Original Research *Highlight Article*

P2RX7 functions as a putative biomarker of gastric cancer and contributes to worse prognosis

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Impact statement

The mechanism of gastric cancer is highly complex, accompanied by a variety of genetic abnormalities. It is of great significance to elucidate the pathogenesis of gastric cancer, find its markers and therapeutic targets in the fight against this fatal disease. In this study, we identified P2RX7 as a putative target of gastric cancer, which was overexpressed in gastric cancer tissues and had relationship with worse prognosis. We also elucidated the roles of P2RX7 on the growth and metastasis of gastric cancer cells, and explored the relationship between it and ERK1/2 pathway, Akt pathway, and epithelial–mesenchymal transition. Our findings begin to offer useful insights into the mechanism of gastric cancer progression and provide clues to novel therapy strategies.

Abstract

P2RX7 has a vital role in promoting proliferation and metastasis and is relevant to worse prognosis in multiple tumors. Nevertheless, P2RX7's prognostic value and unambiguous effect in gastric cancer remain to be further explored. Our study showed that the expression of P2RX7 in human gastric tumor tissue ($n = 80$) was significantly higher than that in normal human gastric tissue ($n = 20$, different cohort). Abnormally high expression of P2RX7 was related to larger tumor size ($P = 0.0473$), higher T stage ($P = 0.0367$), and lymphatic metastasis ($P = 0.0056$). Kaplan–Meier analysis showed that higher expression of P2RX7 was associated with worse overall survival and disease-free survival in gastric cancer patients. Additionally, downregulation of P2RX7 by a specific shRNA suppressed the proliferation and metastasis of gastric cancer cells, while ectopic overexpression P2RX7 had opposite effects. P2RX7 modulated ERK1/2 and Akt pathways and epithelial–mesenchymal transition markers such as E-cadherin, N-cadherin, vimentin, and snail. All in all, our results suggest that P2RX7 represents a prognostic biomarker of patients with gastric cancer and show the importance of P2RX7 as a putative biomarker in gastric cancer.

Keywords: P2RX7, gastric cancer, proliferation, metastasis

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Introduction

Gastric cancer is a phenotypically and genetically complex disease, accounting for 8% of the whole cancer cases and 10% of cancer-related deaths.[1] Even though there are great improvements in surgical and chemotherapeutic treatment, patients in advanced stage with metastasis still suffer from poor prognosis. Therefore, exploration of the mechanisms underneath gastric progression and metastasis and the potential therapeutic targets is of great importance.

Purine receptors are widely distributed on the surface of cell membranes. They are stimulated by extracellular ligands (ATP, etc.) and participate in the regulation of biological functions of various tissues and cells.[2] Purine receptors are composed of P1 type and P2 type receptors. P2 receptors contain two subtypes: receptors P2X and P2Y. With its involvement in the incidence and development of tumors and the possibility of even being a new target for predicting tumor progression and prognosis, P2X7 receptor (P2RX7) in the P2X receptor family has received extensive attention in cancer research over the years.[3,4]

The P2RX7 gene locates on chromosome 12q24.31 encoding a membrane protein consisting of 595 amino acids. P2RX7 is prone to form a trimer and is also distributed as an oligomer on the surface of the cell membrane.[5] P2RX7 receptors mainly function in forming ion channels and water pores. When extracellular low concentrations of

ATP stimulate P2RX7, a transmembrane ion channel which permits the crossing of small ions such as Na+, K+, Ca2+, etc. can be opened in milliseconds. P2RX7 form large non-selective water pores in the presence of low divalent cation (Ca2+, Mg2+, etc.), allowing hydrophilic solute molecules with molecular weight below 900D to pass through.[6] The biological functions of P2RX7 receptors include participation in cell signal transduction, secretion of cytokines, mediating of cell proliferation and apoptosis.[7,8]

In the microenvironment of tumor growth, extracellular high-level ATP activates P2RX7. P2RX7 participates in cell proliferation and tumor metastasis by inducing cells to secret cell cytokines such as interleukin-6 and substance P.[9] Studies have confirmed that P2RX7 is highly expressed in some tumors but low in some other tumors,[10–12] yet its expression and function in gastric cancer are not reported. Herein, we aimed to study the expression and function of P2RX7 and explore the downstream mechanism.

Methods and materials

Patients and specimens

This study was permitted by the ethics review board of Linyi Central Hospital. (Approval No. 201601045). The samples used in immunohistochemistry were randomly chosen from patients who had received excision of gastric cancer at Linyi Central Hospital between 2014 and 2016. Clinicopathological information was obtained from Department of Pathology. All human gastric cancer tissue samples were adenocarcinomas located in gastric subcardia. D2 surgery was performed in all cases. Patients with distant metastasis (who would receive palliative chemotherapy) were excluded, and no patients received neoadjuvant chemotherapy or radiation therapy before surgery. Since many patients received subsequent therapy (adjuvant chemotherapy or radiation therapy) in other hospitals following surgery, additional clinical and survival data could not be obtained for many cases, and thus not included. The samples used in immunohistochemistry were chosen from patients who had received excision of gastric cancer at our hospital when immunotherapy (PD1 and PDL1 markers) was not widely used in China, so PD-L1, MSI, etc. were not included in routine testing. The samples used in real-time PCR were randomly chosen from patients who had received excision of gastric cancer at the Third Peoples's Hospital of Linyi. In this study, Kaplan–Meier survival analysis was based on the cohorts from Kaplan–Meier-plotter (http://kmplot.com/analysis).

Cell lines and antibodies

The Institute of Cell Biology of the Chinese Academy of Sciences (Shanghai, China) provided us with Human gastric cancer cell lines (HGC-27, AGS and SGC-7901) and an immortal normal gastric epithelial cell line (GES-1). All of the cells were cultured in DMEM (Sigma-Aldrich Co., St Louis, MO, USA) supplemented with 10% FBS (HyClone, Shanghai, China) and penicillin/streptomycin(100 U/mL, HyClone, Shanghai, China) at 37°C in a humidified chamber containing 5% CO_2. Antibodies directed against P2RX7 (ab48871), β-actin (ab227387), ERK1/2 (ab115799), p-ERK1/2 (ab223500), Akt (ab8805), p-Akt (ab38449), E-cadherin (ab1416), N-cadherin (ab18203), vimentin (ab137321), and snail (ab53519) were purchased from Abcam.

Immunohistochemical experiments

Tissue paraffin sections were collected (thickness = 5 μm), and deparaffinized by conventional xylene gradient; 0.01 mol/L citrate buffer (pH = 6.0) was used to conduct antigen retrieval. After being blocked with working solution at 37°C for 10 min, primary antibody (1:100) was added to the sample and the sample was incubated at 4°C in the refrigerator overnight. After washing, secondary antibody, horseradish peroxidase-labeled streptavidin working solution, and diaminobenzidine were added on the sections. Microscopic examination was carried out after dehydration and sealing. Two professional pathologists were in charge of the evaluation and scoring of the samples. The staining intensity was evaluated as follows: 0: negative; 1: weak staining; 2: moderated staining; 3: strong staining. The staining distribution was evaluated as follows: 0:<10%; 1: 1–25%; 2: 25–75%; 3: >75%. Histochemistry score was counted by multiplying the staining intensity and distributions. Then the expression of P2RX7 was determined by the histochemistry score: −, not detectable (0); +, weakly positive (ranged from 1 to 3); ++, strongly positive (ranged from 4 to 9).

Real-time PCR

TRIzol reagents (Invitrogen, CA) were used to isolate total RNA from tissues according to the instructions of the manufacturer. Then, 2 μg total RNA of each specimens was used for cDNA synthesis. RT-PCR analyses were conducted with the SYBR Green PCR Master Mix (TaKaRa, Otsu, Shiga, Japan). Each product was repeated three times. The primer sequences are shown as follows: P2RX7 forward: 5′-TACATCGGCTCAACCCTCT-3′; P2RX7 reverse: 5′-CGTCGCCGTCCAGCTCGACCAG-3′; β-actin forward: 5′-CATTAAGGAGAAGCTGTGCT-3′; β-actin reverse: 5′-GTTGAAGGTAGTTTCGTGGA-3′.

shRNA and plasmid transfection

Genechem (Shanghai, China) designed and constructed the shRNA targeting P2RX7 and P2RX7 overexpression plasmid. Lipofectamine 2000 reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was used as the agent to transfect the shRNAs or plasmids according to the manufacturer's instructions. The sequence of the shRNA oligos targeting P2RX7 was as follows: 5′-GATCCCC(GGATCCAGAGCATGAATTA)TTCAAGAGA (TAATTCATGCTCTGGATCC)TTTTTGGAAA-3′.

Western blot

Protein in cells was extracted and protein concentration was measured by the BCA method. After 10% SDSPAGE gel electrophoresis, the sample was transferred to the PVDF membrane with constant current, and then blocked for 1 h at room temperature with skim milk with 10% TBST. And

the corresponding primary antibody (1:1000) was incubated at 4°C overnight, followed by incubating with peroxidase-labeled goat anti-rabbit secondary antibody (1:5000) for 1 h at room temperature, and washed three times with 10% TBST (5 min/time). At last, chemiluminescence phototope-HRP kit (Pierce Biotechnology, USA) was used to detect the signals.

CCK-8 assay

CCK8 cell proliferation experience was carried out with 96-well culture plates at 100 μL/well (approximately 800 cells per well), with three replicate wells per group. Meanwhile, Blank was settled as control (with medium only). Samples were continuously cultivated for one, two, three, and four days. Each well was added with 10 μL of CCK8 reagent before the test, and the culture was stopped after 2 h of incubation at 37°C. The control was adjusted to zero compared with the result of Blank. The microplate reader was used to detect the absorbance value (OD value) at a wavelength of 450 nm. The cell proliferation ability is expressed in terms of the corresponding OD value. The average value of three wells was taken for each group for statistical analysis.

Colony formation assay

Cells from different groups were plated into 60-mm tissue culture dishes. Each dish contained 1000 cells at first. After growing in DMEM medium containing 10% FBS for 14 days, cells were fixed and stained 0.1% crystal violet for 30 min. After being washed by PBS, the number of colonies was counted. Each group was repeated three times in triplicate.

Transwell experiment

The membrane material used in the Transwell chamber was polycarbonate and the sample was tested in a chamber with an 8.0 μm pore size. A single cell suspension with a cell count of 5×10^5/mL was taken; 200 μL of the suspension was added to the inner chamber, while the lower chamber contained 600 μL of complete medium. The sample was cultured at 37°C for 24 h. Afterwards, the chamber was removed and the remaining cells in the lower chamber were fixed by paraformaldehyde, and stained for 30 min with Coomassie blue. The field of view was randomly selected under the microscope to count how many cells had passed through the membrane.

Statistical analysis

GraphPad Prism 8 software was used to conduct statistical analysis. Chi-square test was used to evaluate the results of IHC and determine the relationship of gene expression and the clinicopathologic features. The quantitative data are shown as mean $\pm$ standard deviation, and $P < 0.05$ means there is a statistically significant difference.

Results

P2RX7 expression is abnormally up-regulated in gastric cancer

To analyze the expression of P2RX7 in gastric cancer, we used immunohistochemistry to detect P2RX7 protein in 80 cases of gastric cancer tissues and 20 normal gastric tissues (Figure 1(a) and (b)). The ratio of strongly positive, weakly positive, and negative in gastric cancer tissues is 68.75% (55/80), 17.5% (14/80), and 13.75% (11/80), respectively. Conversely, the percent of the samples of strongly positive, weakly positive, and negative in normal tissues is 20% (4/20), 50% (10/20), and 30% (6/20), respectively (Figure 1(c)). This indicated that the expression of P2RX7 in gastric cancer tissues was significantly higher than that for noncancerous tissues ($P < 0.001$). Next, we verified P2RX7 mRNA by RT-PCR in 10 pairs of gastric cancer tissues and adjacent tissues. We found that, the P2RX7 mRNA in cancerous tissue was increased (>2 folds) in most cases (8/10). Then, we detected P2RX7 protein expression in different cell lines by Western blot. As illustrated (Figure 1(e)), GES-1 cells had significantly lower expression level of P2RX7 than those in gastric cancer cells. Similarly, compared with GES-1 cell, the expression levels of mRNA in all of the three gastric cancer cell lines were increased. Collectively, the results above suggested that P2RX7 had high expression levels in gastric cancer tissues and cell lines.

Association between P2RX7 expression and clinicopathological characteristics of patients with gastric cancer

Aiming at further exploring the role of P2RX7 expression in the aggressive progression of gastric cancer, the 80 gastric cancer samples mentioned above were divided into two groups according to the IHC results of P2RX7 (low expression group: negative staining or weakly positive, $n = 25$; high expression group: strongly positive, $n = 55$), and the associations between P2RX7 expression and the clinicopathologic characteristics of gastric patients were showed (Table 1). The results show that P2RX7 overexpression was correlated with larger tumor size ($P = 0.0473$), higher T stage ($P = 0.0367$), and lymphatic metastasis ($P = 0.0056$). In contrast, no obvious correlation was existed between P2RX7 expression and gender, age, CEA level, differentiation status, or Her-2 status. Our results imply that P2RX7 is significantly related to tumor growth and metastasis of gastric cancer.

High P2RX7 expression had obvious relationships with worse survival in gastric cancer patients

To analyze the value of P2RX7 expression in the diagnosis of patients with gastric cancer, we searched the data in Kaplan–Meier-plotter (http://kmplot.com). The results indicated that patients with high P2RX7 level were associated with worse overall survival (Figure 2(a), $P<0.001$) as well as disease-free survival (Figure 2(b), $P = 0.024$). This further suggested that P2RX7 functioned as a putative

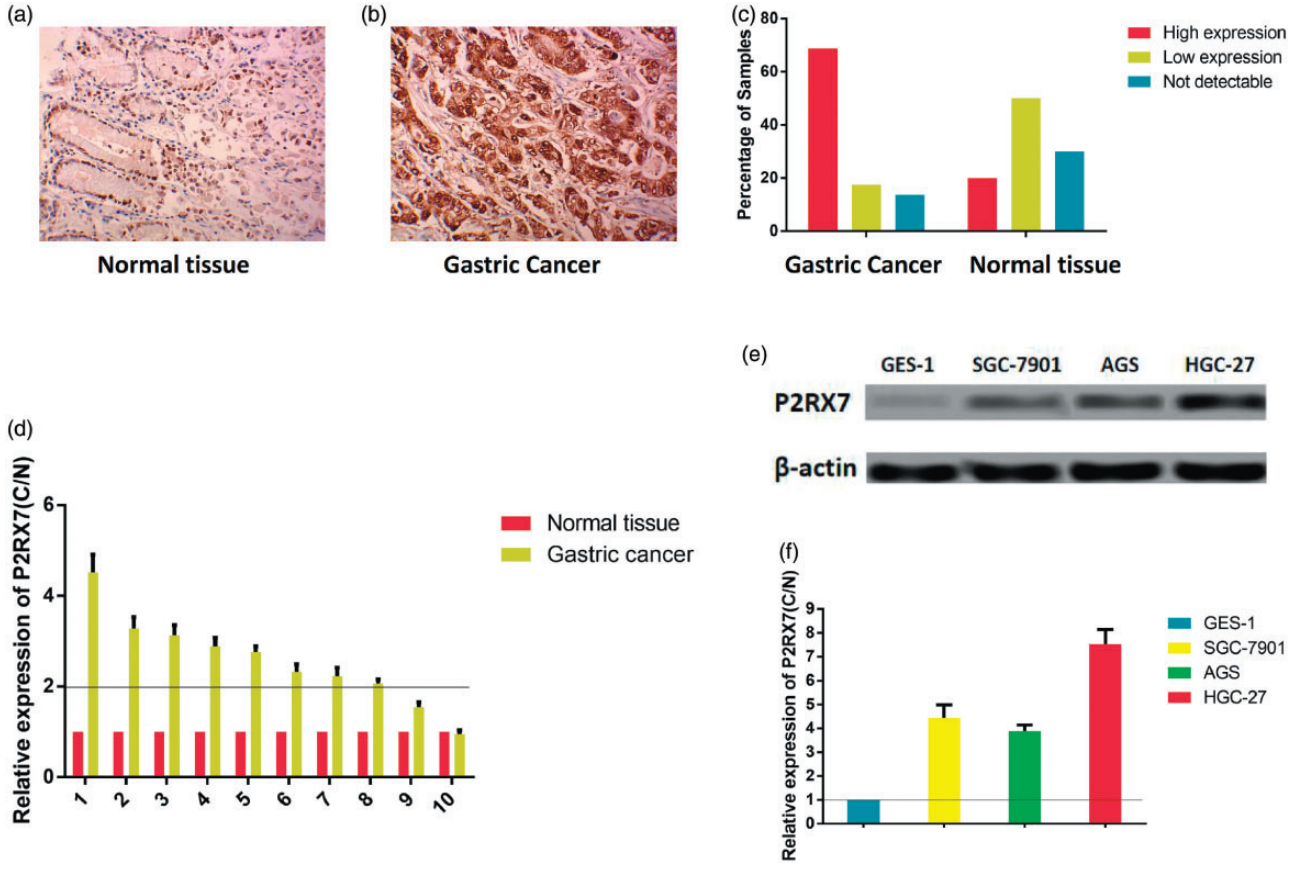

Figure 1. Up-regulation of P2RX7 in gastric cancer tissues and cell lines. (a) Protein expression of P2RX7 (low expression, +) in normal gastric tissue determined by IHC. (b) Protein expression of P2RX7 (high expression, ++) in gastric cancer tissue determined by immunohistochemistry. (c) Distributions of P2RX7 protein expression (−, + and ++) in gastric cancer tissues and normal tissues determined by IHC. (d) P2RX7 mRNA levels were measured by RT-PCR in 10 pairs of GC tissue and corresponding adjacent tissue. β-actin was used as internal control. (e) Western blotting analysis of P2RX7 expression in three gastric cell lines (SGC-7901, AGS, HGC-27) and one immortal normal gastric epithelial cell line (GES-1). β-actin served as the loading control. (f) RT-PCR analysis of P2RX7 expression in GES-1, SGC-7901, AGS, HGC-27. β-actin served as the internal control. (A color version of this figure is available in the online journal.)

biomarker in gastric cancer and could be used as a potential novel prognostic biomarker for GC patients.

P2RX7 may increase the proliferation of gastric cancer cells via activating ERK1/2 and Akt pathways

To decipher the role of P2RX7, its expression was suppressed or increased respectively in GC cell lines (Figure 3(a)). The results revealed that knockdown of P2RX7 caused significant decline in the viability of HGC-27 and AGS cells (Figure 3(b)). In contrast, overexpression of P2RX7 in SGC-7901 cells markedly promoted the proliferation of cells (Figure 3(b)). Additionally, cells with P2RX7 knockdown had a significantly inhibited ability to form colonies, while P2RX7 overexpression helped cancer cells form more and larger colonies (Figure 3(c)). It is reported that, in breast cancer, P2RX7 promoted malignant behaviors of cancer cells via activating ERK1/2 and Akt pathways.[13] Considering the ERK1/2 and Akt pathways are crucial modulator of proliferation and apoptosis,[14] we examined that whether P2RX7 had this mechanism by which it promoted gastric cancer progression. As expected, P2RX7 knockdown inhibited the phosphorylated levels of ERK1/2 and Akt, while P2RX7 overexpression activated the phosphorylation of them (Figure 4(a) and (b)).

P2RX7 enhanced the metastasis of gastric cancer cells via inducing EMT

Transwell assays also demonstrated that P2RX7 knockdown caused suppression of migration and invasion, while P2RX7 overexpression promoted migration and invasion in gastric cancer cells (Figure 5(a) and (b)). Epithelial–mesenchymal transition (EMT) is a process which is associated with tumor initiation, progression, and metastasis,[15] so the markers of the EMT were detected by Western blot. The results revealed that knocking down P2RX7 significantly up-regulated the epithelial marker E-cadherin, while down-regulated the mesenchymal markers N-cadherin, vimentin and snail in HGC-27 and AGS cells (Figure 6(a) and (b)). Conversely, P2PX7 overexpression improved the expression level of mesenchymal markers and inhibited the expression level of E-cadherin (Figure 6 (a) and (b)). These data implied that P2RX7 might exert a crucial function in causing EMT in gastric cancer cells.

Discussion

Studies have shown that P2RX7 play an important role in tumor cell growth and proliferation. In neuroblastoma N2a cells, activated P2RX7 can induce the release of substance P

Table 1. The correlation between P2RX7 expression and pathological characteristics of patients with gastric cancer.

Pathological characteristics	The number of patients	P2RX7 expression		Chi-square value	*P* value
		++	+/−		
Age					
≤55	41	29	12	0.1537	0.6950
>55	39	26	13		
Gender					
Male	48	34	14	0.2424	0.6225
Female	32	21	11		
Tumor diameter, d/cm					
<5	55	34	21	3.9363	0.0473
≥5	25	21	4		
T stage				6.6122	0.0367
T1	23	11	12		
T2	45	35	10		
T3 or T4	12	9	3		
CEA level (μg/L)					
<5	66	47	19	1.0641	0.3023
≥5	14	8	6		
Differentiation status					
Well	11	5	6	3.6076	0.1647
Moderate	43	30	13		
Poor	26	20	6		
Lymphatic metastasis				7.6649	0.0056
No	22	10	12		
Yes	58	45	13		
Her-2 expression				0.0039[a]	0.9503
+++	55	3	52		
++/+/−	25	2	23		

[a]Correction for continuity.

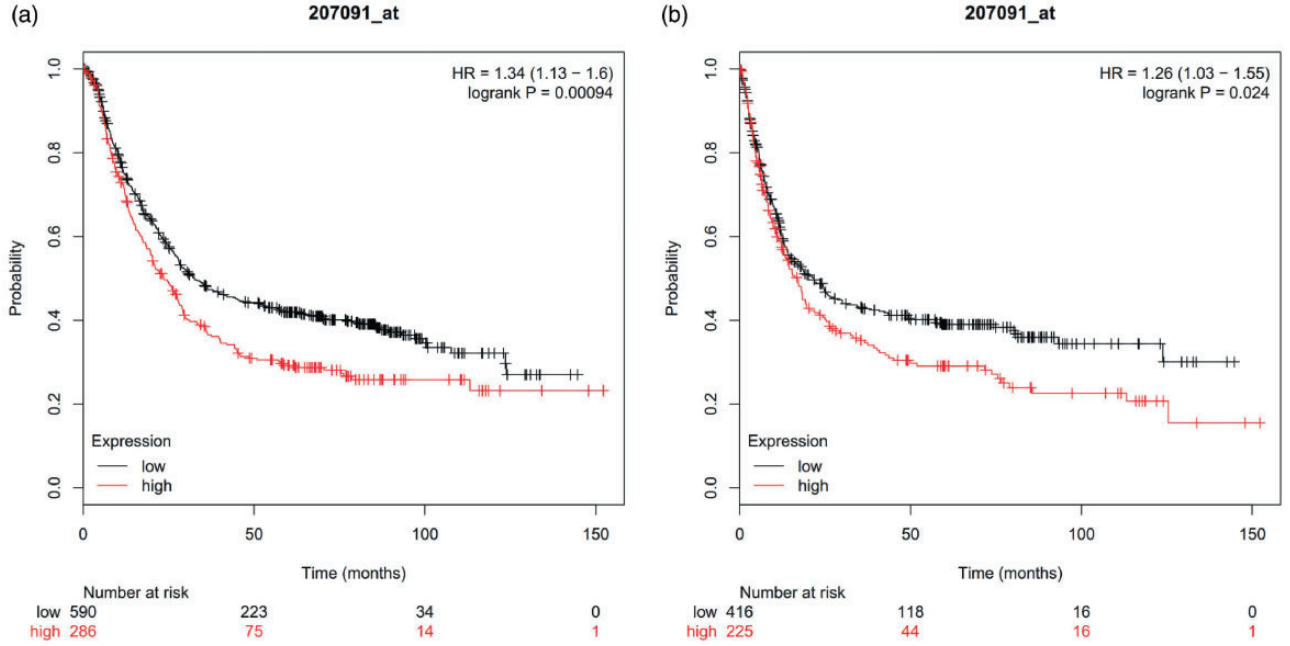

Figure 2. Kaplan–Meier survival analysis of P2RX7. (a) Higher expression level of P2RX7 was associated with shorter overall survival time of patients from k–m plotter ($P<0.001$). (b) Higher expression level of P2RX7 was associated with shorter disease-free survival time of patients from k to m plotter ($P=0.024$). (A color version of this figure is available in the online journal.)

by cells to stimulate tumor growth. After treatment of mouse neuroblastoma with ATP hydrolase or P2RX7 antagonist OATP or BBG, the viability of tumor cell decreased and tumor cell number decreased, indicating that P2RX7 are an important factor in maintaining N2a cell growth and proliferation.[16] In mouse melanoma B16 cells, hypoxia in the tumor center leads to the release of a large amount of ATP to extracellular condition, and extracellular ATP

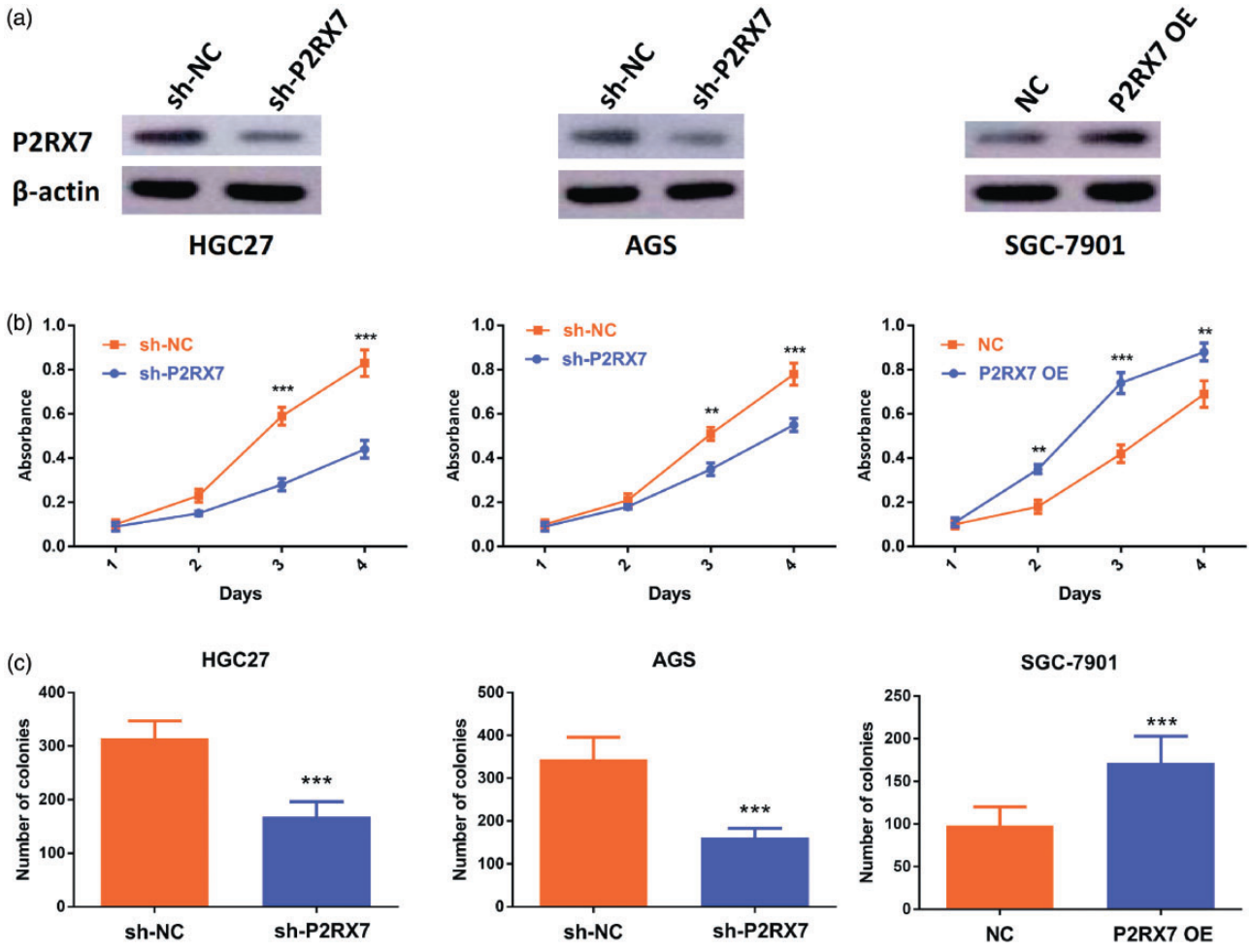

Figure 3. P2RX7 modulated proliferation of gastric cancer cells. (a) Knockdown of P2RX7 in HGC-27 and AGS cell lines by shRNA, and overexpression of P2RX7 in SGC-7901 cell line by ectopic plasmid were confirmed by Western blot. β-actin served as the loading control. (b) Effects of P2RX7 knockdown or overexpression on the proliferation on gastric cancers were determined by CCK-8 assay. From left to right: HGC-27, AGS, and SGC-7901. (c) Effects of P2RX7 knockdown or overexpression on the ability of colony formation on gastric cancers were determined by colony formation assay. From left to right: HGC-27, AGS, and SGC-7901. The bars represent the mean values of three independent tests (mean $\pm$ SD). **, *** represents $P<0.01$ and $P<0.001$, respectively. (A color version of this figure is available in the online journal.)

activates P2RX7. The amount of ATP released represents the degree of proliferation of tumor cells. For such reason, P2RX7 indirectly promote the proliferation of tumor cells.[17]

P2RX7 also enhances the metastasis of cancer cells. In highly invasive breast cancer cells, P2RX7 produce an inward current in the presence of ATP activation to strengthen Ca2+ influx and accelerate the degradation of cysteine cathepsin. In this way, the degradation of extracellular matrix is increased, leading to enhanced ability of invasion of breast cancer tumor cells.[18] In breast cancer cell lines of MCF-7 and MDA-MB-231, P2RX7's expression in tumor cells is increased, and the P2RX7 maintains tumor cell survival and invasion ability by activating ERK1/2 and Akt pathways,[13] which is consistent with our results. We also demonstrated that P2RX7 may regulate the process of EMT of gastric cancer cells, providing novel explanation of clues as to how P2RX7 promoted metastasis of cancer cells.

P2RX7 can also alter the tumor microenvironment. Localization of P2RX7 on the granulocyte-myeloid-inhibiting cell membrane up-regulates the levels of ARG-1, TGF-β1, and reactive oxygen species (ROS) and promotes tumor growth.[19] High expression of P2RX7 in tumors also promotes the expression of vascular endothelial growth factor (VEGF), thereby increasing tumor angiogenesis.[20]

However, P2X7 is specific for its function and role in different tumors. In some other tumors or cell lines, P2X7 exhibits the characteristics of a tumor suppressor. For example, in human colon tumor HCT-8 and Caco-2 cell lines, the survival rate of tumor cells decreased after tumor cells were stimulated with the P2RX7 agonist ATP for 48 h.[21] However, the ability to promote apoptosis is reduced by 50% when these cells were pre-processed with the P2RX7 inhibitor OATP.[21] In glioma cell line GL261, agonists ATP and BZATP inhibit tumor growth and induce apoptosis by activating P2RX7, while inhibitor OATP promotes cell proliferation; these confirm that P2RX7 receptors can inhibit tumor cell proliferation.[22] In a study on colitis-related colonic carcinoma, researchers established a mouse model and studied the role of blocking

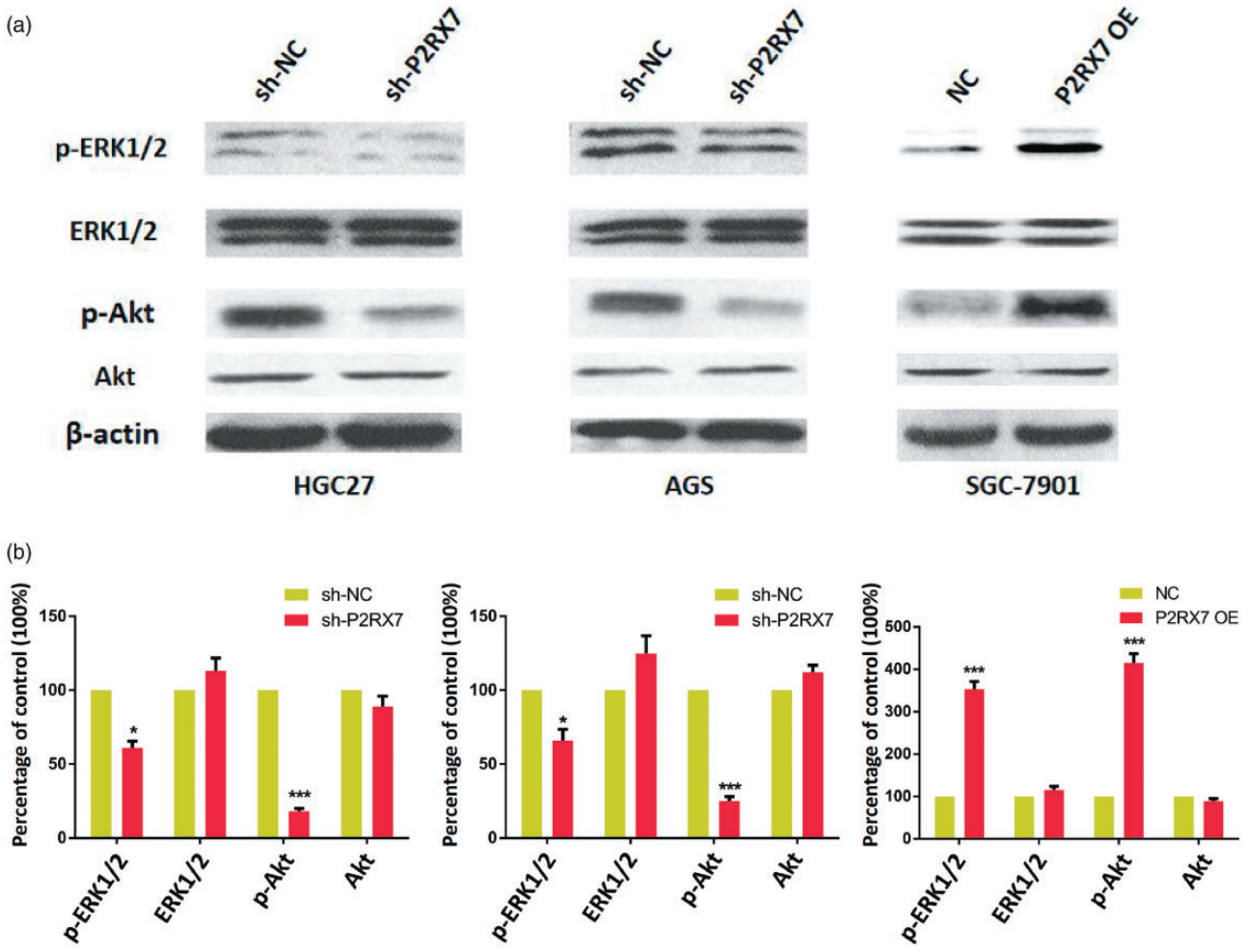

Figure 4. P2RX7 regulated the activation of ERK1/2 and Akt in gastric cancer cell lines. (a) Western blot analysis of the expression of p-ERK1/2, ERK1/2, p-Akt and Akt in HGC-27, AGS, and SGC-7901 cell lines after P2RX7 was inhibited or up-regulated. β-actin served as the loading control. (b) Relative expression levels of p-ERK1/2, ERK1/2, p-Akt, and Akt in (a). The bars represent the mean values of three independent tests (mean $\pm$ SD). *, **, *** represents $P<0.05$, $P<0.01$ and $P<0.001$, respectively. (A color version of this figure is available in the online journal.)

P2RX7. It is demonstrated that blocking P2RX7 can promote intestinal epithelial cell proliferation and inhibit cell apoptosis, thus indicating that P2RX7 can inhibit the tumorigenesis of colitis-associated cancers in mouse models.[23]

As cell membrane receptors, the function of P2RX7 is regulated by various factors such as extracellular ion concentration, pH, ATPase concentration, concentration of agonist and inhibitor. The agonists of P2X7 are mainly ATP and benzoylbenzoyl-ATP (BZATP).[24] Inhibitors of P2X7 include KN-62, Brilliant Blue G, azole compounds A438079, A83997, cyanoguanidine derivatives A740003, A759029, amantadine AZ10606120 and others. In three mesothelioma cell lines, the application of P2RX7′ selective inhibitors inhibits tumor cell proliferation, while the use of BZATP stimulates tumor cell proliferation.[25] Inhibition of P2RX7 by its inhibitors such as KN-62 and A740003 in human breast cancer cell MDA-MB-435s as well as lung adenocarcinoma cell A549 blocks ATP-induced tumor cell migration and invasion.[26] As an emerging anti-tumor drug, P2X7 receptor inhibitor is valuable in clinical pharmacology research and provides new ideas for targeted therapy of tumor.

In conclusion, we firstly demonstrated the overexpression of P2RX7 in both gastric cancer cell lines and tissues. Additionally, up-regulation of P2RX7 was demonstrated to be significantly correlated with several malignant characteristics of gastric cancer. We concluded that P2RX7 could be used as a putative biomarker for patients with gastric cancer. Moreover, our research revealed that P2RX7 enhanced the proliferation, migration, and invasion of gastric cancer cells, potentially via modulating ERK1/2 and Akt pathways and EMT. However, several limitations still remain in this research. Firstly, the present study was a single center study, which was also in lack of enough number of the samples. Secondly, the function of P2RX7 on promoting the proliferation and metastasis of gastric cancer cells was investigated only *in vitro*; *in vivo* studies were essential to be conducted in the future. Furthermore, it is still obscure that how P2RX7 regulates ERK1/2 and Akt pathways and EMT. In-depth studies are needed to illuminate the underlying molecular mechanisms by which P2RX7 regulates other signaling pathways.

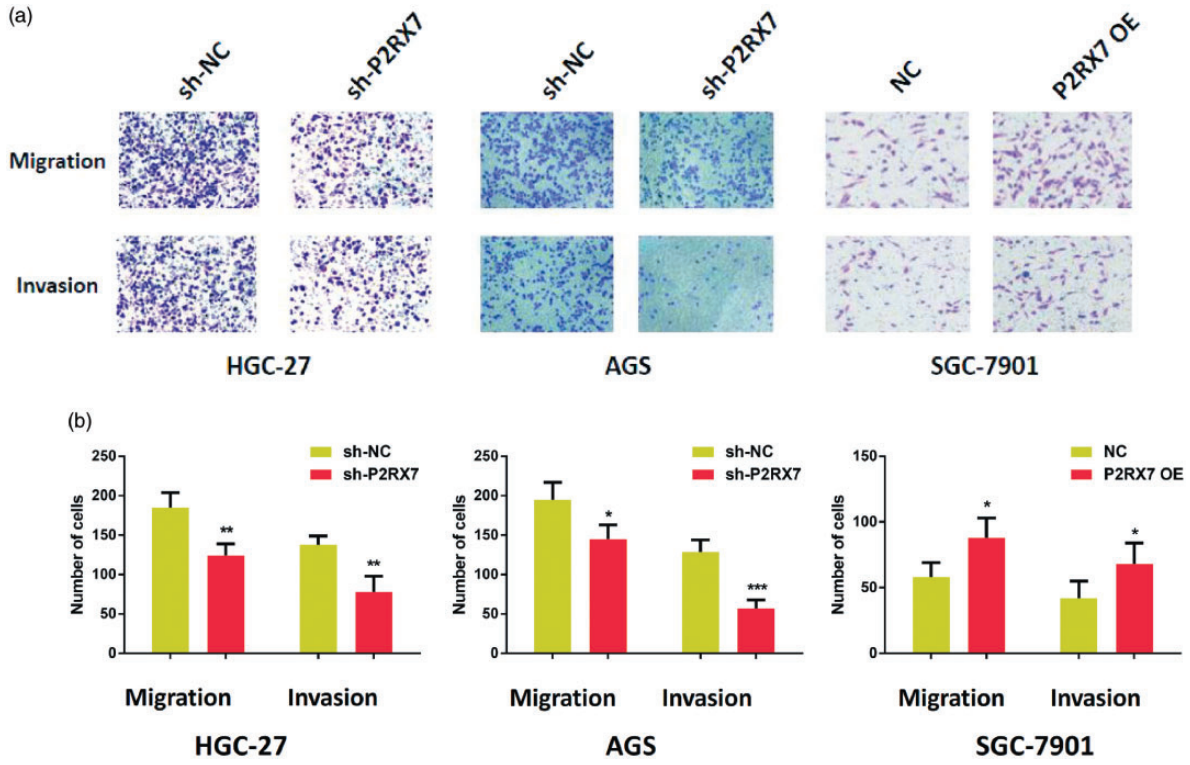

Figure 5. P2RX7 modulated metastasis of gastric cancer cells. (a) Effects of P2RX7 knockdown or overexpression on migration and invasion of gastric cancers were determined by transwell assay. From left to right: HGC-27, and SGC-7901. (b) Results in (a) were quantitatively analyzed. The bars represent the mean values of three independent tests (mean ± SD). **, *** represents $P<0.01$ and $P<0.001$, respectively. (A color version of this figure is available in the online journal.)

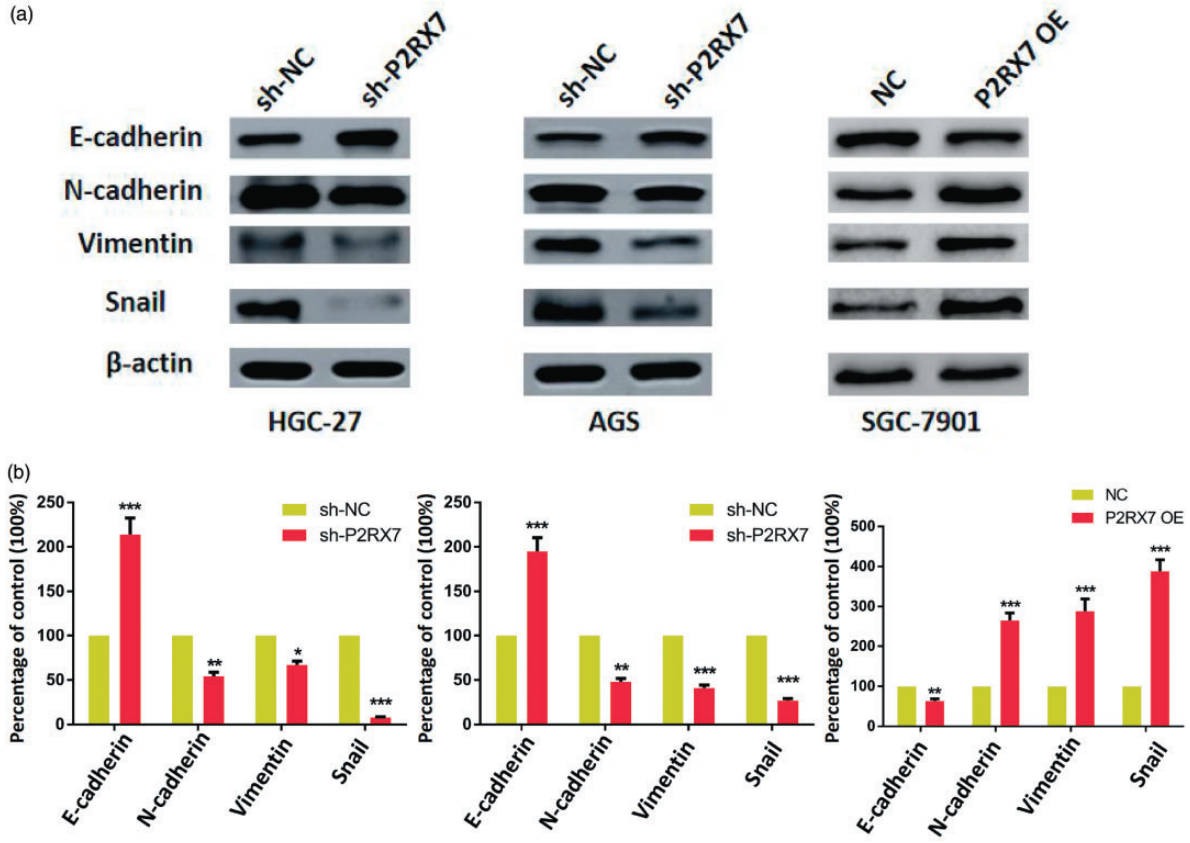

Figure 6. P2RX7 regulated EMT marker expression levels in gastric cancer cell lines. (a) Western blot analysis of EMT-related protein (E-cadherin, V-cadherin, Vimentin and Snail) expressions in HGC-27, AGS, and SGC-7901 cell lines after P2RX7 was inhibited or up-regulated. β-actin served as the loading control. (b) Relative expression levels of EMT markers in (a). The bars represent the mean values of three independent tests (mean ± SD). *, **, *** represents $P<0.05$, $P<0.01$ and $P<0.001$, respectively. (A color version of this figure is available in the online journal.)

Authors' contributions: Conceived and designed the experiments: SYL; Performed the experiments: WLL, LY, WTR, WX; Statistic analysis: WLL, LY; Wrote the paper: LY, WLL.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

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ETHICS STATEMENT

Our study was approved by the ethics review board of the Third People's Hospital of Linyi.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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