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Original Article

High levels of P2RX5 expression predicts a poor prognosis and promotes tumor progression in endometrial cancer

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Abstract: Objective: This study aims to investigate the impact of P2RX5 on the clinical pathological characteristics and prognosis of endometrial carcinoma, and to explore its potential underlying mechanisms. Methods: The clinical samples, including 150 specimens with endometrial carcinoma (Case group) and 100 endometrial samples without cancer (Control group), were collected for analyzing the amount of P2RX5 expression. Cell biology experiments were also used for exploring the effects of P2RX5 expression on biological behaviors of endometrial carcinoma. Results: The results showed that P2RX5 expression in endometrial carcinoma tissues were significantly higher than in endometrial tissues from the control group. Additionally, expression levels showed a positive correlation with tumor size, differentiation grade, and tumor stage, with risk values of 3.57, 6.07, and 9.78, respectively. This increase in risk was statistically significant ($P < 0.001$). Kaplan-Meier survival analysis demonstrated that increasing P2RX5 expression was associated with lower overall survival rates ($P < 0.05$). Functional assays showed that knocking-down P2RX5 expression in the HEC-1B cell line significantly reduced the capacity of cell proliferation, migration, invasion, and clone formation. Following gene knockout, the rate of late apoptosis in tumor cells significantly increased ($P < 0.001$), while the number of cells in the S phase and G2/M phase significantly decreased ($P < 0.05$). Conclusion: These findings suggest a critical role of P2RX5 in endometrial cancer progression and thereby establish the value of P2RX5 as a prognostic biomarker and provide a promising therapeutic target for endometrial cancer treatment.

Keywords: P2RX5, endometrial carcinoma, clinicopathological features, prognosis

Introduction

With the improvement in quality of life, the incidence of endometrial cancer has been gradually increasing both domestically and internationally, becoming one of the most common malignant tumors of the reproductive system that threaten women's health and lives [1]. Previous studies on endometrial cancer have revealed some of the disease's pathogenesis, yet many mechanisms remain unclear. Therefore, further investigation into the mechanisms underlying its occurrence and progression holds significant implications for the treatment of this disease.

Tumor diseases have long plagued humanity. In numerous early studies, many molecules associated with malignant tumorigenesis have been identified. Among these, the purinergic receptor (P2X), a ligand-gated cation channel activated by extracellular adenosine triphosphate (ATP), participates in key processes of cellular energy metabolism. It has been demonstrated to be a crucial component of the tumor microenvironment and garnered extensive academic attention [1].

Recent studies have revealed that purinergic receptors can influence the tumor microenvironment by regulating intracellular and extracellular concentrations as well as their own conformational changes, thereby modulating antitumor immune responses. This mechanism significantly impacts tumor cell proliferation and migration processes, suggesting the criti-

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cal role of purinergic receptors in tumor biology and their value as potential therapeutic targets [1, 2]. P2RX5, as a member of the P2X receptor family, has been shown in studies to exhibit significantly elevated expression levels in tumors such as breast cancer, colorectal cancer, thyroid cancer, and chordoma, and it is capable of promoting the progression of these malignant tumors [3-5].

Despite these insights gained from previous studies, the specific role and clinical significance of P2RX5 in the pathogenesis of endometrial cancer remain largely unknown, representing a gap in current research. Elucidating the role of P2RX5 in endometrial cancer may identify molecular biomarkers associated with clinical outcomes, thereby advancing early diagnosis and personalized treatment to improve patient prognosis. This study aims to compare the expression levels of P2RX5 in endometrial carcinoma tissue versus normal endometrial tissue. By integrating bioinformatics tools, statistical analysis, and in vitro experiments, we elucidate the expression profile of P2RX5 in endometrial carcinoma and its correlation with clinical pathological characteristics. Second, exploring the effects of modulating P2RX5 expression on the functional state of endometrial carcinoma cells aims to deepen our understanding of endometrial carcinoma biology and may contribute to the development of targeted therapeutic strategies utilizing P2RX5 as a biomarker and therapeutic target.

Materials and methods

Study subjects

The study obtained ethical approval from the hospital's ethics committee (NO. 2024110101). This is a hospital-based retrospective study, including 150 cases with endometrial carcinoma (case group) and 100 controls without any cancers. All study subjects were admitted to the Affiliated Hospital of Youjiang Medical University for Nationalities from January 2019 to August 2022. Inclusion criteria for case group: *a.* cases with histopathologically-confirmed endometrial carcinoma; *b.* cases without antitumor treatments (including chemoradiotherapy or hormone therapy) before receiving surgery treatment; *c.* cases with complete clinicopathological data; and *d.* patients who gave informed consent to participate in the study. Exclusion criteria for the case group: *a.* cases with history of other tumors; *b.* cases with insufficient or unavailable paraffin-embedded tissues; *c.* cases with mid-study dropout. All selected cases' clinical and pathological data were collected, including age, ethnicity, personal history, medical history, treatment history, tumor size, tumor differentiation, and staging. Tumor was classified and staged in accordance with the International Federation of Gynecology and Obstetrics (FIGO) system, and survival follow-ups were performed on all patients.

In this study, all subjects from the control group were treated for uterine adenomyosis or uterine fibroids in the same institution within the same period. Inclusion criteria for the control group: *a.* individuals with sufficient paraffin-embedded endometrial tissue; *b.* individuals without the history of any tumors; *c.* individuals without the history of radiotherapy or chemotherapy; and *d.* individuals who gave informed consent to participate in the study. Exclusion criteria for the control group: *a.* individuals with incomplete demographic data; and *b.* individuals with mid-study dropout.

Immunohistochemical staining (IHC)

The paraffin blocks of 150 cases of endometrial carcinoma tissues and 100 cases of control tissues were frozen at -20°C for 10 to 15 minutes. Continuous sections with a thickness of about 4 μm were then prepared using a Leica rotary microtome. After drying the sections at 70°C for 60 minutes, they were dewaxed three times in xylene for 5 minutes each, followed by hydration in 100%, 95%, and 75% ethanol sequentially. Heat-induced antigen retrieval was performed for 2 minutes using EDTA solution, followed by blocking endogenous peroxidase at room temperature with 3% H_2O_2 for 10 minutes. Diluted primary antibody P2RX5 (1:600, Wuhan Shuangxuan Biotechnology) was added and incubated at 37°C for 1 hour, followed by incubation with MaxVision-HRP secondary antibody (Maxin Biology) at room temperature for 15 minutes. After DAB staining (1:20, Maxin Biology), hematoxylin re-staining was performed for 2 minutes, followed by differentiation with hydrochloric acid alcohol, treatment with blueing solution, gradient dehydration with alcohol, and transparency with xylene, finally sealing with neutral gum. Image acquisi-

Table 1. Details of P2RX5 knockdown

Gene Names and IDs	5' to 3' sequence
LV3-P2RX5-Homo-903	GGGTGGCGTGATAGGAATTAA
LV3-P2RX5-Homo-984	TAGCCGTCTGGACAATAAACT
LV3-P2RX5-Homo-288	ATGGGTGTTCCTGATAAAGAA
LV3-NC	TTCTCCGAACGTGTCACGT

tion was performed using an OLYMPUS fluorescence microscope.

The amount of P2RX5 protein expression was quantitatively assessed using the staining reaction integral score (IRS), calculated by multiplying staining intensity (SD) and positive cell ratio (SP). SD was scored as 0 (no staining), 1 (light yellow), 2 (brownish yellow), and 3 (brown). SP was scored as 0 (0%), 1 (≤ 25%), 2 (26-50%), 3 (51-75%), and 4 (> 75%). Two senior pathologists scored independently, and the average was used as the final IRS. Expression levels were classified by median as low (IRS ≤ 2), medium (2 < IRS < 5), and high (IRS ≥ 5).

Cell culture

The HEC-1B cell line, an adherent growth endometrial carcinoma cell line, was procured from Wuhan Punosai Life Science Technology Co., Ltd. The cells (including cell lines with or without P2XR5 knockdown) were cultured in DMEM medium supplemented with 10% FBS and 1% P/S, at 37°C and 5% CO_2 in a constant temperature incubator.

Bioinformatics analysis

To investigate the different expression of P2RX5 mRNA between tissues with and without endometrial carcinoma, the Expression DIY box Plots module in the online database GEPIA2 (http://gepia2.cancer-pku.cn/#analysis) was used. In this analytic model, the parameter settings are first as follows: "Gene A" = "P2RX5", "*P*-value cutoff" = "0.001", "log2FC (Fold change) cutoff" = "1", and "cancer types" = "UCEC". Next, "TCGA normal" and "GTEx" data were selected for matching, and the difference of P2RX5 mRNA expression between tissues with endometrial carcinoma from TCGA database and normal endometrial tissues from GTEx database was obtained.

Construction of P2RX5-knockdown endometrial carcinoma cell line

For ensuring the stability of P2RX5 Knockdown, this study commissioned Suzhou Jimagen Biotechnology Co., Ltd. to develop the lentiviral vector (**Table 1**).

HEC-1B cells at logarithmic growth stage were digested and resuspended to prepare cell suspension, and 4.5×10^5 cells/Wells were inoculated in 6-well plates, then cultured in a 37°C, 5% CO_2 incubator for about 20 h until the cell fusion degree reached 40%-50%. Based on the multiplicity of lentiviral infection (MOI) calculation, which is virus titer (TU/mL) × virus volume (ml)/cell number, 50 MOI was used to transfect the cells, and 5 μg/mL of infection enhancer Polybrene was added. Then cells were placed in a 37°C, 5% CO_2 incubator for further cultivation. After 8 hours, the condition of the cells was observed. If the cells were in poor condition, the medium was promptly changed; if the cells were in good condition, the culture was continued. Subsequently, the medium was changed once at 24 hours and then again at 48 hours. After 72 hours, cells were observed under an inverted fluorescence microscope, revealing an infection efficiency exceeding 80% with cells exhibiting robust growth. To minimize the influence of wild-type cells on subsequent experiments, selection was performed using the puromycin resistance gene in the lentivirus. Cells were treated with 3 μg/mL pyrimethamine to facilitate subsequent selection and amplification, followed by passage for more than three generations. Changes in protein expression levels were verified following P2RX5 gene knockdown via Western blot analysis.

Western blot analyse

The ratio of the efficient RIPA tissue/cells fast pyrolysis liquid (RIPA Lysis Buffer, RIPA) to benzyl sulfonyl fluoride (PMSF) was 100:1 for preparing the cracking liquid to lyse cells. The BCA protein quantification kit (Shanghai Yase Biomedical Technology Co., Ltd.), ready-to-use, was utilized to determine the protein concentration of different stable transmutation strains. A 10% PAGE gel rapid preparation kit (Yisheng Biotechnology (Shanghai) Co., Ltd.) was used to separate the same amount of protein extract (30 μg) by first stage electrophoresis at 80 V and 30 minutes and second stage electrophoresis at 120 V and 60 minutes in agarose gel electrophoresis (WeX). Then the gel protein strips were transferred to PVDF membrane (Beijing Unionic Biotechnology Co., Ltd.)

at 300 mA and 60 minutes. Subsequently, it was placed in a rapid sealing liquid shaker (Shanghai Yase Biomedical Technology Co., Ltd.) for 80 revolutions and 15 minutes at room temperature and then placed on ice. Meanwhile, the shaker was incubated with P2RX5 primary antibody (1:1000, Wuhan Shuangxuan Biological Technology Co., Ltd.) for 80 revolutions and overnight. After the membrane was washed by 1 × TBS (Shanghai Wiao Biotechnology Co., Ltd.), it was incubated in a room temperature shaker at 80 rpm for 1 hour with an anti-rabbit secondary antibody coupled with horseradish peroxidase (HRP) (1:10,000, Shanghai Yase Biomedical Technology Co., Ltd.). A hypersensitive chemiluminescence imager system (Amarsham Imager 600) was used to develop the image. β-actin (1:1000, from Yisheng Biotechnology (Shanghai) Co., Ltd.) served as the internal control. The imaging of the bands was processed using Image J software to generate grayscale values and statistical analysis was conducted using SPSS software.

Cell proliferation assays

HEC-1B cells with or without P2XR5 knockdown were seeded in a 96-well plate at a density of 1.5×10^3 cells/well. After 24, 48, 72, or 96 hours of cell incubation, 10 μL of cell counting reagent -8 (CCK8, MCE, USA) was added to each well, incubated for 2 hours, and the OD value at 450 nm was measured and recorded using. Subsequently, cell proliferation curves were plotted to investigate whether P2XR5 expression altered the proliferative capacity of HEC-1B cells.

Colony-formation analyses

HEC-1B cells with or without P2XR5 knockdown were inoculated into a 6-well plate at a density of 1.5×10^3 cells/well. The fluid was changed every 3 days, and the cell status was observed. The culture was terminated when visible colonies of cloned cells were observed. Then, they were fixed with 4% paraformaldehyde for 15 minutes and stained with 0.1% crystal violet solution for 15 minutes. After that, the amount of clones was counted using an Olympus fluorescence microscope.

Scratch assays

HEC-1B cells with or without P2XR5 knockdown were seeded in a 6-well plate at a density of 2.0×10^6 cells/well. Using a 200 μL pipette, three straight lines were made from left to right to create wounds when the growth density reached more than 90%, and the culture was continued for 48 hours in the medium without FBS. Representative images were taken at 0 h and 48 h using OLMYPUS fluorescence microscopy. ImageJ software was employed to calculate the scratch area, the percentage of scratch healing = (the scratch width at 0 h - the scratch width after 48 h of culture)/the scratch width at 0 h × 100%.

Transwell transfer assays

HEC-1B cells with or without P2XR5 knockdown were starved for 24 hours and then were digested and enumerated. First, the cell density was adjusted to 2×10^6 cells/mL using FBS-free medium. Next, 600 μL of complete medium was added into the lower chamber of the wells and 100 μL of the mixed cell suspension (2×10^5 cells) was injected into the upper chamber of the Transwell plate (with an 8 μm aperture). Finally, the 24-well plate was placed in the cell incubator for culturing. After 48 hours, the upper chamber of Transwell was removed from the 24-well plate. The cells that did not migrate to the lower chamber were wiped off using cotton swabs, whereas the migrated cells were fixed in 4% paraformaldehyde for 30 minutes and stained with 0.1% crystal violet solution for 15 minutes. After cleaning and drying, 5 visual fields (100×) were randomly selected using a microscope, and ImageJ software and SPSS software were employed for quantification and statistical analysis.

Transwell invasion assay

Invasion capacity of cells was tested using a Matrigel invasion assay (BD Biosciences, CA, USA). In brief, the liquefied Matrigel matrix glue was diluted in a pre-cooled serum-free medium at a ratio of 8:1. A total of 60 μL the diluted gel was taken from each well and evenly spread on the membrane at the bottom of the upper chamber of Transwell, and then put in the incubator to make the gel polymerize into a film. The remaining steps are identical to “Transwell transfer assays” described above.

Apoptosis assays

HEC-1B cells with or without P2XR5 knockdown were seeded in six-well plates with a density of

Table 2. Basic information of study subjects

Parameters	Control group	Case group	*P*
Total, *n* (%)	100 (100.0)	150 (100.0)	-
Age (years)[a]	47.04 ± 4.20	52.86 ± 6.89	< 0.001
Ethnicity[a]			0.492
Zhuang, *n* (%)	74 (74.0)	105 (70.0)	
Other, *n* (%)	26 (26.0)	45 (30.0)	
CEA (ng/mL)[a]	1.33 ± 0.08	3.73 ± 1.33	0.074
CA125 (U/mL)[a]	48.54 ± 8.40	53.80 ± 7.84	0.652
CA153 (U/mL)[a]	13.07 ± 0.86	17.14 ± 1.47	0.018

[a]Data showed as means ± S.D.

3×10^5 cells per well. After 72 hours of incubation, the cells were treated with apoptosis detection kit (Yisheng Biotechnology (Shanghai) Co., Ltd.). The cells were first suspended with 100 μL of 1 × Binding Buffer per tube. Then, 5 μL of Annexin V-FITC and 10 μL of PI Staining Solution were added, gently mixed, and reacted at room temperature for 15 minutes in the dark. Finally, 400 μL of 1 × Binding Buffer was added, mixed, and placed on ice, and then detected by flow cytometry (Ulite) within 1 hour.

Cell cycle assays

HEC-1B cells with or without P2XR5 knockdown were seeded in 6-well plates at a density of 4×10^5 cells per well. After 72 hours, cells were collected by trypsinization, centrifuged at 1,000 rpm for 5 min, washed in pre-cooled PBS, and resuspended in pre-cooled 70% ethanol. Cells were processed with the cell cycle detection kit (Yisheng Biotechnology (Shanghai) Co., Ltd.) according to with the kit's protocol. Briefly, cells were first labelled with the mixture of propyl iodide solution and RNase A solution and incubated at 37°C for 30 min in the dark. Next, flow cytometry (Guilin Yulite) was used for analyzing cell cycles.

Statistical analysis

For continuous data (including age, CEA, CA125, CA153, and data from in vitro experiments of cell biological behaviors analyses), data were shown as means ± standard deviation (SD) and the differences in their distribution between the two groups (ex. cases and control group) were tested using *t*-test; whereas the difference among multiple groups were tested using one-way analysis of variance (ANOVA) with post-hoc test. For categorical data (including ethnicity), data were presented as the number of each dummy variable and the difference between groups was tested using chi-square test. A logistic regression model was used to analyze the effects of P2RX5 expression on the risk and clinicopathological features of endometrial carcinoma. A specific risk value was calculated using odds ratios (ORs) and corresponding confidence intervals (CIs) of 95%. Survival models, including Kaplan-Meier survival model and Cox survival regression model, were used for analyzing the effects of P2RX5 expression on the prognosis of endometrial carcinoma. All analyses were conducted with SPSS statistical software (version#32.00), with a P value < 0.05 regarded as statistically significant.

Results

Baseline information of study subjects

As shown in **Table 2**, there were no statistically significant differences in ethnic composition between the two groups, but patients from case group were older on average than those in the control group. Patients in the case group had higher serum CA153 levels, while the differences in serum CEA and CA125 levels were not statistically significant.

P2RX5 expression shows an increase in the endometrial carcinoma

To explore whether the expression of P2RX5 is correlated with the occurrence of endometrial carcinoma, we detected the expression of P2RX5 by IHC in endometrial carcinoma tissues and corresponding control tissues (**Figure 1**). The results showed that P2RX5 had a higher expression in the tissues with endometrial carcinoma (P < 0.001) (**Figure 1A**), and the representative IHC plots showed this difference (**Figure 1B**). Furthermore, we also analyzed the NGS data from the endometrial carcinoma database (**Figure 1C**). Among these, 174 cases of endometrial carcinoma tissue and 91 cases of normal tissue were analyzed. The expression of P2RX5 in tissues with endometrial carcinoma

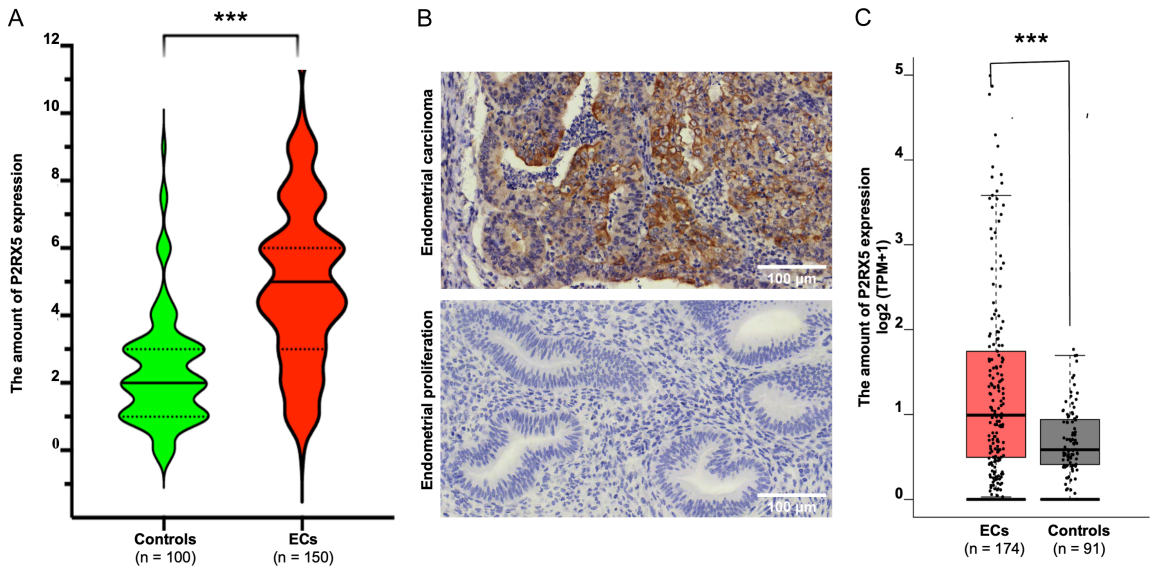

Figure 1. Different expression of P2RX5 between endometrial tissues with and without carcinoma. A. The amount of P2RX5 expression in tissues with endometrial carcinoma or with proliferative endometrial glands was tested by IHC in combination with the IRS integral analysis method, and the differences were evaluated using the Mann-Whitney U test. The horizontal line in the figure indicates the median of the IRS scores, and the dashed line indicates the quartiles of the IRS scores. B. A representative IHC plots showing the differences in P2RX5 expression between tissues with and without endometrial carcinoma. C. The NGS data analysis from the UCEC database (http://gepia2.cancer-pku.cn/#analysis) shows different expression of P2RX5 between endometrial carcinoma tissues and endometrial tissues without cancers. *Abbreviations:* ECs, patients with endometrial carcinoma; Controls, study subjects without endometrial carcinoma. "***" indicates $P < 0.001$.

Table 3. Effects of P2RX5 expression on the risk of endometrial carcinoma

P2RX5	Control group		Case group		OR (95% CI)	Adjusted OR (95% CI)
	n	%	*n*	%		
Low	60	60.0	25	16.7	1	1
Moderate	33	33.0	60	40.0	4.36 (2.32-8.20)**	5.60 (2.44-12.84)**
High	7	7.0	65	43.3	22.29 (8.98-55.29)**	22.93 (7.32-71.82)**

Note: In the table, "adjusted values" of corresponding OR (95% CI) are adjusted for clinical parameters including age, ethnicity, CEA, CA125, and CA153; "**" indicates *P* value < 0.01.

was significantly higher than in normal endometrial tissues.

Growing P2RX5 expression increases endometrial carcinoma risk

To further clarify the specific impact of P2RX5 expression on the risk of developing endometrial carcinoma, the expression levels of P2RX5 were categorized into three groups (*to see Materials and Methods*). Results from logistic regression analyses displayed that as P2RX5 expression increased, the risk of developing endometrial carcinoma progressively escalated (risk values ranging from 4.36 to 22.29) (**Table 3**). This risk effects remained significant even after adjusting for parameters such as age, ethnicity, CEA, CA125, and CA153.

P2RX5 expression significantly correlated with the clinicopathological characteristics of endometrial carcinoma

Table 4 summarize the effects of P2RX5 expressions on the clinicopathological features of endometrial carcinoma. Because of a relatively small sample size, the groups with low and medium expression of P2RX5 were combined into one group (medium/low group). The results showed that P2RX5 expression is not related to parameters such as the age, ethnicity, serum CEA, CA125, and CA153 in patients

Table 4. The association between expression of P2RX5 and clinicopathological characteristics of endometrial carcinoma

Parameters	Medium/low expression		High expression		OR (95% CI)	*P*
	n	%	*n*	%		
Age (years)						
< 53	45	52.9	24	36.9	1	
≥ 53	40	47.1	41	63.1	1.92 (0.99-3.71)	0.052
Ethnicity						
Zhuang	59	69.4	46	70.8	1	
Others	26	30.6	19	29.2	0.94 (0.46-1.90)	0.857
CEA						
Negative	44	51.8	30	46.2	1	
Positive	41	48.2	35	53.8	1.25 (0.66-2.39)	0.496
CA125						
Negative	34	40.0	26	40.0	1	
Positive	51	60.0	39	60.0	1.00 (0.52-2.39)	0.999
CA153						
Negative	74	87.1	55	84.6	1	
Positive	11	12.9	10	15.4	1.22 (0.49-3.08)	0.669
Tumor size						
≤ 5 cm	65	76.5	31	47.7	1	
> 5 cm	20	23.5	34	52.3	3.57 (1.77-7.17)	< 0.001
Tumor differentiation						
Low	23	23.5	4	5.2	1	
Moderate	44	44.9	42	54.5	5.49 (1.75-17.21)	0.004
High	18	31.6	19	40.3	6.07 (1.75-21.02)	0.004
FIGO Staging						
0-Ia	66	60.0	27	37.5	1	
Ib	13	11.8	14	19.4	2.63 (1.09-6.33)	0.031
Ic-II	6	28.2	24	43.1	9.78 (3.60-26.59)	< 0.001

with endometrial carcinoma. However, it is significantly correlated with tumor size, differentiation, and FIGO stage. Through the analysis using logistic regression, it was found that patients with high expression of P2RX5 in cancerous tissues featured a higher risk of tumor proliferation (OR = 3.57), a greater risk of dedifferentiation (with ORs of 5.49 and 6.07 for moderate and low differentiation, respectively), and an elevated FIGO stage (with invasive risk values ranging from 2.63 to 9.78). These results suggest that increasing P2RX5 expression promotes tumor proliferation, dedifferentiation, and invasive capability.

P2RX5 expression influences the prognosis of endometrial carcinoma

To explore whether P2RX5 expression in tumor tissues has prognostic significance for patients with endometrial carcinoma, we conducted a follow-up analysis on all study subjects. Subsequently, after excluding 20 cases lost to follow-up, survival data were finally obtained for 130 patients. We plotted the survival curves for these patients using the Meier-Kaplan survival model (**Figure 2**). Patients with high P2RX5 expression had a slightly shorter overall survival time than those with medium to low expression (51.84 vs. 59.42 months), and this difference was statistically significant (P = 0.040). However, through multivariate Cox regression analysis, we did not observe a statistically significant impact of varying P2RX5 expression levels on the risk of mortality in patients.

Construction and verification of cell lines with P2RX5 knockdown

After culturing, proliferating, and passaging the stable cell lines with each P2RX5 knockdown target, we were ultimately able to obtain stable

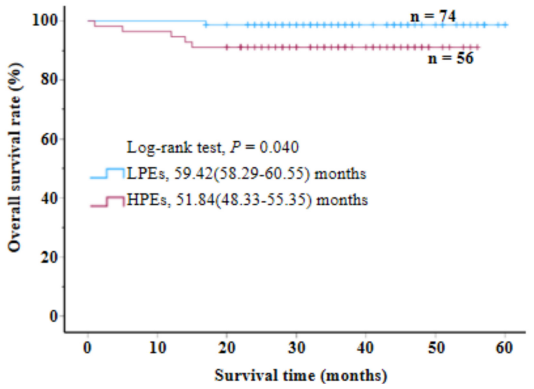

Figure 2. The impact of P2RX5 expression in endometrial cancer tissue on overall survival. The cumulative risk function was plotted using the Kaplan-Meier method, and the *P*-value was calculated using the two-sided log-rank test. *Abbreviations:* LPE, patients with low or moderate P2RX5 expression in endometrial carcinoma; HPE, patients with high P2RX5 expression in endometrial carcinoma.

cell lines corresponding to each P2RX5 knockdown target (**Figure 3A**). Proteins were extracted from each group of cells, and the knockdown efficiency was verified by the western blot method. The results displayed that there was no significant difference in the expression level of P2RX5 protein between HEC-1B cells with empty vector virus (NC group) and without treatment of vector virus (HEC-1B group) (**Figure 3B** and **3C**). This suggests that the viral vector had no significant effects on P2RX5 expression. Compared with the NC group, the amount of P2RX5 expression in HEC-1B with the treatment of vector virus 984 (984 group) were significantly decreased ($P < 0.05$); whereas the expression level in these cells with the treatment of vector virus 288 (288 group) and 903 (903 group) did not alter significantly ($P > 0.05$) (**Figure 3B** and **3C**). Given that the efficiency of knocking down P2RX5 was the most noticeable in 984 group, this group (named as sh-P2RX5 group) and corresponding NC group (named as sh-NC group) were selected for the following functional assays.

Knocking-down P2RX5 expression inhibits the proliferation, migration, and invasion abilities of HEC-1B cells

We evaluated the impact of P2RX5 knockdown on HEC-1B cell proliferation using CCK-8 and colony formation assays. Compared to the sh-NC group, proliferation activity of cells in sh-P2RX5 group was significantly inhibited after 72 hours ($P < 0.001$) (**Figure 4A**). Moreover, the number of colonies formed was markedly reduced following P2RX5 knockdown ($P < 0.001$) (**Figure 4B**). These data indicate that P2RX5 depletion strongly suppresses cell proliferation. Cell migration was assessed using scratch wound healing and Transwell migration assays. The scratch closure was significantly slower in sh-P2RX5 cells than in controls ($P < 0.001$) (**Figure 5**). Consistently, Transwell migration assays showed a notable decrease in migrated sh-P2RX5 cells ($P < 0.001$) (**Figure 6A**), demonstrating that P2RX5 knockdown impedes HEC-1B cell migration. Invasion was assessed using Transwell chambers coated with extracellular matrix gel to simulate interstitial barriers. The number of invading sh-P2RX5 cells was significantly lower than that of sh-NC cells ($P < 0.001$) (**Figure 6B**), indicating that P2RX5 knockdown also attenuates the invasive capacity of HEC-1B cells.

Knocking-down P2RX5 expression affects the cycle and apoptosis of HEC-1B cells

To investigate whether the downregulation of P2RX5 expression induces apoptosis in HEC-1-B cells, cells from the sh-NC group and the sh-P2RX5 group were seeded at equal densities and cultured for 72 hours prior to flow cytometric analysis. The results showed that the apoptosis rate of the HEC-1B cells significantly increased when P2RX5 expression was knocked down ($P < 0.001$) (**Figure 7A**), suggesting that P2RX5 expression might inhibit apoptosis in HEC-1B cells. Furthermore, after the downregulation of P2RX5 expression, the proportion of HEC-1B cells in the S phase and G2/M phase significantly decreased ($P < 0.05$) (**Figure 7B**).

Discussion

To our knowledge, this study represents the first investigation worldwide examining whether P2RX5 expression influences the clinical pathological characteristics and prognosis of endometrial carcinoma. Our results indicate that P2RX5 expression is elevated in endometrial carcinoma tissues. Compared with low-expression cases, intermediate- and high-expression cases exhibit a 4.6-fold and 21.93-fold increased cancer risk, respectively. This elevated P2RX5 expression promotes tumor proliferation, increases tumor dedifferentiation, and serves as an unfavorable prognostic factor

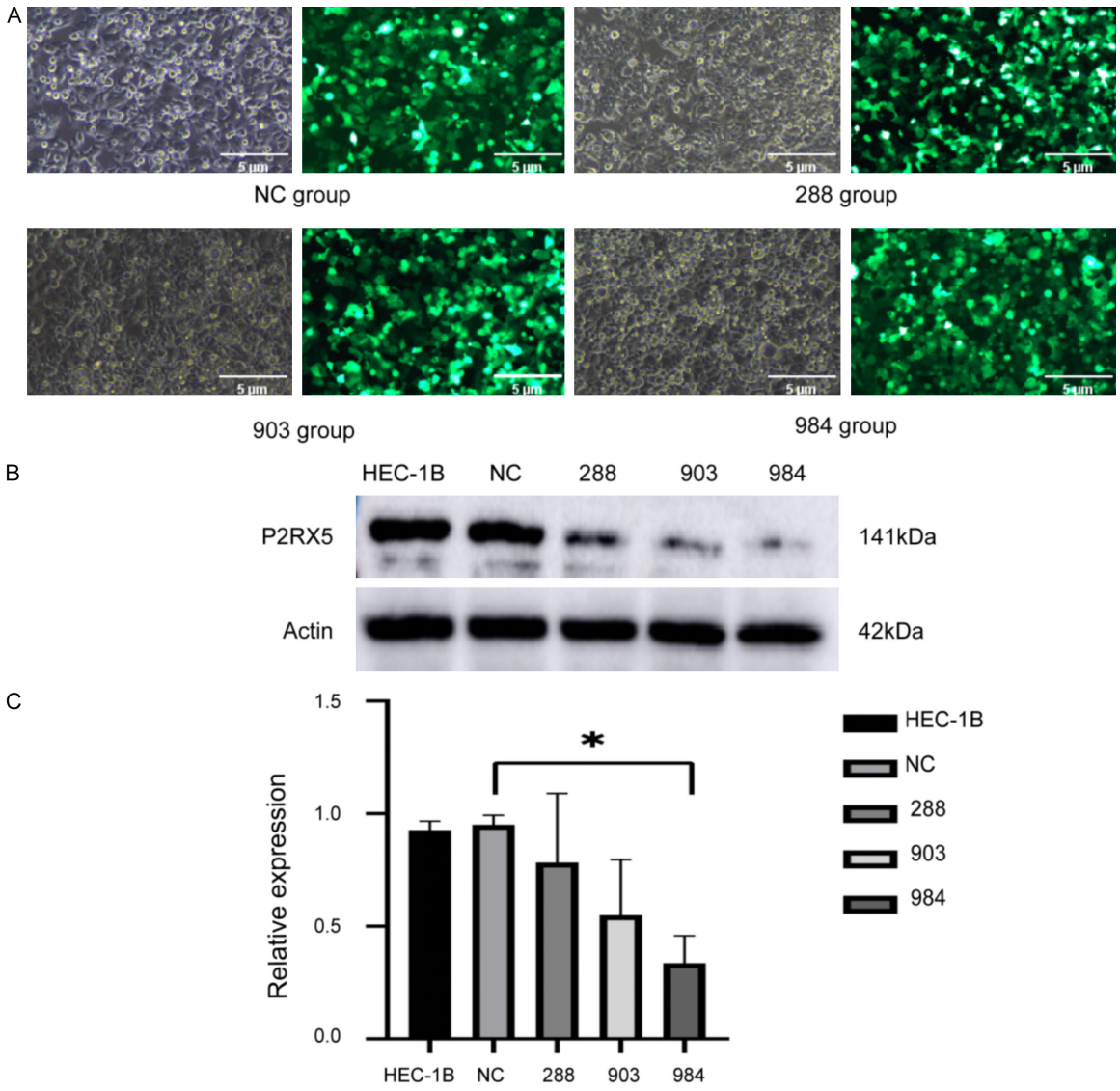

Figure 3. The structure of stable cell lines for each P2RX5 knockdown target. P2RX5 Knockdown endometrial carcinoma cell lines were constructed using the lentiviral vector in the HEC-1B cells (*to see Materials and Methods*). A. Representative dark-field and immunofluorescence images. B, C. Western blot was used for confirming the efficiency of knocking down P2RX5 protein expression. The differences between groups were tested using ANOVA test. In the figures: "HEC-1B" represents the HEC-1B cells without the treatment of vector virus; "NC" represents HEC-1B cells with the treatment of empty vector virus; "288" represents HEC-1B cells with the treatment of vector virus 288; "903" represents HEC-1B cells with the treatment of vector virus 903; "984" represents HEC-1B cells with the treatment of vector virus 984; "*" represents $P < 0.05$.

for tumors. These findings suggest that P2RX5 expression influences the pathological characteristics of endometrial cancer and plays a significant role in tumor progression.

Multiple findings from this study indicate that P2RX5 enhances malignant biological behaviors such as proliferation and invasiveness in endometrial cancer cells. This finding is consistent with previous studies confirming the oncogenic function of P2RX5 family proteins in other solid tumor types. Previous studies have demonstrated that P2RX7 is highly expressed in bladder cancer, prostate cancer, papillary thyroid carcinoma, lung cancer, breast cancer, and basal cell carcinoma, and is associated with malignant progression and poor prognosis [6, 7]. Furthermore, in the various cancer cell lines mentioned above, P2RX7 also exhibits significantly elevated expression levels. This overex-

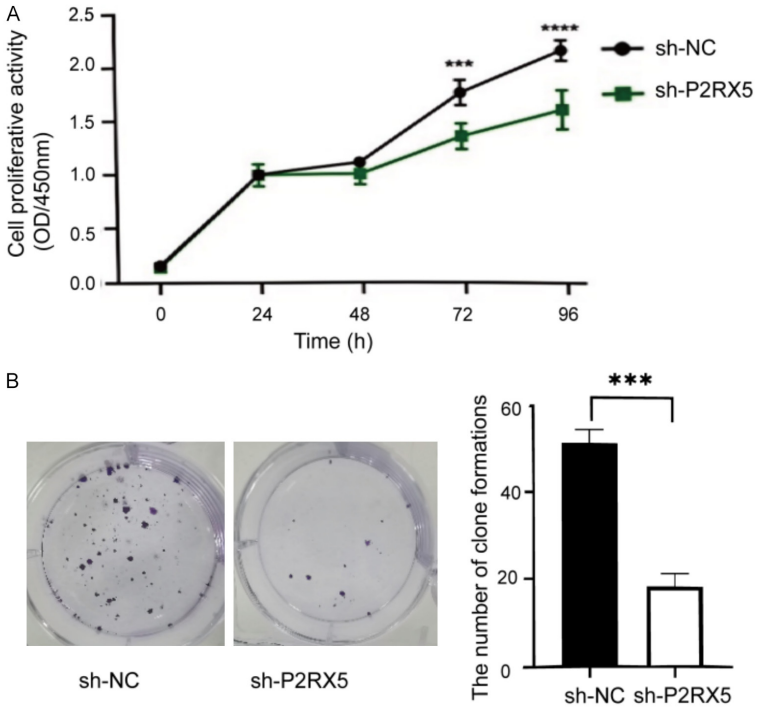

Figure 4. The effects of P2RX5 knockdown on cell proliferation and colon formation. A. The proliferative potential of HEC-1B cells was inhibited under conditions of P2RX5 knockdown. B. P2RX5 knockdown suppressed the colon formation of HEC-1B cells. The differences between groups were tested using *t* test. In the figure: "sh-NC" represents HEC-1B cells with treatment of the empty vector virus; "sh-P2RX5" represents HEC-1B cells with treatment of vector virus 984; "***" indicates $P < 0.001$.

pression is not only closely associated with cancer cell proliferation but also markedly enhances the migratory capacity of cancer cells [8, 9]. Research has also demonstrated that P2RX4 exhibits high expression levels in cholangiocarcinoma, glioma, and prostate cancer. Furthermore, downregulating P2RX4 expression significantly inhibits a series of malignant biological behaviors in cancer cell lines, including proliferation and migration [10-12]. It can thus be concluded that P2RX7 and P2RX4 play significant roles in the onset and progression of cancer. Research has conclusively demonstrated that the P2RX7 and P2RX4 receptors play a significant role in promoting cancer progression. Their mechanism involves regulating the activity of ATP-gated ion channels, thereby influencing intracellular calcium influx and the activation levels of downstream signaling pathways, ultimately achieving their pro-cancer effects [13-15]. Our research indicates that patients with high P2RX5 expression in cancer tissues exhibit tumors characterized by increased proliferation risk, greater dedifferentiation, and higher International Federation of Gynecology and Obstetrics (FIGO) staging. The relationship between P2RX5 expression and tumor invasive characteristics is highly consistent with the roles of P2RX7 and P2RX4 in tumors. Based on this, we boldly hypothesize that as a receptor within the same family, P2RX5 may also promote endometrial cancer through the same pathway. This further supports the potential role of P2RX5 in clinical stratification and prognosis of endometrial cancer patients, similar to P2RX7 and P2RX4. P2RX5 may also serve as a therapeutic target or biomarker.

Additionally, our study found that inhibiting P2RX5 expression significantly reduced the proportion of cells in the S and G2/M phases while increasing the rate of apoptosis. This suggests that P2RX5 may regulate the growth-apoptosis balance in cells via the purinergic signaling pathway, indicating that P2RX5 could serve as a predictive biomarker for early-stage cancer prognosis. P2RX5 is believed to play a role in regulating the inflammatory process, thereby influencing the efficacy of immunotherapy [16-18]. Additionally, in lymphocyte and plasma cell malignancies, CAR-T cell therapy targeting P2RX5 can effectively induce cytotoxic responses against tumor cells. Therefore, P2RX5 also holds potential for regulating immune cell infiltration within the endometrial carcinoma microenvironment, CAR-T cell therapy targeting P2RX5 may also be applicable for precision treatment of endometrial cancer [19]. It is evident that P2RX5 may not only serve as a biomarker for endometrial cancer but also emerges as a significant therapeutic target. This could ultimately enhance the precision of endometrial cancer treatments and markedly improve patient prognosis.

Although this study provides the first histological evidence of high P2RX5 expression, demon-

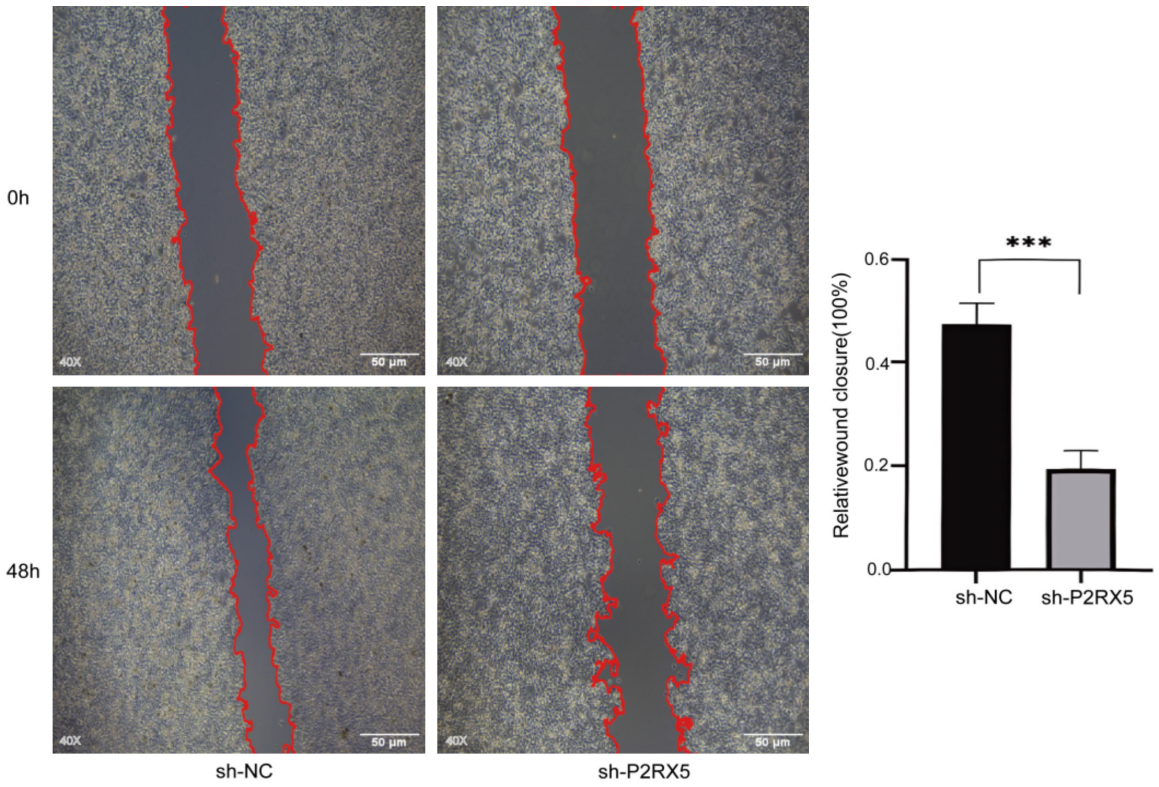

Figure 5. P2RX5 knockdown suppresses the migration ability of HEC-1B cells. In the figure: "sh-NC" represents HEC-1B cells with treatment of the empty vector virus; "sh-P2RX5" represents HEC-1B cells with treatment of vector virus 984; "***" indicates $P < 0.001$.

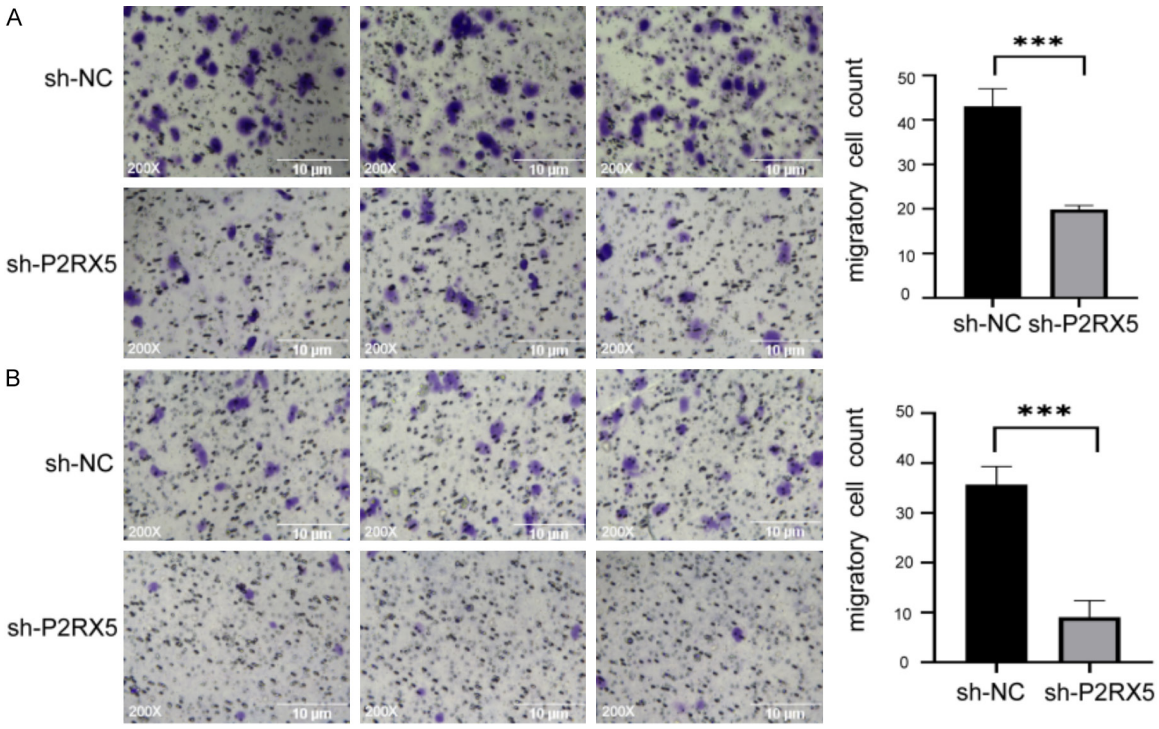

Figure 6. The effects of P2RX5 knockdown on cell migration and invasion. A. The migration potential of HEC-1B cells was inhibited under conditions of P2RX5 knockdown. B. P2RX5 knockdown suppressed the invasion capacity

of HEC-1B cells. The differences between groups were tested using *t* test. In the figure: "sh-NC" represents HEC-1B cells with treatment of the empty vector virus; "sh-P2RX5" represents HEC-1B cells with treatment of vector virus 984; "***" indicates $P < 0.001$.

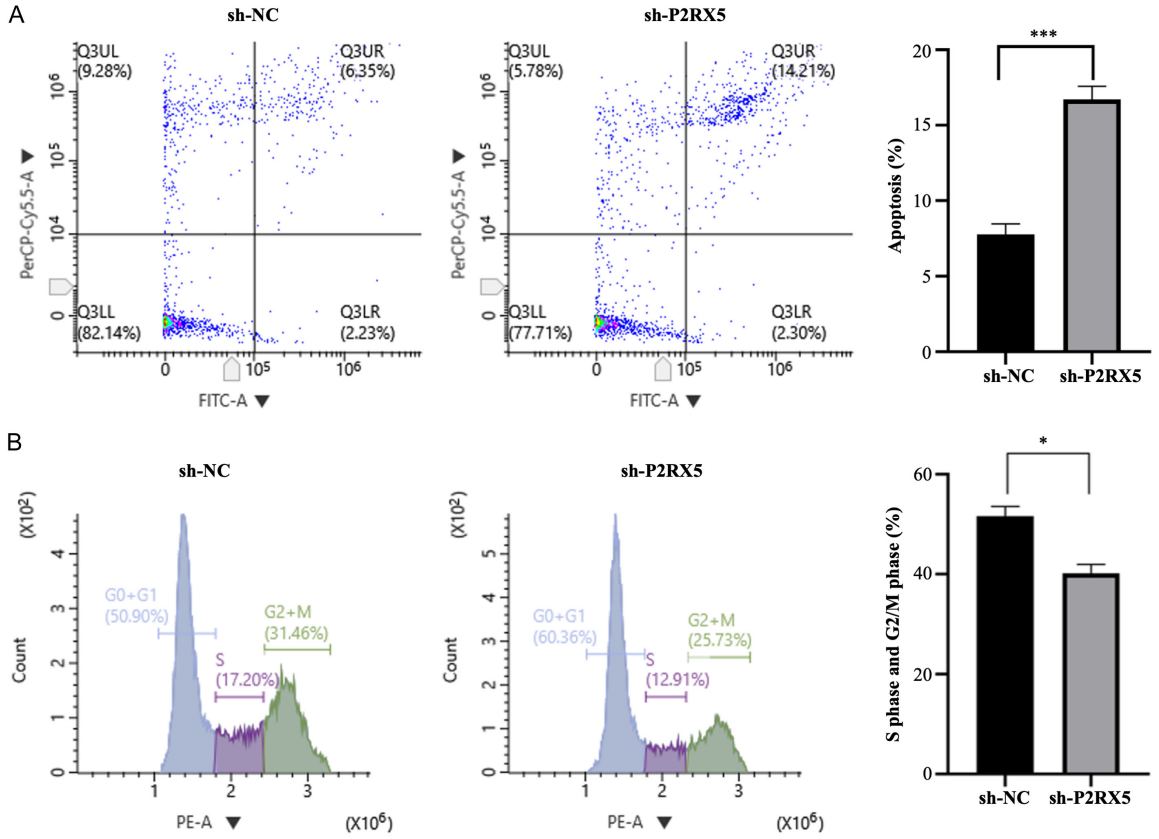

Figure 7. The effects of P2RX5 knockdown on cell apoptosis and cycle. A. The apoptosis potential of HEC-1B cells was increased under conditions of P2RX5 knockdown. B. P2RX5 knockdown significantly modified the cycle of HEC-1B cells. The differences between groups were tested using *t* test. In the figure: "sh-NC" represents HEC-1B cells with treatment of the empty vector virus; "sh-P2RX5" represents HEC-1B cells with treatment of vector virus 984; "*" represents $P < 0.05$; "***" indicates $P < 0.001$.

strating that its expression levels correlate with tumor size, low differentiation, and advanced staging, and that P2RX5 knockdown inhibits invasive characteristics in cell lines - thereby preliminarily validating its pro-cancer function - these findings remain subject to certain limitations. First, the research design was a single-center retrospective analysis that included only 150 cases of endometrial cancer treated at Youjiang Medical University for Nationalities. The sample origin was relatively limited and the number of cases was small. This may affect the statistical power of the results and limit the generalizability of conclusions to a broader population. Secondly, the current understanding of the mechanism by which P2RX5 promotes endometrial cancer progression remains preliminary. While it has been preliminarily demonstrated that P2RX5 knockdown induces cancer cell apoptosis, the specific downstream molecular mechanisms through which it regulates the cell cycle and apoptosis via the purinergic signaling pathway remain unelucidated and require further validation in subsequent studies. Furthermore, this study primarily focused on histological immunohistochemical expression and cellular experiments, failing to validate the specific role of P2RX5 in tumor growth and metastasis through in vivo animal models. Regarding clinical application, the threshold for P2RX5 as a prognostic biomarker requires further validation in multicenter and large-sample cohorts, and should be combined with other molecular markers to enhance the accuracy of stratification.

Future research should focus on several key areas: First, increasing sample sizes and adopt-

ing prospective study designs to validate associations with clinical outcomes [20, 21]. Second, research should evolve to address the molecular subtyping mechanisms of endometrial cancer, utilizing gene editing technologies and animal models to investigate P2RX5-mediated molecular pathways - particularly its synergistic or specific interactions with other P2RX family members such as P2RX7 and P2RX4 [10-15, 22]. Finally, the functional role of P2RX5 within the tumor immune microenvironment should be explored, along with its potential as an immunotherapy target. This includes developing CAR-T cell therapies or small-molecule inhibitors targeting P2RX5 [23]. Such research will advance the translation of P2RX5 from a biomarker to a clinical therapeutic target, ultimately enhancing precision treatment for endometrial cancer.

In summary, this study demonstrates that high expression of P2RX5 in endometrial carcinoma is significantly correlated with aggressive clinicopathological features and unfavorable prognosis. In terms of function, P2RX5 plays a significant role in promoting the proliferation, migration, and invasion of cancer cells. These findings not only establish P2RX5 as a novel prognostic biomarker but also suggest its potential therapeutic applications. A comprehensive investigation of the molecular mechanisms underlying P2RX5 and its roles within the tumor immune microenvironment is essential for advancing precision therapies in endometrial cancer.

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Disclosure of conflict of interest

None.

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References

[1] Nuñez-Rios JD, Ulrich H, Díaz-Muñoz M, Lameu C and Vázquez-Cuevas FG. Purinergic system in cancer stem cells. Purinergic Signal 2025; 21: 23-38.

[2] Manoharan V, Adegbayi OO and Maynard JP. P2 purinergic receptor expression and function in tumor-related immune cells. Purinergic Signal 2025; 21: 393-411.

[3] Roliano GG, Azambuja JH, Brunetto VT, Butterfield HE, Kalil AN and Braganhol E. Colorectal cancer and purinergic signalling: an overview. Cancers (Basel) 2022; 14: 4887.

[4] Do Cao C, Desailloud R, Topolinski H, Crépin M, Flament C, Leteurtre E, Cardot-Bauters C, Frénois F, Ait-Yahya É, Bonte F, Leclerc J and Pigny P. Analysis of genetic predisposition to familial non-medullary thyroid cancer by whole genome sequencing. Ann Endocrinol (Paris) 2025; 86: 102456.

[5] Sarquis M, Moraes DC, Bastos-Rodrigues L, Azevedo PG, Ramos AV, Reis FV, Dande PV, Paim I, Friedman E and De Marco L. Germline mutations in familial papillary thyroid cancer. Endocr Pathol 2020; 31: 14-20.

[6] Huang KC, Chiang SF, Lin PC, Hong WZ, Yang PC, Chang HP, Peng SL, Chen TW, Ke TW, Liang JA, Chen WT and Chao KSC. TNF α modulates PANX1 activation to promote ATP release and enhance P2RX7-mediated antitumor immune responses after chemotherapy in colorectal cancer. Cell Death Dis 2024; 15: 24.

[7] Huang X, Jia Z, Li X, Hu Z, Yu X and Xia J. Asiaticoside hampers epithelial-mesenchymal transition by promoting PPARG expression and suppressing P2RX7-mediated TGF-β/Smad signaling in triple-negative breast cancer. Phytother Res 2023; 37: 1771-1786.

[8] Lara R, Adinolfi E, Harwood CA, Philpott M, Barden JA, Di Virgilio F and McNulty S. P2X7 in cancer: from molecular mechanisms to therapeutics. Front Pharmacol 2020; 11: 793.

[9] Du Y, Cao Y, Song W, Wang X, Yu Q, Peng X and Zhao R. Role of the P2X7 receptor in breast cancer progression. Purinergic Signal 2025; 21: 791-799.

[10] Qiao X, Wang C and Ma J. The role and function validation of P2RX4 as a novel cancer biomarker in pan-cancer analysis. Sci Rep 2025; 15: 11507.

[11] Maynard JP, Lu J, Vidal I, Hicks J, Mummert L, Ali T, Kempski R, Carter AM, Sosa RY, Peiffer LB, Joshu CE, Lotan TL, De Marzo AM and Sfanos KS. P2X4 purinergic receptors offer a therapeutic target for aggressive prostate cancer. J Pathol 2022; 256: 149-163.

[12] He J, Zhou Y, Arredondo Carrera HM, Sprules A, Neagu R, Zarkesh SA, Eaton C, Luo J, Gartland A and Wang N. Inhibiting the P2X4 receptor suppresses prostate cancer growth in vitro and in vivo, suggesting a potential clinical target. Cells 2020; 9: 2511.

[13] Chiarella AM, Ryu YK, Manji GA and Rustgi AK. Extracellular ATP and adenosine in cancer pathogenesis and treatment. Trends Cancer 2021; 7: 731-750.

[14] Rotondo JC, Mazziotta C, Lanzillotti C, Stefani C, Badiale G, Campione G, Martini F and Tognon M. The role of purinergic P2X7 receptor in inflammation and cancer: novel molecular insights and clinical applications. Cancers (Basel) 2022; 14: 1116.

[15] Wang J, Gu Y, Niu Z, Tang F and Li Y. P2RX4 promotes hepatocellular carcinoma progression via calcium-mediated PI3K/AKT activation and immune remodeling. World J Surg Oncol 2025; 23: 363.

[16] Jeong YH, Walsh MC, Yu J, Shen H, Wherry EJ and Choi Y. Mice Lacking the purinergic receptor P2X5 exhibit defective inflammasome activation and early susceptibility to *Listeria monocytogenes*. J Immunol 2020; 205: 760-766.

[17] Di Virgilio F, Vultaggio-Poma V and Sarti AC. P2X receptors in cancer growth and progression. Biochem Pharmacol 2021; 187: 114350.

[18] Wang X, Jiang SH, Ma M, Cai S, Song D and Han B. P2 purinergic receptors in tumor immunity. Cancer Res 2025; 85: 3826-3841.

[19] Ang Z, Annette C, Schimdt C, Hayer K, S Hasanali Z, Diz MD, Sainos PK, Kwok C, Ji C, Krohl P and Sehgal P. P2RX5-directed CAR-T cells for genotype-guided treatment of lymphoid and plasma cell malignancies in patients of African ancestry. Blood 2024; 144: 1033.

[20] Klopp AH, Enserro D, Powell M, Randall M, Schink JC, Mannel RS, Holman L, Bender D, Kushnir CL, Backes F, Zweizig SL, Waggoner S, Bradley KA, Lawrence LD, Hanjani P, Darus CJ, Small W Jr, Cardenes HR, Feddock JM and Miller DS. Radiation therapy with or without cisplatin for local recurrences of endometrial cancer: results from an NRG oncology/GOG prospective randomized multicenter clinical trial. J Clin Oncol 2024; 42: 2425-2435.

[21] Loukovaara M, Bützow R, Staff S, Mäenpää M, Faltinová M, Lassus H, Veijalainen O, Grönvall M, Vaalavirta L, Kuikka E, Haataja M, Urpilainen E, Simojoki M, Anttila M and Auranen A. PErsonalized TReatment for Endometrial Carcinoma (PETREC): study design and methods of a prospective Finnish multicenter trial. Int J Gynecol Cancer 2023; 33: 1807-1811.

[22] Bostan IS, Mihaila M, Roman V, Radu N, Neagu MT, Bostan M and Mehedintu C. Landscape of endometrial cancer: molecular mechanisms, biomarkers, and target therapy. Cancers (Basel) 2024; 16: 2027.

[23] Sluyter R, McEwan TB, Sophocleous RA and Stokes L. Methods for studying P2X4 receptor ion channels in immune cells. J Immunol Methods 2024; 526: 113626.