

Original Article

P300-mediated H3K18 acetylation triggers necroptosis via modulation of KRT18 transcription in diabetic nephropathy

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Received 15 September 2025 Accepted 5 January 2026 Published 10 March 2026

Abstract

Diabetic nephropathy (DN) is a major cause of end-stage renal disease. While glomerular damage is a known aspect of its pathology, tubular epithelial cell necroptosis also plays a crucial role in disease progression. Epigenetic modifications, particularly histone acetylation, have garnered attention for their role in the regulation of kidney damage-related gene expression. This study explores whether the histone acetyltransferase P300 regulates KRT18 expression via histone H3 lysine 18 acetylation (H3K18ac), driving tubular epithelial cell necroptosis and accelerating DN progression. We establish an STZ-induced diabetic nephropathy mouse model and a high glucose-treated HK-2 cell model. Western blot analysis, qPCR, immunohistochemistry, and AO/PI staining are employed to assess the expression levels of P300, H3K18ac, KRT18, and necroptosis-related proteins (RIPK1 and MLKL). Functional validation of the P300-KRT18 axis is performed using shRNA interference, overexpression, and the small molecule inhibitor C646. Both *in vivo* and *in vitro* models show significant upregulation of P300, H3K18ac, and KRT18, coupled with RIPK1/MLKL pathway activation and increased cell death. *P300* knockdown or C646 treatment effectively inhibits H3K18ac and KRT18 expression, reducing necroptosis; *KRT18* knockdown also alleviates P300 overexpression-induced cell death. Co-transfection with P300 overexpression and KRT18 interference demonstrates that KRT18 is a key downstream effector of P300-mediated necroptosis. In conclusion, P300 upregulates KRT18 expression through H3K18 acetylation, subsequently activating the RIPK1/MLKL pathway and promoting tubular epithelial cell necroptosis. The P300-KRT18 axis may serve as a novel epigenetic therapeutic target for DN, suggesting that epigenetic regulation could be a viable intervention strategy to delay DN progression.

Key words P300, H3K18ac, histone acetylation, KRT18, necroptosis, diabetic nephropathy

Introduction

Diabetic nephropathy (DN) is one of the most common and severe chronic complications of diabetes and has become a leading cause of end-stage renal disease (ESRD) [1,2]. Beyond glomerular damage induced by hyperglycemia, increasing evidence indicates that tubulointerstitial injury plays a crucial role in the progression of DN, with programmed cell death of tubular epithelial cells being particularly significant in the pathological process [3,4].

Necroptosis, a form of programmed cell death distinct from apoptosis, is characterized by the involvement of the RIPK1/MLKL

signaling pathway, leading to cell membrane rupture and the release of inflammatory mediators [5–7]. While necroptosis has been noted in several kidney diseases, such as ischemia-reperfusion injury, IgA nephropathy, and DN [8–10], its specific upstream regulatory mechanisms, especially in relation to epigenetic modifications, remain largely unexplored.

P300 is a well-known histone acetyltransferase (HAT) that regulates chromatin conformation and gene transcription through acetylation of histone H3 at lysine 18 (H3K18ac) and other sites [11]. Although the role of P300 in kidney diseases has recently

garnered attention, direct evidence linking P300-mediated histone acetylation to tubular cell necroptosis is still lacking [12,13].

Keratin 18 (KRT18), a major epithelial-specific intermediate filament protein, is well established for its critical roles in maintaining cellular mechanical integrity and serving as a biomarker in various carcinomas [14,15]. However, in contrast to its extensively studied structural and oncogenic functions, the potential role of KRT18 in renal diseases, particularly in DN, remains largely unexplored. Although KRT18 is abundantly expressed in renal tubular epithelial cells [16], it is completely unknown whether and how it participates in tubular necroptosis. Moreover, the possibility that KRT18 could be a direct functional target under epigenetic regulation, thereby contributing to necroptosis, represents a significant knowledge gap.

This study aimed to systematically investigate whether P300 promotes KRT18 expression through H3K18ac acetylation, thereby driving necroptosis in DN, to elucidate its role in DN progression and potential therapeutic implications.

Materials and Methods

Subjects

This study was conducted in accordance with the principles established in the Declaration of Helsinki, and ethical approval was obtained from the Ethics Committee of Huzhou Central Hospital (ethical approval No. 202405039-01). Written informed consent was obtained from each participant with a diagnosis of DN. The estimated glomerular filtration rate (eGFR) of participants in the study was determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), and the DN patients were grouped into stage 2, stage 3 or stage 4 according to the eGFR.

Mouse model of DN

Male C57BL/6J mice, aged 4–6 weeks and weighing 20–25 g, were obtained from Jicui Biotechnology Limited (Nanjing, China). This study was approved by the Shanghai Pudong Hospital Laboratory Animal Welfare and Ethics Committee (ethical approval No. KCZX20250301-001). The mice were randomly allocated to the control group (Con) or DN group (DN). After a one-week acclimation, all mice underwent unilateral nephrectomy under anesthesia with isoflurane (3%–4% for induction, 1.5%–2.5% for maintenance, in 100% oxygen). Two weeks post-surgery, diabetes was induced by intraperitoneal injections of streptozotocin (STZ, 50 mg/kg; Sigma, St Louis, USA) for seven consecutive days. The DN and DN + MLKL^{-/-} groups were fed with a high-glucose and high-fat diet for 16 weeks. Starting from the third week, the mice received intraperitoneal injections of STZ (50 mg/kg) for seven consecutive days. Mice with glucose levels exceeding 16.7 mM were considered successfully modeled. Control mice received injections of an equal volume of citrate buffer (0.1 M, pH 4.5). Mice fed with the diet for 16 weeks constituted the DN model group, while control mice received a standard diet. From week four, DN model mice, following citrate buffer or STZ injections, were administered daily with intraperitoneal injections of the P300 inhibitor C646 (HY-13823; 10 mg/kg; MCE, Shanghai, China) or DMSO (control group) for four weeks to assess the effects of drug intervention.

Cell culture and intervention

Human renal tubular epithelial cells (HK-2; Procell, Wuhan, China) were cultured in DMEM medium (Procell) containing 10% fetal

bovine serum (Bioagrio, Shanghai, China) and 1% penicillin-streptomycin (Beyotime, Shanghai, China) and maintained in a 37°C incubator with 5% CO₂. Cells cultured in high-glucose medium (25 mM) for 72 h were designated the high-glucose group. An additional group with 5 mM glucose and 20 mM mannitol was used to control for osmotic pressure effects. Cells were treated with the MLKL inhibitor NSA (HY-100573; MCE) and the P300 inhibitor C646, and the optimal concentrations of NSA and C646 for significant inhibition of necroptosis were determined.

HE staining, Masson staining, and IHC

Paraffin sections of kidney tissues were incubated in a 60°C oven for 1–2 h and then deparaffinized with xylene and ethanol. Hematoxylin was used to stain the nuclei for approximately 10 min, followed by eosin staining of the cytoplasm for 30 s. Finally, sections were mounted with neutral balsam, dried at room temperature, and examined under an optical microscope. Observations included nephron structure, tubular dilation, and interstitial fibrosis. Masson's trichrome staining and IHC staining were performed as described previously [17]. The primary antibodies used for the IHC analysis are listed in [Supplementary Table S1](#).

Cell death detection (AO/PI double staining)

After various treatments, the cells were stained with a mixture of acridine orange (AO) and propidium iodide (PI) for 5 min and observed under a fluorescence microscope. AO permeates cell membranes, binds to the nuclei of all cells, and emits green fluorescence. PI penetrates only the membranes of dead cells, binding to the nuclei and emitting red fluorescence.

shRNA and plasmid transfection

HK-2 cells were seeded and reached 70%–80% confluence before being transfected with P300 overexpression plasmid (pcDNA3.1-P300) and shRNA vectors (P300-shRNA and KRT18-shRNA were synthesized by GenePharma, Shanghai, China) using Lipofectamine 3000 (Invitrogen, Carlsbad, USA). Empty vectors or non-specific shRNA were used as controls. After 48 h of transfection, the medium was changed, and the cells were further cultured for 24 h before subsequent experiments. The sequences of the shRNAs used in this study are shown in [Supplementary Table S2](#).

Western blot analysis

Cell or tissue lysates were prepared using RIPA buffer with protease inhibitors to extract total protein. Protein concentrations were quantified using a BCA assay kit (Beyotime). Proteins were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, Billerica, USA). The membranes were blocked with 5% non-fat milk for 1 h and then incubated overnight at 4°C with antibodies. The primary antibodies used in this study are listed in [Supplementary Table S1](#). The following day, the membranes were incubated with HRP-conjugated secondary antibodies (Proteintech, Wuhan, China) for 1 h. Detection was performed using enhanced chemiluminescence (ECL) reagent, and band intensities were analyzed with ImageJ software (version 1.54; USA National Institutes of Health, Bethesda, USA).

Quantitative real-time PCR

Total RNA was extracted using a Universal RNA Purification kit (EZBioscience, Roseville, USA), and cDNA was prepared with

HyperScript III RT SuperMix (EnzyArtisan, Shanghai, China). Real-time PCR analysis was performed on an ABI7500 real-time PCR system (Applied Biosystems, Foster City, USA) using 2× S6 Universal SYBR qPCR Mix (EnzyArtisan). The transcript levels were normalized to those of β -actin. The primers used in this study are listed in [Supplementary Table S3](#).

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were conducted to analyze protein-DNA interactions utilizing the ChIP kit (Beyotime) according to the manufacturer's protocol. In brief, cells were subjected to cross-linking with 1% formaldehyde for 10 min, which was subsequently terminated by the addition of 2.5 M glycine. Chromatin was fragmented to an optimal size through ultrasonication (7 cycles of 7-s pulses interspersed with 7-s pauses). Aliquoted samples were immunoprecipitated at 4°C overnight with specific antibodies or an IgG control. The immune complexes were collected with agarose beads, washed, and eluted, followed by reversal of cross-links at 65°C for 5–6 h. The enriched DNA fragments were finally quantified by PCR amplification using specific primers and resolved by agarose gel electrophoresis. The sequences of the primers used in this study are listed in [Supplementary Table S4](#).

High-throughput sequencing

RNA-seq and ChIP-seq were performed to profile transcriptomic and chromatin states. For RNA-seq, total RNA was extracted with a commercial kit (three biological replicates per group). Strand-specific libraries were prepared using the NEBNext Ultra II kit and sequenced on an Illumina NovaSeq 6000 system (150 bp paired-end). Differential expression analysis was conducted with DESeq2 ($|\log_2FC| > 1$, adjusted $P < 0.05$). For ChIP-seq, cells were cross-linked with formaldehyde, and chromatin was sonicated and immunoprecipitated using an anti-H3K18ac antibody. Libraries from enriched DNA were sequenced on the same platform. Reads were aligned to GRCh38 with Bowtie2, and peaks were called using MACS2 ($q < 0.05$).

Pathway enrichment analysis

Enriched signaling pathways were identified using the KEGG database. The extent of enrichment for differentially expressed genes was statistically determined using a two-sided Fisher's exact test.

Statistical analysis

Data were processed using GraphPad Prism 9.0 and SPSS 22.0 software. Cell experiments were repeated at least four times, and animal experiments included at least six mice per group. Data are presented as the mean $\pm$ standard deviation, and comparisons of the means of the two groups were conducted by two-tailed unpaired Student's *t* tests. One-way ANOVA followed by Bonferroni-corrected pairwise comparisons were used to compare the means of more than 2 groups. A *P* value less than 0.05 was considered to indicate statistical significance.

Results

The expressions of P300 and H3K18ac are increased in DN patients and models

The characteristics of the DN patients included in this study are summarized in [Supplementary Table S5](#). Patients were categorized

into stages 2, 3, and 4 based on the eGFR. HE staining of DN patient samples is shown in [Figure 1A](#), with Masson staining indicating increased collagen deposition and interstitial fibrosis as DN progressed ([Figure 1A](#)). IHC results demonstrated that P300 and H3K18ac levels were increased with advancing DN stages in renal biopsy specimens ([Figure 1A](#)).

The serum biochemical indices of the mice in this study are shown in [Supplementary Table S6](#). In the STZ-induced DN mouse model, HE staining revealed noticeable tubular dilation, luminal deformation, and disorganized epithelial cell arrangement, accompanied by inflammatory cell infiltration in the renal interstitium. Masson staining further indicated significant collagen deposition and interstitial fibrosis in the DN group. The levels of P300 and H3K18ac in DN mice were higher than those in controls, supporting pathological tubular structural damage ([Figure 1D](#)). Western blot analysis showed significantly elevated expression of P300 and its catalytic histone acetylation marker H3K18ac in DN kidney tissues compared to controls ([Figure 1B](#)). qPCR further confirmed a significant increase in *P300* mRNA levels in DN mouse kidneys ([Figure 1C](#)).

To verify the localization specificity of these changes, immunohistochemistry was used to assess P300 and H3K18ac distribution in renal tissues. Enhanced staining intensity was observed in the tubular regions, with positive cells primarily in the proximal tubular epithelial cells, displaying a nuclear pattern consistent with their role as histone modification enzymes.

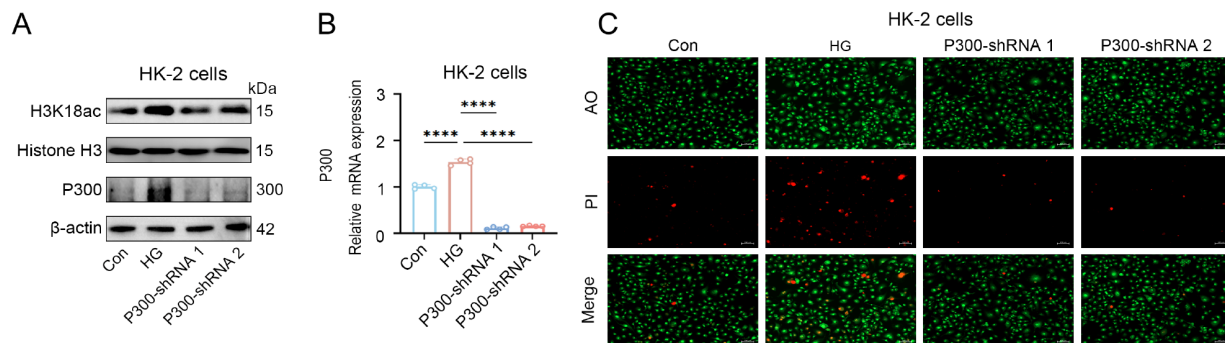
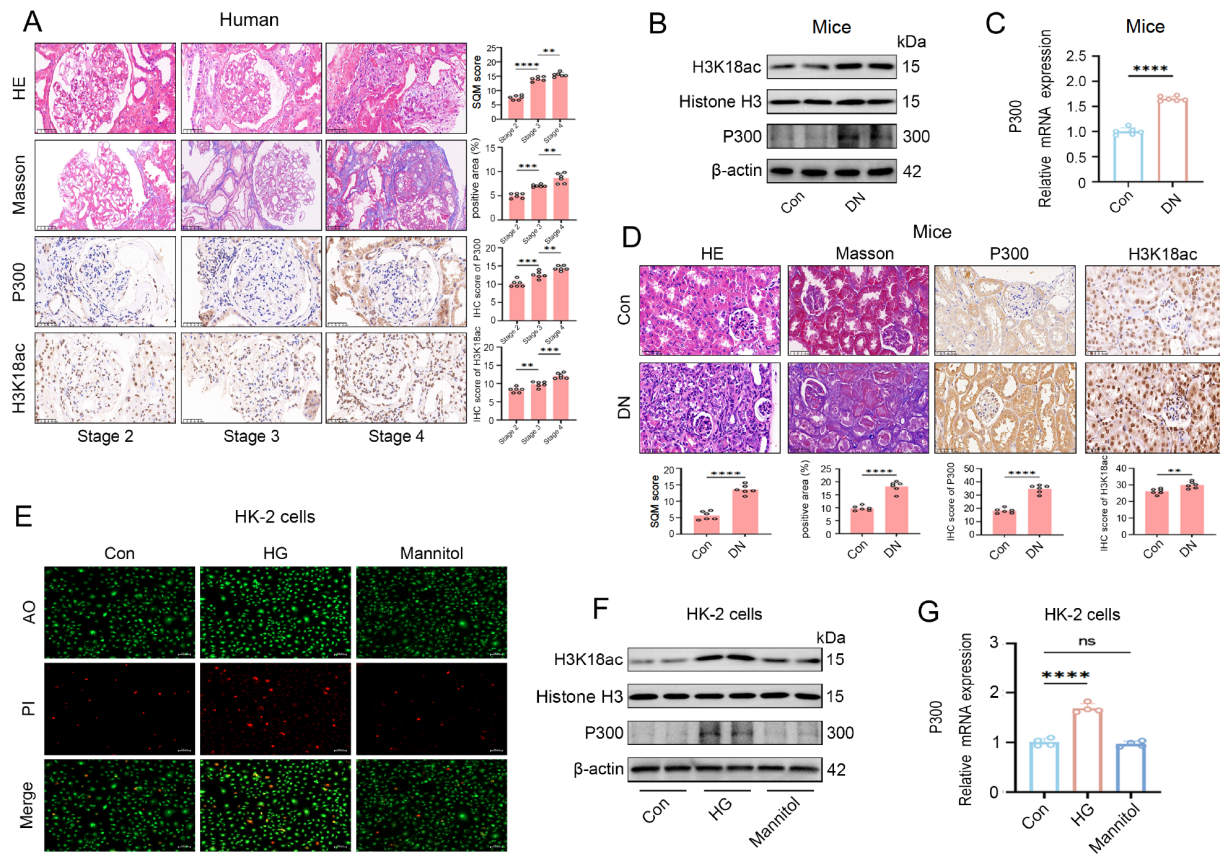
In vitro, HK-2 cells were exposed to high glucose (25 mM D-glucose) to simulate a hyperglycemic environment. Western blot analysis results indicated that high glucose treatment for 48 h significantly induced P300 and H3K18ac expressions, while mannitol treatment as an osmotic control had no effect on P300 and H3K18ac levels, suggesting that the effect was due to hyperglycemia itself rather than osmotic pressure ([Figure 1F](#)). qPCR further validated a marked upregulation of *P300* mRNA in high glucose-stimulated HK-2 cells, with no significant changes in the isotonic control group ([Figure 1G](#)). Additionally, AO/PI double staining showed a notable increase in the proportion of PI-positive cells in the high glucose group, indicating severe cell death, with no such change in the isotonic control group ([Figure 1E](#)).

P300 knockdown inhibits H3K18ac and mitigates high glucose-induced cell death

To investigate the regulatory role of P300 in a high glucose environment, we established stable *P300* knockdown cell lines (shP300-1 and shP300-2). qPCR results confirmed successful *P300* knockdown in the shP300 group ([Figure 2B](#)). Western blot analysis showed that P300 interference significantly reduced the high glucose-induced increase in H3K18ac levels ([Figure 2A](#)). AO/PI staining indicated a significant decrease in the proportion of PI-positive cells in the *P300* knockdown group compared to the high glucose group ([Figure 2C](#)), suggesting that *P300* knockdown alleviated high glucose-induced cell death, supporting its detrimental role in glucose-mediated damage.

High glucose induces tubular cell necroptosis via RIPK1/MLKL pathway activation

To determine the type of cell death, transcriptomic analysis of control and high glucose-treated HK-2 cells identified differential gene enrichment in necroptosis and inflammation pathways ([Figure](#)



3A). We further assessed the expression of the necroptosis-related molecules RIPK1, phosphorylated (p-) RIPK1, MLKL, and p-MLKL in DN patient tissue. IHC results for stage 2, 3, and 4 DN patients showed increasing levels of p-MLKL and p-RIPK1 with disease

progression (Figure 3B). In the DN model mice, IHC revealed enhanced expression of p-RIPK1 and p-MLKL primarily in the tubular regions (Figure 3C). Western blot analysis demonstrated significant upregulation of p-RIPK1 and p-MLKL in both DN mouse

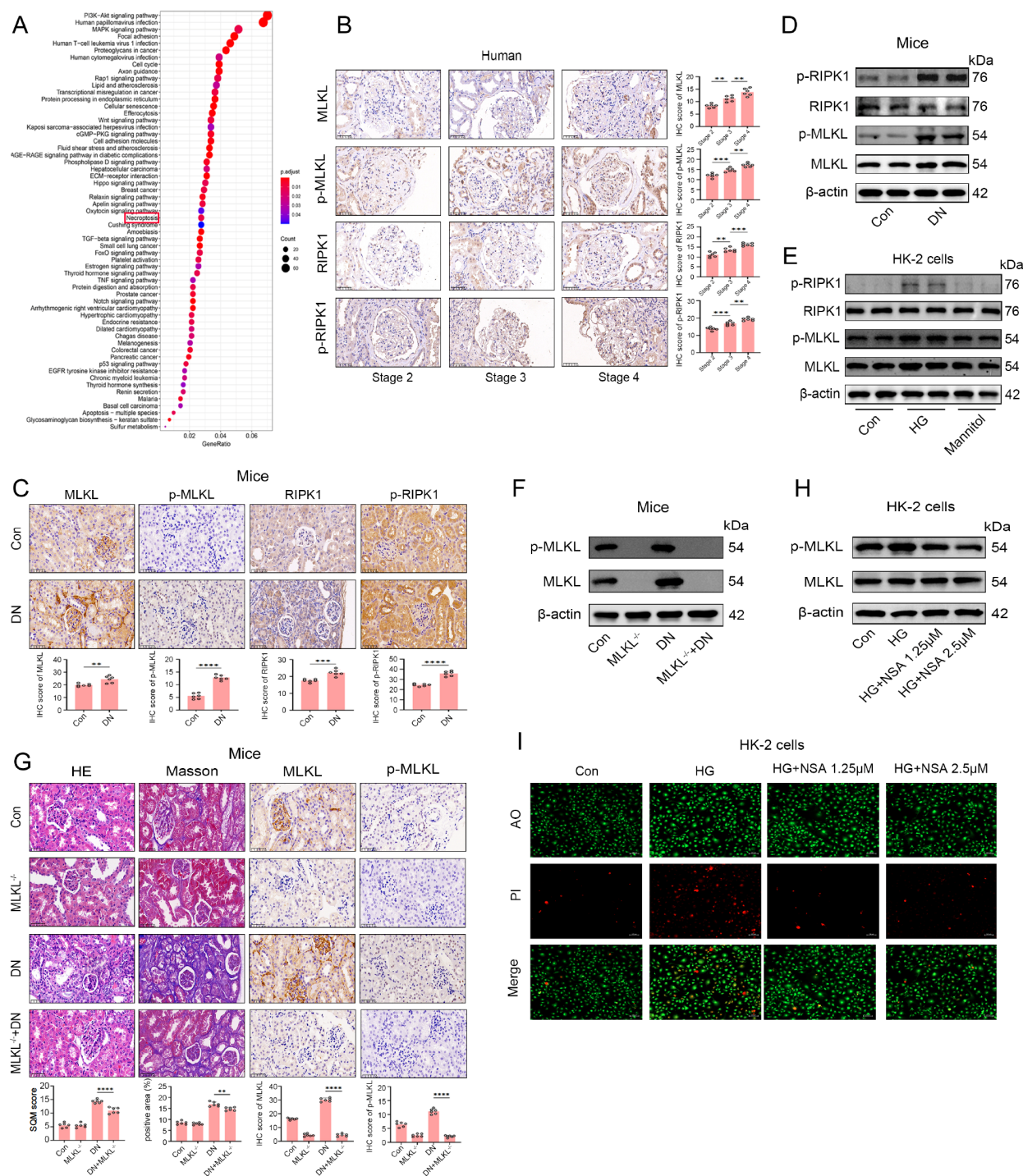


Figure 3. High glucose promotes necroptosis via the RIPK1/MLKL pathway (A) Molecular functions of differentially expressed genes based on KEGG analysis. (B) Representative images of IHC staining for MLKL, p-MLKL, RIPK1 and p-RIPK1 in renal biopsy specimens from DN patients (scale bar: 50 μ m). These results indicated that the levels of p-RIPK1 and p-MLKL were increased as the DN stage progressed. (C) IHC staining indicated that the levels of p-RIPK1 and p-MLKL were increased in DN mice (scale bar: 50 μ m). (D) Western blot analysis indicated that necroptosis induced the death of DN mice. (E) Western blot analysis indicated that necroptosis induced the death of high glucose-exposed HK-2 cells. (F) Western blot analysis was performed on the kidneys of Con, MLKL^{-/-}, DN and DN + MLKL^{-/-} model mice. (G) Representative images of HE staining, Masson's trichrome staining and IHC staining for MLKL and p-MLKL in renal biopsy samples from model mice (scale bar: 50 μ m). (H) Western blot analysis indicated that NSA (necroptosis inhibitor) treatment reduced p-MLKL expression and cell death in HK-2 cells. (I) AO/PI staining shows a significant reduction in PI-positive cells after NSA treatment (scale bar: 50 μ m). ** P < 0.01, *** P < 0.001, and **** P < 0.0001.

kidney tissues and HG-treated HK-2 cells (Figure 3D,E). To ascertain whether cell death occurs predominantly through necroptosis, we generated MLKL^{-/-} knockout mice. HE and Masson staining of DN + MLKL^{-/-} mice indicated reduced tubular dilation, decreased luminal deformation, and organized epithelial cell arrangement compared with wild-type DN mice. Masson staining showed less collagen deposition and interstitial fibrosis, suggesting reduced pathological tubular damage (Figure 3G). Western blot analysis confirmed that MLKL pathway activation induced by the DN model was completely reversed in MLKL^{-/-} mouse kidney tissues (Figure 3F). In HK-2 cells, treatment with the necroptosis inhibitor necrosulfonamide (NSA) significantly reduced p-MLKL expression (Figure 3H), and AO/PI staining showed a decrease in PI-positive cells (Figure 3I), further demonstrating that a high glucose environment induced RIPK1-MLKL pathway-mediated necroptosis.

P300 regulates the necroptosis pathway and P300 overexpression exacerbates cell damage

Under high glucose stimulation, we further explored the regulatory role of P300 in the necroptosis pathway. Western blot analysis

showed that P300 silencing inhibited the activation of the necroptosis pathway in renal tubular epithelial cells under high glucose conditions (Figure 4A). Overexpression of P300 (P300-OE) significantly increased the expression of p-MLKL, while AO/PI staining indicated exacerbated cell death. This effect was partially reversed by treatment with a necroptosis inhibitor (Figure 4B-D), suggesting that P300 promotes necroptosis by enhancing RIPK1/MLKL pathway activity.

Upregulation of KRT18 expression drives necroptosis in high glucose-treated cells

Next, we conducted a combined analysis of H3K18ac ChIP-seq data and RNA-seq, identifying KRT18 as a potential participant in P300-mediated H3K18ac upregulation, driving necroptosis in DN kidney tissues (Figure 5A). Furthermore, KRT18 expression was evaluated in renal biopsy samples from DN patients and mouse models and showed a gradual increase with DN progression (Figure 5B,C). Consistently, KRT18 was upregulated in DN model mouse kidneys (Figure 5D,E) and high glucose-treated cells (Figure 5F,G). Upon P300 knockdown, KRT18 protein and mRNA expressions signifi-

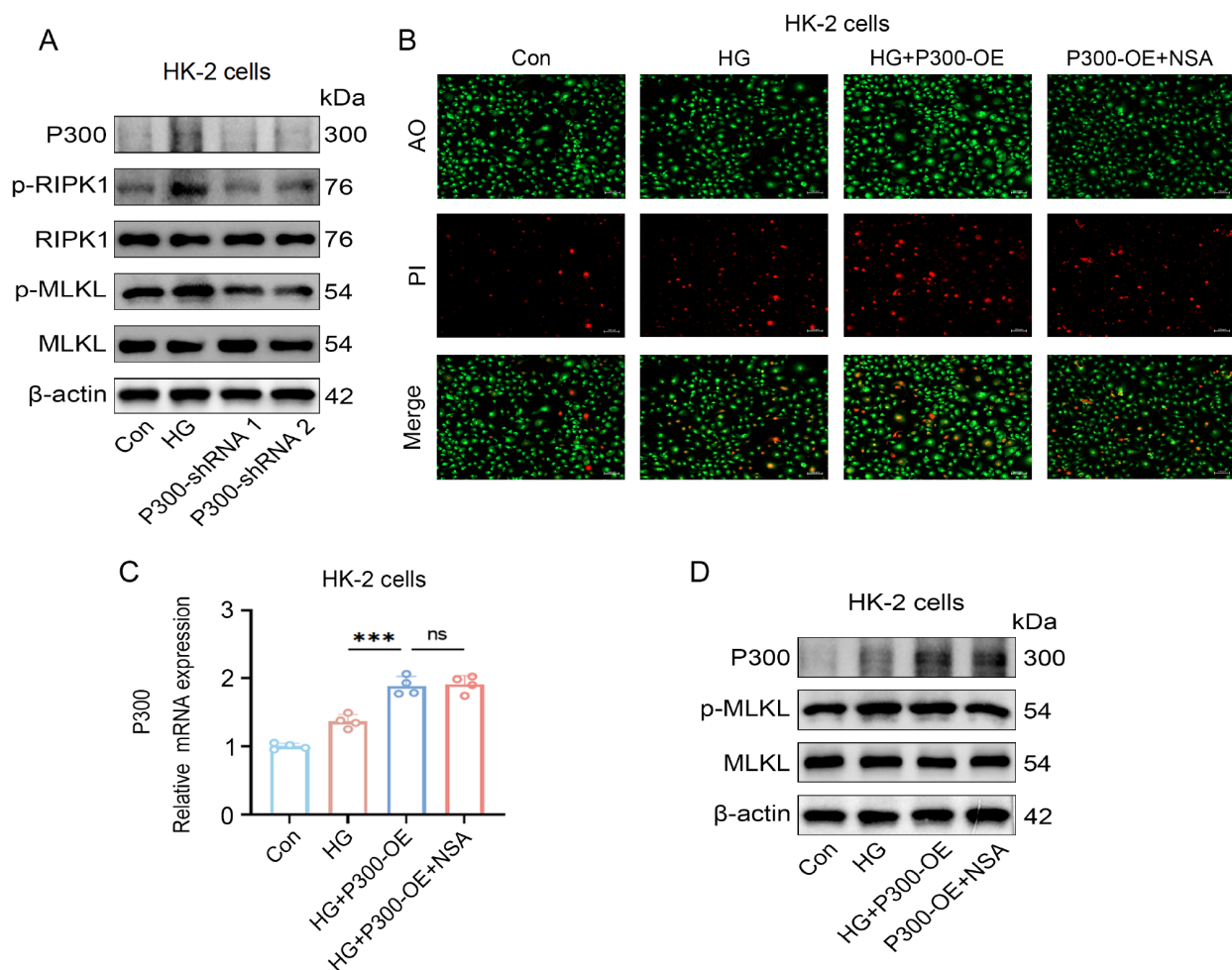


Figure 4. P300 interference reduces high glucose-induced necroptosis, while P300 overexpression enhances cell death (A) Western blot analysis indicated that P300 silencing reduced p-RIPK1 and p-MLKL levels in HK-2 cells treated with high glucose concentrations. (B) AO/PI staining indicates that P300 overexpression exacerbates cell death, which is partially counteracted by NSA treatment. (C) The effects of P300-OE were confirmed by qPCR analysis. (D) Western blot analysis indicated that P300 overexpression enhances p-MLKL expression, with NSA partially mitigating this effect. *** $P < 0.001$. ns, not significant.

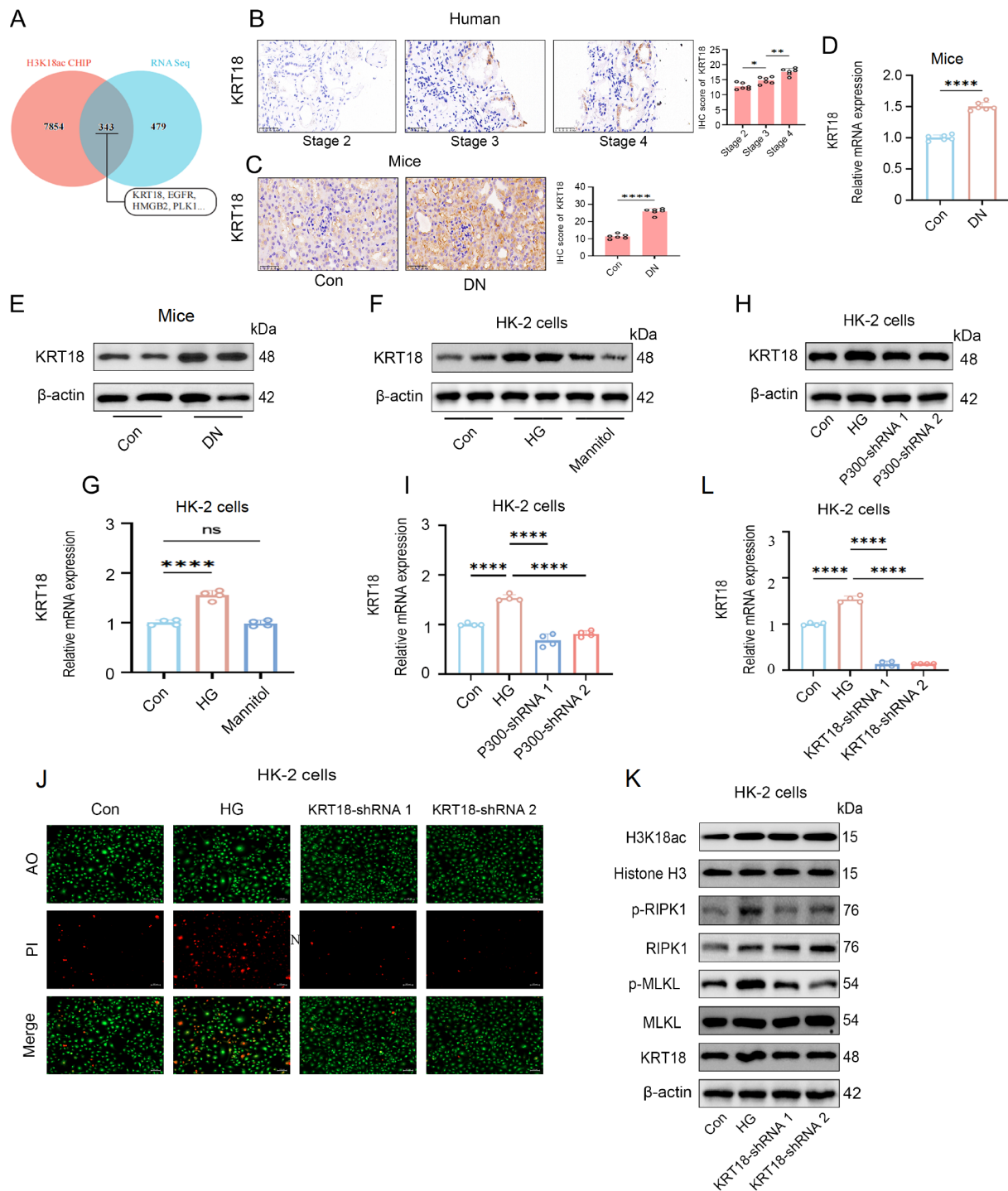


Figure 5. Upregulation of KRT18 expression drives necroptosis in high glucose-treated cells (A) Venn diagram of H3K18ac ChIP-seq data and RNA-seq data from DN mouse kidneys. (B) Representative IHC images of KRT18 in renal biopsy samples from DN patients at stages 2, 3, and 4 (scale bar: 50 μ m). KRT18 expression was increased with DN progression. (C) IHC staining indicated that KRT18 levels are increased in the kidneys of DN model mice (scale bar: 50 μ m). (D) qPCR analysis indicated that the mRNA levels of *KRT18* were increased in DN mice. (E) Western blot analysis indicated that the protein levels of KRT18 were increased in DN mice. (F) Western blot analysis indicated that KRT18 protein expression was increased in HK-2 cells treated with high glucose concentrations. (G) qPCR analysis indicated that *KRT18* mRNA expression was increased in HK-2 cells treated with high glucose concentrations. (H) Western blot analysis revealed that *P300* silencing inhibited the protein level of KRT18 in HK-2 cells. (I) qPCR analysis indicated that *P300* silencing inhibited the mRNA levels of *KRT18* in HK-2 cells. (J) AO/PI staining indicated that KRT18 silencing inhibited the death of hyperglycemic HK-2 cells (scale bar: 50 μ m). (K) Western blot analysis indicated that *KRT18* silencing decreased p-RIPK1 and p-MLKL levels in high glucose-exposed HK-2 cells. (L) The effects of sh-KRT18 were confirmed by qPCR analysis. * $P < 0.05$, ** $P < 0.01$, and **** $P < 0.0001$. ns, not significant.

cantly decreased (Figure 5H,I), suggesting KRT18 as a downstream transcriptional target of P300. To clarify the role of KRT18, we constructed *KRT18* knockdown cell lines. The results showed that *KRT18* knockdown inhibited H3K18ac and high glucose-induced p-RIPK1 and p-MLKL expression, with a significant reduction in PI-positive cells (Figure 5J–L), indicating KRT18's pivotal role in P300-regulated necroptosis.

KRT18 is a key effector in P300-mediated necroptosis

To determine whether the promotion of necroptosis by P300 depends on KRT18, we co-transfected *KRT18*-shRNA under P300 overexpression conditions. Western blot analysis results revealed that *KRT18* knockdown reversed P300-OE-induced p-RIPK1 and p-MLKL upregulation (Figure 6A), and qPCR confirmed the successful overexpression of P300 and the successful knockdown of *KRT18* (Figure 6C,D). Upon P300 overexpression, the proportion of PI-positive cells significantly increased, but this phenomenon was reversed after *KRT18* knockdown (Figure 6B). These findings demonstrate that KRT18 serves as an important mediator of P300-regulated necroptosis, with P300's pro-death effects at least partially mediated through KRT18.

P300 inhibitor C646 mitigates DN-related pathological damage and cell necrosis

ChIP experiments revealed that P300 and H3K18ac were enriched at the *KRT18* promoter region in HK-2 cells (Figure 7B). In STZ-induced DN mice, four weeks of intraperitoneal injection of the P300 inhibitor C646 resulted in improved tubular structure and reduced fibrosis, as shown by HE and Masson staining (Figure 7A). Immunohistochemistry and western blot analysis indicated that C646 effectively suppressed the expression of P300, H3K18ac, KRT18, p-RIPK1, and p-MLKL (Figure 7A,C–E), demonstrating its ability to inhibit necroptosis at the tissue level. *In vitro*, HK-2 cells pretreated with C646 exhibited a weakened response to high glucose stimulation, with AO/PI staining showing a reduction in PI-positive cells and improved cell survival (Figure 7G). Western blot analysis confirmed the downregulation of P300 and KRT18 expression, along with a decrease in necroptosis-related proteins (Figure 7F), further establishing the critical role of P300 activity in high glucose-induced cell death.

Discussion

Worldwide, diabetes affects approximately 415 million people, and

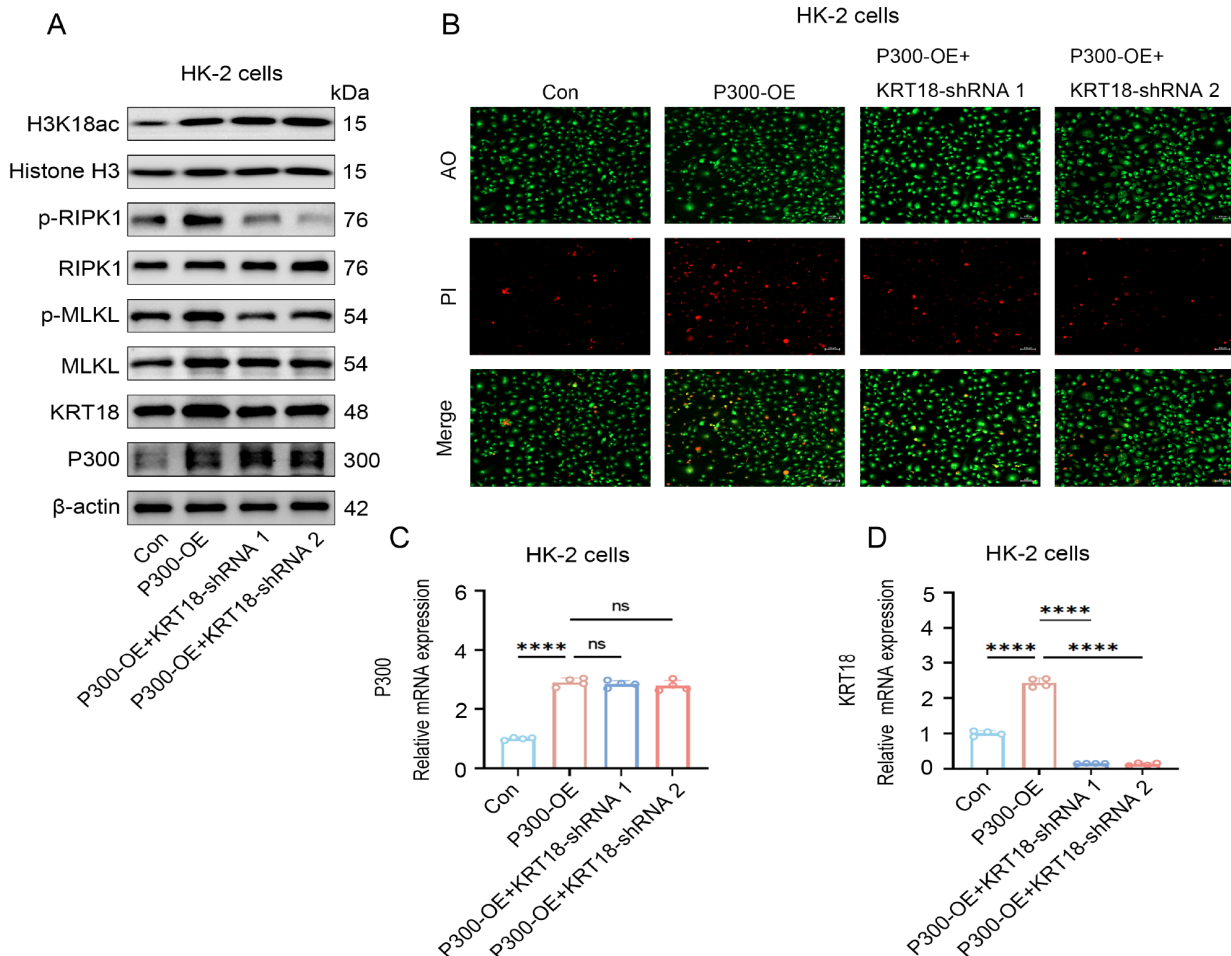


Figure 6. KRT18 is a key downstream effector of P300-mediated necroptosis (A) Western blot analysis indicated that P300 overexpression enhances H3K18ac, p-RIPK1, p-MLKL, and KRT18 expressions in HK-2 cells; this effect is significantly alleviated by *KRT18* interference. (B) AO/PI staining indicated an increase in PI-positive cells after P300 overexpression, which was reduced by *KRT18*-shRNA (scale bar: 50 μ m). (C) qPCR was performed to detect *P300* mRNA expression. (D) qPCR analysis indicated that P300 overexpression increased *KRT18* mRNA expression in HK-2 cells treated with high glucose concentrations, but this change was reversed by *KRT18* silencing. **** $P < 0.0001$. ns, not significant.

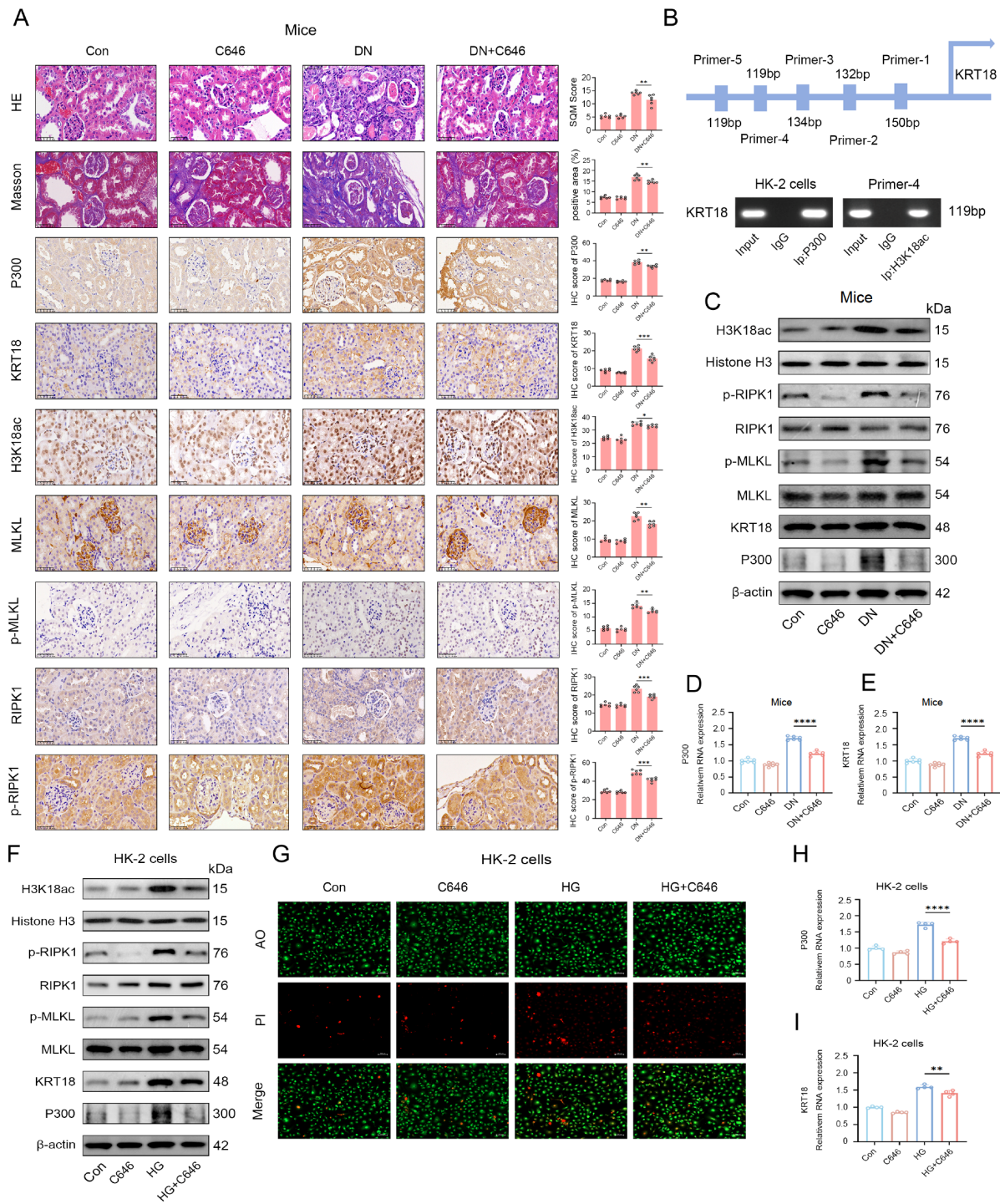


Figure 7. P300 inhibitor C646 significantly alleviates kidney damage and necroptosis in DN mice (A) Representative images of HE, Masson's trichrome staining, and IHC staining for P300, H3K18ac, KRT18, MLKL, p-MLKL, RIPK1, and p-RIPK1 in kidney tissues from Con, C646, DN, and DN + C646 mice (scale bar: 50 μ m). IHC results indicated that C646 improves pathological changes in DN mouse kidneys. (B) ChIP assay demonstrates that both H3K18ac and P300 are enriched at the promoter region of *KRT18*. (C) Western blot analysis indicated that C646 treatment decreased P300 expression, reduced the protein levels of H3K18ac and KRT18, and inhibited necroptosis in the kidneys of DN model mice. (D) qPCR analysis indicated that C646 reduced *P300* mRNA levels in the kidneys of DN model mice. (E) qPCR analysis indicated that *KRT18* mRNA levels were increased in the kidneys of DN model mice, but this change was reversed by C646 treatment. (F) Western blot analysis indicated that C646 treatment reduced the protein levels of P300, H3K18ac, KRT18, p-RIPK1, and p-MLKL in HK-2 cells. (G) AO/PI staining indicated that C646 reduces PI-positive cells, improving cell survival (scale bar: 50 μ m). (H) qPCR analysis indicated that C646 treatment decreased the *P300* mRNA level in HK-2 cells treated with high glucose concentrations. (I) qPCR analysis indicated that C646 treatment decreased the *KRT18* mRNA level in HK-2 cells treated with high glucose concentrations. ** $P < 0.01$, and **** $P < 0.0001$.

its prevalence is steadily increasing [18]. DN, a microvascular complication of diabetes, represents the most common complication and is the leading cause of ESRD globally [19]. The pathogenesis of DN is complex and involves metabolic disturbances, oxidative stress, inflammatory responses, and the activation of multiple signaling pathways. In this study, we uncover a novel epigenetic mechanism by which P300 promotes high glucose-induced necroptosis in renal tubular epithelial cells through histone H3K18 acetylation-mediated upregulation of KRT18 expression. This process effectively inhibits the P300 inhibitor C646, which consequently attenuates renal injury. These findings suggest that targeting P300 may offer a novel therapeutic strategy for DN. Our work not only expands the understanding of DN pathogenesis but also provides important experimental evidence supporting the role of epigenetic regulation in kidney disease.

While previous research on diabetic kidney disease has predominantly focused on glomerular pathologies [20,21], this study reveals that necroptosis in renal tubular epithelial cells represents a critical pathological process that cannot be overlooked in DN progression. We observed significant activation of the RIPK1/MLKL pathway accompanied by increased cell death in both *in vivo* and *in vitro* models. These findings align with recent reports identifying necroptotic features in diabetic kidneys [22,23].

P300, also known as EP300, is a protein initially identified through its interaction with the adenoviral oncoprotein E1A. The histone acetyltransferase (HAT) domains of P300 and CREB-binding protein (CBP) share over 90% sequence identity, placing them within the same protein family and leading to their frequent collective designation as P300/CBP [24,25]. While previous studies have primarily linked P300 to inflammatory cytokine expression, sustained activation of fibrotic genes, and the progression of chronic kidney injury, direct evidence demonstrating its role in driving necroptosis in renal tubular epithelial cells during DN remains limited [26,27]. In our study, both high glucose-stimulated human renal tubular epithelial cells and renal tissues from DN mice exhibited significantly elevated P300 protein levels, accompanied by a concurrent increase in H3K18ac. Further enrichment analysis revealed markedly enhanced occupancy of both P300 and H3K18ac at the *KRT18* promoter region. These findings indicate that P300 directly binds to and remodels the chromatin accessibility of the *KRT18* promoter, thereby facilitating its transcriptional activation.

C646, a competitive and specific inhibitor of p300/CREB-binding protein (CBP) [28,29], has demonstrated protective effects in both *in vivo* and *in vitro* studies [30,31], suggesting that targeting P300 could represent a novel therapeutic strategy for DN. Our findings reveal that under high glucose conditions, the upregulated levels of P300 and H3K18ac were effectively suppressed by C646 treatment. This inhibition subsequently reduced the expression of the downstream target gene KRT18 and significantly alleviated cell death. These results strongly establish P300 as the initial trigger that activates the subsequent pathogenic cascade. The renal protective effects observed with C646 in our models indicate that targeting the HAT activity of P300 may constitute a viable and innovative therapeutic approach.

We identified KRT18 as the critical molecular conduit through which P300 signaling propagates necroptotic activation. While KRT18 is a well-characterized intermediate filament component in epithelial cells with established roles in structural integrity and oncogenesis [32,33], its potential function as an upstream regulator

of necroptosis remains largely unexplored. Our results demonstrate that KRT18 expression is significantly upregulated by high glucose stimulation and similarly induced by P300 overexpression, whereas both P300 knockdown and C646-mediated inhibition effectively suppress its expression. ChIP experiments confirmed the direct binding of P300 and H3K18ac to the *KRT18* promoter, indicating transcriptional activation through specific epigenetic mechanisms rather than nonspecific stress responses. Most importantly, *KRT18* silencing under P300 overexpression conditions substantially attenuated cell death. These findings collectively establish that the enhanced necroptosis susceptibility mediated by P300 is functionally dependent on KRT18.

This study acknowledges several limitations. First, the clinical relevance is constrained by a relatively small sample size and the retrospective nature of the study, which limits statistical power and the ability to fully account for potential confounding factors. Second, while genetic knockdown and pharmacological inhibition of P300 produced concordant phenotypes, the possibility of off-target effects associated with the tool inhibitor C646 cannot be entirely ruled out, warranting further investigation into its precise specificity. Finally, the generalizability and broader pathophysiological significance of the identified P300-H3K18ac-KRT18 axis in other diabetic complications or renal diseases remain to be established in future studies.

This study delineates a complete pathogenic axis in DN, whereby hyperglycemia-activated P300 epigenetically upregulates KRT18 expression through histone H3K18 acetylation. The increased KRT18 heightens the sensitivity of renal tubular epithelial cells to RIPK1/MLKL-mediated necroptosis, resulting in plasma membrane rupture, release of inflammatory signals, and aggravated tubulointerstitial injury and fibrosis. Inhibition of P300 with C646 effectively attenuates this cascade, mitigating cellular damage and supporting the therapeutic potential of targeting the P300-H3K18ac-KRT18 axis.

These findings suggest that targeting epigenetic mechanisms could be a promising strategy to slow the progression of DN, providing a theoretical basis for developing P300-targeted interventions. However, further studies are needed to validate the safety and specificity of long-term P300 inhibition *in vivo*.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

Data Availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2025) in National Genomics Data Center (Nucleic Acids Res 2025), and China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA016583 and GSA: CRA038033) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa-human> and <https://ngdc.cncb.ac.cn/gsa>, respectively. All other data supporting the findings of this study, including raw data and original images, are available from the corresponding authors upon reasonable request.

Funding

This work was supported by the grants from the Tianshan Talent Program for High-Level Personnel Training in Medicine and Health (No. TSYC202301B040) and the Key Research and Development

Program Project of Xinjiang Uygur Autonomous Region (No. 2022B03009-4).

Conflict of Interest

The authors declare that they have no conflict of interest.

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