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ATP6V0B promotes the tumorigenesis of bladder cancer by activating PAQR4/PI3K/AKT signaling

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Abstract

Background ATPase H^+ transporting V0 subunit b (ATP6V0B) is an essential component of the vacuolar ATP multiprotein complex (V-ATPase) associated with energy metabolism. However, information on its role and mechanism of action in bladder cancer (BCa) and other tumors is not clear.

Methods In this study, we evaluated the expression of ATP6V0B in BCa and its correlation with patient survival outcomes by performing public database analysis, as well as, RT-qPCR and Western blotting assays. We also investigated the effect of altering the level of expression of ATP6V0B on the malignant behavior of BCa cells at the cellular level by conducting the CCK-8 assay and Transwell assay. In vivo experiments involved subcutaneous injection of stable ATP6V0B-knockdown BCa cells into nude mice to assess the influence of ATP6V0B on tumorigenesis. Additionally, bioinformatics analysis was combined with other methods to predict that ATP6V0B may modulate signaling pathways.

Results The findings showed that the expression of ATP6V0B increased in BCa tissues, and patients exhibiting high levels of this protein had a poorer prognosis. Additionally, our results showed that ATP6V0B functions as an oncogene and stimulates the proliferation, invasion, and migration of BCa cells in *vitro*. In *vivo* animal studies showed that downregulating ATP6V0B hindered the growth of BCa. Regarding the mechanism of action of ATVP60VB, we found that ATVP60VB can activate the PI3K/AKT signaling pathway through Progestin and AdipoQ Receptor Family Member 4 (PAQR4) -mediated upregulation.

Conclusion To summarize, the results of this study indicated that an increase in the level of expression of ATP6V0B in BCa tissues and cells is associated with unfavorable patient prognosis due to its tumor-promoting effects via upregulation of the PAQR4/PI3K/AKT signaling pathway.

Keywords ATP6V0B, Bladder cancer, V-ATPase, PAQR4, PI3K/AKT signaling

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Introduction

Bladder cancer (BCa) is a commonly occurring malignant tumor in the urinary system and has a high tendency for metastasis and recurrence [1, 2]. Its initial symptoms mostly remain undetected, which leads to treatment delays in many early-stage BCa patients [3]. Lack of monitoring and timely medical attention often causes the disease to progress to advanced stages before diagnosis, thus complicating treatment and resulting in unfavorable prognoses [4, 5]. Additionally, the nonspecific early symptoms of BCa can be misinterpreted as symptoms of common conditions like urinary tract infections and prostate inflammation, causing patients to opt for self-observation or non-professional treatments during the initial stages, further delaying diagnosis and intervention [4]. These factors collectively contribute to high mortality rates among individuals with BCa, emphasizing the importance of early screening, diagnosis, and management [6]. Therefore, the causes and mechanisms of the recurrence and metastasis of BCa need to be determined to prevent and manage this disease.

The Vacuolar ATP multi-protein complex (V-ATPase) is composed of two linked complexes (V0 and V1). It can monitor intracellular pH levels, utilizing ATP as an energy source to transport H^+ ions. It plays a key role in cellular energy metabolism [7, 8]. V-ATPase is commonly upregulated in cancer and primarily exerts oncogenic effects at different stages of cancer, although certain isoforms can suppress tumor growth [9–15]. ATPase H^+ transporting V0 subunit b (ATP6V0B) is part of the V0 complex. It creates the pore spanning the membrane and facilitates the transport of protons across the membrane to the vacuolar compartment [16, 17]. Some studies have shown that ATP6V0B is significantly associated with the autophagy-lysosome system [18, 19]. ATP6V0B may also play a significant role in the development and progression of cancer, particularly hepatocellular carcinoma [20], clear cell renal cell carcinoma [21], gastric cancer [22], and melanoma [23]. However, experimental studies to support this speculation are lacking. Additionally, limited information exists regarding the role of ATP6V0B in cancer, including its involvement in BCa. Thus, further studies are needed to better understand the role of ATP6V0B in BCa and its interactions with other molecules and signaling pathways to develop new and effective methodologies for the diagnosing, treatment, and prognostic assessment of cancer.

In this study, the expression of ATP6V0B was evaluated in BCa tissues, and its correlation with patient survival was determined using publicly accessible databases. Additionally, we investigated the effect of the overexpression or silencing of ATP6V0B on the proliferation, invasion, and migration of BCa cells, followed by an assessment of the underlying mechanisms of action. These findings might provide valuable information to improve clinical treatment strategies for individuals with BCa and achieve better overall survival outcomes.

Materials and methods

Bioinformatics methods

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/, accessed on 12 January, 7 March, and 4 November 2024) serves as an online platform for analyzing gene expression profiles in cancer and normal tissues [24]. It utilizes data from the TCGA and Genotype-Tissue Expression (GTEx) projects to provide profiling and interactive analysis tools. Kaplan-Meier Plotter (https://kmplot.com/analysis/) serves as an online platform for analyzing survival biomarkers [25]. Gene Set Enrichment Analysis (GSEA) (https://www.gsea-msigdb.org/gsea/index.jsp, 8-Mar-2025: GSEA 4.4.0 released.) is a computational method to assure the signal pathways regulated by ATP6V0B [26].

Clinical samples

Tissue specimens were obtained from 30 patients who underwent BCa surgery at Qingdao Municipal Hospital from January 2022 to December 2023. The group comprised an equal number of male and female patients, aged between 45 and 80 years (average age: 61.2 ± 9.8 years). All patients were found to have urothelial carcinoma. These patients were initially diagnosed with BCa following surgery without any preoperative adjuvant therapy. This study was approved by the ethics committee of Qingdao Municipal Hospital, and all participants provided informed consent before participation.

Cell culture

The SV-HUC-1 human normal urinary tract epithelial cells and BCa cells (J82, T24, 5637, and UMUC3) were purchased from the Chinese Academy of Sciences (Shanghai, China). The SV-HUC-1 cells were maintained in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12) supplemented with fetal bovine serum (FBS; Gibco, Carlsbad, USA) and 1% penicillin-streptomycin. The BCa cells (T24, J82, 5637, and UMUC3) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% FBS and 1% penicillin-streptomycin. All cell cultures were incubated at 37 °C with 5% CO_2.

Plasmid construction and cell transfection

The gene fragment of ATP6V0B (NM_004047.5) was synthesized and cloned into the pcDNA3 vector to construct the ATP6V0B-overexpressing vector (pcDNA3-ATP6V0B). Similarly, to construct the ATP6V0B/PAQR4-knockdown plasmids pshR- ATP6V0B/pshR-PAQR4, the knockdown sequences for ATP6V0B (5′-GA

TTTGGGCTTCCGCTTTGATTTCAAGAGAATCAA AGCGGAAGCCCAAATCTTTTTT-3′) /PAQR4 (5′-G CAGTTCAATAAGTTCGTGCTTTCAAGAGAAGCA CGAACTTATTGAACTGCTTTTTT-3′) were synthesized and integrated into the pSilencer 2.1neo vector. All gene sequences were synthesized by GenScript Biotech Corporation.

The BCa cells were plated at a density of 5×10^5 cells in uncoated culture dishes the day before transfection, using an antibiotic-free culture medium. During transfection, the cell fusion rate was determined to be 80%. The plasmids selected for transfection and the transfection reagent were individually diluted, mixed, and added to six-well plates. The cell plate was gently agitated to ensure uniform distribution of the cells, followed by incubation at 37 °C with 5% CO_2 in a cell culture incubator for 24–48 h. Transfection efficiency was evaluated by performing RT-qPCR and Western blotting assays.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

After extracting total RNA from tissues and cells using the Trizol RNA reagent, cDNA was synthesized using a reverse transcription kit. The synthesized cDNA was used as a template for amplifying the primers following the procedures outlined in the RT-PCR reagent kit. The primer sequences used were as follows: for ATP6V0B, the upstream sequence was 5′- CCATCGGAACTACC ATGCAGG −3′ and the downstream sequence was 5′- TCCACAGAAGAGGTTAGACAGG −3′; for GAPDH, the upstream sequence was 5′-GAGCCACATCGCTCA GACAC-3′ and the downstream sequence was 5′-GCC CAATACGACCAAATCC-3′; for PAQR4, the upstream sequence was 5′- TACCTGCACAACGAACTGGG −3′, and the downstream sequence was 5′- AAGAGGTGATA GAGCACGGAG −3′. The relative level of expression of ATP6V0B was calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell counting kit-8 (CCK-8)

After 24 h of cell transfection, the cell suspension was diluted with a serum-containing culture medium to a density of 2×10^4 cells/mL. Subsequently, 0.1 mL of the diluted cell suspension was added to each well of a 96-well cell culture plate, with three replicates per group. At 0, 24, 48, and 72 h post-transfection and after the cells adhered to the plate, 0.01 mL of CCK-8 reagent was added to each well. Then, the plates were incubated for 2 h in an incubator. The absorbance was measured at 450 nm using a microplate reader.

Transwell assay

To determine the migratory ability, 1×10^4 cells resuspended with 200 μL serum-free culture was added to the upper chamber (8.0 μm pore size chamber). To determine the ability to invade, 3×10^4 cells resuspended with 200 μL serum-free culture was added to the upper chamber of matrigel-coated transwells. Then, 600 μL of complete culture was added to the lower chamber to incubate at 37 °C with 5% CO_2 for an appropriate duration. After incubation, the cells were fixed with 4% formaldehyde and stained with crystal violet. Images were captured under the microscope, and cell penetration through the membrane in each well was quantified.

Western blotting assays

Proteins were extracted from tissues and cells using Radio Immunoprecipitation Assay (RIPA) buffer. After determining the protein concentration, the proteins were denatured at 100 °C for 10 min. Subsequently, Western blotting analysis was performed, and the proteins were transferred to a polyvinylidene fluoride (PVDF) membrane following standard procedures. Then, the membrane was blocked and incubated overnight with primary antibodies at 4 °C on a shaker. The primary antibodies used in this study are as follows: ATP6V0B rabbit antibody (Cat No: YN3416, 1:1000, Immunoway, China), β-Tubulin polyclonal antibody (Cat No: AC015, 1:5000, ABclonal, China), p85 monoclonal antibody (Cat No: A11177, 1:1000, ABclonal, China), p-p85 antibody (Cat No: ab191606, 1:1000, Abcam, USA), AKT antibody (Cat No: ab8805, 1:500, Abcam, USA), p-AKT (Ser473) antibody (Cat No: 80455-1-RR, 1:5000, Proteintech, China), E-cadherin antibody (Cat No: EPR16845-108, 1:1000, Abcam, USA), Vimentin (Cat No: A11952, 1:1000, ABclonal, China), and PAQR4 (Cat No: SAB2107001, 1:1000, Sigma-Aldrich, USA). The following day, the biotin-labeled secondary antibodies were applied at room temperature for 1 h. Then, the expression levels of target proteins were visualized by an enhanced ECL luminescence detection kit (Cat No: BL523A, Biosharp, Anhui, China). Finally, grayscale analysis was conducted using the ImageJ software.

In vivo animal experiments

The in vivo animal experiment method was conducted according to the literature we previously published [27]. Following transfection, the J82 cells underwent G418 selection to obtain ATP6V0B-knockdown stable cell lines. In total, 12 nude mice were used for the experiments. They were randomly divided into two groups ($n = 6$ mice per group). The stably transfected cells (2×10^6 cells /100 μL) were injected near the axilla in the right scapular fossa of each mouse. The nude athymic BALB/c mice used in this study were female (weight: 20 ± 2 g; age: six weeks old). All mice were housed and maintained according to SPF guidelines. Three weeks post-injection, all mice were euthanized by intraperitoneal injection of sodium pentobarbital (60 mg/kg, Cat No: 57-33-0,

Sinopharm Group Chemical Reagents (Shanghai) Co., LTD, Shanghai, China). The volume, weight, and size of the tumors were recorded, and tissue samples were collected and photographed.

Immunohistochemistry (IHC)

Paraffin sections were dewaxed in water, and followed by antigen repair with citrate buffer. Subsequently, the sections were blocked with a 5% BSA and incubated at 4 °C overnight with rabbit anti-ATP6V0B antibody (Cat No: YN3416, 1:100, Immunoway, China) and Ki67Ki67 antibody (Cat No: ab15580, 1:500, Abcam, USA). The secondary antibodies from the SABC immunohistochemistry staining kit were applied, followed by using Diaminobenzidine (DAB) Kit (ZSGB-BIO, Beijing, China) and hematoxylin. Finally, the sections were photographed by evaluation under a light microscope.

Statistical analysis

All statistical analyses were conducted using SPSS 22.0. The data were presented as the mean ± standard deviation (mean ± SD). The differences among multiple groups were determined by performing a one-way analysis of variance (ANOVA), whereas the differences between groups were determined by performing two-sample t-tests. All differences among and between groups were considered to be statistically significant at $p < 0.05$.

Results

Upregulation of ATP6V0B was associated with poor prognosis in BCa

To determine the role of ATP6V0B in BCa, we initially assessed its level of transcription in patient tissues using the genotype-tissue expression (GTEx) and gene expression profiling interactive analysis (GEPIA) database. Our results indicated a substantial upregulation of the transcription of ATP6V0B in BCa tissues compared to that in normal tissues (Fig. 1A). The GEPIA and Kaplan-Meier Plotter databases indicated that a high level of expression of ATP6V0B was associated with significantly lower overall survival rates among BCa patients (Fig. 1B and C). To confirm these findings, we assessed the level of expression of ATP6V0B in 30 pairs of BCa patient tissues and corresponding normal tissues obtained from clinical cohorts. The results showed that the ATP6V0B mRNA levels were considerably higher in BCa tissues, which matched the results of the above-mentioned GEPIA database analysis (Fig. 1D). Similar changes were also recorded in the ATP6V0B protein levels across four pairs of BCa tissues and adjacent normal tissues (Fig. 1E). Finally, by analyzing human immortalized urothelial cells SV-Huc-1 and four BCa cell lines (J82, T24, UMUC3, and 5637), we found higher levels of ATP6V0B protein in the BCa cells relative to those in SV-Huc-1 cells (Fig. 1F). These results suggested that ATP6V0B is closely involved in the pathogenesis of BCa.

ATP6V0B acted as an oncogene in BCa

To assess the role of ATP6V0B in BCa, we initially constructed ATP6V0B overexpression plasmids and

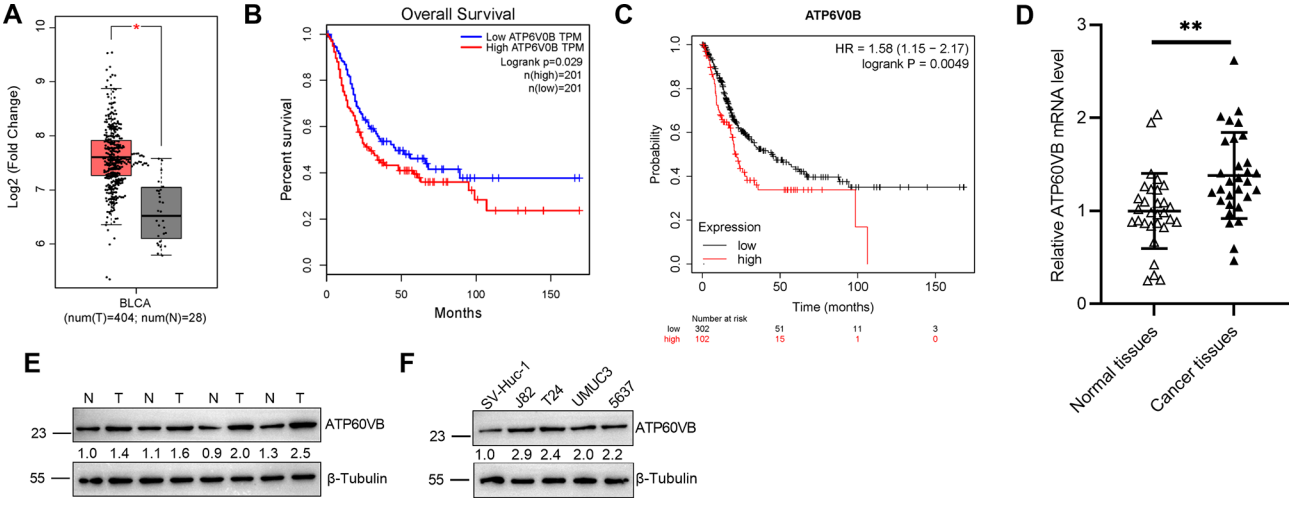

Fig. 1 ATP6V0B was upregulated in BCa. The GEPIA database was used to determine the mRNA level of ATP6V0B (**A**) and the overall survival of BCa patients (**B**). (**C**) The Kaplan-Meier (K-M) plotter was used to analyze the mRNA level of ATP6V0B and survival data. (**D**) RT-qPCR assays were performed to determine the mRNA level of ATP6V0B in BCa tumor tissues and adjacent normal tissues ($n = 30$). (**E**) Western blotting assays were conducted to quantify the expression of ATP6V0B in four paired BCa tumor tissues and adjacent normal tissues. (**F**) Western blotting assays were performed to quantify the expression of ATP6V0B in a non-cancerous immortalized urothelial cell line SV-Huc-1 and four BCa cell lines, including J82, T24, UMUC3, and 5637; *$p < 0.05$ and **$p < 0.01$

knockdown plasmids and transfected them into the BCa cells J82 and T24. The efficacy of transfection was validated by RT-qPCR analysis (Fig. 2A). Next, we evaluated the effect of altering the level of expression of ATP6V0B on the proliferation of BCa cells by conducting a CCK-8 assay. Our results showed that the proliferative capacity of BCa cells increased significantly following the overexpression of ATP6V0B, whereas knocking down ATP6V0B strongly inhibited the proliferation of these cells (Fig. 2B and D). However, whether changes in the expression of ATP6V0B affect the invasion and migration abilities of BCa cells was not clear. Transwell experiments were conducted to address this issue. The results showed that overexpression of ATP6V0B significantly increased the invasion and migration abilities of BCa cells. In contrast, these abilities were suppressed after ATP6V0B was knocked down (Fig. 2E). Further investigations indicated that upregulating ATP6V0B increased the expression of Vimentin but decreased the expression of E-cadherin in BCa cells. In contrast, the level of expression of Vimentin and E-cadherin showed the opposite pattern after ATP6V0B was knocked down (Fig. 2F and I). These findings suggested that ATP6V0B promotes the proliferation, migration and invasion of BCa cells in *vitro*.

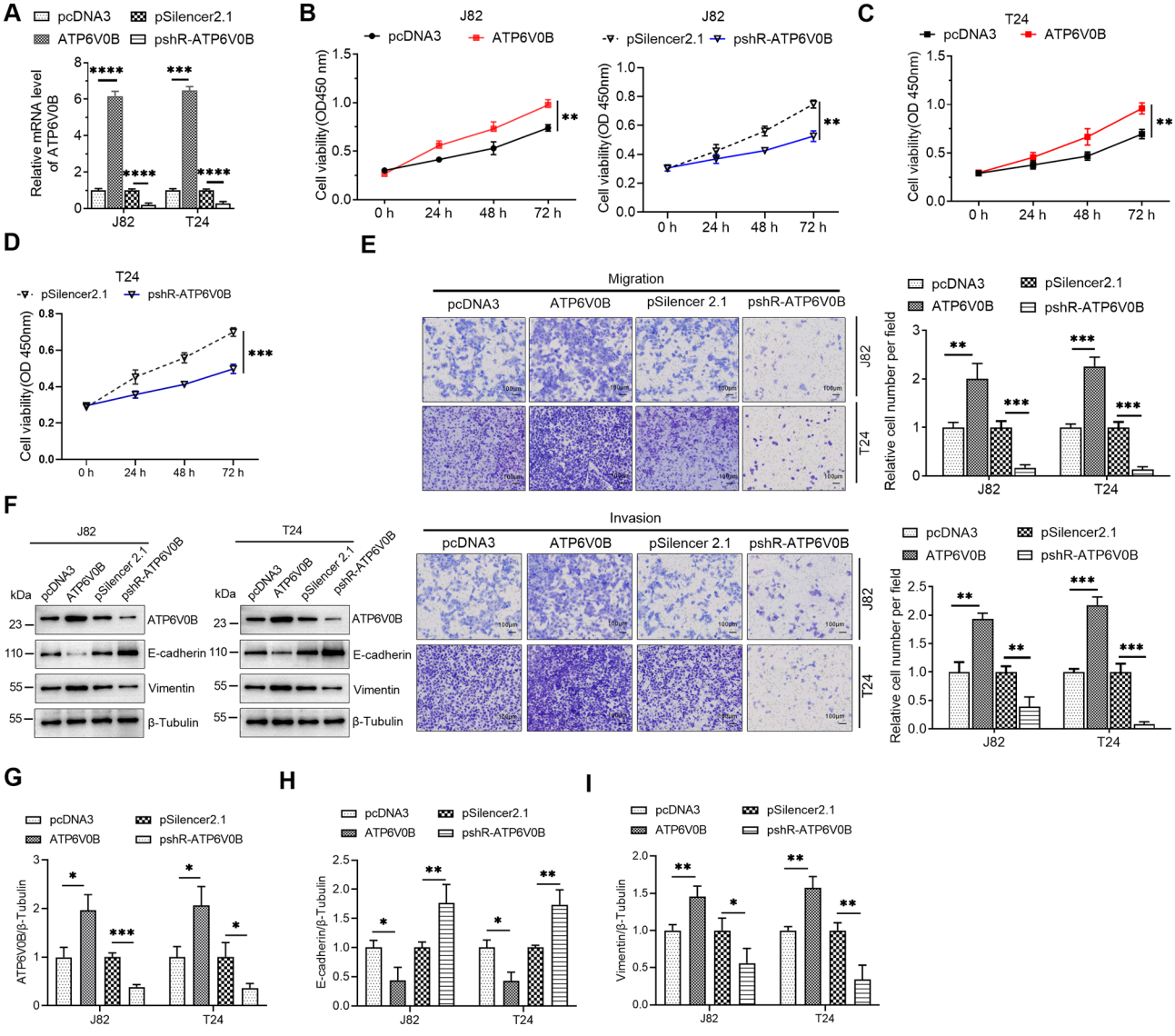

Fig. 2 ATP6V0B increased the proliferation, migration, and invasion abilities of BCa cells in vitro. (**A**) The efficiency of the overexpression and knockdown of ATP6V0B plasmids was evaluated by RT-qPCR assays in BCa cells. (**B** - **D**) The CCK-8 assay was performed to measure the effects of the overexpression and knockdown of ATP6V0B on the proliferation of BCa cells. (**E**) The migration and invasion abilities of BCa cells were evaluated by overexpressing and knocking down ATP6V0B. (**F**) Western blotting analysis was performed to quantify the expression of ATP6V0B, E-cadherin, and Vimentin. (**G** - **I**) Quantification of the protein levels in (**F**); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$

Knocking down ATP6V0B inhibited tumor growth in *vivo*

After determining the role of ATP6V0B in BCa in vitro, we validated its role in vivo by conducting animal experiments. We found that the ability of the BCa cell J82 to form tumors in nude mice was significantly inhibited after ATP6V0B was knocked down (Fig. 3A); additionally, the tumors formed were also smaller in terms of size and weight (Fig. 3B and C). Next, we assessed the level of ATP6V0B mRNA in the tumor tissue and found that the level of transcription of ATP6V0B in the tumors was significantly lower after ATP6V0B was knocked down (Fig. 3D). By performing IHC analysis, we found that the level of expression of ATP6V0B in the ATP6V0B-knockdown tumor tissue was also significantly lower (Fig. 3E). Finally, we evaluated the level of expression of Ki67 (a proliferative cell-related antigen) and found that the expression of Ki67 in the ATP6V0B-knockdown tumor tissue was also significantly lower (Fig. 3F). These results indicated that the ability of BCa cells to grow in nude mice was significantly inhibited after ATP6V0B was knocked down.

ATP6V0B positively regulated the PI3K/AKT signaling pathway in BCa

The mechanism by which ATP6V0B promotes the proliferation and migration of BCa cells is not clear. By conducting GSEA, we found that ATP6V0B is closely related to the PI3K/AKT signaling pathway (Fig. 4A). The results of Western blotting assays showed that the overexpression of ATP6V0B significantly increased the level of p-p85 and p-AKT proteins in cells, whereas knocking down ATP6V0B significantly decreased the level of p-p85 and p-AKT proteins (Fig. 4B and C). Next, we treated ATP6V0B-overexpressing BCa cells with the PI3K inhibitor LY294002 and found that after treatment with LY294002, the level of p-p85 and p-AKT proteins decreased significantly (Fig. 4D and E). These results indicated that ATP6V0B can activate the PI3K/AKT signaling pathway.

ATP6V0B positively regulated the expression of PAQR4 in Bca

To date, the mechanism by which ATP6V0B activates the PI3K/AKT signaling pathway is not clear. Bioinformatics analysis identified 54 genes that are positively correlated with ATP6V0B and highly expressed in BCa (Fig. 5A). Among these, 15 genes exhibited significantly distinct survival curves. Several studies have speculated that PAQR4 might activate the PI3K/AKT signaling pathway [28–30]. Based on our analysis, we found that the mRNA levels of ATP6V0B and PAQR4 were significantly positively correlated with BCa tissues (Fig. 5B). Our results also showed that PAQR4 was highly expressed in BCa tissues, and a high level of expression of PAQR4 was significantly associated with greater survival of BCa patients (Fig. 5C and E). The level of transcription of PAQR4 and its correlation with ATP6V0B in BCa were also confirmed by analyzing 30 pairs of BCa tissues, and the results matched the predicted data (Fig. 5E, F and G). In BCa cells, overexpression of ATP6V0B significantly increased the transcription and protein levels of PAQR4, whereas knocking down ATP6V0B inhibited its expression (Fig. 5H and I). These results suggested that

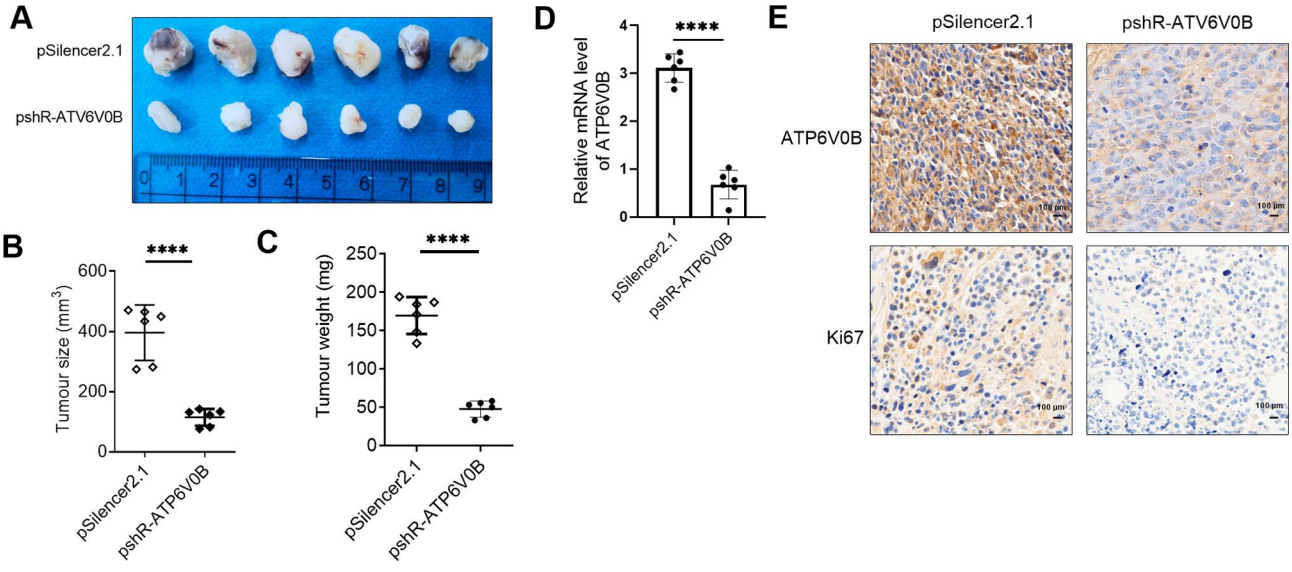

Fig. 3 Knocking down ATP6V0B suppressed tumor growth in vivo. (**A**) The representative photographs of tumors were taken from nude mice 21 days after inoculation ($n=6$). (**B** and **C**) The volume and weight of the tumors were measured after the tumors were isolated. (**D**) RT-qPCR analysis was performed to evaluate the mRNA levels of ATP6V0B in tumor tissues. (**E**) The expression of ATP6V0B in tumor tissues was confirmed by IHC; ****$p<0.0001$

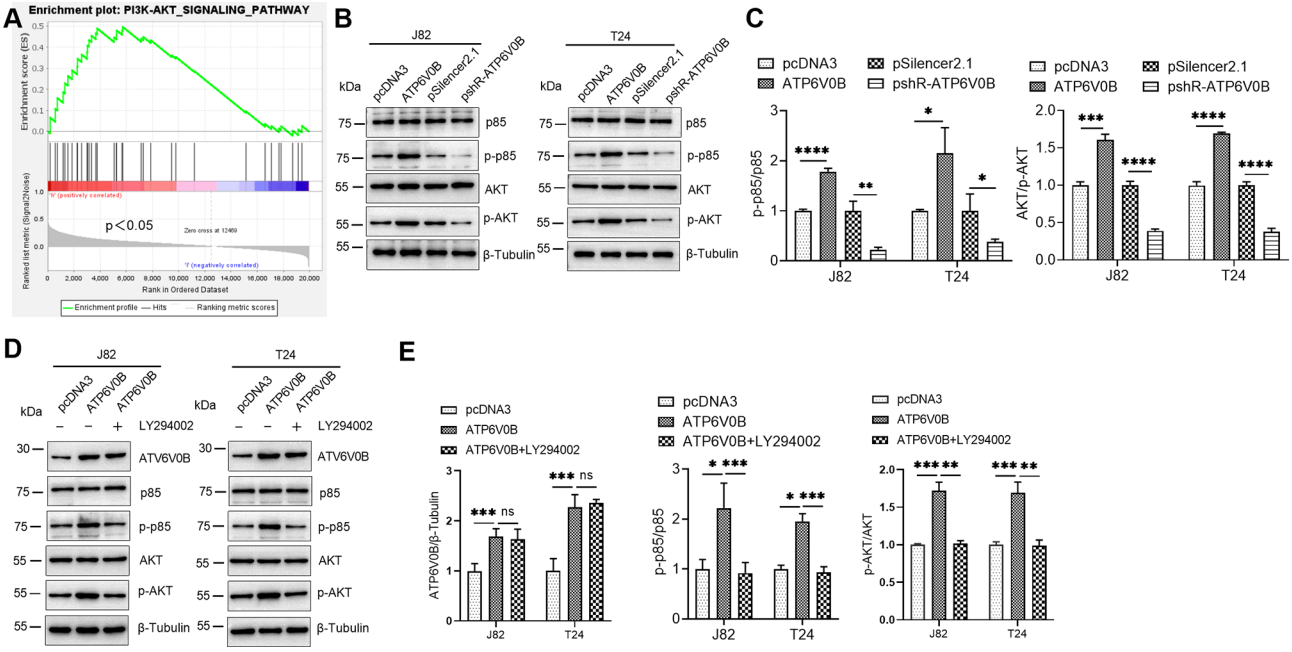

Fig. 4 ATP6V0B activated PI3K/AKT signaling in BCa cells. (**A**) GSEA results showed that the PI3K/AKT signaling pathway was hyperactivated in BCa cells. (**B**) Western blotting assays were performed to determine the indicated protein levels of PI3K/AKT signaling. (**C**) Quantification of the protein expression levels in (**B**). (**D**) The BCa cells were transfected with the pcDNA3 empty vector and the ATP6V0B-overexpressing vector for 24 h, after which the cells were treated with LY294002 (10 μM) for 24 h. Western blotting assays were performed to determine the level of p85, p-p85, AKT, and p-AKT proteins. (**E**) Quantification of the protein levels in (**D**); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$; ns, no significant difference

ATP6V0B can promote the expression of PAQR4 by increasing its mRNA level.

ATP6V0B enhanced the malignant behavior of BCa cells via a PAQR4-mediated mechanism

Whether ATP6V0B can regulate the proliferation, invasion, and migration of BCa cells and activate the PI3K/AKT signaling pathway needs to be confirmed. We found that overexpression of ATP6V0B alone significantly increased the proliferation, invasion, and migration of BCa cells (Fig. 6A and B). We also found that the proliferation, invasion, and migration of BCa cells were partially inhibited when ATP6V0B was overexpressed and PAQR4 was knocked down via co-transfection (Fig. 6A and B). The results of Western blotting assays showed that the levels of p-p85 and p-AKT increased significantly when the cells were transfected with only the ATP6V0B-overexpressing plasmid; however, they were inhibited when the cells were co-transfected with ATP6V0B-overexpressing and PAQR4-knockdown plasmids (Fig. 6C and D). Similarly, we found that the protein levels of E-cadherin and Vimentin could be rescued by knocking down PAQR4 (Fig. 6C and D). These results suggested that ATP6V0B influences the development of BCa by regulating the level of PAQR4.

Discussion

The incidence of BCa is high, and it is prone to recurrence; however, its cause is not known [31]. Invasive BCa is prone to recurrence and metastasis, which is the main cause of deaths related to BCa. Therefore, the characteristics and mechanism of invasion and metastasis of BCa need to be determined for the diagnosis and treatment of BCa. ATP6V0B is an essential component of the V-ATPase complex and plays a key role in energy metabolism [7, 8]. Many members of the V-ATPase family are associated with oncogenic activities in tumorigenesis, but information on the role of ATP6V0B in tumors, especially concerning BCa, is limited [9, 10]. Hence, the function of ATP6V0B in BCa and its underlying mechanism of action need to be investigated.

In this study, we initially investigated the expression of ATP6V0B in BCa tissues using the GEPIA database and found that its level was higher in BCa tissues compared to that in adjacent normal tissues. Additionally, higher expression of ATP6V0B was observed in BCa cells relative to that in normal urothelial cells. By conducting survival analysis using the GEPIA and Kaplan-Meier Plotter databases, we found a correlation between an increase in the expression of ATP6V0B and a poorer prognosis for BCa patients. This suggested that ATP6V0B can be used as a diagnostic and prognostic marker for this condition. Additionally, examining the role of BCa in ATP6V0B showed that its overexpression significantly increased

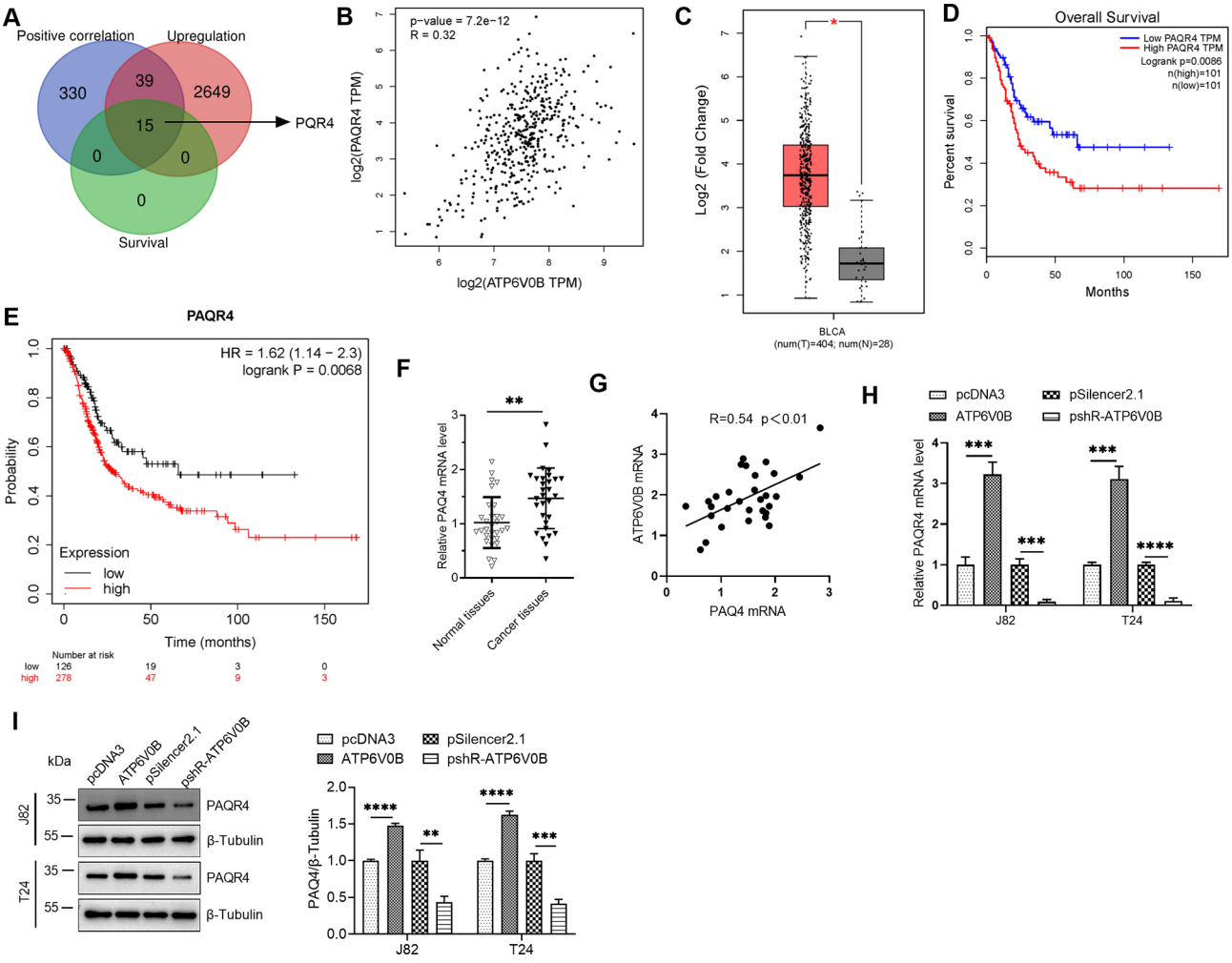

Fig. 5 ATP6V0B upregulated the expression of PAQR4 in BCa. (**A**) The Venn diagram shows that there are 54 genes that are positively correlated with ATP6V0B and upregulated in BCa, and among these 54 genes, 15 genes have significantly different survival curves. (**B** - **D**) The GEPIA database was used to analyze the correlation between ATP6V0B and PAQR4 (**B**), the level of PAQR4 mRNA (**C**), and overall survival, as determined by the level of PAQR4 mRNA (**D**). (**E**) The K-M plotter was used to validate the overall survival of PAQR4. (**F**) RT-qPCR analysis was performed to evaluate the level of PAQR4 mRNA in BCa tumor tissues and adjacent normal tissues ($n = 30$). (**G**) The correlation between ATP6V0B and PAQR4 was analyzed based on the level of mRNA of ATP6V0B and PAQR4. (**H**) BCa cells were transfected with the indicated plasmids, and RT-qPCR analysis was performed to evaluate the level of PAQR4 mRNA 36 h after transfection. (**I**) Western blotting analysis was performed to measure the expression of PAQR4 48 h after transfection; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$

the proliferation, invasion, and migration of BCa cells, whereas silencing the gene led to a considerable decrease in these abilities. These findings suggested that abnormal expression of ATP6V0B can affect the proliferative and invasive abilities of BCa cells. In vivo animal experiments also supported this finding by demonstrating that silencing ATP6V0B inhibited tumor growth in vivo. However, the mechanism underlying the effect of ATP6V0B on cell proliferation and invasion in cancer is not known.

To elucidate the mechanism by which ATP6V0B facilitates the proliferation, invasion, and migration of tumors in BCa, we initially conducted a single-gene GSEA on the TCGA BCa dataset focusing on ATP6V0B. The results showed that ATP6V0B was significantly correlated with the PI3K/AKT signaling pathway, indicating its involvement in regulating this pathway for the development of BCa. The PI3K/AKT signaling pathway is activated in almost all tumors and plays an important role in the development and progression of tumors, as well as in imparting resistance to chemotherapy [32–34]. Therefore, whether ATP6V0B can activate the PI3K/AKT signaling pathway needs to be further determined. The results of Western blotting assays showed that the overexpression of ATP6V0B activated the PI3K/AKT signaling pathway, whereas silencing ATP6V0B inhibited its activity. However, the exact mechanism by which ATP6V0B activates the PI3K/AKT signaling pathway remains unknown.

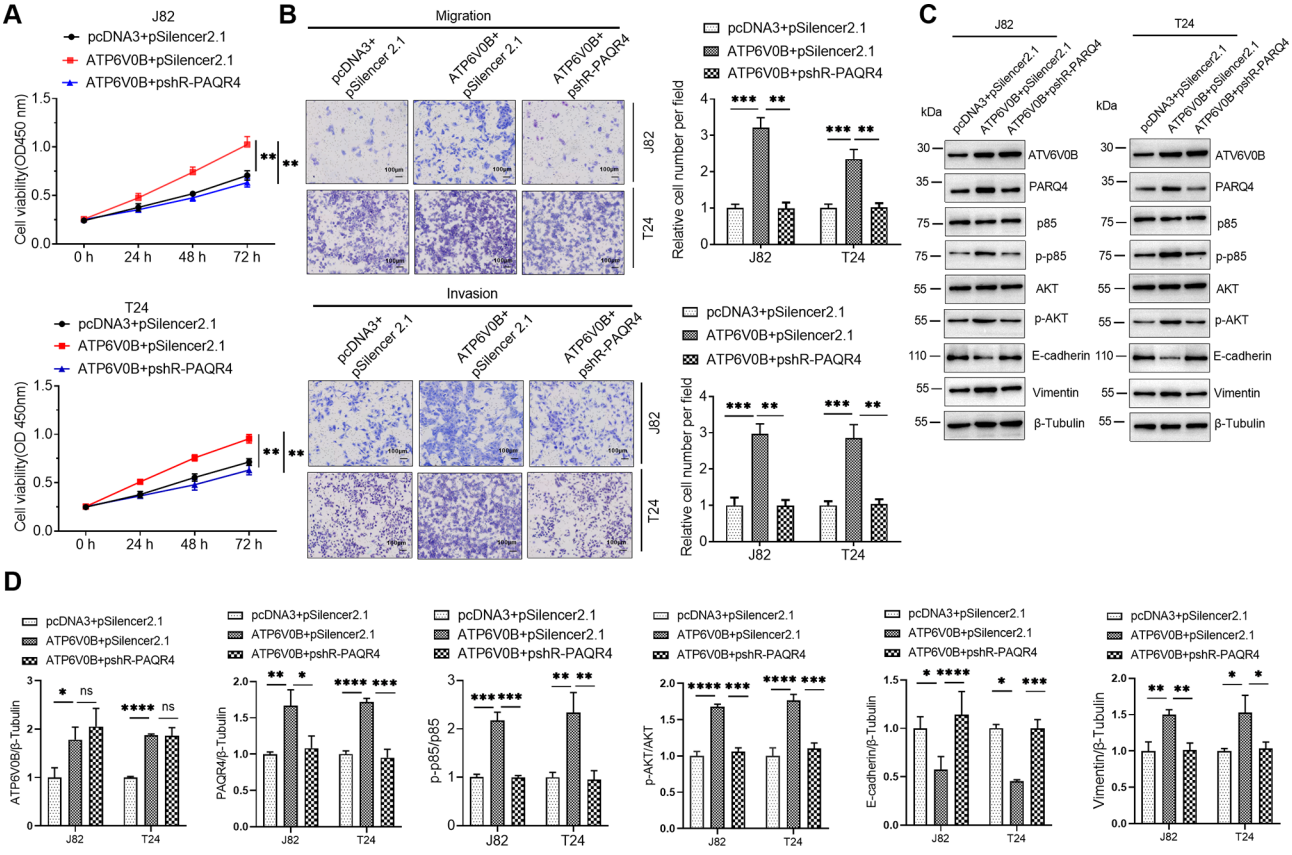

Fig. 6 ATP6V0B increased the malignant behavior of BCa cells and activation of PI3K/AKT signaling by upregulating PAQR4. BCa cells were transfected with pcDNA3 + pSilencer2.1, ATP6V0B + pSilencer2.1, and ATP6V0B + pshR-PAQR4, after which the abilities of the cells to proliferate (**A**), migrate, and invade (**B**) were evaluated. (**C**) Western blotting assays were conducted to determine the levels of the proteins associated with the PI3K/AKT signaling pathway affected by the overexpression of PAQR4. (**D**) Quantification of the protein levels in (**C**); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$; ns, no significant difference

To determine the mechanism, data from the GEPIA database was analyzed, and the results indicated a positive association between ATP6V0B and PAQR4. The level of expression of PAQR4 is high in various types of tumors, and it acts as an oncogene, especially promoting the development of BCa [35–37]. Additionally, PAQR4 plays an important role in activating the PI3K/AKT signaling pathway [28–30]. Through experiments, researchers showed that the overexpression of ATP6V0B increased the mRNA and protein levels of PAQR4. In contrast, silencing ATP6V0B inhibited the expression of PAQR4. These findings suggested that ATP6VOB may activate the PI3K/AKT signaling pathway by interacting with PAQR4. Finally, rescue experiments provided preliminary evidence that modulation by ATVP60VB can influence the expression of PAQR4 to promote the proliferation, invasion, and migration of BCa cells. Further investigation also showed that ATVP60VB can regulate PAQR4, leading to the activation of the PI3K/AKT signaling pathway. Although our research has initially demonstrated that ATP6V0B can up-regulate the expression of PAQR4 by increasing its mRNA level, our study still has certain limitations. We have not yet proven the specific mechanism by which ATP6V0B affects the level of PAQR4 mRNA, whether it is by regulating the expression level of transcription factors to promote the transcription of PAQR4 or by regulating the stability of PAQR4 mRNA. Future resolution of these issues will further deepen our understanding of the regulatory mechanism of ATP6V0B.

Conclusion

To summarize, ATVP60VB was overexpressed in BCa tissues and cells and was associated with an unfavorable prognosis for patients. It promoted the proliferation, invasion, and migration of BCa cells. Subsequent investigations suggested that its mechanism of action probably involves the activation of the PAQR4/PI3K/AKT signaling pathway. Our results indicated that ATVP60VB contributes to the progression of BCa. This study might provide valuable information for clinical intervention to increase overall survival rates for patients with this condition.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14183-z.

Supplementary Material 1

Author contributions

Xinsheng Wang and Yong Jia: Writing– original draft, Writing– review & editing, Project administration, Methodology, Investigation, Funding acquisition. Yanqing Qu, Yanbo Sun, Tong Yang, Wei Wang, and Xinmeng Dou: Formal analysis, Data curation, Conceptualization, Software, and Resources.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Before the initiation of this study, all the procedures and protocols used in this study was strictly reviewed and finally approved by the Ethics Review Committee of the Qingdao Municipal Hospital (Appr. No.: 2024-LW-105). All the performance of the experiments in this study strictly followed the Declaration of Helsinki. All the patients enrolled in this study signed the written inform consent form before the study. For animal experiments, all the procedures and protocols used in animal experiments were approved by the Nankai University (Appr. No.: 2024-SWDWLL000149). All the performances of animal experiment were conducted according to the guidelines for the care and use of experimental animals.

Consent for publication

Informed consent was obtained from participants or their legal representatives.

Competing interests

The authors declare no competing interests.

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