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ORIGINAL RESEARCH

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Knockdown of PRR14 inhibits the growth and migration of gastric cancer cells

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Abstract

Background: Although proline-rich protein (PRR) 14 has been implicated in various malignancies, its involvement in stomach cancer is yet unclear. In order to improve therapeutic approaches for gastric cancer, this study intends to explore the ways in which PRR14 controls the growth and metastasis of gastric cancer cells. **Methods**: PRR14 expression levels in gastric cancer were analyzed using the The University of Alabama at Birmingham CANcer data analysis Portal (UALCAN) database, while the Kaplan-Meier Plotter database was used to evaluate the impact of PRR14 expression on overall survival in gastric cancer patients. After PRR14 knockdown, cell proliferation was evaluated using colony formation and cell counting kit-8 (CCK8) assays. Using flow cytometry, the rates of cell apoptosis were determined. Cell migration and invasion were assessed using transwell and wound healing assays. Protein expression in the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway was evaluated by Western blotting. **Results**: Significant expression of PRR14 was discovered in stomach cancer tissues, and it was associated with poor prognosis. Knockdown of PRR14 significantly reduced cell viability, colony formation, migration and invasion, while promoting apoptosis in gastric cancer cells. Furthermore, the expression levels of p-PI3K and p-Akt were markedly decreased following PRR14 knockdown. **Conclusions**: PRR14 knockdown inhibits the proliferation, migration and invasion of gastric cancer cells while promoting apoptosis, possibly via blocking the PI3K/Akt signaling pathway. According to these results, PRR14 might be a viable target for treatment in gastric cancer.

Keywords

Gastric cancer; PRR14; Proliferation; Migration; PI3K/Akt

1. Introduction

One of the most prevalent malignant tumors in the world is gastric cancer (GC). It originates from the gastric mucosal epithelium, and is commonly associated with genetic predisposition, chronic atrophic gastritis and *Helicobacter pylori* infection [1]. The incidence of GC is significantly higher in men than in women, a pattern observed globally. These similarities make early detection challenging, and many cases are diagnosed only after metastasis has occurred [2]. Despite continuous improvements in surgical, chemotherapy, radiation and immunotherapy techniques, the prognosis for stomach cancer is still dismal because of the limited effectiveness of these treatments [3]. Therefore, the identification of novel therapeutic targets is crucial for improving outcomes in gastric cancer research and treatment. A member of the proline-rich protein (PRR) family, proline-rich protein 14 (PRR14) has nuclear localization signals at both its N- and C-termini in addition to a proline-rich domain [4]. The development of several types of cancer has been linked to PRR14. In squamous cell carcinoma, it has been determined that PRR14 is a possible therapeutic target and prognostic biomarker [5]. Similarly, in lung cancer, PRR14 could enhance cell proliferation, an effect that can be suppressed through PRR14 knockdown. In lung cancer, PRR14 has also been found to act on the PI3K pathway [6].

The development of many malignancies, including gastric cancer, is linked to the PI3K/Akt pathway. Regulation of this pathway can significantly influence the invasion, metastasis and proliferation of gastric cancer cells [7].

There has been comparatively little research done on PRR14's function and related pathways in gastric cancer. According to The Cancer Genome Atlas, PRR14 is substantially overexpressed in gastric cancer and is associated with poor prognosis for patients. Functional studies showed that PRR14 knockdown causes apoptosis and prevents gastric cancer cells from proliferating, migrating, and invading. The PI3K/Akt signaling pathway may be regulated to mediate these effects.

2. Methods

2.1 Bioinformatics analysis

UALCAN is an online platform used for analyzing cancer omics data, primarily for evaluating gene expression profiles in publicly available databases such as The Cancer Genome Atlas (TCGA). In this study, the UALCAN database was used to assess PRR14 expression levels in gastric cancer tissues. The Kaplan-Meier Plotter is an integrated online tool that combines clinical and gene expression data from multiple public sources, including TCGA, Gene Expression Omnibus (GEO) and European Genome-phenome Archive (EGA), to evaluate the prognostic relevance of specific genes. In this study, the relationship between PRR14 expression and overall survival in patients with gastric cancer was examined using the Kaplan-Meier Plotter.

2.2 Cell culture and transfection

Roswell Park memorial institute (RPMI)-1640 media supplemented with 10% fetal bovine serum (FBS) and 1% double resistance was used to cultivate the human gastric adenocarcinoma cell line (AGS and MKN45), which were verified to be contamination-free by short tandem repeat (STR) profiling. The cells were kept at 37 °C with 5% CO_2. 24 hours before transfection, AGS/MKN45 cells were seeded into 6-well plates (2×10^5/well) to ensure that the cell density reached 60–70% confluence at the time of transfection. Transfection complexes were prepared by placing a mixture of 50 pmol of siRNA targeting PRR14: (si-PRR14#1: AAGGTTTCTCGATTCAGAATACG; si-PRR14#2: AAGCTTACAGGGGAAGGAAAAGC) or negative control siRNA: (si-control (NC): UUCUCCGAACGUGUCACGU) was mixed with Lipofectamine 3000. The resulting transfection complexes were added dropwise to the 6-well plates, and the transfection efficiency and downstream assays were conducted 48 hours post transfection.

2.3 Cell Counting Kit-8 (CCK8) assay

Five replicate wells per group were created by seeding cells at a density of 2×10^3 cells per well onto 96-well plates. The plates were incubated for 24, 48, 72 and 96 hours at 37 °C in a humidified environment with 5% CO_2. Ten microliters of CCK8 solution were combined with new culture media at each time point to replace the aspirated culture medium. After that, the plates were incubated for two hours at 37 °C in the dark. A microplate reader was used to detect absorbance at 450 nm in order to assess cell viability.

2.4 Colony formation

Following transfection, cells were digested with trypsin, neutralized with complete medium and resuspended as a single-cell suspension. To enable colony development, 500 cells were cultured for 14 days. After being fixed for 15 minutes at room temperature with 4% paraformaldehyde, colonies were dyed with 0.1% crystal violet for 30 minutes. After staining, excess dye was washed off with water and colonies were counted manually. A colony was defined as a cluster containing at least 50 cells.

2.5 Flow cytometry

To create a single-cell suspension, cells were harvested using 0.25% trypsin without ethylenediaminetetraacetic acid (EDTA). The supernatant was then removed by centrifuging the cells after they had been twice washed with cooled phosphate-buffered saline (PBS). Following a resuspension in 100 μL of 1× Binding Buffer, the concentration of cells was adjusted to 1×10^6 cells/mL. Subsequently, each sample received 5 μL of Annexin V-fluorescein isothiocyanate (FITC) and 5 μL of propidium iodide (PI). After giving the combination a gentle vortex, it was allowed to sit at room temperature for fifteen minutes in the dark. Following incubation, the samples were placed on ice and shielded from light before Binding Buffer was added. The apoptotic rate was measured immediately.

2.6 Wound healing assay

After being collected and counted, transfected cells were planted at a density of 5×10^5 cells per well into 6-well plates. The cells were cultivated for 24 to 48 hours at 37 °C until 90 to 100% of them formed a confluent monolayer. A marker was used to create a straight reference line on the underside of each well. A sterile 200 μL pipette tip was then used, held perpendicular to the plate surface, to gently scratch the monolayer along the reference line, creating a wound of uniform width. Detached cells were removed by gently rinsing with PBS three times. The culture medium was then replaced with serum-free RPMI-1640 to minimize the influence of cell proliferation on migration. Baseline images (0 h) of 3–5 fixed fields of view were captured immediately after scratching using an inverted microscope. The plates were then returned to the incubator, and images of the same fields were taken again after 24 hours to evaluate wound closure.

2.7 Transwell assay

After being broken down and reconstituted in serum-free media, the transfected cells' concentration was brought down to 5×10^5 cells/mL. The cell solution is located in the Transwell chamber's upper chamber. The lower chamber is filled with full culture medium that contains 10% fetal bovine serum. For 24 to 48 hours, the cells were cultured at 37 °C. A cotton swab was used to carefully remove any non-migrated or non-invaded cells that remained on the membrane's upper surface after incubation. Following a PBS wash, the cells were fixed with 4% paraformaldehyde for 30 minutes and stained for 20 minutes with 0.1% crystal violet. The plates were allowed to air dry after any excess stain was removed by rinsing them with PBS until the background was clear. ImageJ software was used to quantify the number of cells that had invaded or moved through the membrane after five randomly selected fields of view were photographed under a microscope.

2.8 Western blotting

After lysing the cells with radioimmunoprecipitation assay (RIPA) buffer, they were incubated for half an hour on ice. After sonicating the lysates to guarantee total cell destruction. Protein samples were standardized to equal quantities using

lysis buffer and then denatured for five minutes in a boiling water bath. Electrophoresis was used to separate protein that were put onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. Proteins were then applied to polyvinylidene fluoride (PVDF) membranes. The primary antibodies were incubated at 4 °C for the entire night after the membranes were blocked for an hour at room temperature using 5% skim milk. After cleaning the membranes, horseradish peroxidase (HRP)-conjugated secondary antibodies were added and allowed to sit at room temperature for an hour. A chemiluminometer and an enhanced chemiluminescence (ECL) chemiluminescence detection kit were used to photograph the protein bands. ImageJ software was used to quantify band intensities. The following primary antibodies were used: PRR14 (NovoPro, Shanghai, China, #114242, 1:1000), p-Akt (Cell signaling, Danvers, MA, USA, #13038, 1:1000), Akt (Cell signaling, Danvers, MA, USA, AF3242, 1:1000), PI3K (Affinity, Cincinnati, OH, USA, AF6241, 1:1000) and β-actin (Affinity, Cincinnati, OH, USA, AF7018, 1:3000).

2.9 Statistical analysis

SPSS version 22.0 (IBM Corp, Armonk, NY, USA) was used for all data analysis, one-way analysis of variance (ANOVA) for comparisons between several groups. Every experiment was independently conducted at least three times. The statistics are shown as the mean $\pm$ standard deviation (SD), with a p-value of less than 0.05 considered statistically significant.

3. Results

3.1 PRR14 is highly expressed in gastric cancer

PRR14 expression patterns were examined by analyzing data from the UALCAN database. In contrast to normal gastric tissues, the data demonstrated that PRR14 was markedly elevated in gastric cancer tissues (Fig. 1A). In addition, patients with high PRR14 expression had a substantially poorer overall survival rate, according to survival analysis performed using the Kaplan-Meier Plotter database (Fig. 1B). These results raise the possibility that PRR14 could be a prognostic biomarker for gastric cancer.

3.2 Knockdown of PRR14 suppressed the growth of gastric cancer cells

Western blot analysis confirmed reduced PRR14 protein levels in both si-PRR14#1 and si-PRR14#2 groups, with si-PRR14#1 showing a more efficient knockdown (Fig. 2A). After PRR14 knockdown, the CCK8 assay showed markedly reduced cell viability in AGS and MKN45 cells (Fig. 2B). As demonstrated by colony formation assays, the si-PRR14#1 and si-PRR14#2 groups consistently had significantly fewer colonies than the si-NC group (Fig. 2C). These results indicate that PRR14 knockdown suppressed the growth of gastric cancer cells.

3.3 PRR14 knockdown increased the apoptosis of gastric cancer cells

In contrast to the si-NC group, the si-PRR14#1 and si-PRR14#2 groups exhibited significantly increased apoptotic rates of MKN45 and AGS cells (Fig. 3). This suggests that PRR14 knockdown enhanced apoptosis in gastric cancer cells.

3.4 Knockdown of PRR14 inhibited migration and invasion of gastric cancer cells

Following si-PRR14 transfection, Transwell and wound healing tests were used to evaluate cell migration and invasion. The ability of AGS and MKN45 cells to migrate and invade was markedly reduced by PRR14 knockdown. Wound healing assays revealed that the si-PRR14#1 and si-PRR14#2 groups had wider scratches (Fig. 4A). Similarly, the Transwell assays revealed a decreased number of invaded cells in the PRR14-silenced groups (Fig. 4B).

3.5 PI3K/Akt signaling pathway downregulation by PRR14 knockdown

The expression of important proteins in the PI3K/Akt signaling pathway was evaluated using Western blot. The results showed that the p-PI3K and p-Akt expression of the si-PRR14#1 and si-PRR14#2 groups were significantly lower than those of the si-NC group (Fig. 5). According to these findings, PRR14 knockdown prevented the PI3K/Akt pathway from being activated.

4. Discussion

Because of risk factors like smoking, drinking alcohol, males are more prone than women to acquire GC. The treatment of gastric cancer has entered a new era of comprehensive gastric care management, emphasizing both precision and standardization. In this context, molecular classification and personalized treatment, particularly gene-targeted therapies are showing significant therapeutic promise. Nevertheless, the identification of novel therapeutic targets with clinical translational potential remains a major goal and ongoing challenge in gastric cancer research.

Based on analysis of the TCGA and Kaplan-Meier Plotter databases, this study showed that A poor patient prognosis is associated with increased expression of PRR14, which is highly expressed in gastric cancer tissues. Mechanistically, PRR14 may accelerate the growth of stomach cancer by encouraging cell invasion, migration and proliferation. Therefore, a viable treatment approach to enhance outcomes for patients with gastric cancer may involve targeting PRR14 or its downstream signaling pathways.

Abnormal, unchecked growth is one of the basic traits of cancer cells. The formation of malignant tumors is characterized by a breakdown of the dynamic equilibrium between the proliferation and death of tumor cells [8]. Invasion and migration, recognized as the “cardinal hallmarks of cancer”, are particularly critical in driving metastasis and significantly contribute to cancer-related mortality, especially in GC [9].

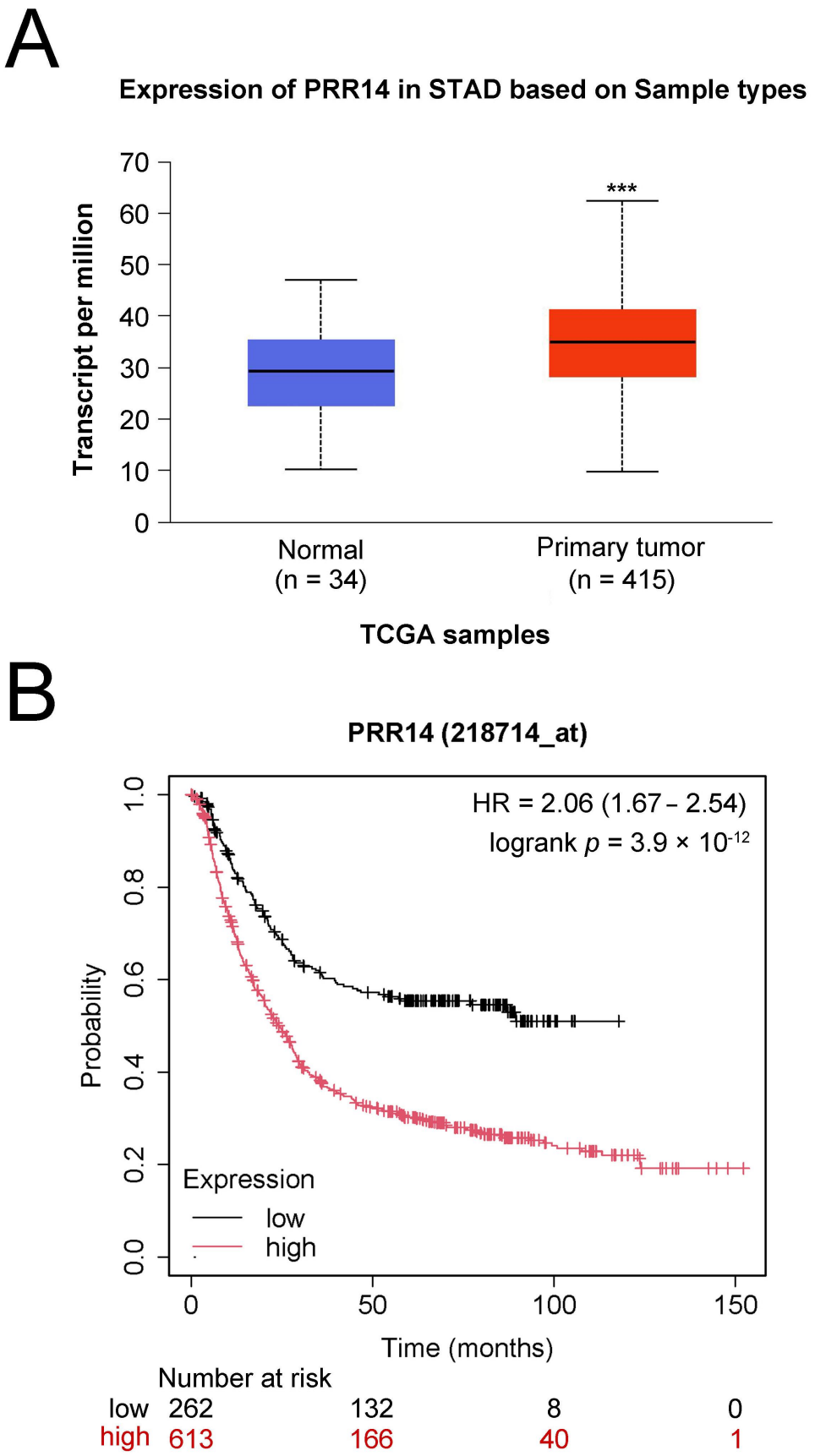

FIGURE 1. PRR14 is highly expressed in gastric cancer. (A) Query the expression of PRR14 in gastric cancer using the UALCAN database. (B) The Kaplan-Meier Plotter database shows PRR14 expression's effect on patients' overall survival with gastric cancer. *vs.* Normal, ***: $p < 0.001$. PRR14: proline-rich protein 14; TCGA: The Cancer Genome Atlas; STAD: stomach adenocarcinoma; HR: hazard ratio.

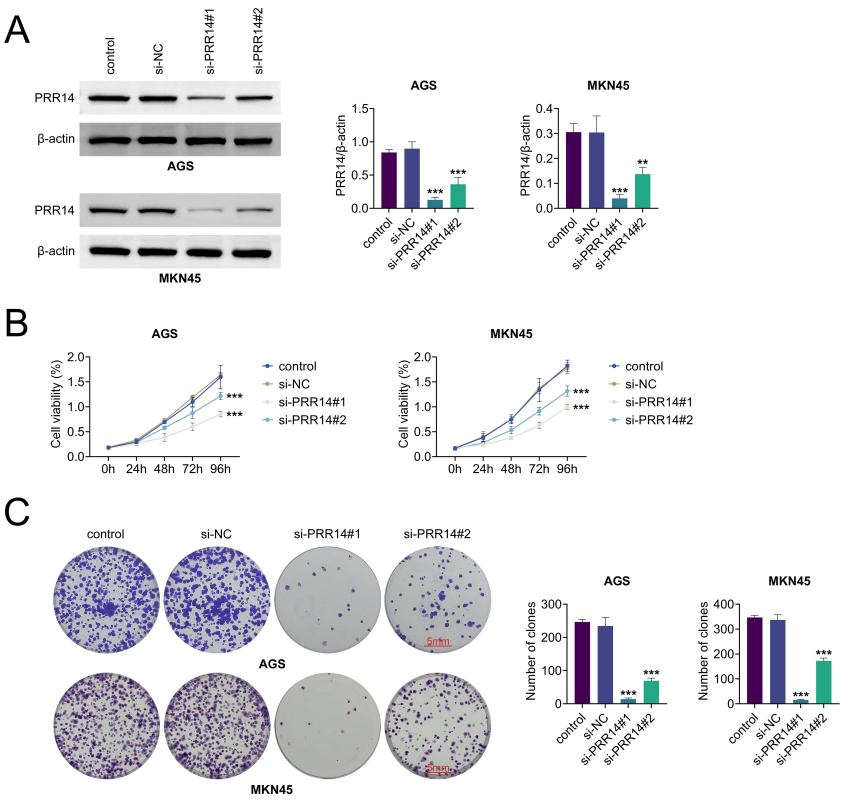

FIGURE 2. Knockdown of PRR14 suppressed the growth of gastric cancer cells. (A) Protein expression of PRR14 in cells transfected with si-PRR14 was detected by western blot. (B) CCK8 was used to detect the cell viability of cells transfected with si-PRR14 at different time points. (C) The number of crystal violet in cells transfected with si-PRR14 was detected by clone formation assay. *vs.* control, **: $p < 0.01$, ***: $p < 0.001$. si-NC: si-control; PRR14: proline-rich protein 14; β-actin: beta-Actin.

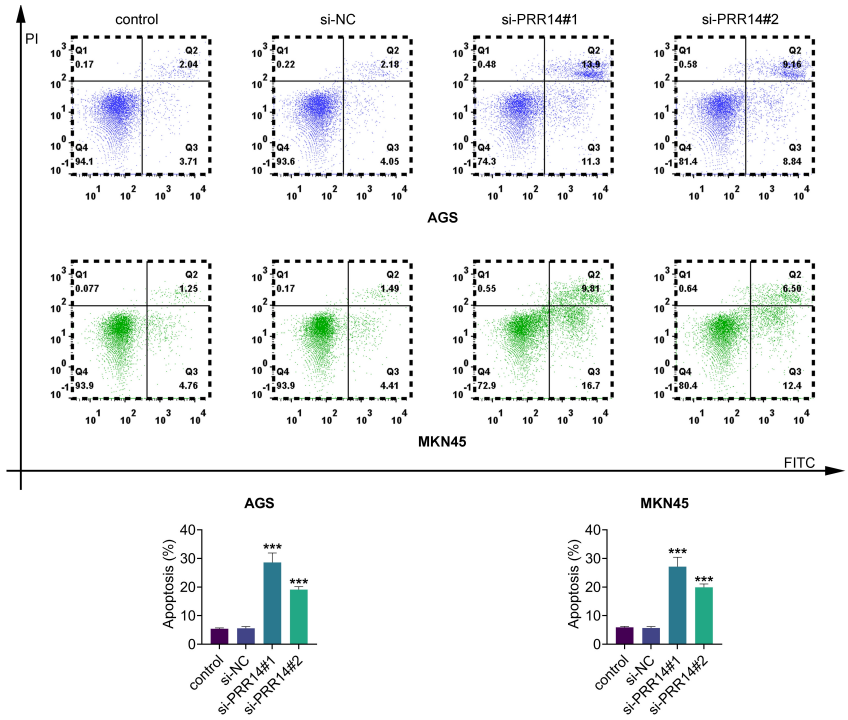

FIGURE 3. Knockdown of PRR14 promoted apoptosis of gastric cancer cells. Following si-PRR14 transfection, the apoptotic rate of the cells was measured by flow cytometry. *vs.* control, ***: $p < 0.001$. si-NC: si-control; PRR14: proline-rich protein 14; PI: propidium iodide; FITC: fluorescein isothiocyanate.

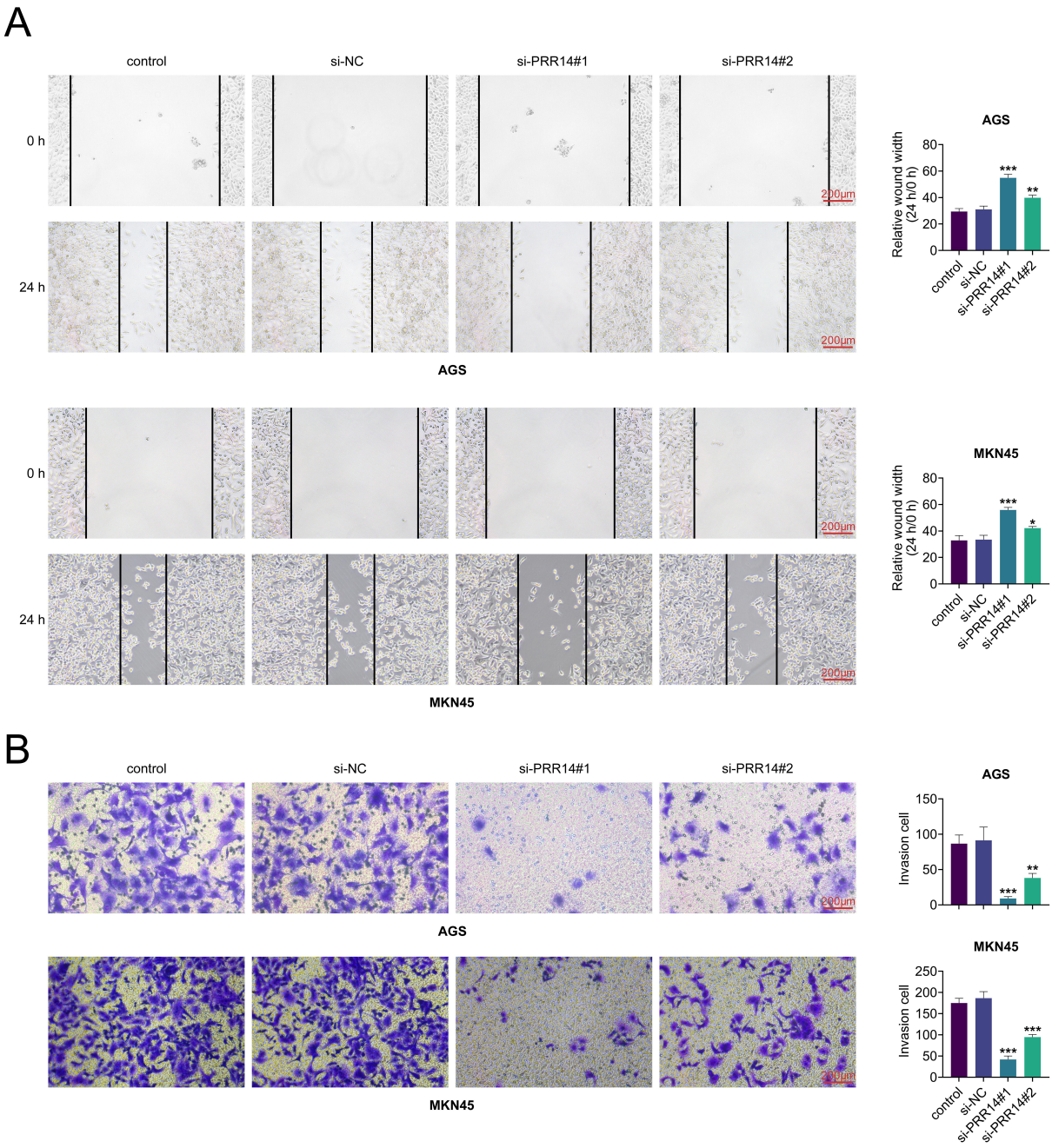

FIGURE 4. Knockdown of PRR14 inhibited migration and invasion of gastric cancer cells. (A) Assay to identify cell migration following si-PRR14 transfection. (B) Transwell assay to detect the invasion of cells after transfection with si-PRR14. *vs.* control, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. si-NC: si-control; PRR14: proline-rich protein 14.

To examine PRR14's function in the biological activity of gastric cancer cells, si-PRR14 was transfected into AGS and MKN45 cells. Functional assays demonstrated that PRR14 knockdown enhanced apoptosis and inhibited cell proliferation, migration, and invasion. These findings are in line with previous studies implicating PRR14 in tumor progression. Tumor node metastasis (TNM) stage, distant metastasis, and tumor size were all positively connected with PRR14 expression, which was shown to be substantially expressed in colon cancer tissues. PRR14 knockdown inhibited colon cancer cells' migration, invasion, proliferation, p-Akt levels, production of cell cycle-related proteins and epithelial-mesenchymal transition (EMT) processes, according to functional assessments [10].

Many oncogenes and growth factor receptors can promote the PI3K/Akt signaling pathway, which is commonly active in cancer. By phosphorylating downstream targets, this route regulates cell survival, proliferation and metabolism [11]. In the present study, si-PRR14 transfection dramatically reduced the expressions of p-PI3K and p-Akt in AGS and MKN45 gastric cancer cells, suggesting that PRR14 knockdown inhibited the PI3K/Akt pathway. These results are consistent with earlier research demonstrating that PRR14 activates the PI3K/Akt pathway to inhibit apoptosis and promote proliferation in breast cancer cells [12].

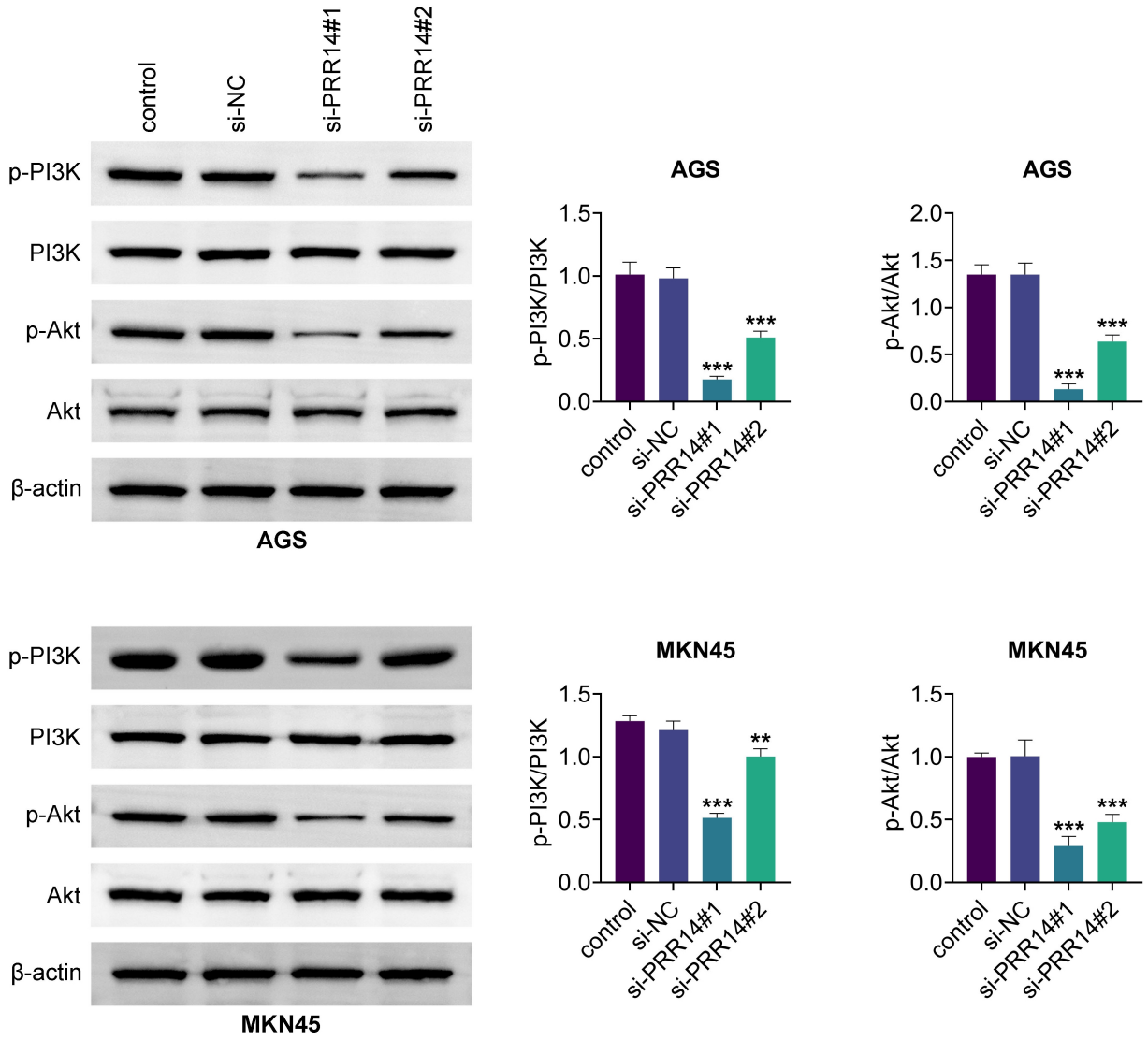

FIGURE 5. Knockdown of PRR14 downregulated the PI3K/Akt signaling pathway. Proteins linked to the PI3K/Akt pathway (p-PI3K, PI3K, p-Akt and Akt) were analyzed by Western blot in gastric cancer cells transfected with si-PRR14 in comparison to the si-NC group. **: $p < 0.01$, ***: $p < 0.001$. si-NC: si-control; PRR14: proline-rich protein 14; PI3K: phosphatidylinositol 3-kinase; Akt: protein kinase B; β-actin: beta-Actin.

5. Conclusions

The biological role of PRR14 in gastric cancer was validated by this study, and it offered a possible guideline for gene-targeted treatment, especially in male patients who were at high risk for the disease. However, this study has several limitations. Due to practical constraints, tumor tissues from gastric cancer patients were not collected to validate PRR14 expression in clinical samples. Additionally, no *in vivo* experiments were conducted to confirm the functional role of PRR14 in animal models. These limitations will be addressed in future studies.

AVAILABILITY OF DATA AND MATERIALS

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

AUTHOR CONTRIBUTIONS

MZY, HG—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. MZY, FT, JL, XMY, HG—supervised the data collection; analyzed the data; interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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