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Original Article

PRR11 promotes growth and progress of colorectal cancer via epithelial-mesenchymal transition

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Abstract: Objective: Proline-rich protein 11 (PRR11) is a relatively new tumor-promoting gene, which can accelerate the progress of some cancers, but its roles in colorectal cancer remain unclear. Hence, this study aims to establish the effect of PRR11 on biological functions in colorectal cancer and further explore the underlying mechanisms. Methods: Immunohistochemistry methods were used to examine the PRR11 expression in colorectal cancer tissues and analyze the relationship of the PRR11 expression with the clinical parameters and prognosis. CRISPR/Cas9 PRR11 gene-knockout system and lentivirus-mediated PRR11 overexpression vectors were constructed. PCR, Western blot, CCK-8, colony-formation assay, wound-healing assay, and Transwell assay detected that PRR11 in the proliferation, migration, and invasion of colorectal cancer in vitro. Results: The expression of PRR11 in colorectal cancer tissues was significantly higher than that in normal colorectal samples. The high expression of PRR11 associates with the poor prognosis of patients with colorectal cancer. CRISPR/Cas9 system and lentivirus-mediated PRR11 overexpression vectors were successfully constructed. PRR11 knockout reduces the proliferation, migration, and invasion of sw480 and HCT116 colorectal cancer cells. Cell proliferation, migration, and invasion were regained after HCT116-PRR11-KO cells recovered their PRR11 protein expression. Silencing PRR11 in colorectal cancer cells increases the expression of E-cadherin and decreases the expression of the mesenchymal marker vimentin. Conclusion: Our data demonstrated that PRR11 promotes the growth and progress of colorectal cancer, possibly through the mechanisms of epithelial-mesenchymal transition.

Keywords: Colorectal cancer, proline-rich protein 11, CRISPR/Cas9, proliferation, migration, invasion

Introduction

Colorectal cancer (CRC) remains a very common disease with characteristics of highly variable presentation. CRC is ranked as one of the most common malignancies worldwide [1-3]. Resection is still the most effective and most likely curative treatment for CRC. The recurrence and metastasis of CRC are the vital reasons of death after curative excision [4]. However, the molecular mechanisms of occurrence, recurrence, and metastasis of CRC are poorly understood.

The whole-length gene sequence of proline-rich protein 11 (PRR11) was uploaded in 2000 and named as FLJ11209. The American National Institute of Health Mammalian Gene Collection Team renamed FLJ11029 into PRR11 (Accession BC008669) in 2002. Weinmann et al. [5] found that PRR11, as a novel oncogene, associates with E2FI and E2F4 to regulate tumor progression. Ji Y et al. [6] observed the overexpression of PRR11 at both mRNA and protein levels in lung cancer tissues as compared with normal lung tissues and indicated that high expression of PRR11 was significantly correlated with poor prognosis in lung cancer patients. Moreover, PRR11 knockdown suppresses lung cancer cellular proliferation, migration, and invasion both in vitro and in vivo.

Our team studied the functions of overexpressed PRR11 gene in gastrointestinal tumors and found that PRR11 is a biomarker of poor prognosis in gastric cancer tissues [7]. PRR11

expression is remarkably associated with gastric wall invasion, lymph node metastasis, tumor progression, and poor differentiation. Our results showed that PRR11 expression significantly increases with progression of human gastric cancer [7]. Chen Ying et al. [8] showed that PRR11 overexpression is associated with adverse clinical outcomes in patients with hilar cholangiocarcinoma. The knockdown of PRR11 caused inhibition of cell growth and tumor survival by inhibiting the activity of key regulators involved in cell migration and invasion. Zhou Fanhan et al. [9] reported that the levels of PRR11 protein were remarkably elevated in breast cancer. Research on the preliminary mechanism showed that epithelial-mesenchymal transition (EMT) is important for PRR11 to promote the progression of breast cancer.

The findings listed above strongly support the oncogenic role of PRR11 in lung cancer, gastric cancer, hilar cholangiocarcinoma, and breast cancer, indicating that inhibition of PRR11 may be a future therapeutic target in the treatment of carcinoma patients.

However, the role of PRR11 regulating the growth migration and invasion of colorectal cancer cells and its detailed mechanisms are largely unknown. The precise mechanisms underlying PRR11 promotion of colorectal cancer growth and invasion are also not well understood. Therefore, the present study aims to establish the role of PRR11 in colorectal cancer. We also analyzed the biological functions and underlying mechanisms of PRR11 in human colorectal cancer tissue samples and colorectal cancer cell lines in this study.

Materials and methods

Patients tissues and data collection

This study of human colorectal cancer sample was approved by the Ethics Committee of the first hospital affiliated to Fujian medical university. 80 formalin-fixed and paraffin-embedded colorectal cancer tissues and 23 samples of normal colorectal tissues were used as controls. The samples were provide by Pathology Department and Department of gastrointestinal surgery 2 section of the First Hospital Affiliated to Fujian Medical University. The patient group samples were conserved during the period from 2009 to 2010. All colorectal cancer patients included in this study had not been given any preoperative chemotherapy or other therapy such as radiotherapy. Postoperative chemotherapy was indicated for patients who owned the indications of chemotherapy with intravenous infusion of 5-fluorouracil-based chemotherapy. Follow-up was done every three months for the first 2 years and then annually thereafter.

Immunohistochemistry and evaluation

Immunohistochemistry and evaluation were performed as described before [10]. Rabbit polyclonal anti-human PRR11 antibody (1:300) (from Abcam biotechnology) was used in the experiment.

Cell lines and materials

The two colorectal cancer cell lines (sw480, HCT116) were obtained from cell bank of the Chinese Academy of Sciences (Shanghai, China). Both cell lines were cultured in RPMI 1640 with L-glutamine supplemented with 10% fetal bovine serum in 5% CO_2. The primers of PRR11 yielded 156 bp and full-length gene as follows: PRR11-156 bp forward 5'-ATCTGCGGAAACTGCTTA-3' and reverse 5'-TTGGGCTT CTAGGGTGAG-3'; PRR11-full-length forward 5'-GC GAATTCGCC ACCATGCCCAAGTTCAAACAACG-3' and reverse 5'-GCGGATCCT CAGTTTTGTTCATCAAAGC-3' which were containing EcoR I and BamH I. The primers of β-actin are forward 5'-CTGTCTGGCGGCACC ACCAT-3' and reverse 5'-GCAACTAAGTC ATAGTCCGC-3'.

RNA preparation, reverse transcription, and real-time PCR amplification

Semiquantitative RT-PCR was used to detect the expression of PRR11 gene in colorectal cancer cell lines SW480 and Hct116 respectively. And the RNA preparation, reverse transcription and qPCR amplification and the analysis results were carried out as described previously [10].

Western-blot analysis

Western blot and detection of the blotted product were carried out as described previously [11]. The following primary antibodies were used from the different companies under listing respectively: Rabbit polyclonal anti-human PRR11 antibody (1:1000) (from Abcam biote-

chnology), E-cadherin polyclonal rabbit anti-human antibody (1:1000) (from Cell Signaling Technology, Danvers, MA, USA), Rabbit polyclonal anti-human Vimentin antibody (1:1000) (from Abcam biotechnology), β-actin monoclonal mouse anti-human antibody (1:1500) (from Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA). Primary antibodies behind figures in brackets stand for the antibody dilution multiple.

Cell proliferation assay, cell migration and invasion assay

Cells were seeded onto 96-well plates at a density of 1,500 cells per well and detected using the cell counting kit CCK-8 (Donjindo, Kumamoto, Japan) by a microplate reader (Bio-Tek, Winooski, VT, USA) for 5 days. The data of absorbance at a wavelength of 450 nm was collected to analyze cell proliferation. Transwell chamber (8 μm, 24-well format; FALCON) and Matrigel coated transwell chamber (BD Bioscience) were inserted into 24 well cell culture plates to measure migration and invasion. 9×10^4 cells in 0.3 mL of serum-free medium were added in the upper chamber, while 0.8 mL of RPMI 1640 containing 10% FBS was added to the lower chamber. Cells were cultured for 24 hours. Then cells with transwell chambers were fixed in methanol for 5 minutes and stained with crystal violet and counted in 3 random fields under microscope.

Colony formation assay

Cells were seeded onto 3.5 cm cell-plates at a density of 800 cells per well and cultured for 10 days. Then cells were fixed by methanol for 5 minutes and stained with crystal violet. Colonies of 50 or more cells were counted.

Wound healing assay

Cells were seeded onto 6-well plates and cultured until convergence degree reached approximately 100%. Wounds were scratched on the monolayer cells using 20 μL pipette tips. The plates were photographed at the time of 0 hour, 24 hours, 48 hours after cells seeding in FBS-free RPMI 1640.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system (CRISPR/Cas9)-mediated knockout of PRR11 plasmids construction. According to the sgRNA design principle, three suitable sgRNA target PRR11 gene sequences (sg-1, sg-2 and sg-3) were synthesized as follows respectively: sg-1 forward: 5'-CACCGTCTCTGCACGATTCGC TCTT-3' reverse: 5'-AAACAAGAGCGAATCGTGCAGAGAC-3' sg-2: forward: 5'-CACCGATTCGCTCTTCGGATGGTGT-3' reverse: 5'-AAACACACCATCCGAAGAGCGAATC-3' Sg-3: forward: 5'-CACCG AGGCGCTTCTGGTATGAATA-3' reverse: 5'-AAACTATTCATACC AGAAGCGCCTC-3'. The sg-1, sg-2 and sg-3 oligonucleotides, annealing and ligation were performed using gradient cooling method. And, sg-1, sg-2 and sg-3 sequences were jointed in the backbone vector of pSpCas9(BB)-2A-Puro(PX-459) plasmids. And all the plasmids were verified by DNA sequencing. The sw480 and HCT116 cells were cultured in appropriated medium supplemented with 10% FBS. When the cells were at approximately 90% confluency, we transfected the plasmids into these cells using Lipofectamine 3000 (Life technologies). And PX459 plasmids were transfected into sw480 and HCT116 cells as control. We using puromycine screened the resistant colorectal cancer cells. Using single cells got the monoclonal colorectal cancer cells. Western-blot assay confirmed the PRR11 gene knockout in the colorectal cancer cells for the further experiments.

Construction of PRR11 expression lentiviral vectors and infection

The methods of PRR11 lentiviral expression vectors construction were carried out as described previously [12]. Briefly, we ligated the PRR11 DNA sequence with the digested of pCDH-CMV-MCS-EF1-RFP plasmids by the incision enzyme of EcoR I and BamH I. And then pCDH-PRR11 vectors and pMK-VSVG, pMDL-G/P-RRE and pRSV-REV plasmids co-transfected the 293T cells using Lipofectamine 3000. Sw480 and HCT116 cells were infected by virus using the supernatant of 293T cells. And the microscope was used to confirm the infection efficiency by observing the RFP positive in the cells.

Statistical analysis

All statistical analyses used GraphPad Prism 5 software. Data were analyzed by one-way ANOVA or Student's *t*-test. Data were expressed as mean ± standard deviation (SD). Survival curves were calculated by Kaplan-Meier method and compared using log-rank test. A *P*-value

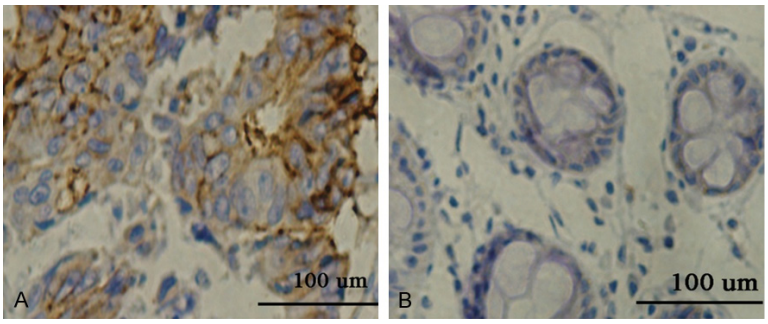


Figure 1. Different expression of PRR11 in the human colorectal cancer tissues and normal colorectal samples. A: Positive or strong expression of PRR11 in colorectal cancer tissues (400×). B: Negative or mild expression of PRR11 in normal colorectal samples (400×).

Table 1. The expression of PRR11 immunohistochemistry results of colorectal cancer tissues and normal colorectal samples

Tissues	N	PRR11 Expression (MOD±SD)[a]	P
Colorectal cancer tissues	80	0.4236±0.1943	<0.001
Normal colorectal tissues	23	0.2480±0.1765	

[a]MOD±SD: Mean Optical Density ± Standard Deviation.

Table 2. Relationship between PRR11 expression and colorectal carcinoma clinicopathological paramet

Factors	N	PRR11 Expression (MOD±SD)[a]	P
Age (year)			
<60	33	0.4421±0.1264	0.6971
≥60	47	0.4116±0.1721	
Gender			
Male	37	0.4324±0.1642	0.4133
Female	43	0.3921±0.2230	
Tumor size (cm)			
≤3	51	0.3839±0.2823	0.078
>3	29	0.4957±0.1983	
Clinical stage[b]			
I~II	25	0.2963±0.0986	0.0261*
III~IV	55	0.4596±0.1721	
Lymph node metastasis			
No	33	0.2783±0.2091	0.0017*
Yes	47	0.4269±0.1685	
Histological grade			
Poorly	23	0.4932±0.1476	0.0321*
Moderately	27	0.3246±0.1921	
Well	30	0.2109±0.2108	

[a]MOD±SD: Mean Optical Density ± Standard Deviation. [b]The 7 th edition of TNM staging criteria of colorectal cancer. *P<0.05.

of <0.05 was considered to indicate a statistically significant difference.

Results

High PRR11 expression in colorectal cancer tissues

PRR11 expression was assessed by immunohistochemistry in colorectal cancer tissues and normal colorectal tissues. Low expression of PRR11 protein was visible during immunostaining of normal colorectal tissues (**Figure 1B**), whereas high expression of PRR11 protein immunostaining was more apparent in colorectal cancer tissues (**Figure 1A**). The mean optical density (MOD) of PRR11 in colorectal cancer tissues (0.4236±0.1943) was significantly higher than that in normal colorectal tissues (0.2480±0.1765; P<0.001; **Table 1**). This finding suggests that PRR11 expression increased in colorectal cancer tissues.

Colorectal cancer patients with high PRR11 expression had worse outcome

Relationship between PRR11 expression and some clinicopathological parameters (**Table 2**) reveals that age, gender, and tumor size are not notably different. However, the high PRR11 expression was significantly associated with clinical stage (I~II vs. III~IV; P=0.0261), lymph node metastasis (negative vs. positive; P=0.0017), and histological gr-ade (poor, moderate, and well; P=0.0312). This finding indicates that the high expression of PRR11 may be involved in the progression of colorectal cancer.

We further analyzed the PRR-11 expression levels and the prognosis of colorectal cancer patients. The 80 colorectal cancer tissues were split into two

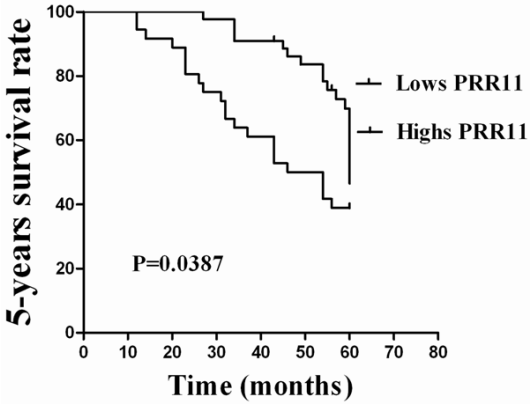


Figure 2. Relationship between PRR11 expression of patients with colorectal cancer and prognosis. The survival and prognosis of the expression of PRR11 group was notably better than the high expression of PRR11 group (P<0.05).

groups in accordance with the MOD expression levels and the following principle: low PRR11 expression group (<0.4236, which is the average MOD values of 80 colorectal cancer tissues) and a high PRR11 expression level group (≥0.4236). The 5-year survival rate and prognosis of the low PRR11 expression group were markedly better than those of the high PRR11 expression group (P<0.05) (**Figure 2**). These data also implied that PRR11 can promote colorectal cancer progression.

PRR11 mRNA and protein expression in colorectal cancer cell lines

We used real-time PCR and Western blot to detect the expression of PRR11 in human colorectal cancer cell lines (sw480 and HCT116). PRR11 mRNA and protein were expressed in both sw480 and HCT116 cell lines. The expression quantities of PRR11 mRNA and protein in sw480 and HCT116 cells were not remarkably different (**Figure 3**). Thus, we used both sw480 and HCT116 cells in further experiments and tested the biological roles of PRR11 in colorectal cancer.

Knockout of PRR11 reduced colorectal cancer cell proliferation, migration, and invasion

To obtain insights into the role of PRR11 in the development and progression of colorectal cancer, we generated PRR11 knockout cells by using CRISPR/Cas9 mediated gene editing technology in colorectal cancer sw480 and HCT116 cell lines. We constructed three sgRNAs (sg-1, sg-2, and sg-3) targeting different regions in the first exon of the human PRR11 gene by using bioinformatics software (**Figure 4A**) and ligated the sgRNAs into PX459 plasmids. We transfected the plasmids into sw480 and HCT116 cells by using Lipofectamine 3000. Selection was performed with 1.5 μg/mL HCT116 and 2 μg/mL sw480 cells. We used 96-well plates to obtain a single-cell clone for further experiments. Western blot was used to test the effect of CRISPR/Cas9-mediated sgRNAs on PRR11 knockouts in sw480 and HCT116 cells. **Figure 4B** shows the complete absence of the PRR11 protein in the sg-1-d, sg-1-c, sg-2-a, sg-2-b,d, sg-3-b, and sg-3-b,d single clones of colorectal cancer cells. We named the knockout of PRR11 colorectal cancer cells as sw480-PRR11-KO/HCT116-PRR11-KO cells; the control cells using the empty PX459 plasmids obtained sw480-control and HCT116-control cells.

sw480-PRR11-KO/HCT116-PRR11-KO cells exhibited poorer proliferation than their sw480-control/HCT116-control counterpart. Accordingly, we observed a notable decrease in the viability of sw480-PRR11-KO/HCT116-PRR11-KO cells compared with sw480-control/HCT116-control cells as tested by CCK-8 assay (**Figure 5A**, **5B**) (P<0.05). Similarly, PRR11 gene knockout in colorectal cancer cells significantly decreased colony formation via colony-formation assay compared with the control group (**Figure 5C**, **5C1**, **5D**, **5D1**) (P<0.05). Transwell assay indicated that the total population of cells in sw480-PRR11-KO/HCT116-PRR11-KO groups that migrated and invaded through the Transwell polycarbonate filter were significantly fewer than the sw480-control/HCT116-control groups in both colorectal cancer cell lines (migration: **Figure 5E**, **5E1**, **5F**, **5F1**; invasion: **Figure 5G**, **5G1**, **5H**, **5H1**) (P<0.05). To affirm the roles of PRR11 in the sw480 and HCT116 cell migration, we conducted wound-healing assays to test the ability of colorectal cancer cells to migrate. As a result, the migration demonstrates a large decrease of sw480-PRR11-KO/HCT116-PRR11-KO cells compared with the sw480-control/HCT116-control groups in the sw480 and HCT116 cells after 36 h of cell seeding (**Figure 5J**, **5J1**, **5K**, **5K1**) (P<0.05). Collectively, our results suggest that PRR11 knockout tremendously impairs the prolifera-

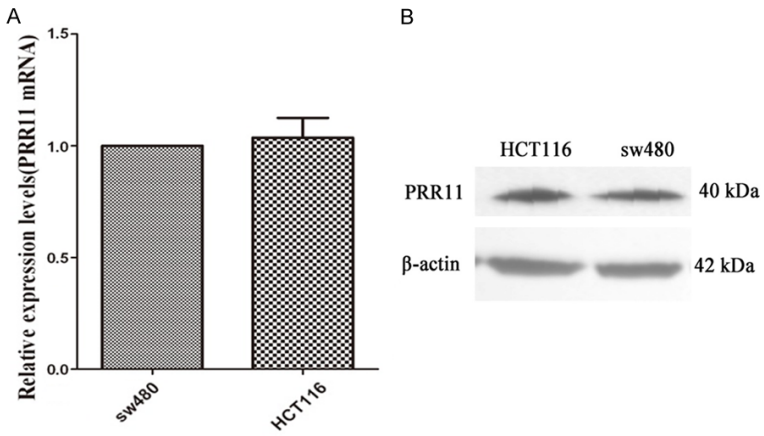


Figure 3. mRNA and protein expression of PRR11 in the colorectal cancer cell lines sw480 and HCT116. A: Colorectal cancer cells were evaluated by real time PCR using PRR11 primers. β-actin was amplified as an internal control. PRR11 mRNA expression quantities in sw480 and HCT116 cells are no different from each other. B: PRR11 protein in colorectal cancer cell lines of sw480 and HCT116 were detected by western blotting using β-actin as a positive control.

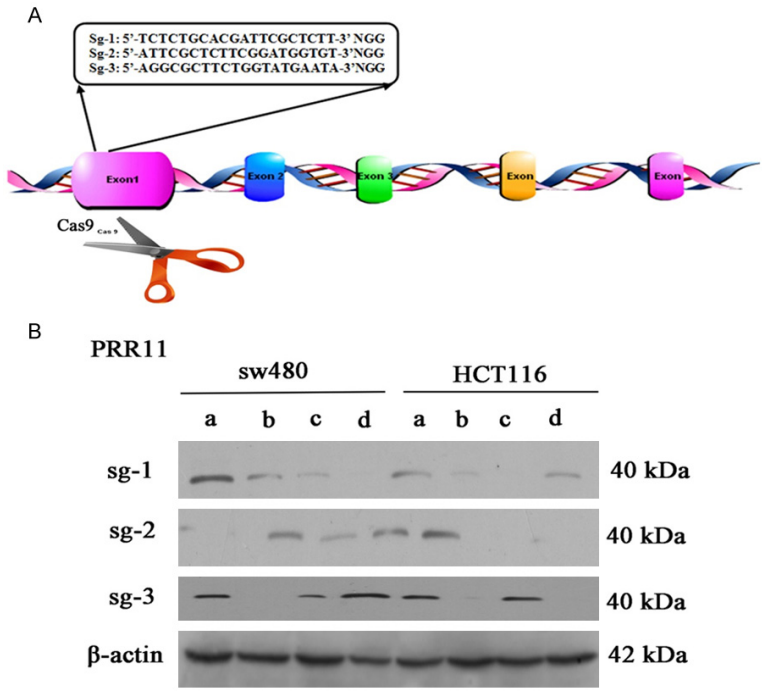


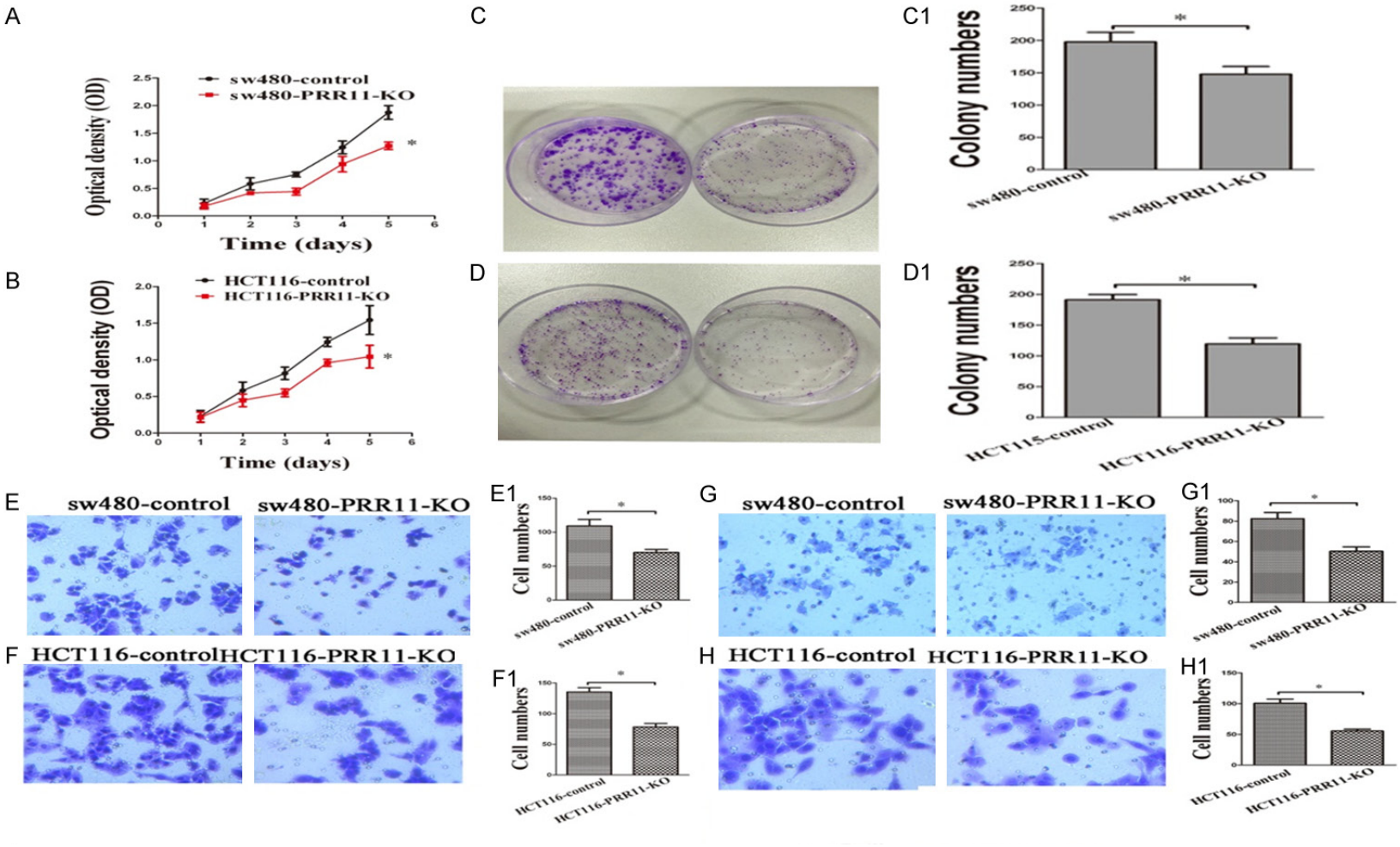
Figure 4. CRISPR/Cas9 system-mediated knockout of PRR11 plasmids construction and the effect of detection using western blotting. A: The sketch map of targeted PRR11 gene exon1. The siRNA leading sequences are shown in the block diagram. The scissors referred to the Cas9 protein which cuts off the DNA sequences. B: Western blot detects the effect of CRISPR/Cas9 knockout PRR11 gene. The results showed that the complete absence of the PRR11 protein in the sg-1-d, sg-1-c, sg-2-a, sg-2-b,d, sg-3-b, and sg-3-b,d single clones of colorectal cancer cells.

tion, migration, and invasion of sw480 and HCT116 cells.

Overexpression of PRR11 rescue colorectal cancer cell proliferation, migration, and invasion

To further confirm our experiments, we constructed the overexpression vector of PRR11 in the pCDH-CMV-MCS-EF1-RFP plasmids by using 293T cells obtained from the lentiviral-mediated PRR11 overexpression supernatant. The supernatant infected the HCT116-PRR11-KO cells and renamed as HCT116-PRR11-KO-LV-PRR11 cells, and the control groups were prepared by infecting HCT116-PRR11-KO cells with empty pCDH group. Transfection efficiency was quantified by counting the cells under a fluorescent microscope after 72 h infection. The virus vectors, expression of red fluorescent protein (RFP), and the efficiency of HCT116-PRR11-KO and HCT116-PRR11-KO-LV-PRR11 cells were greater than 90% (**Figure 6A**, **6B**). Western blot analysis revealed that PRR11 is detectable in HCT116-PRR11-KO-LV-PRR11 cells, and HCT116-PRR11-KO cells do not express PRR11 at detectable levels (**Figure 6C**). PRR11 protein expression was recovered in the HCT116-PRR11-KO-LV-PRR11 cells, which suggested that the endogenous PRR11 protein was recovered in HCT116-PRR11-KO-LV-PRR11 cells.

CCK-8 assay show that compared with the HCT116-PRR11-KO group, the cell growth in the HCT116-PRR11-KO-LV-PRR11 colorectal cancer cells was faster (**Figure 6D**) ($P<0.05$). Colony-formation assay also showed the proliferative ability was recovered in the HCT116-PRR11-KO-LV-PRR11 group compared with the HCT-


A
sw480-control
sw480-PRR11-KO
Optical density (OD)
Time (days)
B
HCT116-control
HCT116-PRR11-KO
Optical density (OD)
Time (days)
C
C1
Colony numbers
sw480-control
sw480-PRR11-KO
D
D1
Colony numbers
HCT115-control
HCT116-PRR11-KO
E
sw480-control
sw480-PRR11-KO
E1
Cell numbers
F
HCT116-control
HCT116-PRR11-KO
F1
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H1
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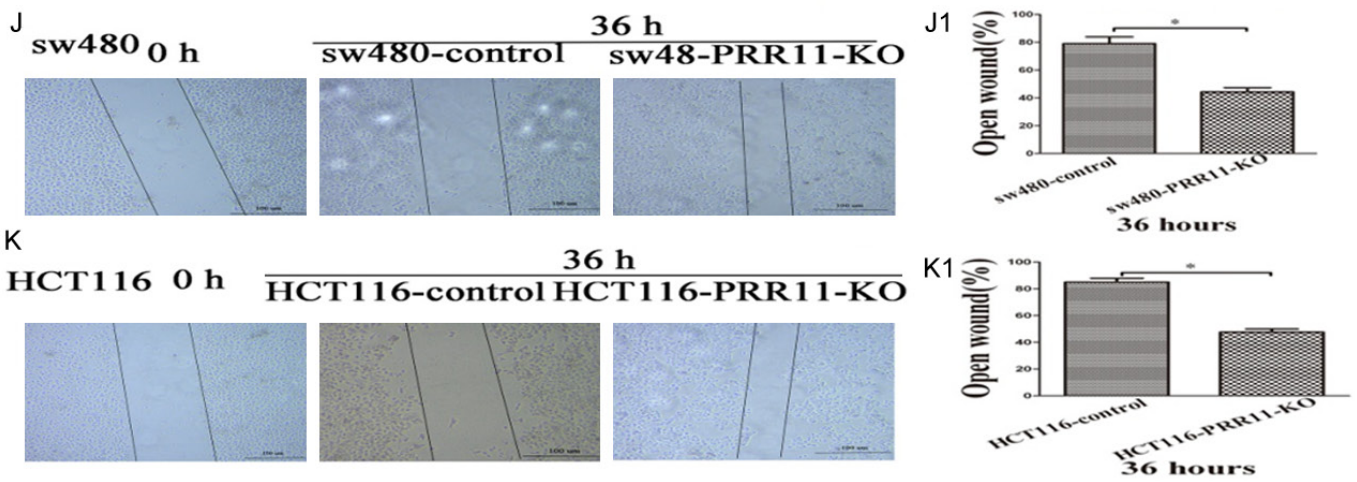
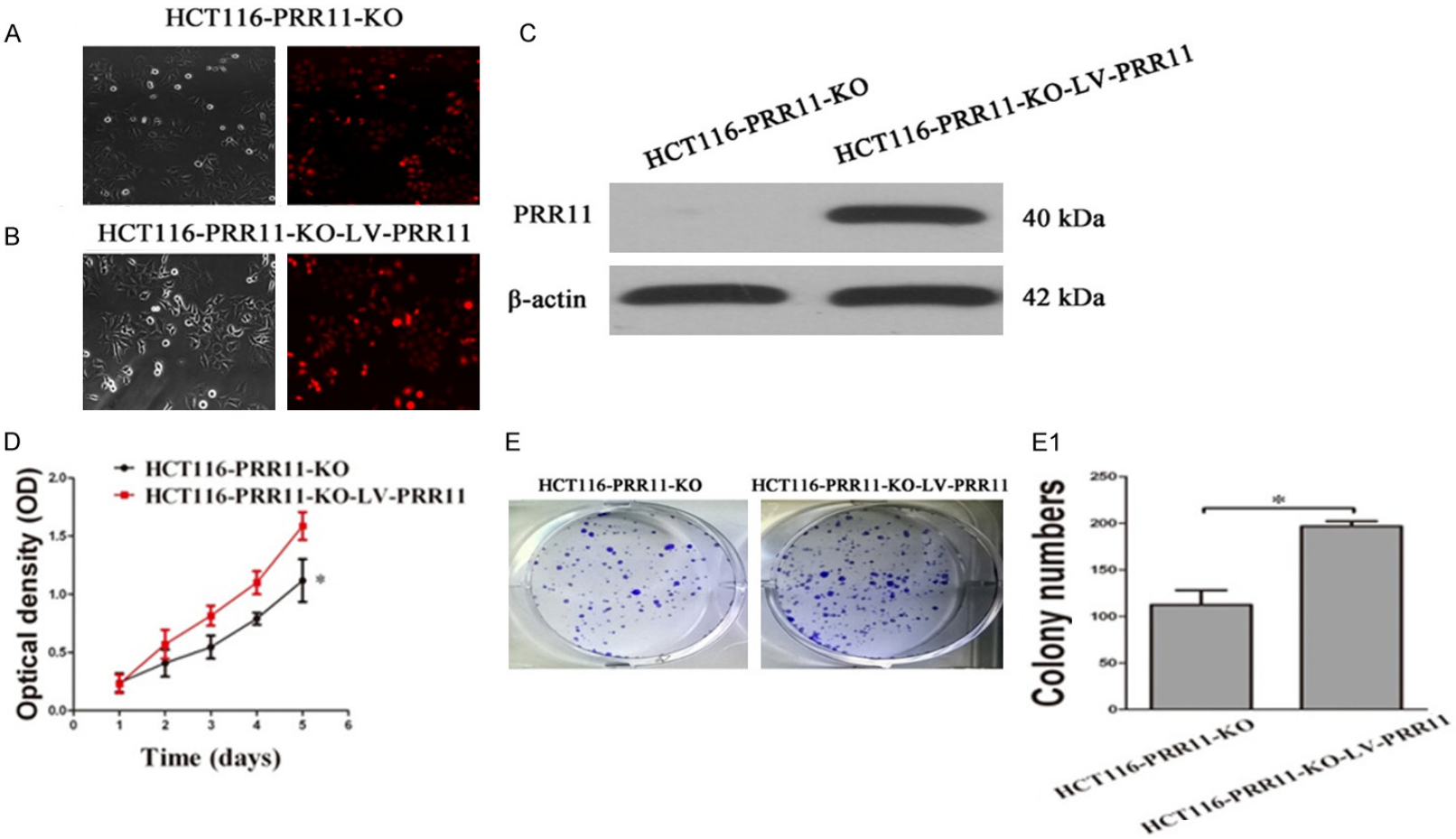


Figure 5. Knockout of PRR11 decreased colorectal cancer cells proliferation, migration and invasion. (A and B) The CCK-8 assay results showed that the proliferation of sw480-PRR11-KO/HCT116-PRR11-KO cells are significantly slower than sw480-control/HCT116-PRR11-KO cells ($P<0.05$) (C and D, respectively). The colony formation assays revealed that the sw480-PRR11-KO/HCT116-PRR11-KO cells have lower colony formation numbers compared with the sw480-control/HCT116-control cells ($P<0.05$); (C1 and D1), respectively: shown are quantitative evaluation of the results (the histogram in the right of colony formation figure) and representative the sw480-control/HCT116-control groups cells and sw480-PRR11-KO/HCT116-PRR11-KO groups cells (E and F, respectively). Migration assay showed that a number of sw480-PRR11-KO/HCT116-PRR11-KO cells numbers that migrate across the Transwell polycarbonate filter were significantly fewer than the sw480-control/HCT116-control groups cells, respectively (E1 and F1) show quantitative results using column graph ($P<0.05$). (G and H) Invasion assay shows that the number of sw480-PRR11-KO/HCT116-PRR11-KO cells invading the Transwell polycarbonate filter is significantly fewer than the sw480-control/HCT116-control group cells (G1 and H1). Shown are quantitative evaluation of the results using column graph ($P<0.05$) (J and K, respectively). Wound-healing assay results indicated that the ability of sw480-PRR11-KO/HCT116-PRR11-KO group cells to migrate demonstrated a large decrease compared with the sw480-control/HCT116-control groups in the sw480 and HCT116 cells after 36 h seeding, respectively. (J1 and K1) Show quantitative results using column graph ($P<0.05$).


A
HCT116-PRR11-KO
B
HCT116-PRR11-KO-LV-PRR11
C
HCT116-PRR11-KO
HCT116-PRR11-KO-LV-PRR11
PRR11
40 kDa
β-actin
42 kDa
D
HCT116-PRR11-KO
HCT116-PRR11-KO-LV-PRR11
Optical density (OD)
Time (days)
E
HCT116-PRR11-KO
HCT116-PRR11-KO-LV-PRR11
E1
Colony numbers
HCT116-PRR11-KO
HCT116-PRR11-KO-LV-PRR11

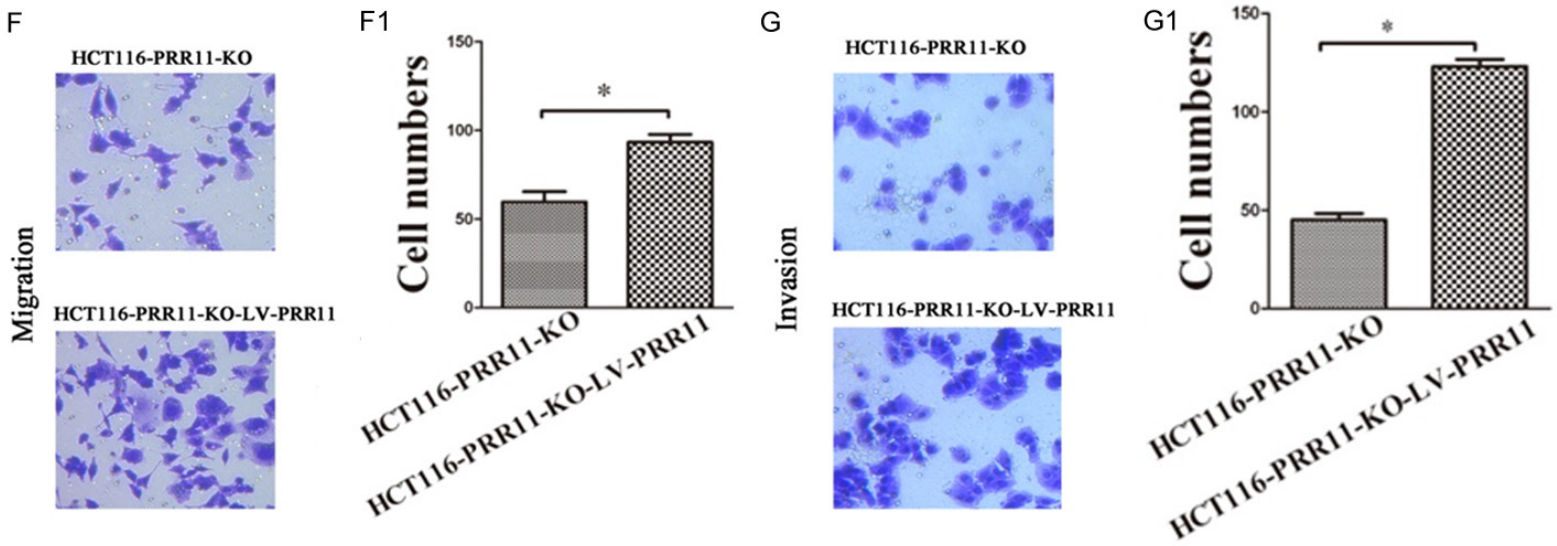


Figure 6. Building the rescue cell line in the HCT116-PRR11-KO cells and detecting biological functions of the rescue cell line. A and B: The efficiency of HCT116-PRR11-KO and HCT116-PRR11-KO-LV-PRR11 cells were greater than 90% under white light and green light visual field of fluorescent microscope respectively. C: Western blotting reveals that PRR11 protein expression is detectable in HCT116-PRR11-KO-LV-PRR11 cells, and HCT116-PRR11-KO cells that do not express PRR11. D: CCK-8 assay results show that the cells proliferation of the HCT116-PRR11-KO-LV-PRR11 group was faster in the colorectal cancer cells ($P<0.05$) compared with HCT116-PRR11-KO group. E and E1: The colony formation assay shows that the ability of proliferation recovers in the HCT116-PRR11-KO-LV-PRR11 cells, compared with HCT116-PRR11-KO group ($P<0.05$). F, F1, G, G1: Migration and invasion assays indicate that the numbers of cell penetrating the basal membrane from the HCT116-PRR11-KO-LV-PRR11 group were notably more than the HCT116-PRR11-KO group ($P<0.05$).

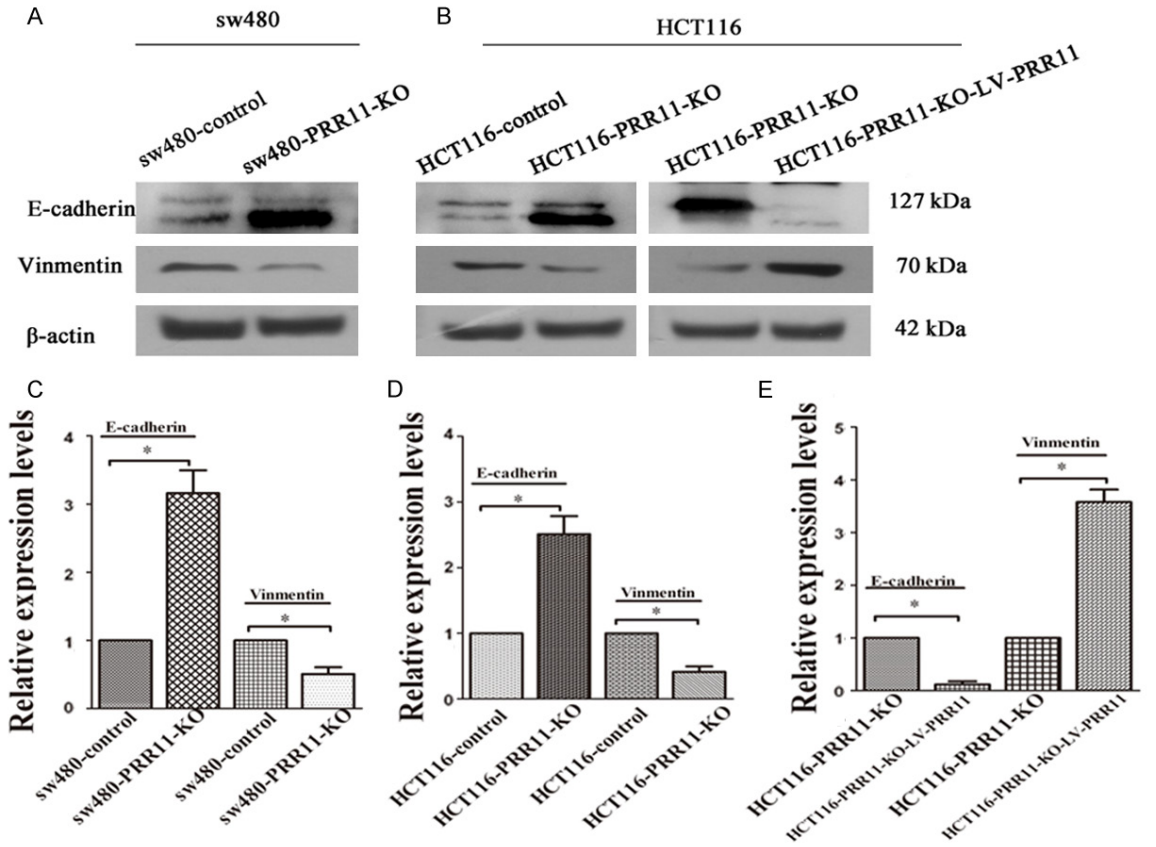


Figure 7. Assessment in the changes of E-cadherin and Vimentin protein levels (A and left of B). Western blot analysis revealed that the E-cadherin protein levels increase in both sw480-PRR11-KO and HCT116-PRR11-KO cells when compared with the sw480-control and HCT116-control cells, respectively (P<0.05). The Vimentin protein levels decrease in both sw480-PRR11-KO and HCT116-PRR11-KO cells when compared with the sw480-control and HCT116-control cells, respectively (P<0.05). The right of (B) The E-cadherin protein expression levels are lower in HCT116-PRR11-KO-LV-PRR11 cells when compared with HCT116-PRR11-KO cells. By contrast, the Vimentin protein levels increase in the HCT116-PRR11-KO-LV-PRR11 cells when compared with HCT116-PRR11-KO cells (P<0.05). (C-E) show quantitative results using column graph (P<0.05).

116-PRR11-KO group (**Figure 6E**, **6E1**) (P< 0.05). The in vitro migration and invasion assays indicated that the numbers of cells that penetrated the basal membrane from the HCT116-PRR11-KO-LV-PRR11 group were notably higher than the HCT116-PRR11-KO group (**Figure 6F**, **6F1**, **6G**, **6G1**). Taken together, these results suggest that the recovery of expressed PRR11 protein is very vital for function restoration in the HCT116 cells.

PRR11 affects the landmark proteins E-cadherin and vimentin and expression of EMT in sw480 and HCT116 cells.

To investigate the effects of PRR11 on the proliferation, migration, and invasion of colorectal cancer cells, we assessed the changes in the protein levels of E-cadherin and vimentin. Both E-cadherin and vimentin are the landmark proteins of EMT. Western blot analysis revealed that the E-cadherin protein levels increase in both sw480-PRR11-KO and HCT116-PRR11-KO cells compared with the sw480-control and HCT116-control cells, respectively (P<0.05). On the contrary, the vimentin protein levels decrease in both sw480-PRR11-KO and HCT116-PRR11-KO cells when compared with the sw480-control and HCT116-control cells, respectively (P<0.05). In addition, the E-cadherin expression levels are lower in HCT116-PRR11-KO-LV-PRR11 cells than in HCT116-PRR11-KO cells, whereas the vimentin levels increased in HCT116-PRR11-KO-LV-PRR11 cells compared with HCT116-PRR11-KO cells (P<0.05) (**Figure 7**). This finding indicates that PRR11 affects the biological function of colorectal cancer cells via EMT.

Discussion

PRR11 gene is a relatively new gene that was identified in chromosome 17q22. The PRR11 protein is associated with cell-regulated protein degradation. Thus, PRR11 is a tumor-control gene [6]. However, little is known about its distribution and clinical value in colorectal cancer. In the current study, immunohistochemical methods established the relationship between expression levels of PRR11 and clinicopathological parameters and prognosis in patients with colorectal cancer by evaluating the PRR11 expression levels in colorectal cancer tissues. We also explored the roles of PRR11 in promoting cell proliferation, migration, and invasion of colorectal cancer and the mechanistic effects of PRR11 on the changes of biological functions in colorectal cancer cells.

In our study, we detected higher expression levels of PRR11 in human colorectal cancer tissues than in normal colorectal tissues. The high expression level of PRR11 is significantly associated with clinical stage, lymph node metastasis, and histological grade. The 5-year survival rate and prognosis of the low PRR11 expression group are remarkably better than those of the high PRR11 expression group. Chen Ying et al. [8] previously reported that PRR11 was overexpressed in colorectal cancer tissues and indicated the significant differences of PRR11 expression levels between normal tissues and colorectal cancer tissues. However, information about the roles of PRR11 lacks details in the previous literature. In addition, high expression levels of PRR11 were only reported in some tumors, such as gastric cancer [7, 13], lung cancer [6], and breast cancer [9].

However, the roles of PRR11 in colorectal cancer remain unclear. The CRISPR system consists of regularly interspaced short palindromic repeat sequences and the Cas gene in bacterium and archaea, which were thought to comprise the adaptive immune system of bacterium [14]. The CRISPR/Cas9 system offers users the greatest ease and modularity for design and execution of any genetic engineering experiments [15-18]. The CRISPR/Cas9 system can edit any site of DNA sequence as long as the sequence follows the NGG (PAM) locus. Given that NGG is abundant in the human genome, the CRISPR/Cas9 system can edit all human genes [19]. Compared with RNAi technology, CRISPR/Cas9 can completely and permanently knockout the target gene [20]. In the present study, we constructed the CRISPR/Cas9 system targeting the PRR11 gene. We transfected a single-cell colony by using CRISPR/Cas9 plasmids, resulting in undetectable levels of PRR11 upon Western blot analysis.

To date, the role of PRR11 in colorectal cancer cells in vitro has not been reported. Thus, we confirmed that PRR11-KO suppresses cell proliferation, migration, and invasion in sw480 and HCT116 cells. Furthermore, we recovered the PRR11 protein expression by using lentivirus-mediated overexpression vectors in the PRR11-KO HCT116 cells and found that HCT116 cells regain their ability of cell proliferation, migration, and invasion. These data suggest that PRR11 promotes the tumor progression of colorectal cancer. This finding is consistent with a previous study by Song Z et al. [13], who reported that silencing the PRR11 gene made the gastric cancer cells proliferate and grow slowly. Zhao Q [21] revealed that the depletion of PRR11 leads to a dramatic massive apoptotic cell death, resulting in a significant decrease in growth and viability of lung cancer cell lines. Knockdown of PRR11 gene also reduces the proliferation, migration, and invasion of hilar cholangiocarcinoma and breast cancer cells [8, 9]. These studies demonstrate that PRR11 is crucial in the growth, migration, and invasion of colorectal cancer cells.

EMT is not only an important mechanism for tumor invasion and metastasis ability of cancer cells but also in the conferring apoptotic resistance and stemness [22]. EMT also alters E-cadherin and vimentin proteins [22, 23]. In breast cancer, silencing PRR11 in metastatic breast tumor cells promotes a shift toward an epithelial morphology concomitant with elevated with increased E-cadherin expression and decreased mesenchymal marker-vimentin expression [9]. The expression of vimentin mRNA and protein is also downregulated in cholangiocarcinoma from silencing of PRR11, indicating a potential role of PRR11 in the initiation of EMT, which is supported by the finding indicating that the knockdown increases the expression of E-cadherin [8]. Hence, we assessed the changes of E-cadherin and vimentin levels in the present study. E-cadherin protein levels increase in both sw480-PRR11-KO and HCT-

116-PRR11-KO cells, whereas the vimentin protein levels decreased in both sw480-PRR11-KO and HCT116-PRR11-KO cells. By contrast, the vimentin protein levels are more abundant in HCT116-PRR11-KO-LV-PRR11 cells than in HCT116-PRR11-KO cells. This finding suggests that PRR11 possibly affects the biological function of colorectal cancer cells through EMT mechanisms.

In conclusion, our study has shown that PRR11 expression levels are significantly higher in colorectal cancer tissues than in normal colorectal samples, and the high expression of PRR11 is associated with poor prognosis of patients with colorectal cancer. Additionally, we used the CRISPR/Cas9 system to successfully construct the targeted PRR11 gene of PX459 vectors and obtained the sw480 and HCT116 cells of PRR11 gene knockout. Furthermore, we provided evidence indicating that PRR11 knockout reduced the proliferation, migration, and invasion of sw480 and HCT116 colorectal cancer cells. HCT116-PRR11-KO cells can regain proliferation, migration, and invasion only after recovering PRR11 protein expression. We also proved that silencing PRR11 in colorectal cancer cells increased the expression of E-cadherin and decreased the expression of mesenchymal marker-vimentin. Hence, our data demonstrated that PRR11 promotes the growth and progress of colorectal cancer through the possible mechanisms of EMT.

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Disclosure of conflict of interest

None.

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