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Study on effect of pancreatic kininogenase on diabetic nephropathy-induced fibrosis via Notch1/Hes1/Pten/Akt signaling pathway

Mingjie Qing[2], Ximei Zhang[3], Qiangxiang Li[1,4*] and Canqun Yan[5*]

Abstract

Objective To elucidate the mechanism by which pancreatic kininogenase (PKase) impacts renal fibrosis in diabetic nephropathy through modulation of the Notch1/Hes1 and Pten/Akt pathways.

Methods This study employed in *vivo* models and cellular assays to investigate PKase's effects on cellular viability, apoptosis, and oxidative stress. Assay kits were used to assess these parameters, while protein expression levels were measured via Western Blot and RT-qPCR. Histological changes in kidney tissues were analyzed using HE and Masson's staining. Fibrosis markers—including E-cadherin, vimentin, α-SMA, Collagen I, TGF-β, and fibronectin—were evaluated through immunofluorescence and immunohistochemistry.

Results After eight weeks of PKase treatment, significant improvements in blood glucose levels and associated symptoms were observed in diabetic nephropathy rats. Both in vivo and in vitro results demonstrated that PKase treatment inhibited the expression of diabetic nephropathy markers, including vimentin, α-SMA, FN, Collagen I, and TGF-β, while increasing the expression of E-cadherin. Additionally, the expression of Notch1, Hes1, and phosphorylated Akt (p-Akt) was upregulated, and Pten expression was suppressed, all of which were reversed by PKase treatment. Furthermore, both analyses indicated that PKase alleviated Jagged1-induced apoptosis and oxidative stress, and mitigated tubulointerstitial fibrosis.

Conclusion PKase appears to ameliorate diabetic nephropathy-induced renal fibrosis by activating the Pten/Akt pathway and inhibiting the Notch1/Hes1 pathway, suggesting its potential as a therapeutic agent in diabetic nephropathy.

Clinical Trial Number Not applicable.

Keywords PKase, Diabetic nephropathy, Renal fibrosis, Notch1/Hes1 pathway, Pten/Akt pathway

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Introduction

Diabetic nephropathy (DN) is a chronic kidney disease resulting from prolonged glucose metabolism disorders in diabetes patients. Elevated blood glucose levels impose an increased metabolic burden on the kidneys, leading to renal function impairment and damage [1, 2]. Renal fibrosis, a key pathological feature of DN, involves abnormal extracellular matrix (ECM) deposition and the activation of epithelial-mesenchymal transition (EMT) processes [3, 4]. Due to prolonged hyperglycemia and metabolic abnormalities, ECM in renal tissue increases aberrantly. The ECM, primarily composed of proteins like collagen and fibronectin (FN), normally maintains the structure and function of glomeruli and renal tubules. However, excessive deposition disrupts tissue architecture and causes renal fibrosis [5, 6]. The EMT process engages various signaling pathways and molecules, some associated with inflammation and oxidative stress [7, 8]. In DN, prolonged hyperglycemia activates these pathways, inducing EMT in renal tubular epithelial cells and transforming them into fibroblasts, thus promoting renal fibrosis. For instance, as blood glucose metabolism becomes disrupted, the TGF-β pathway is activated, leading to a reduction in E-cadherin levels and stimulating the expression of mesenchymal markers such as α-SMA and vimentin, facilitating the onset of EMT [9, 10].

The Notch1 signaling cascade is crucial during embryonic development and tissue healing, with its dysregulation linked to renal pathogenesis [11, 12]. Aberrant activation of the Notch1/Hes1 pathway is closely associated with renal fibrosis. Upon activation, Notch1 increases the expression of the downstream transcription factor Hes1 [13]. Hes1 plays a crucial role in EMT by suppressing E-cadherin expression, weakening epithelial cell adhesion [14], and promoting mesenchymal markers such as vimentin, thereby facilitating the transformation of epithelial cells into mesenchymal-like cells, which contributes to DN progression [15]. The Notch1/Hes1 pathway enhances ECM synthesis and deposition, leading to increased accumulation of FN and Collagen I, resulting in renal tissue fibrosis. Thus, targeting this pathway may serve as a significant therapeutic strategy in DN.

Pten functions as a lipid phosphatase that inhibits the Akt (protein kinase B) signaling cascade under normal conditions [16]. However, in DN, elevated blood glucose can lead to abnormal activation of the Akt pathway, resulting in deleterious physiological alterations affecting renal structure and function. Research suggests that Hes1 can directly regulate Pten expression, indicating that modulation of the Notch1/Hes1 and Pten/Akt pathways may be vital for therapeutic interventions in DN [17].

Pancreatic kininogenase (PKase) has been shown to ameliorate fibrosis in DN by reducing levels of fibrotic-associated proteins. Additionally, PKase is considered to possess antioxidant properties, reducing oxidative stress and protecting renal cells from oxidative damage [18, 19]. Further comprehensive research is warranted to uncover the exact mechanism and therapeutic potential of PKase in diminishing renal fibrosis by regulating the Notch1/Hes1-mediated Pten/Akt signaling pathway in DN.

Experimental design and methods

Cell culture

Human renal tubular cells (HK-2) (SNL-165, SUNNCELL, Wuhan, China) are cultured in DMEM/F-12 medium supplemented with 10% FBS and 100 U/mL penicillin-streptomycin antibiotics, and maintained in a CO_2 incubator at 37 °C with 5% CO_2. The cells are passaged every 2–3 days and used for experiments during the logarithmic growth phase. Cells were subjected to a 72-hour incubation in DMEM medium supplemented with high glucose (25 mmol/L, HG) to establish the model. After successful modeling, PKase (6 pg/mL, Shanghai, Kanglang) and Jagged1 (Notch1 activator, It binds to the Notch receptor, activating the Notch signaling pathway. 10 μM, Med Chem Express, HY-P1846A) were separately added to the cells for treatment. The control group is cultured at normal glucose concentration (5.5 mmol/L). The cells are divided into five groups: HK-2, HG, PKase, Jagged1, and PKase + Jagged1.

CCK-8

Inoculate HK-2 cells in logarithmic growth phase into 96-well plates, with a cell density of 5×10^3 cells per well. Following 48 h of continuous adherence, analyze cell viability at 0, 24, and 48-hour time intervals using the CCK-8 assay (Med Chem Express, SF4139-10mM). Calculate the optical density value at 450 nm and plot the viability curve using GraphPad Prism 8.0 software.

Flow cytometry

The Annexin-V-FITC/propidium iodide (PI) staining assay was used for analysis (Elabscience, Wuhan, E-CK-A211). Before harvesting cells, pre-chilled PBS was used to rinse HK-2 cells, and a cell suspension was generated with FITC binding buffer at a density of 1×10^6 cells/mL (AmyJet, WuHan). Next, an EP tube was loaded with 100 μL of the cell suspension. Subsequently, Annexin-V-FITC and PI were sequentially added, followed by incubation in a dark room at room temperature for 20 min. Cell apoptosis rate was assessed through flow cytometry analysis (Beckman Coulter, USA).

Immunofluorescence

The HK-2 cells in culture were subjected to fixation using 4% paraformaldehyde, and subsequently, permeabilization was achieved using 0.1% Triton X-100 (Shanghai Lianmai Biological Engineering Co., LTD) to enhance

cellular penetration. Target antibodies (anti-E-cadherin from Fien Biology Technology, anti-vimentin from Omer Biotechnology, anti-α-SMA from BioLayb Biotechnology, anti-Collagen I from Qincheng Biotechnology, and anti-TGF-β antibody from Abcam) were added to the culture medium containing HK-2 cells and allowed to incubate overnight at 4%. Following the PBS buffer wash of the cells, the slides were subjected to an incubation with the secondary antibody (anti-rabbit secondary antibody, sourced from Abcam, catalog number abs50029) at room temperature for a duration of 1 h. Fluorescent nuclear staining reagent DAPI (Servicebio, WuHan) was used to label the cell nuclei, and finally, the cell membrane was sealed using an anti-fluorescence quenching sealing agent (purchased from Xinbosheng Biotechnology). The cells' fluorescent signals were visualized using a fluorescence microscope (Japan, KEYENCE).

Animal experiment

Purchase 6–8 week-old, approximately 200 g male Sprague-Dawley rats (Purchased from HuaFuKang Biology.), and after one week of normal feeding, divide them into five groups (Control group, Model group, PKase group, Jagged1 group, PKase + Jagged1 group). The control group rats were provided with a standard diet, while the remaining groups received a diet rich in high fat and sugar (HFD). The rat were anesthetized by inhalation of a mixture (1: 49, 1.5 L/min) of isoflurane (792632, Sigma, USA) and pure oxygen (851086, Sigma, USA) prior to administration. Streptozotocin (STZ, 40 mg/kg, Solarbio, S8050) was administered through intraperitoneal injection after dissolving it in chilled sodium citrate buffer (0.1 M, pH 4.4). Control group rats received a comparable volume of buffer solution injection. After 3 days, fasting blood glucose levels were assessed via tail blood using a glucometer. The modeling is considered successful when the blood glucose concentration in the model group is > 16.7 mmol/L. Except for the model group, PKase (6 pg/mL), Jagged1 (0.5 mg/kg), and PKase + Jagged1 were administered separately for a duration of eight weeks. At the end of eight weeks, 24-hour urine samples were collected and rats were euthanized by intraperitoneal injection of high-dose pentobarbital sodium (100 mg/kg, Sigma-Aldrich, P3761) [20], and serum and kidney specimens were collected. Subsequently, rats' blood glucose levels were monitored biweekly following the successful establishment of the model. All procedures were carried out in compliance with the *Guidelines for the Care and Use of Laboratory Animals*.

Assessment of renal function

Fasting blood glucose, SCr, BUN, TG, TC and 24-hour urine microalbuminuria were assessed using a biochemical analyzer (Seamaty, Chengdu).

Oxidative stress

Utilizing SOD assay kit (provided by Science Ab Co., Ltd), MDA assay kit (provided by Seapea BioTech Co., Ltd), and ROS assay kit (provided by Shanghai Fanrui BioTech Co., Ltd), the levels of SOD, MDA and ROS in serum of DN rats were assessed following the protocols provided by the respective assay kits.

HE staining

Renal tissues were rinsed with PBS, underwent fixation in 4% paraformaldehyde (G-CLONE, BeiJing) for 24 h, and subsequently underwent paraffin embedding. Paraffin Sect. (4 μm) were deparaffinized, followed by rehydration. Kidney sections were stained with standard HE staining to assess tissue histopathological renal injury.

Masson's staining

After fixation with 4% paraformaldehyde for 24 h, renal tissues were embedded in paraffin for sectioning. Subsequently, the tissue sections were subjected to Masson's staining, and the degree of fibrosis was semi-quantitatively analyzed using ImageJ software (version 1.44, NIH).

Immunohistochemistry

To prepare tissue sections, begin by subjecting them to an initial pre-incubation step in a sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH = 6.0) that has been heated to boiling. This pre-incubation facilitates antigen retrieval. Block the slides using 0.1% Triton X-100 and 0.5% BSA (Shanghai, Aiyan) to reduce nonspecific binding, allowing them to incubate for 1 h. Following that, individually incubate with primary antibodies targeting E-cadherin, vimentin, α-SMA, FN, Collagen I and TGF-β at 4℃ for 24 h. After washing with PBS, incubate with secondary antibodies at 37℃ for 30 min. Finally, employ DAB (ShangHai, Kamar) staining, counterstain with hematoxylin, and observe the staining under a Nikon TS2 microscope (BeiJing, Omiget).

Western blot (WB)

The RIPA lysis buffer is utilized for total protein extraction, while the BCA protein assay kit (mlbio, Shanghai) is employed to quantify protein concentration. The complete protein content underwent separation via SDS/PAGE and was then transferred onto a PVDF membrane (Sigma-Aldrich, IPFL00010). The membrane underwent blocking using 5% skim milk in PBST (Phosphate-Buffered Saline with Tween 20) for 1 h before being incubated with the primary antibody overnight at 4℃. Subsequently, the membrane underwent washing before being incubated with anti-rabbit IgG antibody at room temperature for 1 h. Protein bands were detected using an ECL detection kit (TW-reagent, Shanghai). Signal intensity was analyzed using ImageJ software (NIH).

RT-qPCR

Total RNA was isolated with TRIzol reagent (Applygen, Beijing, R1030), and then subjected to reverse transcription using a cDNA synthesis kit (Vazyme, NanJing). RT-qPCR was performed using the SYBR Green PCR Master Mix (Phygene, Shanghai, PH0120) on an Applied Biosystems 7300 Sequence Detection System (HZbiotech, Hangzhou). GAPDH was employed as an endogenous control for the purpose of normalization. The $2^{-\Delta\Delta Ct}$ method was employed for analysis. The primer sequences are provided below:

Gene	Forward primer	Reverse primer
Notch1	ATGCTGAGTGTGCTCCATGT	TCTGCTGAGCTGCTCTTG
Hes1	TCTGGACGACACCGTCAAGT	GAGGTGATGCCGTCTGGTTT
Pten	ATGTTGCCACCTTAGTGATG	TCACAGTGCAGGGTCTGG
Akt	ATGGAAGACCTCACAGGAGG	TCAGTGTAGCCATTGATTCC
GAPDH	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGA

Statistical analysis

Statistical analysis was performed utilizing Student's t-test or one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. The results are expressed as the mean ± standard deviation (SD) of three separate experiments. A significance level of $P < 0.05$ was used to denote statistically significant differences.

Results

The influence of PKase on the viability and apoptosis of HK-2 cells

The results showed that in the CCK-8 assay, compared to the HG group, the PKase group enhanced the survival capability of HK-2 cells (Fig. 1A). To further investigate whether Jagged1 (a Notch1 agonist) affects the cell status and subsequent diabetic nephropathy-related markers through the Notch1 signaling pathway, we performed Jagged1 treatment. The results revealed that, compared to the HK-2 group, the HG group exhibited an increase in the apoptosis rate, which was further exacerbated by Jagged1 treatment. However, PKase treatment significantly reduced the apoptosis rate (Fig. 1B-C). Western blot analysis was performed to assess the levels of apoptosis-related proteins Bax and Bcl2 in HK-2 cells. The outcomes demonstrated that the addition of PKase to the cells reduced the upregulation of Bax induced by HG and Jagged1 treatment, while also restoring the expression of Bcl2 (Fig. 1D-F).

The effect of PKase on the Pten/Akt signaling pathway regulated by Notch1/Hes1 in HK-2 cells

The results revealed that protein expression levels of Notch1, Hes1, and Akt increased in the HG group compared to normal HK-2 cells, while Pten expression decreased. The Jagged1 group exhibited a more pronounced effect than the HG group. However, PKase

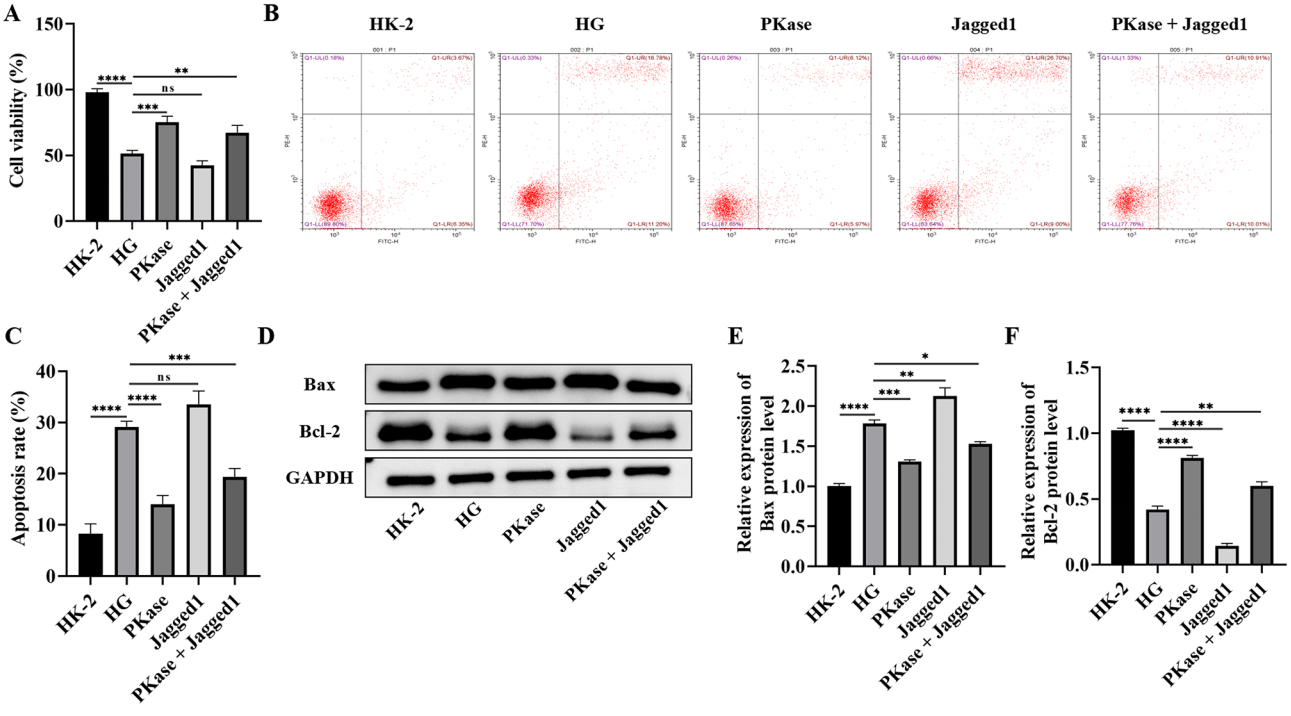

Fig. 1 The influence of PKase on the viability and apoptosis of HK-2 cells. **A.** Assessment of HK-2 cell viability. **B-C.** Flow cytometric analysis of apoptosis rate in HK-2 cells. **D-F.** WB analysis was used to assess the levels of apoptotic proteins in HK-2 cells. $N = 3$, $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{**}P < 0.0001$

treatment reduced protein expression levels of Notch1, Hes1, and Akt, while enhancing Pten expression (Fig. 2A-H).

PKase's effect on the expression of fibrotic-associated proteins in HK-2 cells

The immunofluorescence results demonstrate that in the HK-2 cells of the model group, E-cadherin decreased, while the expression of vimentin, α-SMA, Collagen I, TGF-β and FN increased. The Jagged1 group further exacerbates these changes. However, in the PKase treatment group, the expression of E-cadherin is partially restored, while the expression of Vimentin, α-SMA, Collagen I, TGF-β and FN decreases (Fig. 3A-L). These findings indicate that PKase mitigates the progression of renal fibrosis in DN.

Establishment of diabetic nephropathy model and evaluation of renal function

Fasting blood glucose levels were assessed in DN rat models, and the results indicated that the blood glucose concentrations of the control group rats remained within the normal range, while those in the DN group were consistently higher than 16.7mmol/L, validating the effective creation of the model (Fig. 4A). Within the subsequent eight weeks after successfully establishing the DN rat model, fasting blood glucose levels were monitored. The results demonstrated a gradual decrease in blood glucose levels within the PKase intervention cohort in comparison to the model set (Fig. 4B). Moreover, biochemical analyses were performed on kidney function-related indicators (SCr, BUN, TG, TC and 24-hour urinary microalbumin) in the serum and urine. The results showed varying degrees of elevation in these indicators in the model group compared to the control group. In contrast, PKase intervention effectively lowered the levels of SCr, BUN, TG, CHOL and 24-hour urinary microalbumin, thereby partially ameliorating kidney function (Fig. 4C-G). The model group's HE-stained images revealed enlarged glomerular volume, unclear and disorganized structure, widened mesangial area, and evident mesangial cell proliferation. The interstitial area of the kidney expanded. The addition of Jagged1 further exacerbated these changes. The PKase-treated group exhibited a relatively restored renal tissue structure with a more orderly arrangement, reduced interstitial fibrosis, and relatively normal cell morphology. These findings unequivocally demonstrate the therapeutic potential of PKase, effectively ameliorating the kidney functional damage in diabetic rats (Fig. 4H).

The influence of PKase on modulating Notch1/Hes1 pathway and its influence on the Pten/Akt signaling pathway in the diabetic nephropathy model

PCR and WB analyses demonstrated notable increases in the levels of Notch1, Hes1 and p-Akt proteins within

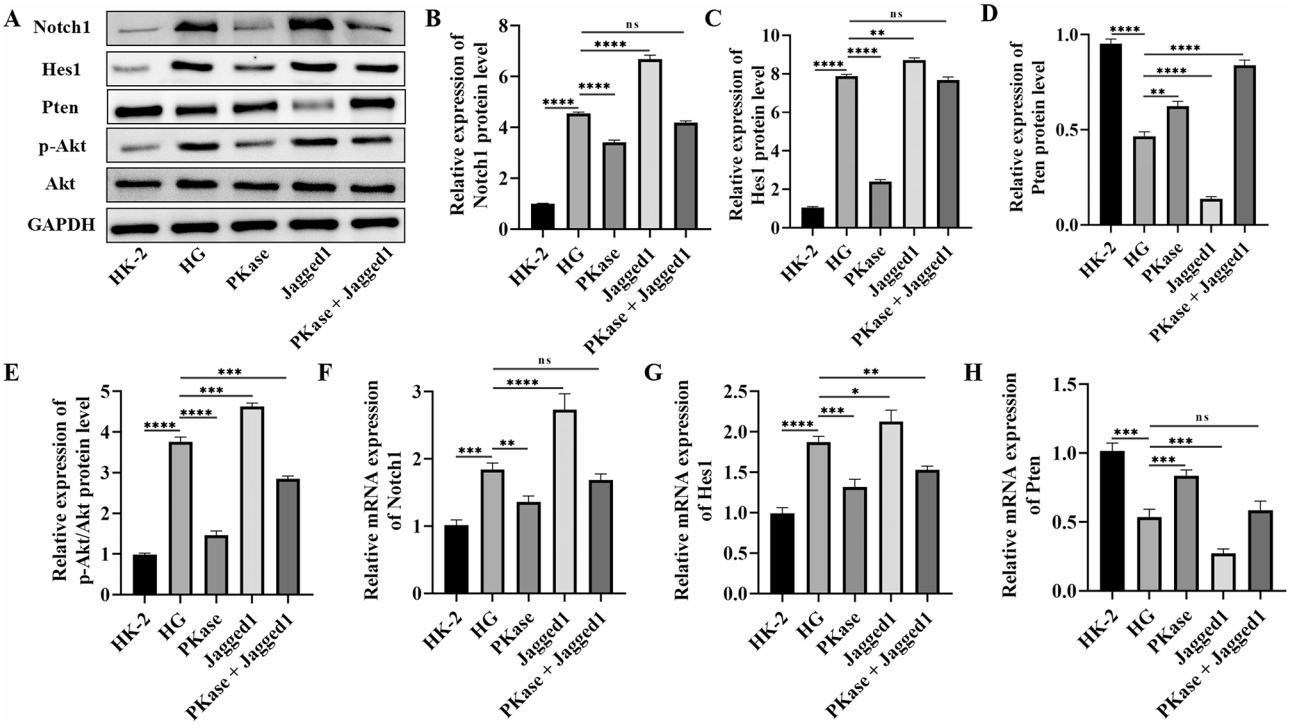

Fig. 2 The effect of PKase on the Pten/Akt signaling pathway regulated by Notch1/Hes1 in HK-2 cells. **A-E** WB analysis of pathway protein expression levels. **F-H** RT-qPCR assessment of relative expression levels of pathway proteins. $N = 3$, * $^{ns}P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, **$P < 0.0001$

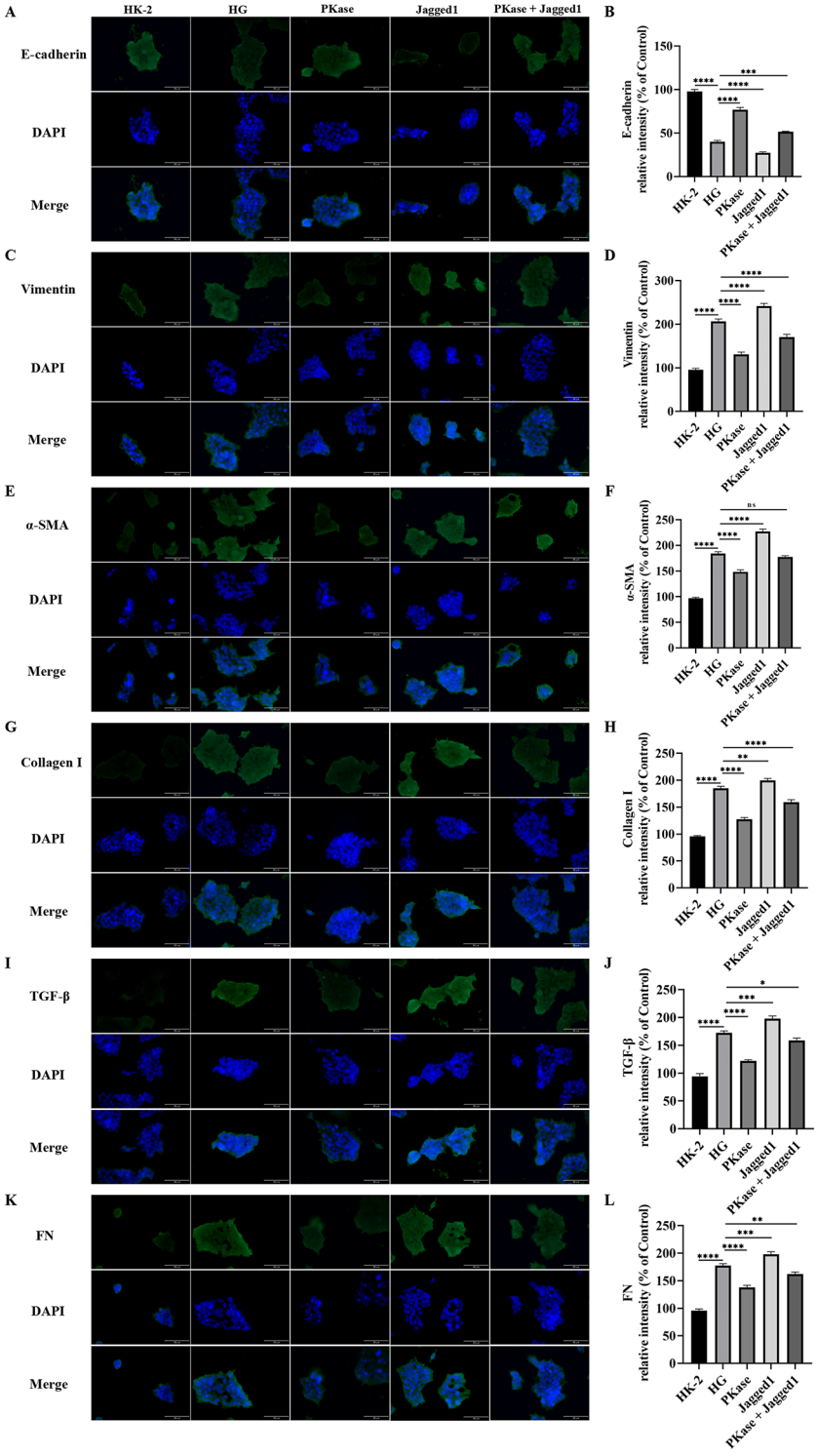

Fig. 3 PKase's effect on the expression of fibrotic-associated proteins in HK-2 cells. **A-L** Expression of E-cadherin, Vimentin, α-SMA, Collagen I, TGF-β and FN in HK-2 cells, Scale = 50 μm. $N = 3$, $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{**}P < 0.0001$

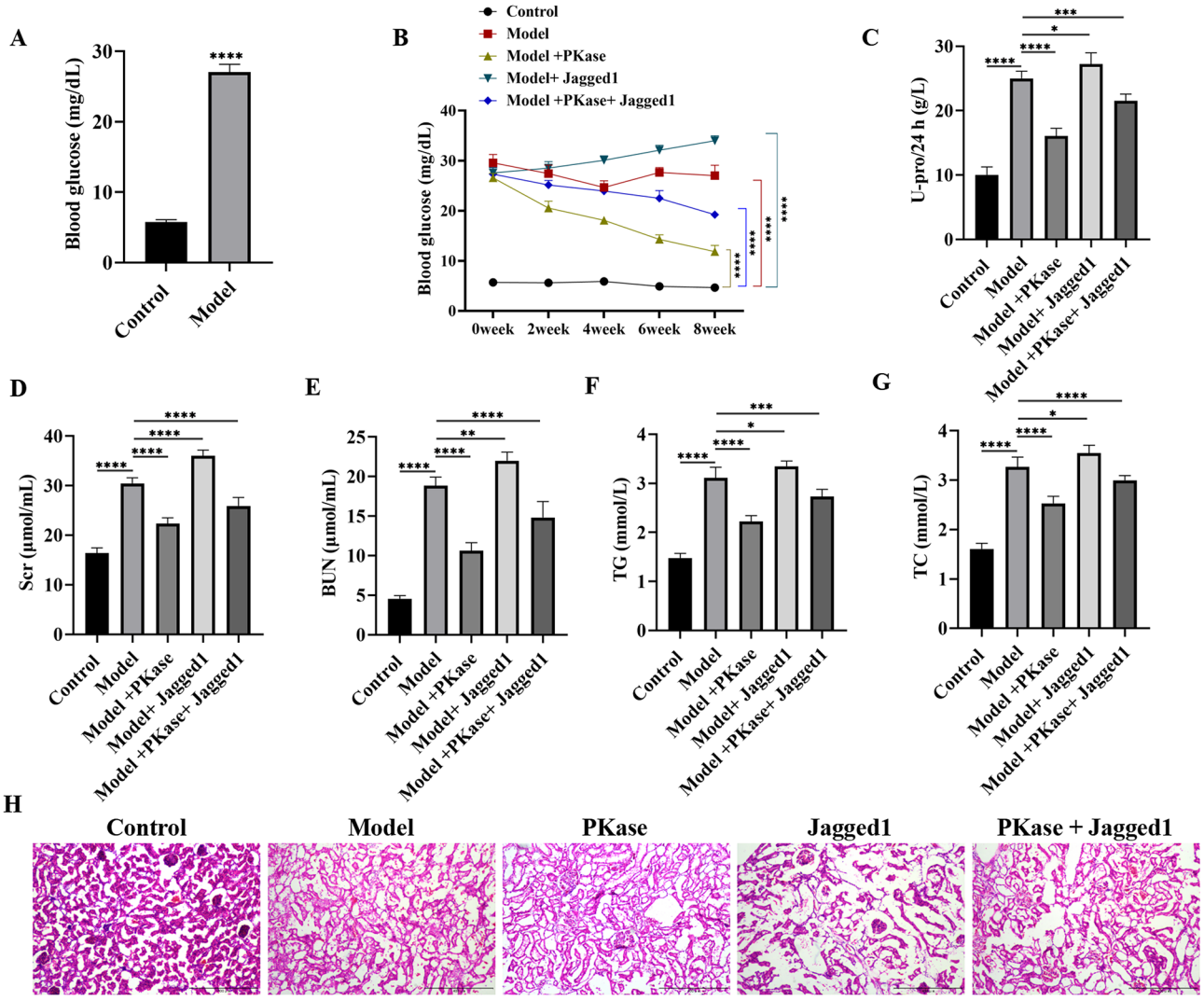

Fig. 4 Establishment of diabetic nephropathy model and evaluation of renal function. Illustrates the evaluation of PKase's influence on renal function within the diabetic model. (**A**) Evaluation of blood glucose levels in the model group and control group rats. (**B**) Measurement of blood glucose levels in rats of each group within eight weeks. **C-G** Determination of concentrations of SCr, BUN, TG, TC in rat serum and 24-hour microalbuminuria in urine after eight weeks. **H** HE staining, Scale = 400 μm. $N = 6$, $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{**}P < 0.0001$

the model group, coupled with a decrease in Pten protein expression. Contrasting with the model group, the PKase intervention group displayed reduced levels of Notch1, Hes1 and p-Akt proteins, while witnessing an increase in Pten protein expression. Contrasting with the model group, the Jagged1-treated group demonstrated noteworthy enhancements in the quantities of Notch1, Hes1 and p-Akt proteins. Nevertheless, subsequent to PKase intervention, there was a noticeable reduction observed in the expression levels of these proteins (Fig. 5A-H). The results suggest that PKase administration efficiently dampens the levels of Notch1, Hes1 and Akt pathway proteins in DN rats, primarily accomplished by curbing the expression of Notch1 signaling components.

The impact of PKase on apoptotic proteins and oxidative stress in DN renal tissues through the modulation of the Notch1/Hes1-regulated Pten/Akt signaling pathway

WB analysis revealed increased levels of the apoptosis-associated proteins Bax in both the model and Jagged1 groups, along with a reduction in the anti-apoptotic protein Bcl-2. Nevertheless, PKase treatment significantly mitigated the Bax protein levels while simultaneously enhancing the Bcl-2 protein content (Fig. 6A-C). The results indicate that PKase effectively reduces the expression of apoptotic proteins in DN tissues.

At the same time, the test kit results showed that compared with the control group, the activity of ROS and SOD in the kidneys of the model group rats increased, while the level of MDA decreased. Compared with the model group, the Jagged1 group showed worsening of

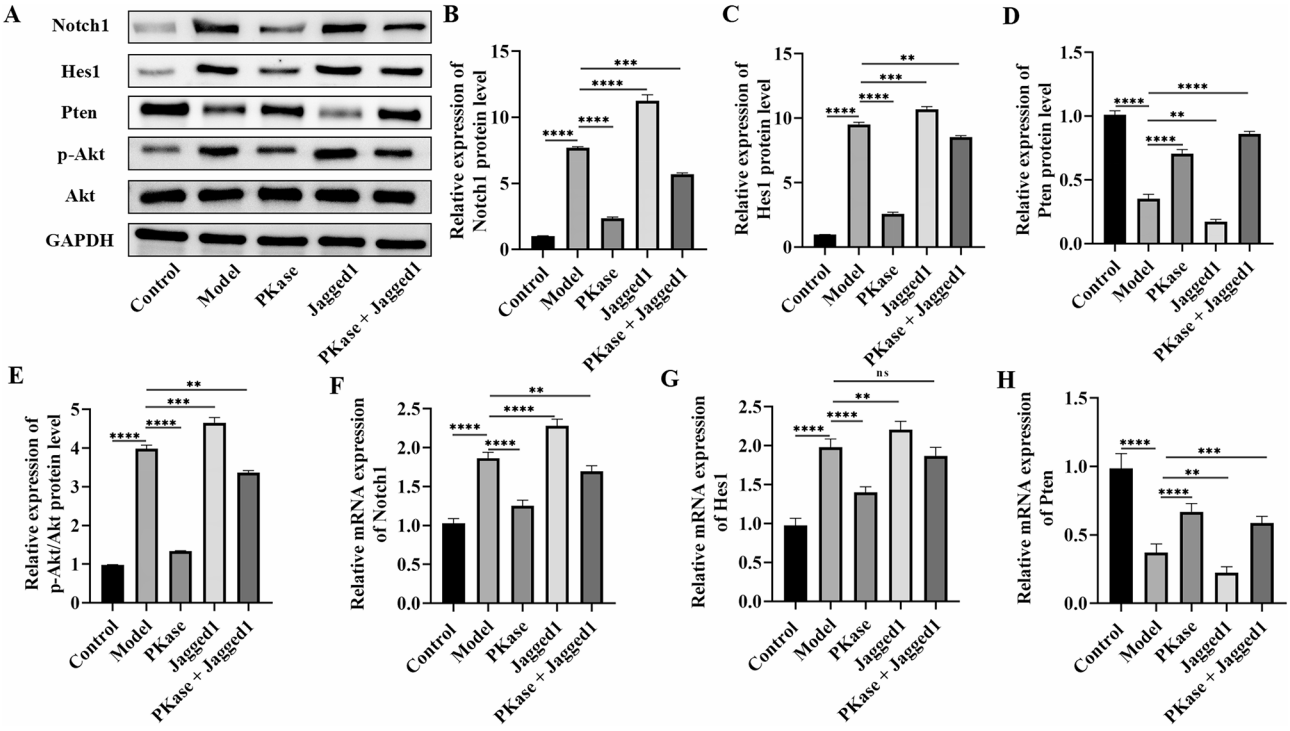

Fig. 5 The influence of PKase on modulating Notch1/Hes1 pathway and its influence on the Pten/Akt signaling pathway in the diabetic nephropathy model. Illustrates the examination of pathway protein expression in the diabetic model. **A-E**. WB analysis of the expression levels of Notch1, Hes1, Pten and p-Akt. **F-H** RT-qPCR assessment of the expression of Notch1, Hes1 and Pten. $N = 6$, * $^{ns}P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, **$P < 0.0001$

the condition, but after PKase treatment in both groups, ROS and SOD levels decreased and MDA expression increased (Fig. 6D-F). These findings suggest that PKase treatment can reduce oxidative stress damage in diabetes nephropathy.

PKase mediates the effect of Pten/Akt signaling pathway on renal fibrosis in DN through Notch1/Hes1

The immunohistochemical results demonstrate that, when compared to the control group, the model group exhibited a significant increase in the expression of Vimentin, α-SMA, Collagen I, TGF-β, and FN, along with a decrease in E-cadherin levels. The addition of Jagged1 led to an augmentation of fibrosis-promoting proteins within the model group, while administration of PKase notably reduced the expression of fibrosis-related markers (Fig. 7A-G).

Masson's staining exhibited a more intense blue color in the renal interstitium of model group rats compared to the control group, indicating a higher extent of fibrosis characterized by enhanced extracellular matrix deposition and densely arranged fibrous structure. In the Jagged1-treated group, the degree of interstitial fibrosis was further intensified, resulting in a more pronounced and intense deep blue staining in the Masson's staining. In the PKase treatment group, the results demonstrated a relative reduction in the intensity of deep blue color in the renal interstitium, along with a significant decrease in the size of the fibrotic area and reduced deposition of extracellular matrix (Fig. 7H-I). This indicates that PKase treatment led to a certain degree of alleviation of fibrotic changes in the renal interstitium and inhibition of extracellular matrix accumulation.

Discussion

DN, also known as Kimmelstiel-Wilson syndrome, is a chronic kidney disease caused by diabetes [21]. Due to long-term hyperglycemia leading to kidney damage, early diabetic nephropathy often results in dysfunction of renal tubules and glomeruli, leading to the entry of large molecules such as proteins into urine [22, 23]; In the later stage of DN, renal function gradually declines, leading to the accumulation of waste, body fluids, and electrolytes in the body, resulting in further damage to the kidneys [24]. In the DN cell model we constructed, the vitality of HK2 cells significantly decreased, the apoptosis rate significantly increased, and the expression of fibrosis related proteins significantly increased. This is consistent with the research results of Liu et al. [25]. In the DN rat model we constructed, we also observed impaired renal function, high degree of renal tubular fibrosis, and elevated levels of apoptosis and oxidative stress in DN rats. This further validates the results in the cell model. Previous research reports have shown that the clinical

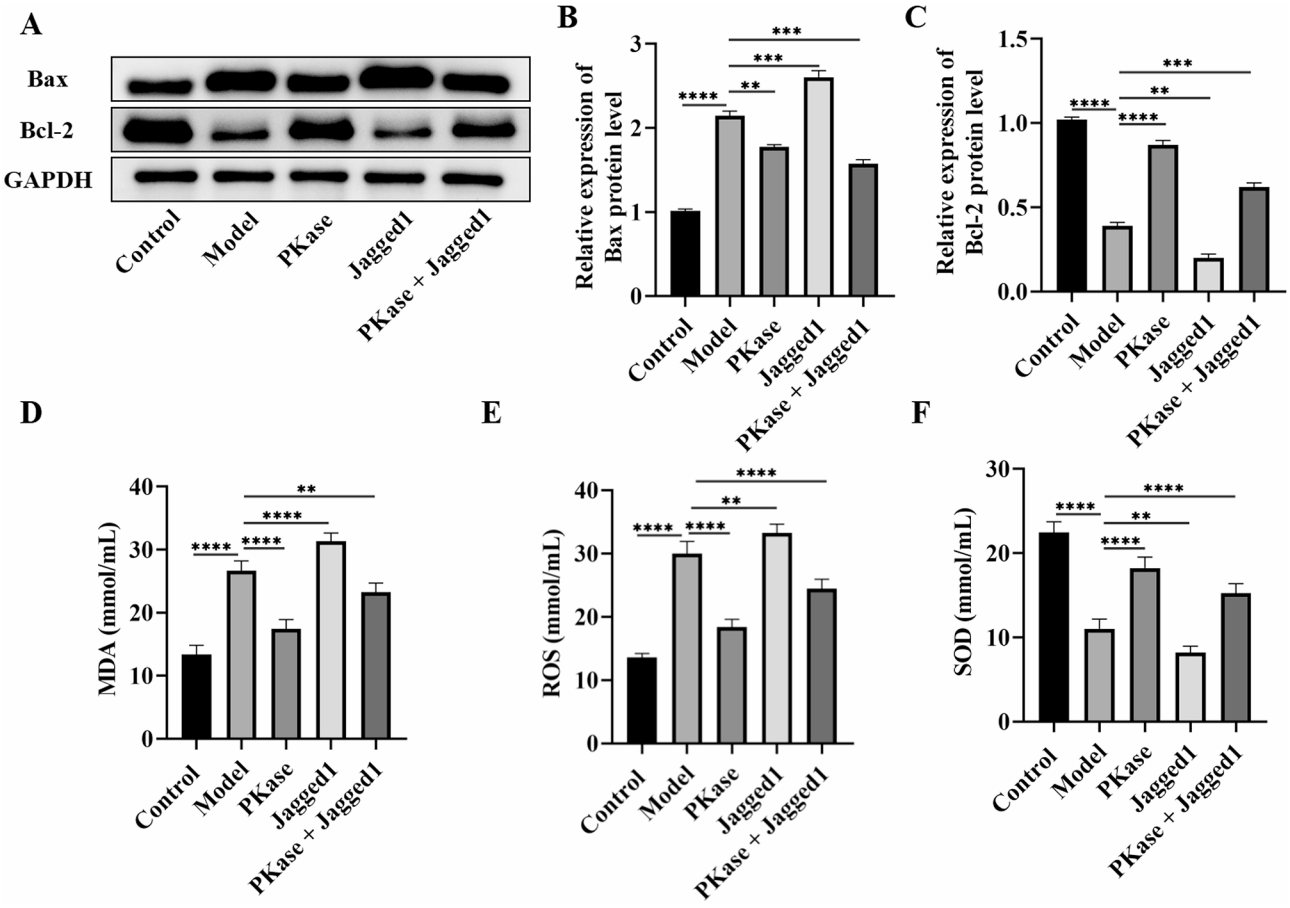

Fig. 6 The impact of PKase on apoptotic proteins and oxidative stress in DN renal tissues through the modulation of the Notch1/Hes1-regulated Pten/Akt signaling pathway. PKase's influence on the expression of apoptotic proteins in kidney tissues. **A-C** WB analysis of apoptosis protein expression. **D-F** The levels of oxidative stress factors were assessed using assay kits. $N=6$, $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{**}P < 0.0001$

manifestations of DN mainly include urinary albumin excretion and renal tubular fibrosis [26, 27]. In a high sugar environment, the kidneys produce a large amount of ROS, which exacerbates the oxidative stress response of the kidneys [28], causing a large number of cell apoptosis, tubular fibrosis, and increased glomerular permeability in the kidneys [25, 29], ultimately leading to renal dysfunction and loss of normal filtration and excretion functions [24].

PKase, a protease, is commonly used in clinical treatment of cardiovascular and thrombotic disorders. In the context of renal fibrosis research, PKase has also garnered some attention. Although studies are relatively limited, certain research suggests that it may have an impact on renal fibrosis under specific circumstances. Some early studies suggest that PKase may exert a protective effect on renal fibrosis through its anti-inflammatory, antioxidant, and antifibrotic actions [19, 30, 31]. In this study, it was also found that PKase can significantly inhibit DN fibrosis in vitro and in vivo, improve cell viability and kidney damage, and have a certain alleviating effect on oxidative stress. At the same time, it was also found that PKase has a regulatory effect on the Notch1/Hes1-Pten/Akt signaling pathway.

The Notch signaling pathway is an evolutionarily conserved transmembrane signaling pathway that holds a critical function in regulating cell fate determination, differentiation, proliferation, and apoptosis [32]. The Notch1/Hes1 signaling pathway has a promoting effect on renal tubulointerstitial fibrosis in DN [17]. The expression of PTEN is negatively regulated by the Notch1/Hes1 signaling pathway [17, 33], and the downregulation of PTEN is an important inducing factor for the activation of the AKT signaling pathway, which has a regulatory effect on cell proliferation in many tumors [34, 35]. In this study, the activation of the Notch1/Hes1-Pten/Akt signaling pathway by Jagged1 significantly reversed the therapeutic effect of PKase on DN, and Jagged1 treatment further exacerbated renal function damage and fibrosis in DN. According to reports, the Notch1 signaling pathway has a certain regulatory effect on cell apoptosis and oxidative stress [36, 37]; The abnormal activation of the Notch1 signaling pathway will lead to DN fibrosis [38],

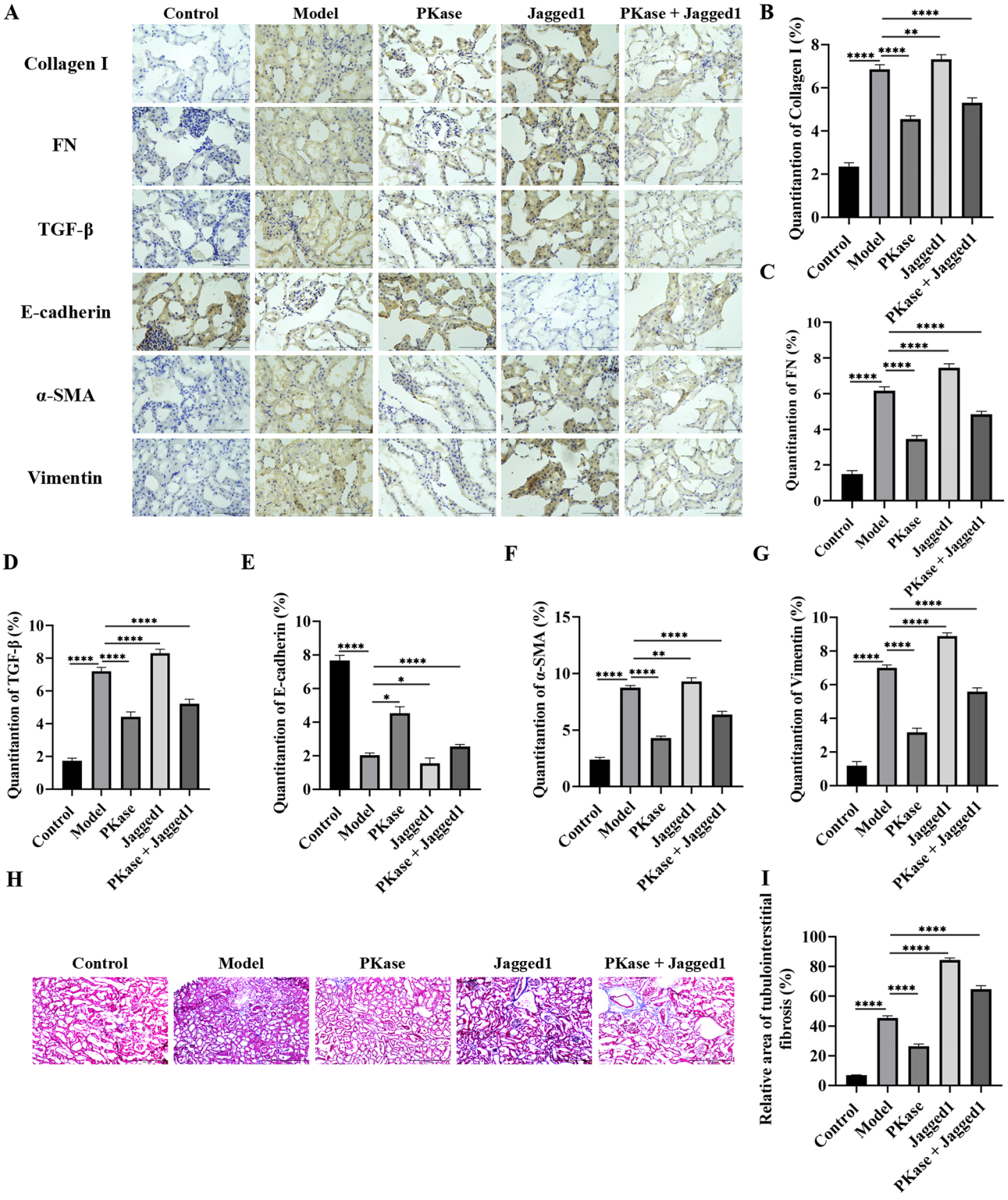

Fig. 7 PKase mediates the effect of Pten/Akt signaling pathway on renal fibrosis in DN through Notch1/Hes1. **A-G** Immunohistochemical examination of the expression of kidney tissue-related proteins associated with renal fibrosis, Scale = 100 μm. **H-I** Assessment of renal fibrosis extent using Masson's staining, Scale = 400 μm. $N = 6$, $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{**}P < 0.0001$

and Notch1 also has a certain regulatory effect on inflammation and fibrosis processes in DN.

In summary, our research suggests that PKase can alleviate the progression of DN renal fibrosis and improve renal function by inhibiting the activation of the Notch1/Hes1-Pten/Akt signaling pathway. Although we have validated that PKase can exert its effects on DN through the Notch1/Hes1-Pten/Akt signaling pathway, research on PKase is still limited and more studies are needed to demonstrate the presence or absence of side effects, and drugs typically have multiple regulatory pathways. Further research is needed to investigate the mechanism of action of PKase in the future. However, this study is beneficial as it is the first to investigate the relationship between PKase and the Notch1/Hes1-Pten/Akt signaling pathway.

Conclusion

The results imply that PKase enhances the Pten/Akt signaling pathway expression in DN through the suppression of the Notch1/Hes1 signaling pathway. This subsequently improves cellular apoptosis and oxidative stress, leading to a reduction in renal fibrosis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12882-025-04050-1 .

Supplementary Material 1

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Not applicable.

Author contributions
Mingjie Qing and Ximei Zhang contributed equally to the conception and design of the research study, acquisition of data, analysis and interpretation of the data, and drafting of the manuscript. Qiangxiang Li provided technical support in the acquisition of data, performed statistical analysis, and critically revised the manuscript for important intellectual content. Canqun Yan provided expertise in the field of study, contributed to the interpretation of the data, and critically revised the manuscript for important intellectual content. All authors have read and approved the final manuscript, and have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

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Data availability
Data Availability declarationThe datas used and analyzed during the current study are available from the corresponding author.

Declarations

Ethical approval
This study was approved by the ethics committee of The Second Affiliated Hospital, Hengyang Medical School, University of South China (YK2023011).

Consent to participate and consent to publish
This study was conducted in accordance with the guidelines provided by the ethics committee and adhered to all relevant ethical standards for the care and use of laboratory animals. The animals used in this study were provided by Huafukang Bio, and informed consent was obtained from all participants prior to their inclusion in the study. Throughout the study, animal care and handling were in compliance with ethical guidelines to ensure their well-being.

Competing interests
The authors declare no competing interests.

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