

Long Non-Coding RNA *PITPNA-AS1* Accelerates the Progression of Colorectal Cancer Through *miR-129-5p/HMGB1* Axis

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Background: Long non-coding RNAs (lncRNAs) are essential regulators in colorectal cancer (CRC) progression. This work aimed to delve into the characteristics of lncRNA *PITPNA-AS1* in CRC.

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was adopted to examine *PITPNA-AS1*, *miR-129-5p*, high-mobility group box-1 (HMGB1) mRNA expressions in CRC tissues and cell lines. Functionally, cell counting kit-8 (CCK-8) and 5-ethynyl-2-deoxyuridine (EdU) incorporation assays were employed to examine cell proliferation; wound healing assay was utilized to detect cell migration; and flow cytometry was used to detect the cell apoptosis. Luciferase reporter assay, RNA immunoprecipitation assay and Western blot were conducted to detect the regulatory relationships among *PITPNA-AS1*, *miR-129-5p* and HMGB1.

Results: *PITPNA-AS1* and HMGB1 mRNA expressions were observably elevated in CRC tissues and cell lines. Knocking down *PITPNA-AS1* could significantly inhibit cell proliferation and migration, and promote apoptosis of CRC cells. *PITPNA-AS1* could serve as a competitive endogenous RNA, and up-regulate HMGB1 expression by adsorbing *miR-129-5p*.

Conclusion: *PITPNA-AS1* can expedite CRC cell proliferation and migration, and inhibit apoptosis through *miR-129-5p/HMGB1* axis.

Keywords: colorectal cancer, *PITPNA-AS1*, *miR-129-5p*, HMGB1

Introduction

Colorectal cancer (CRC) is a common tumor globally with its incidence still on the rise.¹ Although great progress has been made in surgery, chemotherapy, radiotherapy and targeted therapy, CRC patients still suffer from poor prognosis due to relapse, metastasis and drug resistance.² Hence, it is still essential to investigate the underlying mechanisms of CRC progression to provide novel insights for the diagnosis and treatment of CRC.

Long non-coding RNAs (lncRNAs) are RNA molecules with over 200 nt in length. At present, more and more studies find that lncRNA can be a participant in diverse physiological and pathological processes, and it plays a regulatory role in cancers.³ It is confirmed that some lncRNAs participate in the CRC progression, such as *MALAT1*,⁴ *SNHG3*,⁵ etc. *PITPNA* antisense RNA 1 (*PITPNA-AS1*) is a newly discovered lncRNA, which serves as a cancer-promoting molecule in hepatocellular carcinoma.⁶ Instead, the role and potential mechanism of *PITPNA-AS1* in CRC still require a clearer interpretation.

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MicroRNAs (miRs), known as non-coding RNAs with about 20 nt, are implicated in tumor progression by suppressing target gene expressions.⁷ Accumulating evidence proves that miRs take part in the progression of CRC, such as *miR-143* and *miR-145*.⁸ As a tumor suppressor, *miR-129-5p* participates in various tumors, like rectal adenocarcinoma⁹ and gastric cancer.¹⁰ It is reported that *miR-129-5p* expression is declined in CRC cell lines, and *miR-129-5p* can impede cancer cell proliferation and apoptosis.⁴ Nonetheless, the mechanism of *miR-129-5p* dysregulation in CRC is unclear.

High-mobility group box-1 (HMGB1) partakes in the carcinogenesis and development of cancers, and is regarded as a new diagnostic marker and therapy target.¹¹ Besides, HMGB1 is highly expressed in diverse tumors, CRC included.¹² Moreover, lymph node metastasis, distant metastasis and poor prognosis in patients with CRC are related to the high expression of HMGB1.¹³

Recent evidence authenticates that lncRNAs figure prominently in tumorigenesis and cancer progression by acting as competing endogenous RNAs (ceRNAs), which means that lncRNAs act as molecular sponges to reduce the availability of miRNAs, and modulate the expressions of target genes.¹⁴ In this work, we analyzed *PITPNA-AS1* expression in CRC tissues and cell lines and its relationship with clinical pathological characteristics. Then, we investigated the role of *PITPNA-AS1* in regulating CRC cell proliferation, migration and apoptosis. Subsequently, the regulatory function of *PITPNA-AS1* on *miR-129-5p*/HMGB1 axis was explored. It was demonstrated that *PITPNA-AS1* could function as a ceRNA, by repressing *miR-129-5p* expression to up-regulate HMGB1 expression, to facilitate the progression of CRC.

Materials and Methods

Ethics Approval

This project complied with the principles of the Declaration of Helsinki and was endorsed by the Research Ethics Committee of the Xinyu People's Hospital (No.201508005). All subjects involved signed written informed consent.

Patient Tissues Collection

From 2015 to 2018, the tumor and paracancerous tissues from 66 patients with CRC who had been surgically resected were randomly collected from the Xinyu People's Hospital. After being verified by two

pathologists, all samples were frozen in liquid nitrogen and then stored at -196°C .

Cell Culture

The American Type Culture Collection (ATCC, Rockville, MD, USA) was the provider of human CRC cancer cell lines, including DLD-1, HCT116, SW480, HT-29 cells, and the normal human intestinal epithelial NCM460 cells. The above cells were cultured in Dulbecco's modified Eagle's medium (DMEM, HyClone, South Logan, UT, USA) with 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), 100 U/mL penicillin, 100 $\mu\text{g/mL}$ streptomycin (Hyclone, Logan, UT, USA) in 5% CO₂ at 37°C.

Cell Transfection

The small interfering RNA (siRNA) against *PITPNA-AS1* (si-*PITPNA-AS1*) and the negative control (si-NC) were available from GeneChem (Shanghai, China). *miR-129-5p* mimic (*miR-129-5p*), *miR-129-5p* inhibitor (*miR-129-5p* in) and corresponding negative control oligonucleotides (miR-NC) were synthesized by GenePharma (Shanghai, China). They were transfected into the cells with LipofectamineTM2000 (Invitrogen, Carlsbad, CA, USA). Cells were harvested for subsequent experiments 48 h after transfection.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The TRIzol reagent (Invitrogen, Carlsbad, CA) was used to extract RNA from tissues and cells. AMV reverse transcriptase (Promega, Madison, WI, USA) was adopted to reversely transcribe 2 μg total RNA into cDNA, and then qRT-PCR was performed with SYBR Green RealTime PCR Master Mix kit (Roche, Basel, Switzerland) in ABI 7900 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA), and finally, relative RNA expression was estimated by the $2^{-\Delta\Delta\text{Ct}}$ method. The primer sequences are listed in Table 1.

Cell Counting Kit-8 (CCK-8) Assay

Cell counting kit-8 (Dojindo, Kumamoto, Japan) was applied to evaluate CRC cell proliferation. Transfected cells were inoculated into 96-well plates ($2 \times 10^3/\text{well}$). Next, 10 μL CCK-8 solution (Dojindo Molecular Technologies, Japan) was dripped into each well at 0 h, 24 h, 48 h, 72 h, respectively, with which the cells were

Table 1 The Primers Used in This Study

	Forward	Reverse
GAPDH	5'-AGAAGGCTGGGGCTCATTTG-3'	5'-AGGGGCCATC CACAGTCTTC-3'
U6	5'-CTCGCTTCGGCAGCACACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
miR-129-5p	5'-CGGCGGTTTTTTGCGGTCTGGGCT-3'	5'-AGCCCAGACCGCAAAAAACCGCCG-3'
PITPNA-AS1	5'-GCAGGGTGGATAAAGAGGA-3'	5'- CCTACTGACAGGATGTCCT-3'
HMGB1	5'-GATGGGCAAAGGAGATCCTA-3'	5'-CTTGGTCTCCCCTTTGGGGG-3'

incubated at 37°C for 2 h. Ultimately, a microplate reader (BioRad, Hercules, CA, USA) was used to record the absorbance of each well at 450 nm.

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

Cell proliferation was examined by EdU assay kit (RiboBio, Guangzhou, China). To be specific, transfected cells were inoculated into 96-well plates. Then, 50 µM EdU labeling medium was loaded into the wells and incubated for 2 h. Subsequently, 4% paraformaldehyde was dripped into each well for 30 min to fix the cells, followed by the addition of 1 × Apollo[®] reaction cocktail (100 µL). Thirty-minute later, 100 µL of Hoechst 33,342 staining solution was added to stain the nuclei, and then a fluorescent microscopy (Nikon, Japan) was adopted to observe the cells in 5 random wells.

Wound Healing Assay

Transfected cells were inoculated into 6-well plates. After the wells were fully covered by the cells, a 200 µL pipette tip was employed to draw a straight line on the surface of the mono-layer cells to form a clear cell-free zone, and then the cells were cultured in serum-free medium for 24 h. Wound closure was observed at 0 h and 24 h, respectively, under the microscope.

Flow Cytometry

Annexin V-FITC/PI Apoptosis Detection Kit (KeyGEN BioTECH, Jiangsu, China) was adopted to measure the apoptosis rate. Transfected cells were stained with Annexin V-FITC staining solution and propidium iodide (PI) solution to detect the early and late apoptotic cells, thus calculating the total apoptotic rate.

Fluorescent Staining of Nuclei

Transfected cells were cultured in 6-well plates. Then, the cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, St. Louis, MO) and visualized using a fluorescence microscope (Nikon, Japan).

Luciferase Reporter Assay

The sequences of *PITPNA-AS1* and 3'UTR of *HMGB1* were inserted into the pmirGLO luciferase vector (Promega, Madison, WI, USA), namely, *PITPNA-AS1*-wild type (WT) or *HMGB1*-WT reporter vector, and then the *PITPNA-AS1*-WT or *HMGB1*-WT binding site was mutated to produce *PITPNA-AS1*-mutant type (MUT) or *HMGB1*-MUT reporter vector. Subsequently, the reporter vectors and *miR-129-5p*/miR-NC were co-transfected into HKE-293T cells employing Lipofectamine[™] 2000 (Invitrogen, Carlsbad, CA, USA). Forty-eight-hour later, the activity of luciferase was measured by Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

RNA Immunoprecipitation (RIP) Assay

EZ-Magna RIP Kit (Millipore, Bedford, MA, USA) was utilized for RIP experiments. In brief, HEK-293T cells were harvested and then lysed in RIP lysis buffer. The cell extracts were co-immunoprecipitated with RIP buffer containing anti-Ago2 antibody conjugated with magnetic beads or normal mouse IgG. Subsequently, samples were incubated with Proteinase K. Eventually, qRT-PCR was adopted to measure the expressions of *PITPNA-AS1* and *miR-129-5p*, and HMGB1 mRNA and *miR-129-5p* in immunoprecipitated RNA.

Western Blot Assay

RIPA buffer (Sigma-Aldrich, St. Louis, MO) was adopted to prepare protein samples. After 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, proteins were transferred to polyvinylidene fluoride membranes (PVDF, Millipore, Billerica, MA, USA) and then blocked by 5% skim milk. Next, the membranes were incubated with primary antibodies at 4°C overnight, and then with horse-radish peroxidase-conjugated secondary antibodies at 37°C for 2 h. Ultimately, enhanced chemiluminescence

reagents (ECL-Plus; Thermo Fisher Scientific, Waltham, MA) were used to detect protein expressions.

Statistical Analysis

Statistical analysis was completed employing GraphPad Prism 6.0 (GraphPad Software, Inc, CA, USA). Data were expressed as means $\pm$ standard deviation, and Student's *t*-test or one-way ANOVA was applied for analyzing differences. Chi-square test was performed to evaluate the relationship between *PITPNA-AS1* expression and clinical characteristics. Pearson's correlation coefficient was calculated to analyze the correlations. $P < 0.05$ indicated statistical significance.

Results

PITPNA-AS1 Expression Was Increased in CRC Tissues and Cell Lines, and PITPNA-AS1 Was Negatively Interrelated with miR-129-5p Expression and Positively Associated with HMGB1 Expression

We first detected *PITPNA-AS1*, *miR-129-5p* and HMGB1 expressions in CRC tissues and cell lines, and qRT-PCR and Western blot assays denoted that compared with that in paracancerous tissues or NCM460 cell lines, *PITPNA-AS1* expression in CRC tissues and cell lines was observably higher (Figure 1A and B), while *miR-129-5p* expression in

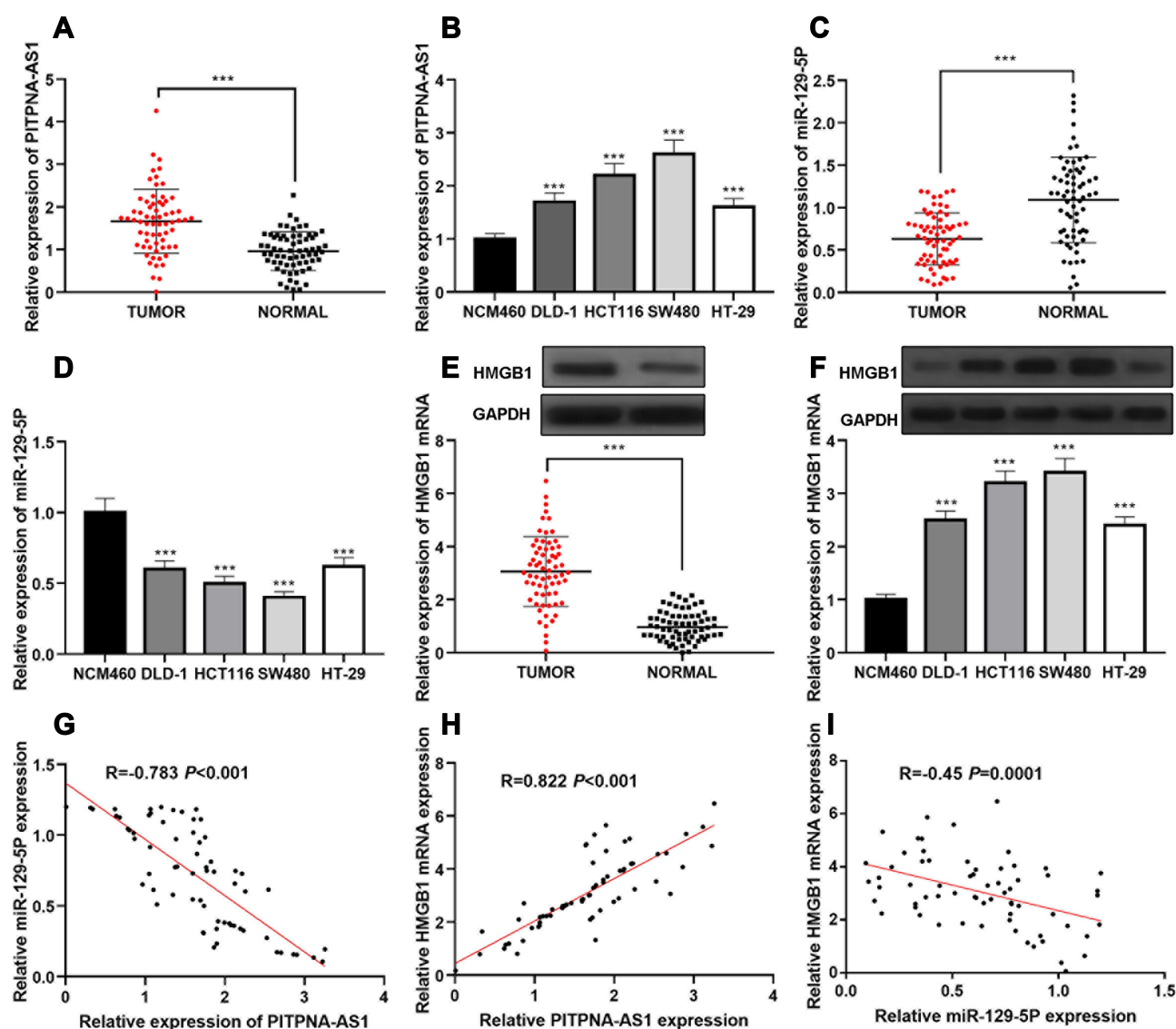


Figure 1 *PITPNA-AS1* and HMGB1 expressions were significantly upregulated in CRC, while *miR-129-5p* expression was markedly downregulated. (A and B) qRT-PCR was used to detect the expression of *PITPNA-AS1* in CRC tissues and cell lines. (C and D) qRT-PCR was used to detect the expression of *miR-129-5p* in CRC tissues and cell lines. (E and F) qRT-PCR and Western blot assays were adopted to detect the expression of HMGB1 in CRC tissues and cell lines. (G–I) Correlation analysis for *PITPNA-AS1*, *miR-129-5p* and HMGB1 in CRC tissues. *** $P < 0.001$.

CRC tissues and cell lines was lower (Figure 1C and D); HMGB1 expression was remarkably higher in CRC tissues and cell lines (Figure 1E–F). In addition, in CRC tissue, *PITPNA-ASI* expression was negatively interrelated with *miR-129-5p* expression (Figure 1G) and positively associated with HMGB1 mRNA (Figure 1H), and *miR-129-5p* expression was negatively associated with HMGB1 mRNA expression (Figure 1I). In order to evaluate the clinical significance of *PITPNA-ASI* expression in CRC, we divided the CRC patients into a high *PITPNA-ASI* expression group and a low *PITPNA-ASI* expression group based on the median, and analyzed the relationship between *PITPNA-ASI* expression and clinical characteristics of CRC patients, the findings of which depicted that *PITPNA-ASI* expression was related to clinical stage and lymph node metastasis (Table 2). These results suggested that the overexpression of *PITPNA-ASI* might be associated with CRC progression.

PITPNA-ASI Facilitated the Proliferation and Metastasis of CRC Cells, and Impeded Apoptosis

To expound on the function of *PITPNA-ASI* in CRC, we used *PITPNA-ASI* siRNA#1 and *PITPNA-ASI* siRNA#2 to silence *PITPNA-ASI* expression in HCT116 and SW480 cell lines (Figure 2A). The cell proliferation changes in CRC cells

were analyzed using CCK-8 and EdU experiments. It was revealed that the proliferation of HCT116 and SW480 cells in si-*PITPNA-ASI*#1 group and si-*PITPNA-ASI*#2 group was markedly lower than in si-NC group (Figure 2B–D). The migratory ability of CRC cells was determined using wound-healing assay. In comparison to the control group, inhibited cell migration was observed in HCT116 and SW480 with *PITPNA-ASI* knockdown (Figure 2E). Additionally, as shown, after the nuclei of CRC cells were stained with DAPI, in *PITPNA-ASI* knockdown groups, apoptotic morphological characteristics obviously appeared, such as pyknosis and karyorrhexis (Figure 2F). Consistently, flow cytometry analysis uncovered that the apoptosis rate was dramatically higher than that in *PITPNA-ASI* knockdown group (Figure 2G). All findings showed that *PITPNA-ASI* promoted cell proliferation and migration, and inhibited cell apoptosis in CRC.

PITPNA-ASI Could Sponge miR-129-5p and Negatively Regulate Its Expression

To pinpoint the molecular mechanism by which *PITPNA-ASI* promotes CRC progression, we obtained a binding site in the sequence of *PITPNA-ASI* for *miR-129-5p* from LncBase Predicted v.2 database (Figure 3A). Subsequently, dual-luciferase report assay was performed, the findings of which depicted that the luciferase activity in the co-transfection group of *PITPNA-ASI*-WT and *miR-129-5p* was markedly lower than that of HEK-293T cells co-transfected with *PITPNA-ASI*-MUT and *miR-129-5p* (Figure 3B). RIP experiment found that *PITPNA-ASI* and *miR-129-5p* were rich in Ago2-containing immunoprecipitate, suggesting that *PITPNA-ASI* was directly interacted with *miR-129-5p* (Figure 3C). The dual-luciferase report assay and RIP experiment demonstrated that *PITPNA-ASI* could directly bind to *miR-129-5p*. Moreover, we further confirmed the regulatory relationship between *PITPNA-ASI* and *miR-129-5p*, and *miR-129-5p* expression in CRC cells with *PITPNA-ASI* knockdown was remarkably increased (Figure 3D). Hence, it was verified that *PITPNA-ASI* could directly regulate *miR-129-5p* in CRC cell lines. The results indicated that *PITPNA-ASI* could sponge *miR-129-5p* in CRC.

MiR-129-5p Inhibition Could Reverse the Effect of PITPNA-ASI Knockdown on CRC Cells

To further elaborate on that *PITPNA-ASI* takes part in CRC cells through *miR-129-5p*, we co-transfected

Table 2 Correlation Between Clinicopathological Features and *PITPNA-ASI* Expression in CRC (n = 66)

Variables	n	<i>PITPNA-ASI</i> Expression		P value
		High (n = 33)	Low (n = 33)	
Age(years)				
≥60	30	16	14	0.590
< 60	36	17	19	
Gender				
Male	32	14	18	0.869
Female	34	19	15	
Tumor stage				
III–IV	41	27	12	0.045*
I–II	19	4	15	
Tumor size				
≥5cm	32	16	16	0.861
< 5cm	34	17	17	
Lymph node metastasis				
N1–2	41	30	11	0.032*
N0	19	3	16	

Notes: * $P < 0.05$, chi-square test.

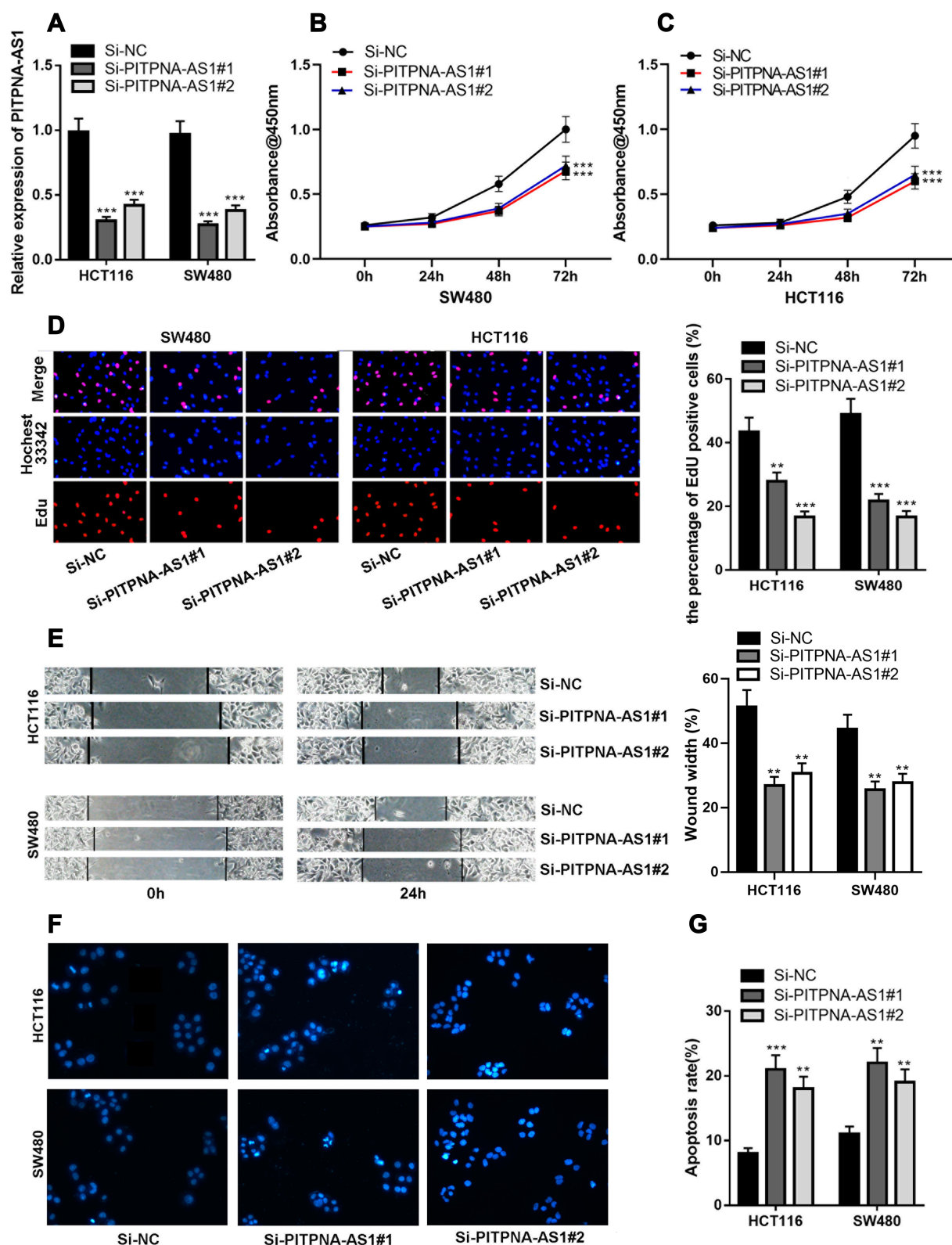


Figure 2 *PITPNA-AS1* promoted CRC cell proliferation and migration, and inhibited apoptosis. (A) qRT-PCR was used to detect *PITPNA-AS1* expression after CRC cells were transfected with *PITPNA-AS1* siRNAs. (B and C) CCK-8 assay was used to detect the cell proliferation after CRC cells were transfected with *PITPNA-AS1* siRNAs. (D) EdU assay was used to detect cell proliferation after CRC cells were transfected with *PITPNA-AS1* siRNAs. (E) Wound healing assay was used to detect cell migration after CRC cells were transfected with *PITPNA-AS1* siRNAs. (F) Immunofluorescence was used to show the morphologic change of nuclei after CRC cells were transfected with *PITPNA-AS1* siRNAs. (G) Flow cytometry assay was used to detect cell apoptosis rate after CRC cells were transfected with *PITPNA-AS1* siRNAs. ** $P < 0.01$ and *** $P < 0.001$.

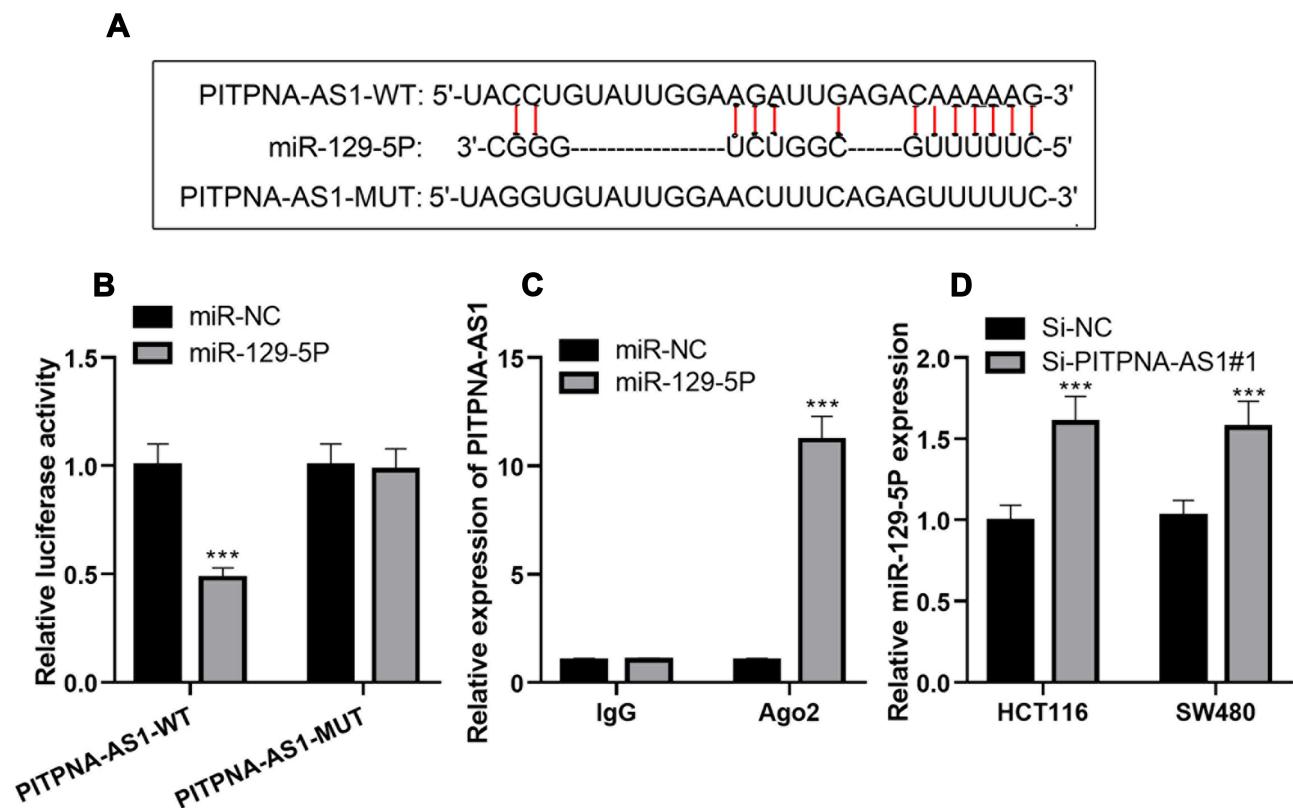


Figure 3 *miR-129-5p* was a target of *PITPNA-AS1*. **(A)** In LncBase database, we found that *PITPNA-AS1* contained a *miR-129-5p* binding site. **(B and C)** Luciferase reporter assay **(B)** and RIP experiment **(C)** confirmed the binding site between *miR-129-5p* and *PITPNA-AS1*. **(D)** qRT-PCR was adopted to detect *miR-129-5p* expression after CRC cells were transfected with *PITPNA-AS1* siRNA. *** $P < 0.001$.

PITPNA-AS1 siRNA#1 and *miR-129-5p* inhibitors into HCT116 and SW480 cell lines, and CCK-8 and EdU experiments unmasked that *miR-129-5p* inhibitors could partially counteract the inhibitory effect of *PITPNA-AS1* knockdown on CRC cell proliferation (Figure 4A–C). Wound healing assay manifested that *miR-129-5p* inhibitors could partially reverse the inhibitory effect of *PITPNA-AS1* on the migration of CRC cells (Figure 4D). Flow cytometry depicted that *miR-129-5p* inhibitors could offset the promotion of *PITPNA-AS1* knockdown on the apoptosis of CRC cells (Figure 4E). Therefore, we confirmed that *PITPNA-AS1* promoted the progression of CRC through regulating *miR-129-5p*.

PITPNA-AS1 Could Promote HMGB1 Expressions via Repressing *miR-129-5p* Expression

To delve into the ceRNA network among *PITPNA-AS1*, *miR-129-5p*, and their target gene in CRC, we searched the TargetScan database and found that *HMGB1* was

a downstream target gene of *miR-129-5p* (Figure 5A). Dual-luciferase reporter experiments confirmed that *miR-129-5p* mimics could reduce the luciferin activity of pmirGLO-HMGB1-WT. However, it had no influence on the luciferase activity of pmirGLO-HMGB1-MUT (Figure 5B). The RIP experiment demonstrated that HMGB1 mRNA and *miR-129-5p* were rich in Ago2-containing immunoprecipitate, which suggested that HMGB1 mRNA and *miR-129-5p* interacted directly in CRC cells (Figure 5C). In addition, it was found that *miR-129-5p* inhibitors could significantly improve HMGB1 mRNA expression (Figure 5D). Next, we co-transfected *PITPNA-AS1* siRNA#1 and *miR-129-5p* inhibitors into HCT116 and SW480 cell lines, and observed that as against si-NC group, HMGB1 expression in *PITPNA-AS1* siRNA#1-transfected cells decreased markedly; the expression of HMGB1 in *miR-129-5p* inhibitors-co-transfected cells was higher than that in si-*PITPNA-AS1*#1 group (Figure 5E). These data suggested that *PITPNA-AS1*/*miR-129-5p* axis modulated the expression of HMGB1 to participate in the progression of CRC.

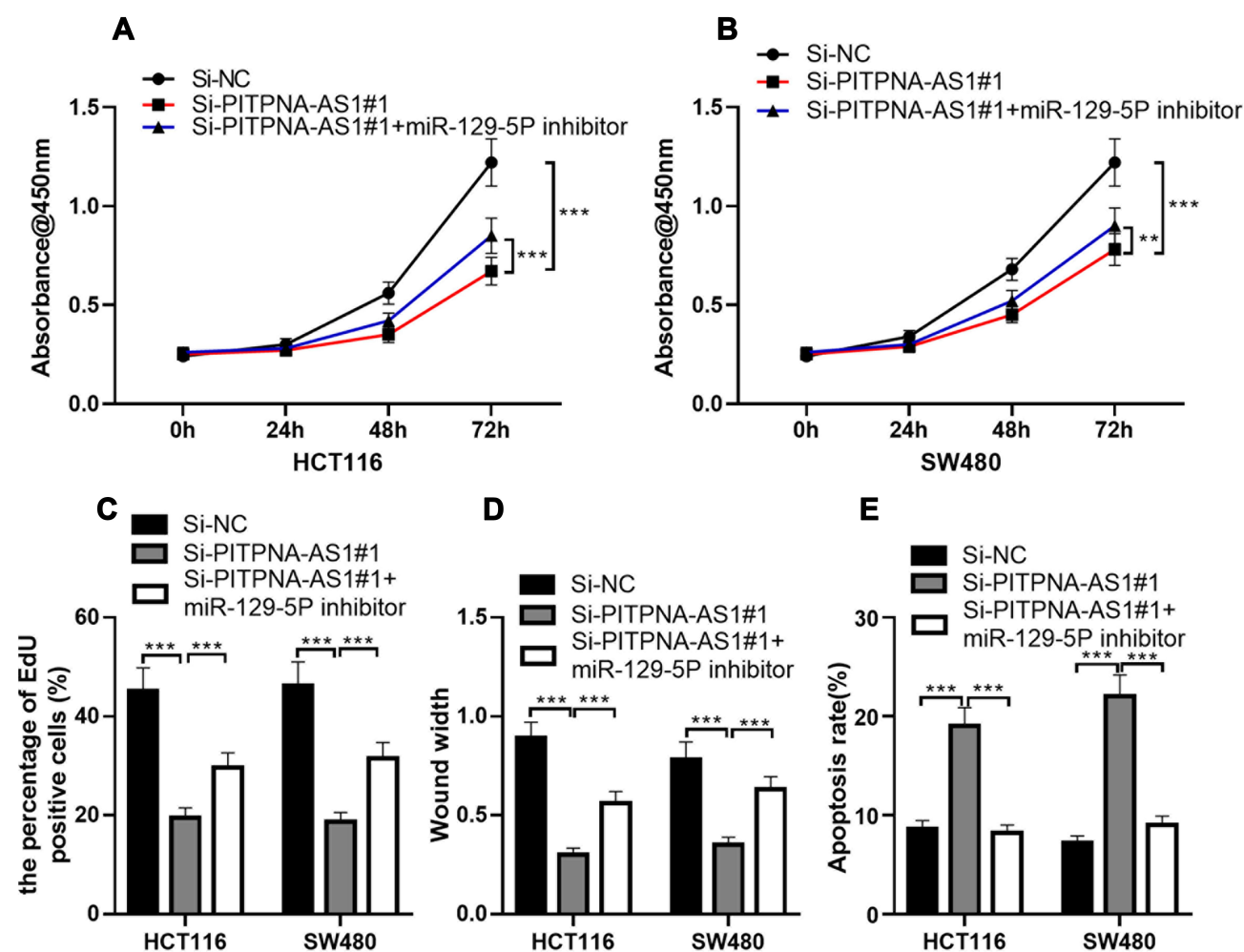


Figure 4 *MiR-129-5p* inhibitor could reverse the effect of *PITPNA-AS1* knockdown on cells. (A–E) After CRC cells were transfected with scrambled siRNA or *PITPNA-AS1* siRNA, or co-transfected with *PITPNA-AS1* siRNA and *miR-129-5p* inhibitor, CRC cell proliferation, migration and apoptosis were detected by CCK-8 assay (A and B), EdU assay (C), wound healing assay (D) and flow cytometry analysis (E), respectively. ** $P < 0.01$ and *** $P < 0.001$.

Discussion

CRC is a common gastrointestinal cancer. Although the current treatments have been greatly enhanced, the 5-year survival rate of patients is still not high.¹⁵ Hence, it is fundamental to deeply probe the pathological mechanism of CRC and to find new therapeutic targets. Mounting research shows that lncRNAs are crucial regulators in human cancers, including CRC. For example, *ZEB1-AS1* expression is up-regulated in CRC tissues and cell lines, and *ZEB1-AS1* can promote cell proliferation and inhibit apoptosis through the *miR-205/YAP1* axis.¹⁶ *SNHG3* facilitates the proliferation, migration and invasion of CRC cells by modulating the *miR-539/RUNX2* axis.⁵ *LINC00520* is highly expressed in CRC tissues and cell lines, and can function as a ceRNA for *miR-577*, thus increasing HSP27 expression and promoting cell proliferation, migration and invasion.¹⁷ A recent study implies that

PITPNA-AS1 expression is markedly elevated in hepatocellular carcinoma, and *PITPNA-AS1* can increase the expression of WNT5A by inhibiting *miR-876-5p* expression to promote cell proliferation and migration.⁶ However, its role in CRC is still unknown. Our study found for the first time that *PITPNA-AS1* was markedly overexpressed in CRC tissues and cell lines, and that high expression of *PITPNA-AS1*, interrelated with higher clinical stage of patients and positive lymph node metastasis, could promote cell proliferation and migration, and suppress apoptosis. Therefore, we confirmed that, in CRC *PITPNA-AS1* could function as a tumor promoter.

We also explored the underlying mechanism of *PITPNA-AS1* in CRC, and luciferase report assay and RIP experiment confirmed that *miR-129-5p* was one of the targets of *PITPNA-AS1*. Accumulating studies manifest that *miR-129-5p* has an anti-tumor effect, and for example, *miR-129-5p*

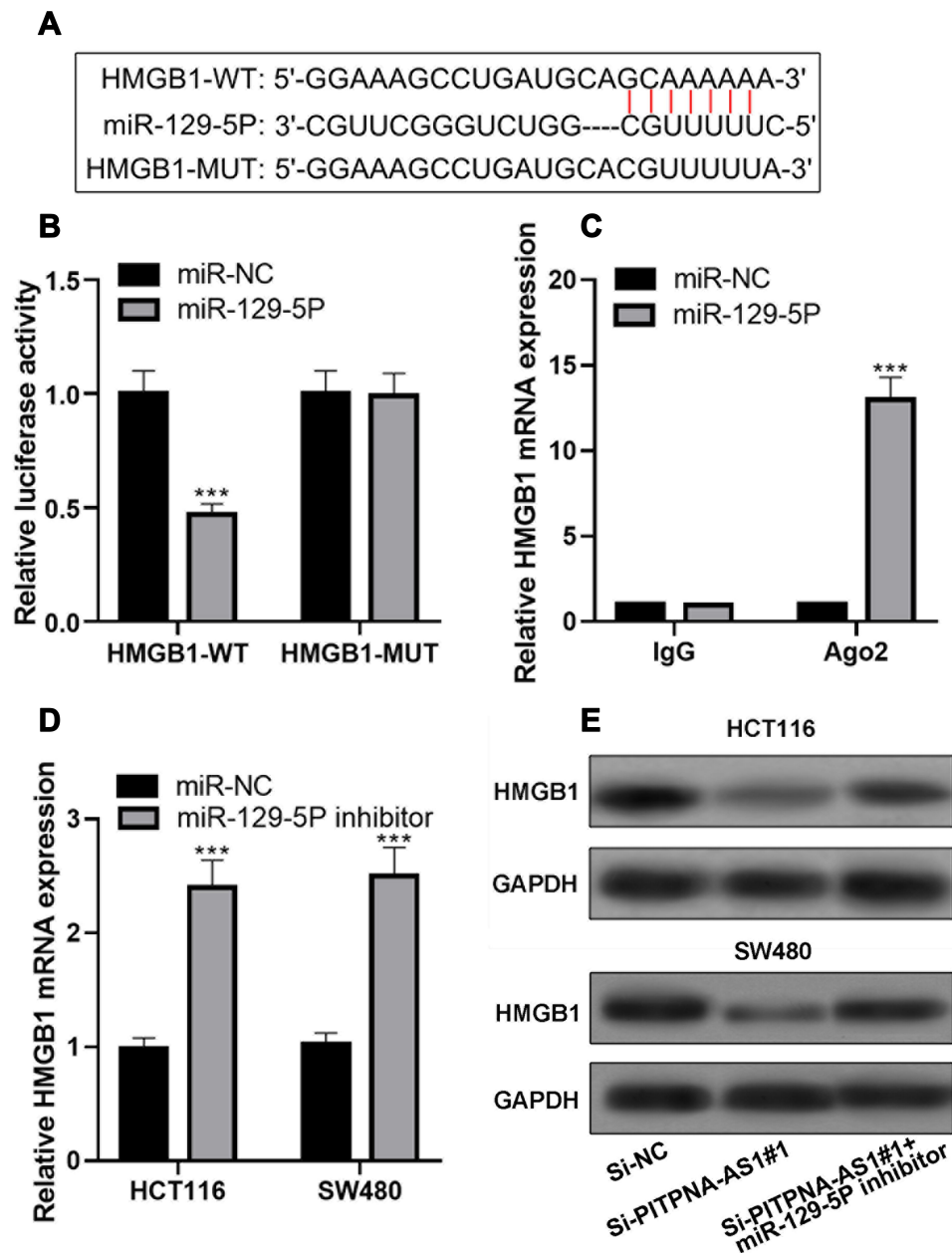


Figure 5 *PITPNA-AS1* regulated the expression of HMGB1 through *miR-129-5p*. **(A)** In TargetScan database, it was predicted that the 3'UTR of HMGB1 contained a *miR-129-5p* binding site. **(B–C)** Luciferase reporter assay **(B)** and RIP experiment **(C)** confirmed the predicted binding site. **(D)** qRT-PCR was used to detect the expression of HMGB1 after cells were transfected with *miR-129-5p* inhibitor. **(E)** Western blot assay was used to detect HMGB1 protein expression after CRC cells were transfected with *PITPNA-AS1* siRNA, or co-transfected with *PITPNA-AS1* siRNA and *miR-129-5p* inhibitor. *** $P < 0.001$.

expression is significantly declined in gastric cancer cell lines and *miR-129-5p* can suppress the proliferation and invasion of gastric cancer cells through targeting COL1A1.¹⁰ *MIR-129-5p* expression is significantly decreased in rectal adenocarcinoma cell lines and *miR-129-5p* can impede cell proliferation, migration and invasion via targeting E2F7.⁹ Another study manifests that *miR-129-5p* expression is remarkably declined in CRC cell line, and *miR-129-5p* can impede cell proliferation and promote apoptosis via targeting HMGB1.⁴

In this study, we unearthed that *miR-129-5p* expression was dramatically decreased in CRC tissues and cell lines, and mechanistically, it was confirmed that *PITPNA-AS1* could adsorb *miR-129-5p*, which might partly explain the mechanism of *miR-129-5p* dysregulation in CRC.

Next, we searched for downstream genes of *miR-129-5p*. Bioinformatics analysis, luciferase reporter assay and RIP experiment demonstrated that HMGB1 was a target gene of *miR-129-5p*. HMGB1 is a versatile regulator that is

originally thought to be a nuclear protein involved in the regulation of gene transcription, by which it can regulate inflammation and infection responses.¹⁸ In recent years, accumulating studies confirm that HMGB1 features prominently in the tumorigenesis of multiple cancers including liver cancer, lung cancer, breast cancer, prostate cancer, cervical cancer and CRC.¹⁹ In CRC, it is demonstrated that HMGB1 overexpression can promote cancer cell proliferation and metastasis, and HMGB1 can also serve as a predictor for the poor prognosis of the patients.²⁰ Another study reports that the HMGB1 expression is associated with lymph node metastasis and distant metastasis of CRC patients, and HMGB1 inhibits the apoptosis of cancer cells by inducing the activation of ERK signaling and c-inhibitor of apoptosis protein 2.¹² Besides, HMGB1 promotes CRC cell growth, migration, invasion via JAK/STAT3 pathway.²¹ Moreover, HMGB1 mediates the proliferation and migration of CRC by increasing the phosphorylation of ERK1/2, ras-related C3 botulinum toxin substrate 1 and Akt, and promoting the expressions of inducible nitric oxide synthase and matrix metalloproteinase 9.²² In this work, we confirmed that HMGB1 expression was markedly raised in CRC tissues and cell lines, and *PITPNA-AS1* could modulate its expression via targeting *miR-129-5p*.

In conclusion, in the present work, we verify the function and downstream mechanism of *PITPNA-AS1* in CRC. Specifically, *PITPNA-AS1* can adsorb *miR-129-5p* to modulate HMGB1 expression, and thus exert its indirect cancer-promoting role in CRC. Nonetheless, this work has certain limitations. First of all, these data are only obtained from in vitro experiments and the conclusion needs to be confirmed with animal experiments in the following work. Secondly, whether *PITPNA-AS1* can promote other malignant biological behaviors of CRC cells, such as chemoresistance, requires further investigation. Last but not the least, there are other candidate downstream miRNAs of *PITPNA-AS1*, which remain to be screened and verified.

Disclosure

The authors declare no conflicts of interest.

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