets for colorectal cancer.

Materials and Methods

Clinical Samples

Fifty-five paired colorectal cancer specimens (n = 55) and noncancerous tissue specimens (n = 55) were gathered from 55 colorectal cancer patients who underwent surgery in First Hospital of Jiaxing from June 2022–June 2023. All tissue samples were immediately frozen and preserved at –80 °C. None of the colorectal cancer patients received anticancer therapy before surgery.

Cell Lines and Transfection

A human normal colon epithelial cell line (NCM460 cells) and colorectal cancer cell lines (HCT-116 and LOVO cells) were used for this study. NCM460 (IM-H445) and HCT-116 (IM-H098) were obtained from IMMOCELL (Xiamen, China), and LOVO cells (SCSP-514) were provided by the Cell Bank of the Chinese Academy of Science (Shanghai, China). All cell lines were detected by short tandem repeat (STR) profiling, and tested as mycoplasma-free before our experiments. NCM460, HCT-116, and LOVO cells were cultured in Dulbecco's modified Eagle's medium (DMEM, D0819, Sigma-Aldrich, St. Louis, MO, USA) containing penicillin-streptomycin (100 U/mL, 15140122, Gibco, Gaithersburg, MD, USA), and fetal bovine serum (FBS, 10%, 10099-141, Gibco). The incubator was set at 37 °C and 5% CO_2.

The plasmids including *ZNF667-AS1*-overexpressed vector (*ZNF667-AS1*) and the scrambled control (pcDNA), and the oligonucleotides including siRNA targeting *PNRC2* (si-PNRC2) and control siRNA (si-NC), were constructed by the GenePharma Co. Ltd (Shanghai, China). The sequences for si-PNRC2 and si-NC were 5′-GGACCAGUAGAUUGUUGAAAG-3′ and 5′-UUCUCCGAACGUGUCACGUTT-3′, respectively. Transfection with these plasmids or oligonucleotides was performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Reverse Transcriptase Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

TRIzol reagent (15596018CN, Invitrogen, Carlsbad, CA, USA) was employed to collect total RNA from colorectal cancer tissues and cells. Next, the extracted RNA was reverse-transcribed into cDNA using PrimeScript RT reagent kits (RR036A, Takara, Otsu, Japan). RT-qPCR analysis was carried out using the SYBR® Premix Ex TaqTM II reagent kit (RR820A, Takara) in line with the requirements of the manufacturer (ABI7500, ABI Company, Foster City, CA, USA). The levels of *ZNF667-AS1* and *PNRC2* were calculated utilizing the $2^{-\Delta\Delta Ct}$ method. U6 served as the internal reference when calculating the expression of *ZNF667-AS1*; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference in the analysis of *PNRC2* expression. The primers were displayed as follows: *ZNF667-AS1*, 5′-GGGAGTGTCCGCCATAAAGT-3′ (F) and 5′-AGATCGTAGCAGGGTCCAGT-3′ (R); U6, 5′-ATTGGAACGATACAGAGAAGATT-3′ (F) and 5′-GGAACGCTTCACGAATTTG-3′ (R); *PNRC2*, 5′-CAAAGTTGGAATTCTAGCTTATCAG-3′ (F) and 5′-GGGAAGAACACTTGGTGATGGC-3′ (R); GAPDH, 5′-ATCATCAGCAATGCCTCC-3′ (F) and 5′-CATCACGCCACAGTTTCC-3′ (R).

Cell Counting Kit-8 (CCK-8) Assay

HCT-116 and LOVO cells were plated on 96-well plates and incubated overnight. 48 h after cell transfection, each well was supplemented with CCK-8 (10 µL; Dojindo, Kumamoto, Japan) was added to each well. After 2 h of incubation, optical density (450 nm) was assessed using a microplate reader (Synergy H1, BioTek Instruments, Winooski, VT, USA).

5-Ethynyl-2′- Deoxyuridine (EdU) Assay

The transfected colorectal cancer cells were incubated with EdU solution (10 µM), and then fixed, permeabilized, and dyed with Click Additive Solution. Subsequently, the treated cells were stained using Hoechst and observed EdU-positive cells by fluorescence microscopy (IX73, Olympus, Tokyo, Japan). Lastly, the EdU-positive HCT-116 and LOVO cells were analyzed.

Colony Formation Assay

The transfected cells were seeded into the 6-well plates (5×10^3 cells per well) and cultivated for 14 days. After 20 minutes of methanol fixation, the colonies (more than 50 cells) were viewed by a light microscope (BX53, Olympus, Tokyo, Japan).

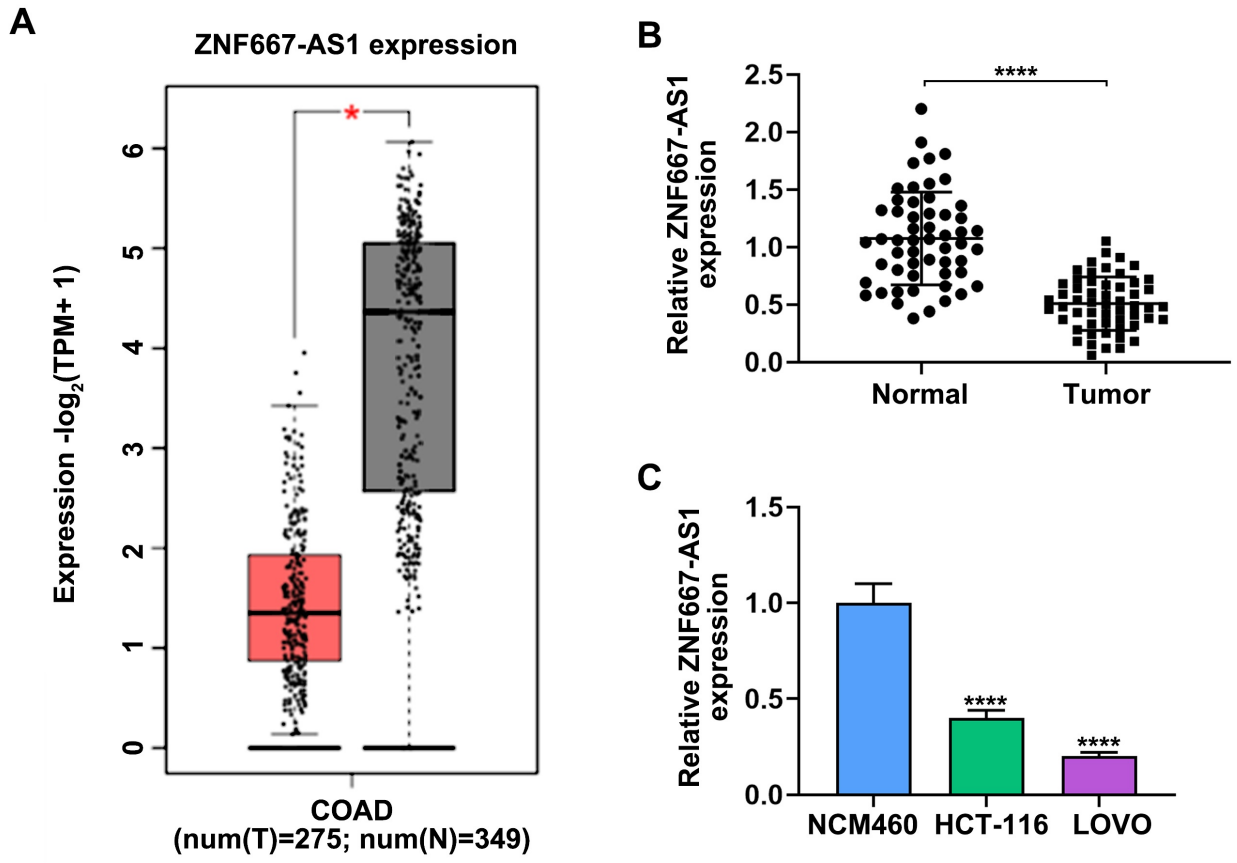

Fig. 1. *ZNF667-AS1* was lowly expressed in clinical tissues and cells of colorectal cancer. (A) The cancer genome atlas (TCGA) database was adopted to explore the expression level of *ZNF667-AS1* in colorectal cancer patients (red column) and healthy participants (gray column), *$p < 0.05$. (B) The *ZNF667-AS1* expression level in colorectal cancer (n = 55) and adjacent normal tissues (n = 55) from 55 colorectal cancer patients were detected by RT-qPCR, ****$p < 0.0001$. (C) The *ZNF667-AS1* expression level was determined using the mean of RT-qPCR in NCM460, HCT-116 and LOVO cells (n = 3). ****$p < 0.0001$ compared with NCM460. *ZNF667-AS1*, zinc finger protein 667-antisense RNA 1; RT-qPCR, reverse transcriptase real-time quantitative polymerase chain reaction; COAD, Colon adenocarcinoma; T, tumor; N, normal.

Flow Cytometry

The transfected colorectal cancer cells were subjected to trypsin (Gibco, USA), and then centrifuged (1000 g, 5 min). After discarding the supernatant, the cells were washed with phosphate buffer saline. Next, the apoptosis cells were detected following the instructions of the Apoptosis Detection Kit (C1062, Beyotime, Beijing, China). Cells were staining with Annexin V-FITC (5 µL; Beyotime, Shanghai, China) and propidium iodide (10 µL) for 15 min (keep away from light and room temperature). Finally, apoptotic cells were detected by Flow Cytometry (FACScalibur, BD Biosciences, Franklin lakes, NJ, USA).

Transwell Invasion Assay

Diluted Matrigel (356234, BD Biosciences, USA) was added to membranes inserted on the upper transwell chamber, and then 1×10^5 HCT-116 or LOVO cells in DMEM medium with no serum were plated on the upper chamber. The bottom chamber added DMEM supplemented with 10% FBS. After 24 h, invading cells that reached the bottom chamber were dyed using 0.5% crystal violet (G1062, Solarbio, Beijing, China). Finally, the invaded HCT-116, or LOVO cells were photographed through a light microscope (BX53, Olympus, Japan).

Bioinformatics Analysis

The cancer genome atlas (TCGA) database was utilized to retrieve *ZNF667-AS1* expression in colorectal cancer. The interaction probabilities of *ZNF667-AS1* and PNRC2 were predicted using the RNA-Protein Interaction Prediction database (http://pridb.gdcb.iastate.edu/RPISeq/#) [20].

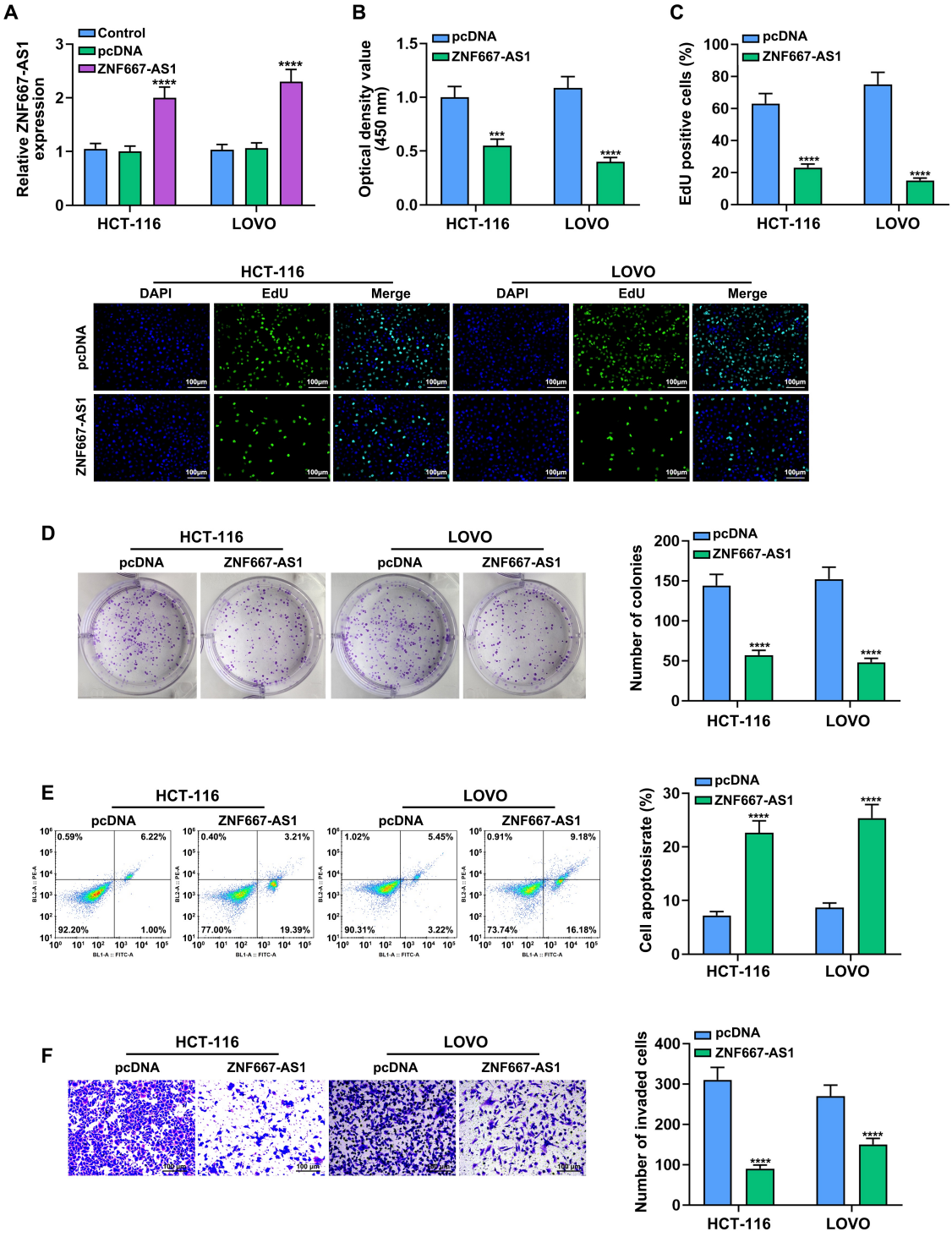

Fig. 2. *ZNF667-AS1* overexpression restrained cell proliferation and invasion, and enhanced apoptosis in colorectal cancer cells. (A) Colorectal cancer cells, containing HCT-116 and LOVO cells, were transfected with pcDNA and *ZNF667-AS1*; qRT-PCR was to examine *ZNF667-AS1* expression to verify transfection efficiency. ****$p < 0.0001$ compared with Control. (B–D) HCT-116 and LOVO cells were transfected with pcDNA and *ZNF667-AS1*, CCK-8 (B), EdU (C), and colony formation assays (D) were utilized to assess the cell proliferation after transfection. (E) Flow cytometry to test the apoptotic rate of transfected HCT-116 and LOVO cells. (F) A transwell invasion assay was carried out to evaluate the invasive capability of transfected HCT-116 and LOVO cells. n = 3, ***$p < 0.001$, ****$p < 0.0001$ compared with pcDNA group. CCK-8, cell counting kit-8; EdU, 5-Ethynyl-2′- deoxyuridine; DAPI, 4′,6-diamidino-2-phenylindole.

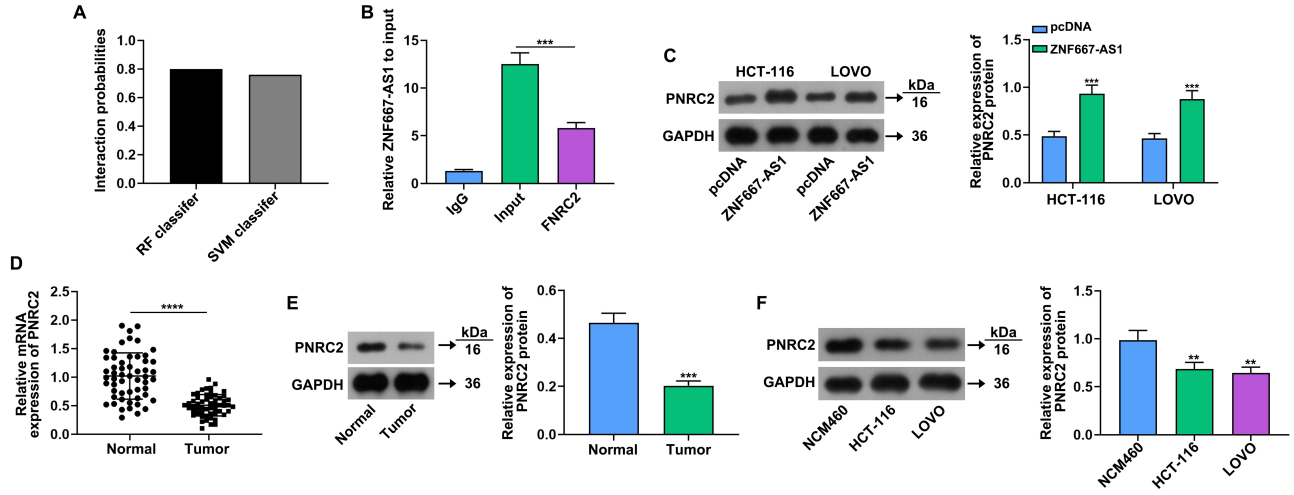

Fig. 3. *ZNF667-AS1* targeted *PNRC2* in colorectal cancer cells. (A) Bioinformatics was applied to predict the interaction probabilities of *ZNF667-AS1* and *PNRC2* through RNA-protein interaction prediction. Positive predictions were set to a probability >0.5. (B) RNA immunoprecipitation (RIP) assay was to detect the binding of *ZNF667-AS1* and *PNRC2* in HCT-116 cells. n = 3, ***$p < 0.001$. (C) Western blot showed that *ZNF667-AS1* overexpression significantly up-regulated PNRC2 protein expression level, n = 3, ***$p < 0.001$ compared with pcDNA. (D,E) RT-qPCR (D) and western blot (E) were conducted to determine the mRNA and protein expression level of PNRC2 in clinical tissues, including tumor and normal tissues, n = 3, ***$p < 0.001$, ****$p < 0.0001$. (F) PNRC2 protein expression in NCM460, HCT-116, and LOVO cells was measured by the approach of western blot, n = 3, **$p < 0.01$ compared with NCM460. PNRC2, proline-rich nuclear receptor co-activator protein 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

RNA Immunoprecipitation (RIP) Assay

A Magna RIP Kit (17-701, Millipore, Billerica, MA, USA) was used for the RIP assay. Briefly, a lysis buffer containing an RNase inhibitor and protease inhibitor cocktail was utilized for lysing cells. Then, RIP buffer containing Ago2 antibodies-coated magnetic beads was added to the HCT-116 or LOVO cell lysates at 4 °C for 2 h, and immunoglobulin G (IgG) served as a reference. RT-qPCR was used to detect the coprecipitated RNA.

Western Blot

Protein was extracted by Radio Immunoprecipitation Assay (RIPA) buffer, separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and next transferred onto the polyvinylidene difluoride (PVDF) membranes. After 1.5 h blocking by 5% non-fat milk, the membranes were incubated with anti-PNRC2 (ab235599; 1:2000, Abcam, Cambridge, UK) and anti-GAPDH (ab181602; 1:1000, Abcam) at 4 °C overnight. After washing, membranes were incubated with secondary antibody [goat anti-rabbit IgG (H+L) (ab205718; 1:20,000, Abcam)] for 2 h at ambient temperature. The protein bands were viewed with an enhanced chemiluminescence (ECL) kit (PE0010, Solarbio, Beijing, China). The grayscale value was analyzed using Image J software (1.8.0, Media Cybernetics, Silver Spring, MD, USA). GAPDH was used as an internal reference to calculate protein expression.

Tumor Xenografts in Mice

A total of twelve Bagg albino (BALB)/c nude male mice (4–6 weeks; 15–20 g) were purchased from the Beijing Vital River Laboratory Animal Technology (Beijing, China). The tumor xenografts were described in previous studies [21,22]. All mice in this study were successfully constructed xenograft models. Specifically, HCT-116 cells were transfected with pcDNA and *ZNF667-AS1*. nude mice were given injections of transfected cells. A total of two animal groups were set: pcRNA group and *ZNF667-AS1* group, 6 mice per group. Tumor volume was measured per week. 4 weeks later, all animals were euthanized by cervical dislocation under isoflurane anesthesia (2%, Sigma-Aldrich, USA) by Rodent anesthesia machine (ABS, Yuyan Corporation, Shanghai, China). The tumor tissues were extracted and weighed.

Statistical Analysis

Data analysis was carried out using SPSS 21.0 software (IBM Corp., Armonk, NY, USA). The data were expressed as mean ± standard deviation. Differences between the two groups were compared using *t*-tests, and analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparing among multiple groups. p values < 0.05 were considered statistically significant.

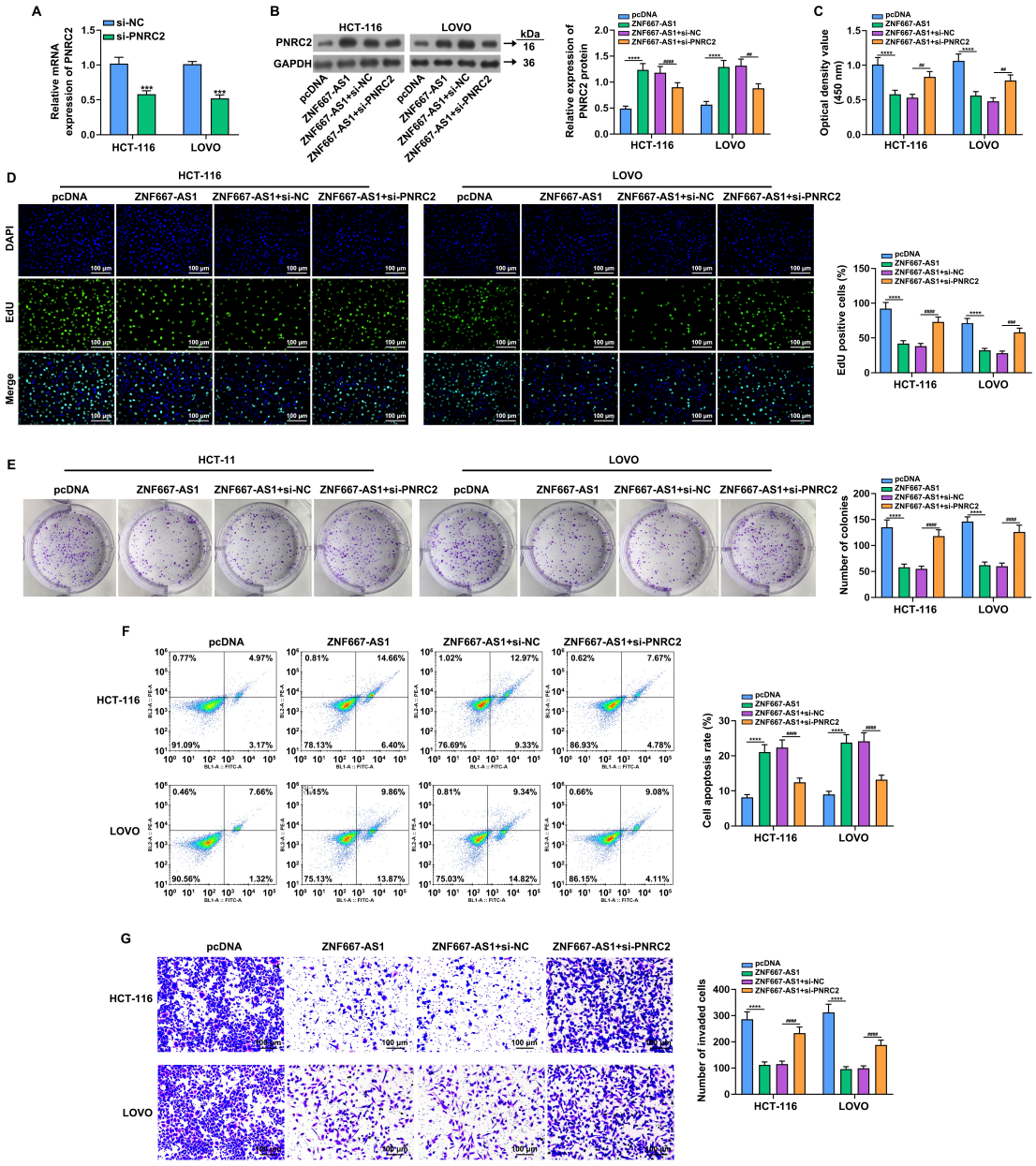

Fig. 4. *ZNF667-AS1* overexpression inhibited the development of colorectal cancer by upregulating PNRC2. (A) RT-qPCR was to verify the knockdown efficiency of si-PNRC2, ***$p < 0.001$ compared to the si-NC group. (B–G) HCT-116 and LOVO cells were transfected with pcDNA, *ZNF667-AS1*, *ZNF667-AS1*+si-NC, and *ZNF667-AS1*+si-PNRC2. (B) The PNRC2 protein expression was assessed using a western blot. (C–E) CCK-8 (C), EdU (D), and colony formation assays (E) were employed to test cell proliferation. (F) Flow cytometry was employed to analyze cell apoptosis. (G) The invasive ability of HCT-116 and LOVO cells was measured by transwell invasion assay. n = 3, ****$p < 0.0001$ compared with pcDNA; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ compared to *ZNF667-AS1*+si-NC.

Results

ZNF667-AS1 was Lowly Expressed in Clinical Tissues and Cells of Colorectal Cancer

The TCGA database indicated that *ZNF667-AS1* expression was lower in colorectal cancer tissues relative to that in normal colorectal tissues ($p < 0.05$, Fig. 1A). Our data also demonstrated that *ZNF667-AS1* was lowly expressed in tumor tissues of colorectal cancer patients (n = 55) in comparison to normal tissues (n = 55) ($p < 0.0001$, Fig. 1B). Similarly, HCT-116 and LOVO cells showed a diminished *ZNF667-AS1* expression level in comparison to NCM460 cells ($p < 0.0001$, Fig. 1C).

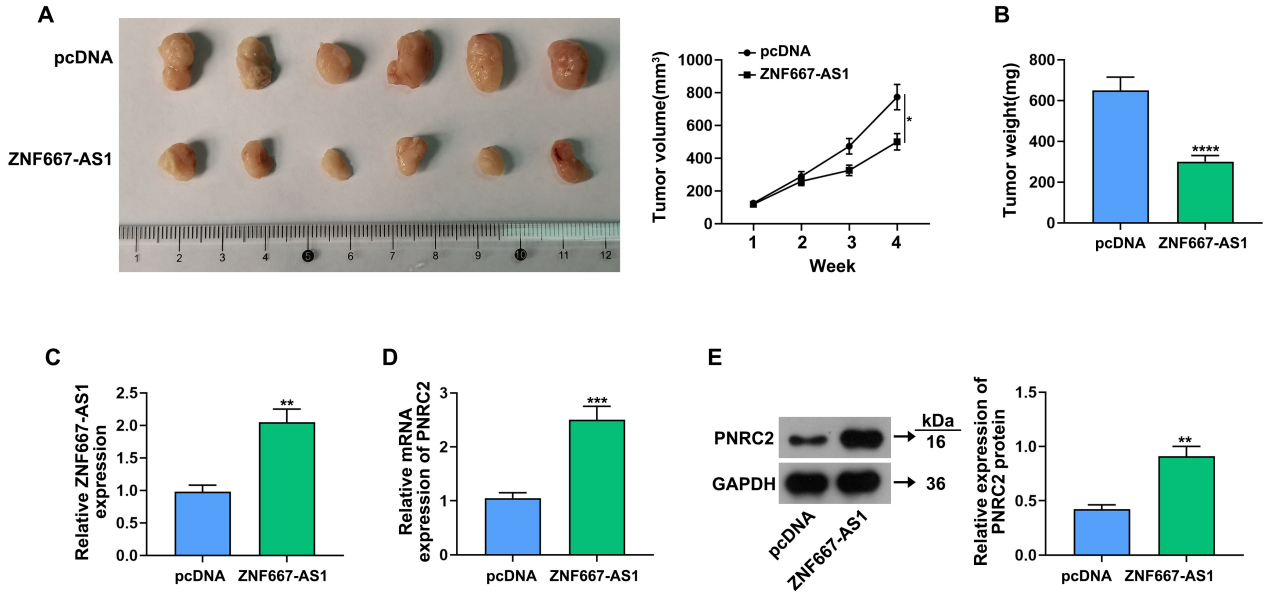

Fig. 5. *ZNF667-AS1* overexpression repressed tumor growth in mice. (A,B) Tumor volume and weight were evaluated. (C) RT-qPCR was to analyze *ZNF667-AS1* expression in tumor tissues obtained from nude mice. (D,E) RT-qPCR (D) and western blot (E) were employed to assess PNRC2 mRNA and protein expression levels in tumor tissues from nude mice. n = 6, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

ZNF667-AS1 Overexpression Restrained Cell Proliferation and Invasion, and Enhanced Apoptosis in Colorectal Cancer Cells

As represented in Fig. 2A, the level of *ZNF667-AS1* was remarkably elevated in *ZNF667-AS1*-transfected HCT-116 and LOVO cells ($p < 0.0001$), suggesting the successful overexpression efficiency of *ZNF667-AS1*. CCK-8 assay results indicated that *ZNF667-AS1* upregulation markedly suppressed cell viability in both HCT-116 and LOVO cells ($p < 0.001$, Fig. 2B). The EdU-positive cells were also significantly decreased by *ZNF667-AS1* overexpression ($p < 0.0001$, Fig. 2C). Moreover, the number of cell colonies was significantly decreased after *ZNF667-AS1* transfection ($p < 0.0001$, Fig. 2D). Flow cytometry demonstrated that the cell apoptotic were enhanced by *ZNF667-AS1* upregulation ($p < 0.0001$, Fig. 2E). Additionally, cell invasion was inhibited by upregulating *ZNF667-AS1* ($p < 0.0001$, Fig. 2F).

ZNF667-AS1 Targeted PNRC2 in Colorectal Cancer Cells

Bioinformatics analysis indicated that PNRC2 might be the binding protein of *ZNF667-AS1* (as the support vector machine (SVM) or random forest (RF) score >0.5) (Fig. 3A). The data in RIP assay verified that *ZNF667-AS1* directly bound to PNRC2 in HCT-116 cells (Fig. 3B). Moreover, the results from western blot revealed that *ZNF667-AS1* overexpression markedly upregulated PNRC2 protein expression level in both HCT-116 and LOVO cells ($p < 0.001$, Fig. 3C). Additionally, PNRC2 mRNA and protein expression levels were both remarkably lessened in colorectal cancer tissues ($p < 0.001$, Fig. 3D,E). The lower protein level of PNRC2 was also found in cells of colorectal cancer (HCT-116 and LOVO cells) in comparison to NCM460 cells ($p < 0.01$, Fig. 3F).

ZNF667-AS1 Overexpression Inhibited the Development of Colorectal Cancer by Upregulating PNRC2

As shown in Fig. 4A, transfection of si-PNRC2 markedly downregulated the mRNA level of *PNRC2* in both HCT-116 and LOVO cells ($p < 0.001$). To investigate whether PNRC2 participated in the inhibitory effects of *ZNF667-AS1* overexpression on the advances of colorectal cancer, HCT-116 and LOVO cells were transfected with pcDNA, *ZNF667-AS1*, *ZNF667-AS1*+si-NC, and *ZNF667-AS1*+si-PNRC2. *ZNF667-AS1* overexpression significantly increased the protein expression of PNRC2 ($p < 0.0001$), which was reduced by *PNRC2* knockdown in HCT-116 and LOVO cells ($p < 0.01$, Fig. 4B). The suppressive effects of *ZNF667-AS1* overexpression on cell proliferation (Fig. 4C–E) and invasion (Fig. 4G), and the promotion effect on cell apoptosis (Fig. 4F) were blocked by PNRC2 downregulation in HCT-116 and LOVO cells ($p < 0.001$).

ZNF667-AS1 Overexpression Suppressed Tumor Growth in Colorectal Cancer in Mice

Tumor volume and weight in nude mice were significantly suppressed by *ZNF667-AS1* overexpression ($p < 0.05$, Fig. 5A,B). The *ZNF667-AS1* and PNRC2 expression levels were both markedly boosted in *ZNF667-AS1*-overexpressed nude mice ($p < 0.01$, Fig. 5C–E).

Discussion

Colorectal cancer ranks second among tumor-associated deaths globally [23]. Based on analysis of thousands of identified noncoding transcripts, accumulating evidence has demonstrated the modulatory roles of noncoding RNAs in various diseases [24–26]. Multiple noncoding transcripts exert their effects through the regulation of tumorigenesis and progression, offering new opportunities for the diagnosis, therapy, and improved prognosis of malignancies [27]. LncRNA *ZNF667-AS1* is a newly identified noncoding RNA with remarkable controlling functions, and its downregulated expression has been proved in various malignancies, like nasopharyngeal carcinoma, melanoma, and gastric cancer [16,28,29]. Accordingly, our data displayed that *ZNF667-AS1* was expressed at a low level in the tumor tissues and cell lines of colorectal cancer. Moreover, *ZNF667-AS1* overexpression significantly restrained tumor growth in mice, and also suppressed cell metastasis and proliferation and enhanced cell apoptosis of colorectal cancer cells *in vitro*. Molecular mechanism studies revealed that *ZNF667-AS1* upregulation increased the level of PNRC2 to exert its suppressive role in colorectal cancer development.

An increasing number of studies have suggested that lncRNAs function as oncogenes or suppressors to take part in controlling tumorigenesis and malignancy progression via numerous pathways, such as regulating transcription at the posttranslational and epigenetic levels [27,30,31]. For instance, as an upregulated lncRNA in colorectal cancer, Chemoresistance Associated Colorectal cancer lncRNA (CACClnc) increased the chemoresistance of colorectal cancer through the regulation of RecA Homolog, *E. coli* (RAD51) [31]. Moreover, lncRNA colon cancer-associated transcript 2 (CCAT2) was highly expressed in colorectal cancer tissues and cells. Its high level was related to the poor prognosis of colorectal cancer, and CCAT2 was able to accelerate proliferation and hamper apoptosis of colorectal cancer cells [32]. In our study, *ZNF667-AS1* was reduced in the tumor tissues and cell lines of colorectal cancer. *In vitro* experiments further showed that *ZNF667-AS1* upregulation significantly reduced cell proliferation and invasion, as well as increased cell apoptosis, of colorectal cancer cells. In animal experiments, *ZNF667-AS1* overexpression markedly impeded tumor growth in colorectal cancer mice. Our results were in line with the previous study for cervical cancer in which *ZNF667-AS1* restrained cancer cell metastasis and propagation [33]. In nasopharyngeal carcinoma, *ZNF667-AS1* also played a suppressive role via adsorbing miR-1290 to upregulate actin-binding LIM protein 1 (ABLIM1) expression [29]. Additionally, *ZNF667-AS1* regulated the miR-523-3p/ Kinesin superfamily protein 5C (KIF5C) pathway to impede the progression of colon cancer [34].

Next, we further explored the mechanisms underlying *ZNF667-AS1* in colorectal cancer development. According to the bioinformatics analysis, the interaction probabilities between *ZNF667-AS1* and PNRC2 were RF = 0.8 and SVM = 0.76. RIP assay verified that *ZNF667-AS1* directly targeted PNRC2, and our data also confirmed that *ZNF667-AS1* positively regulated PNRC2 expression in colorectal cancer cells. Previous study explored the PNRC2 expression in meningioma, and found the low PNRC2 level in malignant patients, and it was linked to the malignant progression [19]. In this study, we also found that PNRC2 was significantly downregulated in colorectal cancer tissues and cells, and PNRC2 was bound to *ZNF667-AS1* and positively regulated by *ZNF667-AS1* in colorectal cancer cells. Gain- and loss-of-function experiments suggested that the knockdown of PNRC2 counteracted *ZNF667-AS1* overexpression-mediated suppressive effects on cellular processes in colorectal cancer.

Conclusion

In summary, our findings suggest that *ZNF667-AS1* was lowly expressed in colorectal cancer and restrained colorectal cancer development by directly regulating PNRC2. Our results confirmed that *ZNF667-AS1* exerted a suppressive role in colorectal cancer, providing a potential therapy target for colorectal cancer treatment.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

YL and HL designed the research. HL and XS performed the experiments. XS carried out the data analysis. All authors were involved in the drafting and critical revision of the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by The First Hospital of Jiaxing Ethics Committee (2024-LY-498) and complies with the Helsinki Declaration, all patients were informed and con-

sented to participate in our study. Animal trials were approved by the Ethics Committee and all procedures complied with approved protocols. The animal experiment in this study was approved by the Experimental Animal Ethics Committee of The First Hospital of Jiaxing (No. JXYY2024-023).

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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