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Original Article

PAR2 promotes malignancy in lung adenocarcinoma

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Abstract: Proteinase-activated receptor-2 (PAR2) is closely linked to tumor malignancy, but its biological role in cancer remains underexplored. In this study, we assessed PAR2 expression in lung adenocarcinoma (LUAD) and normal lung tissues, analyzed associations between clinicopathological features and survival rates, and confirmed that PAR2 promotes apoptosis resistance and reduces cisplatin-induced cytotoxicity in lung cancer cells. Using TCGA datasets, western blotting, qPCR, and immunohistochemistry (IHC), we observed a significant increase in PAR2 levels in LUAD samples compared to normal tissues ($P<0.05$), with high PAR2 expression correlating with poor differentiation and lymphatic invasion ($P<0.05$). Upregulated PAR2 was associated with reduced survival. Additionally, PAR2 inhibition increased the BAX/BCL-2 axis and contributed to cisplatin-induced endoplasmic reticulum stress and apoptosis in H1299 cells. However, PAR2 inhibition reduced cisplatin-induced ATF4 expression. Overall, PAR2 upregulation is strongly associated with poor postoperative survival, differentiation, and lymphatic metastasis in LUAD and modulates cisplatin cytotoxicity.

Keywords: Lung cancer, PAR2, survival, lymphatic metastasis, cisplatin

Introduction

Lung carcinoma originates in the bronchial mucosa or lung glands [1]. According to global cancer statistics, lung cancer accounts for more cancer-related deaths than any other type worldwide, with over 2 million new cases diagnosed each year [2, 3]. While the incidence of lung cancer in the United States has declined by approximately 1-3% annually over the past decade [4], new cases have risen most rapidly in China, and approximately half of all new cases globally are in Asia [3, 5, 6]. Although targeted therapies have partially improved survival rates in lung cancer, the overall prognosis remains poor. Therefore, it is urgent to uncover new molecular mechanisms to reduce lung cancer mortality and incidence.

Protease-activated receptors (PARs) are a subfamily of G protein-coupled receptors (GPCRs), consisting of PAR1-4, and they play critical roles in tissue hemostasis, thrombosis, wound healing, inflammation-related diseases, fibrosis, and cancer. PAR activation occurs through extracellular cleavage by specific serine proteases, exposing N-terminal ligands that bind to and activate the receptor. Unlike other PAR family members that are activated by thrombin, PAR2 can be activated by trypsin and the FXa-tissue factor (TF) complex [7]. Once cleaved, PAR2 signals within the cell via classical G proteins and β-arrestin [8-10], and synergizes with other signaling pathways, including toll-like receptors, NOD-like receptors, ion channels, and cargo receptors, to promote fibrosis and tumorigenesis [8]. PAR2 is highly expressed in various tumor-derived cell lines, including those from lymphocytic leukemia, lung cancer, colon cancer, prostate cancer, pancreatic tumors, gastric tumors, hepatocellular carcinoma, breast cancer, and cervical cancer.

Evidence indicates that kallikrein-related peptidase 6 (KLK6) and trypsin activate LUAD cell proliferation through PAR2 [11]. Thus, PAR2 may contribute to lung cancer progression through regulation by serine proteases. PAR2 has

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Table 1. Clinicopathological features of lung cancer cases (N = 44)

Group	Characteristics	Number (%)
Gender	Male	13 (29.50)
	Female	31 (70.50)
Age	<60	15 (34.10)
	≥60	29 (65.90)
Smoking	Yes	10 (22.73)
	No	34 (77.27)
Differentiation degree	High-differentiated	10 (22.73)
	Middle-differentiated	21 (47.73)
	Low-differentiated	13 (29.55)
Clinical staging	IA-IIB	34 (77.30)
	IIIA-IV	10 (22.70)
Pleural invasion	Absent	36 (81.8)
	Present	8 (18.2)
Lymphatic invasion	Positive	29 (65.9)
	Negative	15 (34.1)

also been shown to induce migration and inactivate glycogen synthase kinase 3β (GSK3β), promoting slug-mediated epithelial-mesenchymal transition (EMT) in LUAD cells [12]. Monocyte chemoattractant protein-1 (MCP-1) is induced by metalloproteinase-1 (MMP-1) via PAR2 activation [13]. Moreover, PAR2 methylation has been linked to malignant progression in LUAD, affecting cell proliferation, migration, and invasion [14]. Additionally, PAR2 is associated with resistance to osimertinib and gefitinib [15, 16], as its inhibition can potentially reactivate osimertinib by limiting ERK-mediated EMT and subsequently reducing EGFR transactivation.

Most existing studies focus on PAR2's mechanism in lung cancer but lack analyses linking PAR2 with clinicopathologic features. In this study, we identified an upregulation of PAR2 in lung cancer cells and examined the significance of this upregulation and its potential as a clinical marker. PAR2 may become a valuable biomarker for LUAD malignancy progression, aiding in the diagnosis and treatment of LUAD.

Materials and methods

Bioinformatic analysis

Expression profile data and clinical information for lung tumors were obtained from The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga). The files singleGene.Symbol.pl and human.gtf were used to convert the probe matrix into a gene matrix, saved as an mRNA matrix file. The R packages "BiocManager" and "limma" were used to extract PAR2 expression data, which were saved as individual gene fragments. PAR2 expression in normal and LUAD samples was analyzed using the "Ggpubr" R package. We applied singleGene.preparePariedPlot.pl and the Wilcoxon test for paired testing of PAR2 expression in normal and cancer groups, represented in red and blue, respectively.

Cell lines and cell culture

Human A549 and H1299 non-small cell lung cancer (NSCLC) cell lines, along with BEAS-2B lung epithelial cells, were sourced from Pricella (CL-0016, CL-0165, and CL-0496, China). Additional A549 and H1299 cells were obtained from the Cell Bank of the Institute of Basic Medical Sciences, Chinese Academy of Sciences (Shanghai, China). A549, H1299, and BEAS-2B cells were cultured in DMEM/F12, RPMI-1640, and DMEM media, respectively, with 10% FBS and 1% streptomycin-penicillin. Cultures were maintained in an incubator at 37°C with 5% CO_2 and 95% humidity.

Tissue samples

Lung carcinoma and adjacent non-malignant tissues were obtained from 44 patients with primary lung cancer who underwent surgery at People's Hospital of Jilin Province in Changchun, China. The study followed Institutional Review Board guidelines, and all participants provided informed consent. All patients were diagnosed with lung adenocarcinoma by pathological examination and staged according to the tumor-lymph node metastasis (TNM) classification International Association for the Study of Lung Cancer (IASLC, 7th edition, 2009) and World Health Organization guidelines, 3rd edition. Participants included 13 males and 31 females aged 35-72 years (median = 62 years). Clinical characteristics and clinicopathologic factors were retrospectively assessed (**Table 1**).

Table 2. Sequences of the primers for PCR

Gene name	Primer sequence (5'-3')
PAR2	F: CATTGGCATCTCCCTGGCAA
	R: ACAGGTCGTGATGTTCAGGG
BAX	F: ACCAGGGTGGTTGGGGG
	R: AAAGTAGGAGAGGAGGCCGT
BCL-2	F: GAACTGGGGGAGGATTGTGG
	R: GCCGGTTCAGGTACTCAGTC
Caspase-3	F: CCTGGTTCATCCAGTCGCTT
	R: TCTGTTGCCACCTTTCGGTT
ATF4	F: ATGACCGAAATGAGCTTCCTG
	R: GCTGGAGAACCCATGAGGT
CHOP	F: GGAAACAGAGTGGTCATTCCC
	R: CTGCTTGAGCCGTTCATTCTC
S18	F: CATTCGAACGTCTGCCCTAT
	R: GATGTGGTAGCCGTTTCTCA

Immunohistochemistry (IHC)

Tissue sections were treated with 0.3% Triton X-100 for 10 minutes, followed by antigen retrieval and blocking with goat serum for 15 minutes. Sections were incubated with PAR2-specific antibodies (12160-1-AP, Proteintech, China), then imaged with a Nikon 80i light microscope (Japan) to quantify positively stained cells using ImageJ. Five high-power fields were randomly selected, and positive cell percentages were scored: <5% (score 0), 5-25% (score 1), 26-50% (score 2), 51-75% (score 3), and 76-100% (score 4). Staining intensity was scored as follows: no color (score 0), pale yellow (score 1), light brown (score 2), and dark brown (score 3). The final score, based on the product of cell percentage and staining intensity, categorized cells as negative (score 0), low expression (scores 1-4), moderate expression (scores 5-8), or high expression (scores 9-12).

qPCR analysis

Total RNA was extracted from tissues and cells using the RNA Isolator Total RNA Extraction Reagent (R401-01, Vazyme, Nanjing, China). RNA concentrations were measured with a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, USA). cDNA was synthesized from 1 μg of total RNA using the 5xHiScript III qRT SuperMix (R323-01, Vazyme, Nanjing, China), following the manufacturer's protocol. qPCR was performed in triplicate with ChamQ SYBR qPCR Master Mix (Q311-02/03, Vazyme, Nanjing, China) on a Gentier 96R Real-Time PCR system (Xian, China). Cycling conditions were 40 cycles of 95°C for 10 minutes, 95°C for 30 seconds, 64°C for 34 seconds, and 72°C for 30 seconds. Relative mRNA expression was normalized to S18 using the 2-ΔΔCt method. Primer sequences are listed in **Table 2**.

Western blot analysis

Protein expression levels in lung tissue, A549, H1299, and BEAS-2B cells were assessed via western blot. Frozen lung tissue and cell samples were lysed in RIPA buffer, and protein concentrations were measured using a BCA protein assay kit. Equal amounts of protein (20 mg) were loaded onto 10% SDS-PAGE gels and transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were blocked at room temperature for 1 hour and then incubated overnight at 4°C with primary antibodies, including anti-PAR2 (12160-1-AP, Proteintech, China), BAX (AB32503, Abcam, UK), BCL-2 (002611GA01, Cusabio, China), GAPDH (sc-166574, Santa Cruz Biotechnology, USA), Caspase-3 (9662, CST, USA), and β-actin (sc-81178, Santa Cruz Biotechnology, USA). Secondary antibodies (HRP-conjugated anti-mouse or anti-rabbit) were applied for 2 hours. Bands were visualized using a gel imaging system (01600MF, Bio-Rad, USA).

Evaluation of apoptosis

Apoptosis was measured using the Annexin V-FITC/PI Apoptosis Kit (APExBIO, K2003, USA). After trypsinization, cells were centrifuged and resuspended in 500 μl of 1× Binding Buffer (at a cell density of 1 × 10^5 cells), incubated with 5 μl Annexin V-FITC and 5 μl Propidium Iodide for 10-20 minutes in the dark, and immediately analyzed using a flow cytometer.

PAR2 antagonist treatment

PAR2 antagonist (FSLLRY-NH2 TFA) was obtained from Selleck (S991501, USA). Cells were treated with FSLLRY-NH2 TFA (100 μM) for 24 hours before protein or RNA extraction. Protein concentrations were determined using the BCA assay kit (Shanghai Binyuntian Biotechnology Co., Ltd., China), and RNA concentrations and purity were measured with a UV spectrophotometer (Thermo Fisher Scientific, USA).

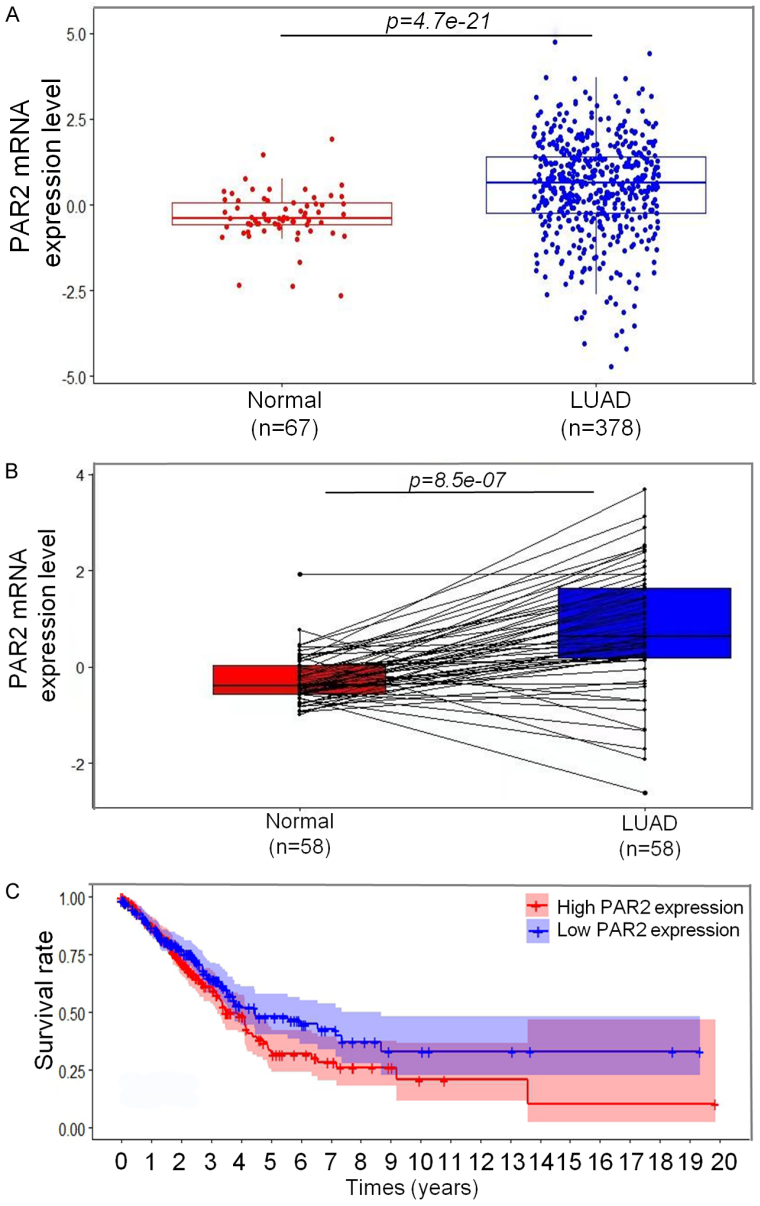

Figure 1. Predicted role of PAR2 in LUAD based on bioinformatics analysis (A) Higher PAR2 levels in LUAD samples compared to normal samples or (B) Paired adjacent lung tissues. (C) LUAD patients with a high PAR2 expression was related to poorer prognosis. The analysis of the above data from the TCGA database was performed by the R language. PAR2, protease-activated receptor-2; LUAD, Lung adenocarcinoma; TCGA, The Cancer Genome Atlas.

CRISPR-Cas9 genome-edited cells

PAR2-specific sgRNAs (AGTGCGGACACTTCGGCAA, GACCTGCCTCAGTGTGCAG) were inserted into the Bsb1 site of PX330. H1299 cells were transfected with 0.5 μg of PX330 construct for 24 hours using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA). Stable genome-edited clones were selected with 1 μg/mL puromycin and isolated with cloning discs. Knockout was confirmed via western blot.

Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparisons among multiple groups. Qualitative data were expressed as percentages, and categorical variables were compared using either the chi-square (χ^2) test or Fisher's exact test. Statistical analyses were conducted using IBM SPSS Statistics 25 (IBM Corp., Armonk, NY, USA). Bar graphs were plotted with GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $P<0.05$.

Results

Bioinformatics prediction

Based on the TCGA dataset, PAR2 expression in lung adenocarcinoma (LUAD) samples was significantly higher than in normal tissues (**Figure 1A**). Paired sample analysis yielded similar results (**Figure 1B**). Survival analysis indicated that LUAD patients with higher PAR2 expression had reduced survival times compared to those with lower PAR2 levels (**Figure 1C**). These bioinformatics predictions suggest that PAR2 may play an oncogenic role in LUAD.

Upregulation of PAR2 in LUAD

To investigate PAR2's role in NSCLC, we assessed PAR2 expression in lung cancer tissues and cells. Initial IHC analysis revealed that PAR2 predominantly localized to the cell membrane (**Figure 2A** and **2B**). Quantitative analysis via western blotting and qPCR confirmed that PAR2 expression was significantly elevated in lung cancer tissues compared to adjacent non-

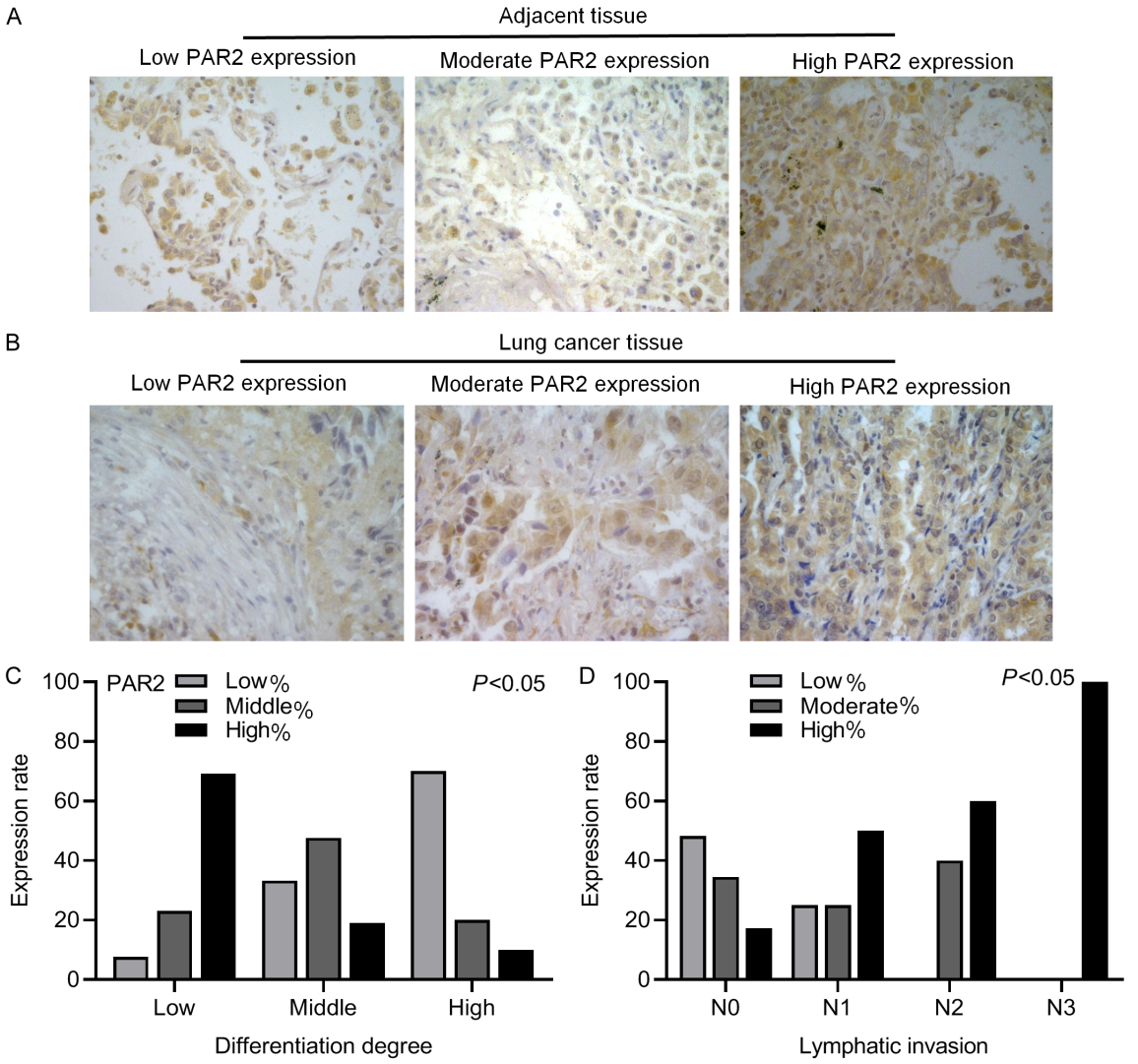

Figure 2. Correlation between pathologic grade and the expression of PAR2 in lung cancer IHC analysis of PAR2 in lung cancer and adjacent tissues (IHC ×400). A. Low, moderate and high staining of PAR2 respectively in adjacent tissues. B. Low, moderate and high staining of PAR2 respectively in lung cancer tissues. C. Upregulation of PAR2 was observed in poorly differentiated lung cancer tissues compared with well-differentiated tissues (*P<0.05*). D. High expression of PAR2 was positively correlated with the degree of lymphatic metastasis (*P<0.05*). PAR2, protease-activated receptor-2; IHC, Immunohistochemistry.

carcinoma tissues (**Figure 3A** and **3C**). PAR2 mRNA levels also showed an upward trend in lung cancer tissues (**Figure 3E**). Additionally, PAR2 expression in A549 and H1299 lung cancer cell lines was markedly higher than in normal lung BEAS-2B cells (**Figure 3B**, **3D** and **3F**).

Correlation ofPAR2 with clinicopathologic factors

We analyzed PAR2 protein levels in 44 lung cancer and adjacent normal tissue samples using IHC. PAR2 was highly expressed in 14 cancer cases but was present in only one normal lung tissue sample (2.28%) out of 44, with significantly elevated expression in cancer tissues compared to normal tissues (P<0.05) (**Table 3**). The association between clinicopathologic factors and PAR2 expression is summarized in **Table 4**. As shown in **Figure 2C**, PAR2 was expressed at high levels in 69.23% of poorly differentiated carcinomas, compared to 10.0% in well-differentiated tissues (P<0.05). Furthermore, the rate of high PAR2 expression was positively correlated with lymphatic metastasis (P<0.05) (**Figure 2D**). Among

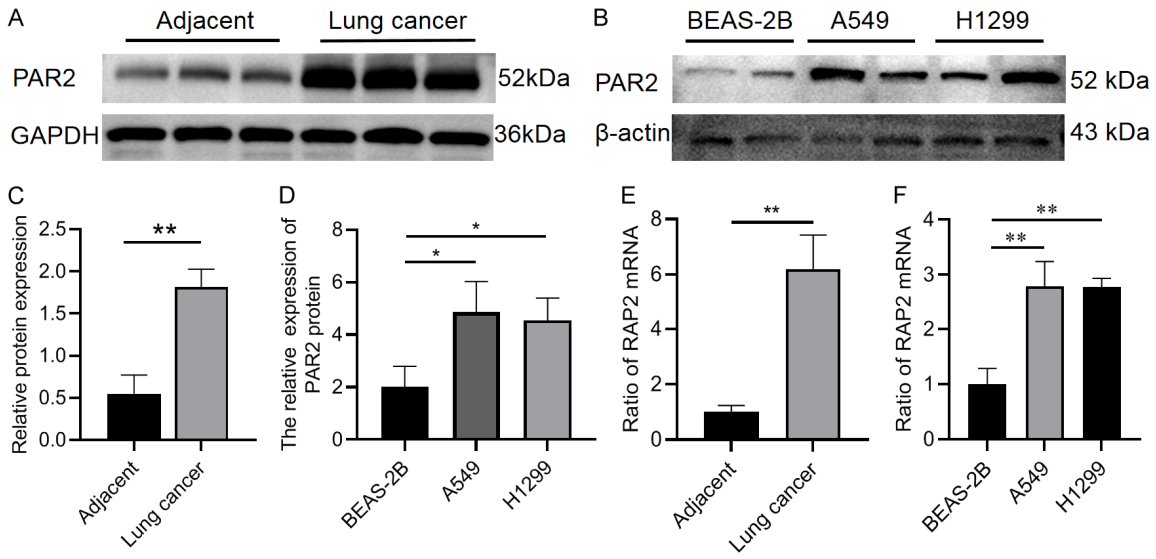

Figure 3. Increased PAR2 expression in LUAD tissues and cells. The expression of PAR2 in LUAD tissues was remarkably higher than adjacent tissues assessed by western blotting (A, C) and qPCR (E). Relative protein levels were normalized to β-actin (n = 3), ***P<0.01* versus adjacent group. High expression of PAR2 in A549 and H1299 cells compared to BEAS-2B cells which was detected by western blot (B, D) and qPCR (F). Data are presented as mean ± SD (n = 3), **P<0.05, **P<0.01* versus the BEAS-2B group.

Table 3. Expressions of PAR2 in lung cancer tissues and adjacent-cancer normal tissues

Groups	N	Expression of PAR2				
		Low (%)	Moderate (%)	High (%)	χ^2 value	*p value*
Adjacent	44	32 (72.70)	11 (25.00)	1 (2.28)	36.29	0.003
Cancer	44	15 (40.91)	15 (31.82)	14 (27.28)		

Table 4. Correlation between the expression of PAR2 and clinicopathologic factors

Parameter	Group	N	Expression of PAR2				
			Low (%)	Moderate (%)	High (%)	χ^2 value	*p value*
Gender	Male	13	4 (30.77)	4 (30.77)	5 (38.46)	0.458	0.842
	Female	31	11 (35.48)	11 (35.48)	9 (29.03)		
Ages	<60	15	4 (26.67)	3 (20.00)	8 (53.33)	5.004	0.081
	≥60	29	11 (37.90)	12 (41.40)	6 (20.70)		
Lymphatic invasion	N0	29	14 (48.28)	10 (34.48)	5 (17.24)	12.964	0.01
	N1	4	1 (25.00)	1 (25.00)	2 (50.00)		
	N2	10	0 (0.00)	4 (40.00)	6 (60.00)		
	N3	1	0 (0.00)	0 (0.00)	1 (100.00)		
Differentiation degree	High	10	7 (70.00)	2 (20.00)	1 (10.00)	14.808	0.003
	Middle	21	7 (33.33)	10 (47.62)	4 (19.05)		
	Low	13	1 (7.69)	3 (23.08)	9 (69.23)		
pTNM	IA-IIB	34	15 (44.10)	12 (35.30)	7 (20.60)	10.274	0.001
	IIIA-IV	10	0 (0.00)	3 (30.00)	7 (50.00)		

patients with stage IIIA-IV lung cancer, 50% exhibited high PAR2 expression, which was significantly greater than those with moderate to low PAR2 expression, suggesting a potential association between PAR2 and tumor stage (P<0.05). However, PAR2 expression did not

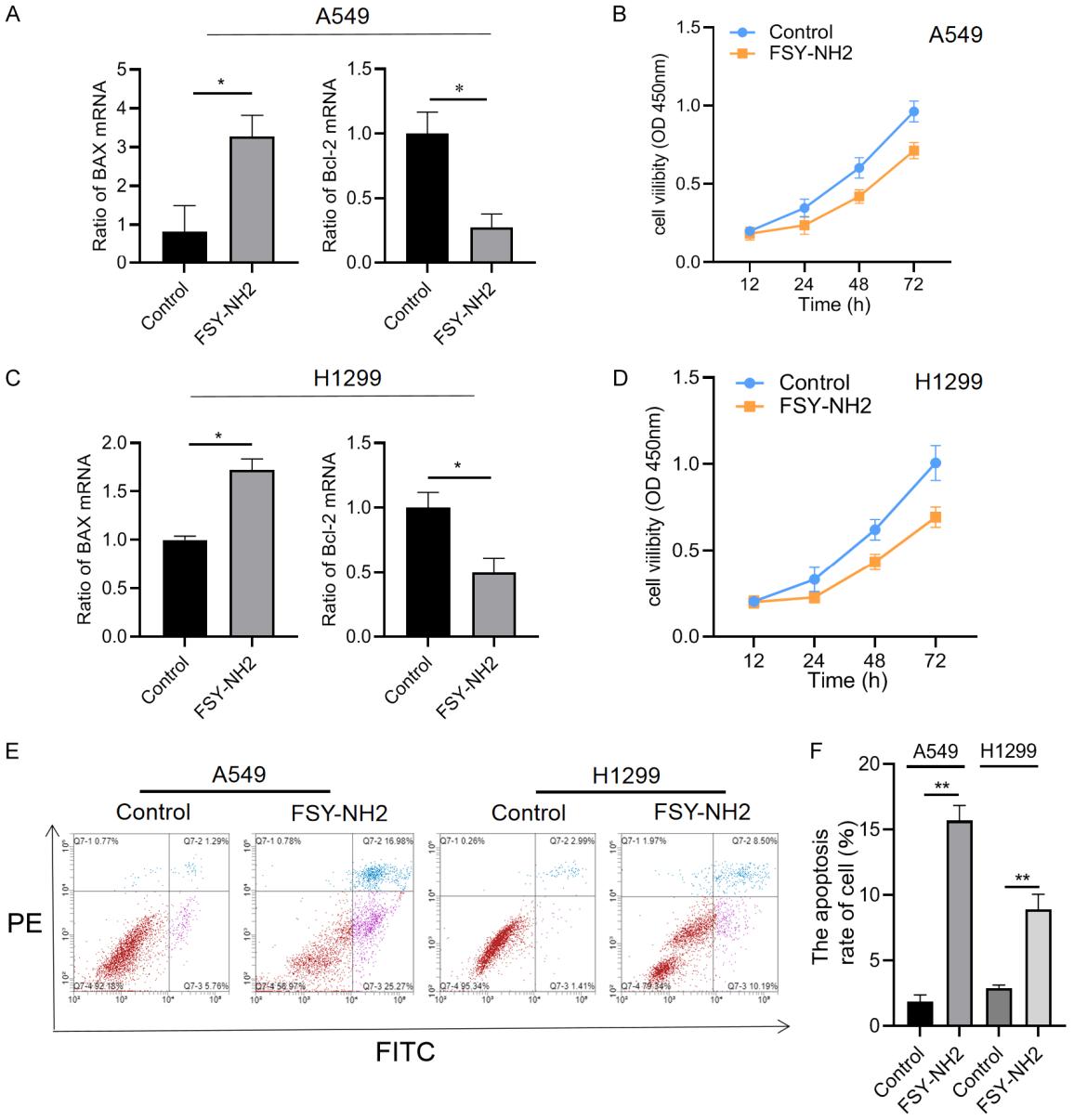

Figure 4. Inhibition of PAR2 attenuated the apoptosis resistance in A549 and H1299 cells. A, C. The inhibition of PAR2 increased BAX and decreased BCL-2 expression determined by qPCR in A549 and H1299 cells respectively. B, D. Inhibition of PAR2 suppressed the proliferation of A549 and H1299 cells as verified by CCK8 assay. E, F. Inhibition of PAR2-promoted apoptosis of LUAD cells evaluated by flow cytometry. **P<0.05, **P<0.01* versus control group.

correlate with other clinicopathologic factors such as gender or age.

Impairment of apoptosis resistance in LUAD cells by PAR2 inhibition

To further elucidate PAR2's role in apoptosis resistance in lung cancer cells, we inhibited PAR2 expression in A549 and H1299 cells using the PAR2 antagonist FSLLRY-NH2 TFA. Given that impaired apoptosis in lung cancer cells is often marked by a reduced BAX/BCL-2 ratio, we measured mRNA levels of BAX and BCL-2. Additionally, the effects of PAR2 down-regulation on lung cancer cell proliferation and apoptosis were assessed using the CCK-8 assay and Annexin V-FITC/PI flow cytometry, respectively. As shown in **Figure 4A-F**, qPCR analysis revealed significant upregulation of BAX and downregulation of BCL-2 in PAR2-inhibited A549 and H1299 cells (**Figure 4A** and **4C**). The CCK-8 assay indicated a notable

reduction in lung cancer cell proliferation upon PAR2 downregulation (**Figure 4B** and **4D**). In line with these results, suppression of PAR2 in A549 and H1299 cells led to a marked increase in apoptosis (**Figure 4E** and **4F**).

PAR2 inhibition promotes cisplatin-induced endoplasmic reticulum stress and apoptosis in LUAD cells

Cisplatin-based radiotherapy remains the standard of care for lung cancer, and PAR2-induced EGFR transactivation has been implicated in chemoresistance in cervical cancer cells [17]. We therefore examined the impact of PAR2 on cisplatin cytotoxicity in H1299 cells. Cisplatin treatment effectively reduced PAR2 expression (**Figure 5A** and **5C**). To further explore whether PAR2 plays a role in cisplatin's effects on LUAD cells, CRISPR-Cas9 was employed to downregulate PAR2 expression (**Figure 5B** and **5D**). PAR2 knockout (PAR2^-/-) H1299 cells were then treated with cisplatin for 24 hours. The results demonstrated that PAR2 deletion significantly reversed the cisplatin-induced upregulation of ATF4/CHOP (**Figure 5E** and **5F**). Furthermore, levels of caspase-9 and caspase-3 were reduced compared to the cisplatin-treated group (**Figure 5E** and **5G**). Notably, the BAX/BCL-2 ratio was higher in the cisplatin + PAR2 inhibition group than in the cisplatin-only group (**Figure 5G**).

Discussion

Lung cancer is a major health issue and the leading cause of cancer-related mortality worldwide. NSCLC accounts for approximately 80% of all lung cancers, with LUAD as the predominant subtype [1, 18]. Recent data indicate that about 631,000 people die from lung cancer annually in China, with its incidence rate still rising, in contrast to a decline observed in some southern countries. Such high incidence and mortality rates place a significant burden on society [18]. Therefore, understanding the molecular mechanisms underlying LUAD and developing effective treatments are crucial to improving prognosis.

The tumor microenvironment (TME) both drives and results from tumor progression. Tissue factor (TF) is involved in generating local thrombin and fibrin deposition within the tumor matrix. The TF-VIIa binary complex triggers various pro-angiogenic and immunomodulatory cytokines, chemokines, and growth factors that reshape the TME by activating PAR2 [19]. Additionally, proteases such as KLK6 and trypsin, secreted by cancer and adjacent non-cancerous cells, activate PAR2, promoting cancer cell proliferation and metastasis [20]. Beyond its critical impact on the TME, PAR2 also has direct effects on tumor biology. Previous studies have identified PAR2 as a novel regulator of TGF-β signaling in cancer.

Many studies have demonstrated the significance of PAR2 in cancers such as breast, colorectal, and liver cancer, particularly in vitro. However, few have explored the link between PAR2 expression and clinicopathologic factors through bioinformatics or patient tissue studies. Our bioinformatics analysis identified significant upregulation of PAR2 in LUAD tissues and cells, consistent with IHC and western blotting results, showing that PAR2 is markedly overexpressed in LUAD samples compared to paired normal tissues. Moreover, LUAD patients with higher PAR2 expression exhibited shorter survival times than those with lower PAR2 levels, and PAR2 expression was significantly correlated with the degree of malignancy in LUAD. Specifically, 69.20% of poorly differentiated cancer tissues expressed high PAR2 levels, compared to only 10.0% in well-differentiated tissues.

The critical role of PAR2 in chronic inflammatory diseases has spurred interest in developing selective PAR2 agonists and antagonists. Several agonists, such as GB110 and IK187, demonstrate drug-like properties and are more selective for PAR2 over PAR1. Notably, IK187 exhibits PAR2-dependent G protein activation and β-arrestin2 recruitment [21]. However, certain agonists, including DF253, AY77, and AY254, show a preference for Gαq/11 activation over β-arrestin2 recruitment [22]. Recently, a range of PAR2 antagonists has been developed, encompassing peptides, peptoids, and antibodies. For example, FSLLRY-NH2 blocks trypsin-mediated PAR2 activation and inhibits synthetic peptide activation of PAR2 by binding to the receptor docking site on the extracellular loop-2 (ECL-2) of the pegylated receptor ligand [23, 24].

In this study, we used FSLLRY-NH2 as a PAR2 inhibitor to examine the effect of PAR2 on apop-

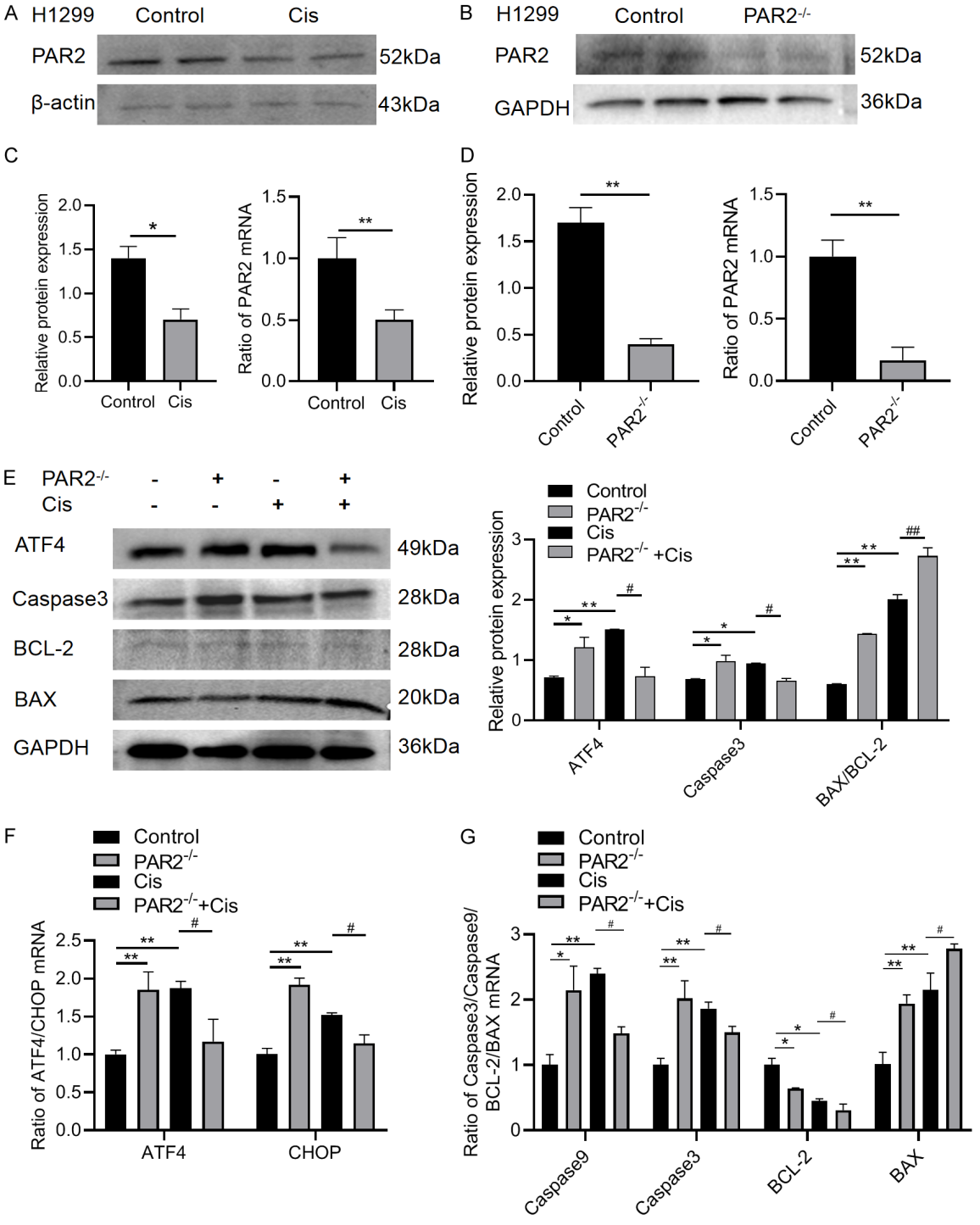

Figure 5. Inhibition of PAR2 promotes cisplatin-induced endoplasmic reticulum stress and apoptosis. A, C. Cisplatin promoted PAR2 expression determined by western blotting and qPCR in H1299. B, D. Knockdown of PAR2 in H1299 cells as verified by western blotting and qPCR. E. ATF4, Caspase3, BCL-2 and BAX evaluated by western blotting. F, G. ATF4, CHOP, Caspase3, Caspase9, BCL-2 and BAX evaluated by qPCR. Relative protein levels were normalized to β-actin or CAPDH, the date were presented as $\bar{x} \pm s$ (n = 3), **P<0.05, **P<0.01* versus control group, #P*<0.05,* ##*P<0.01* versus Cis+PAR2$^{-/-}$ group.

tosis resistance in LUAD cells. Our findings showed that PAR2 downregulation reduced apoptosis resistance in A549 and H1299 cells. Additionally, PAR2 promoted tumor-associated

angiogenesis and VEGFA expression through stimulation of the EGFR pathway [25]. Previous studies have shown that PAR2 enhances ERK-1/2 phosphorylation, thereby promoting proliferation and migration in colon cancer cells [26]. PAR2 has also been shown to increase MMP-2 and MMP-9 activity, facilitating tumor cell metastasis to surrounding tissues in PC3 and LNCap human cell lines [27]. Thus, PAR2 may act as a novel regulator in multiple tumor-related signaling pathways. However, further research is needed to elucidate its specific mechanisms of action.

This study has certain limitations. First, a large sample size assessment was not conducted to analyze PAR2 and clinical indicators in patients. For instance, the number of male samples and the sample sizes for patients in stages III and IV were limited. Second, the specific regulatory network through which PAR2 influences apoptosis resistance was not fully explored. These limitations should be addressed in future research.

In summary, the upregulation of PAR2 expression in LUAD patients may enhance apoptosis resistance in A549 and H1299 cells, indicating that PAR2 inhibitors could serve as a promising new target for lung cancer therapy.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

PAR2, protease-activated receptor-2; LUAD, lung adenocarcinoma; IHC, immunohistochemistry; PARs, Protease-activated receptors; GPCRs, G protein-coupled receptors; KLK6, kallikrein-related peptidase 6; TF, tissue factor; GSK3β, glycogen synthase kinase 3β; EMT, epithelial-mesenchymal transition; MMP-1, Matrix Metalloproteinase-1; MCP-1, Monocyte Chemoattractant Protein-1; NSCLC, non-small cell lung cancer; TNM, tumor-lymph node metastasis.

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