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NEPHROLOGY - ORIGINAL PAPER

The protective effect of Cordycepin on diabetic nephropathy through autophagy induction in vivo and in vitro

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Abstract

Background Diabetic nephropathy (DN) is one of the most serious chronic complications of diabetes mellitus (DM). Autophagy is an important physiological function for podocytes to maintain stability of intracellular environment. In this study, we planned to clarify the effect of Cordycepin, a traditional Chinese medicine, on DN and the related mechanisms.

Methods All rats were randomly divided into normal control group, diabetic controls, low-dose group (10 mg/kg), medium-dose group (100 mg/kg), and high-dose group (500 mg/kg). The level of cholesterol, blood sugar, triglyceride, creatinine, and urine protein was examined through an automatic biochemistry analyser. Enzyme-linked immunosorbent assay (Elisa) was used to detect the level of IL-1β, IL-6, and IL-18. HE staining was used to examine histopathologic changes. TUNEL staining was used to detected cell apoptosis. The expression of fibrosis markers α-SMA, t-TG, and TIMP-1, apoptosis-related proteins cleaved-caspase3, Bax and Bcl-2, autophagy markers Beclin1, light chain 3 (LC3)I/II, and p62 were evaluated by western blot.

Results The level of cholesterol, blood sugar, triglyceride, creatinine, and urine protein in the diabetic controls was much higher than that in the normal control group. Obvious histopathology injuries were also found in DN model group. After Cordycepin treatment, all the above indexes were improved compared with the DN group and tissue damages were also alleviated. Further studies showed that Cordycepin suppressed cell apoptosis and renal fibrosis and rescued cell autophagy in DN rat model. Moreover, the results of our in vitro experiments showed that the addition of 3-methyladenine (3-MA, specific autophagy inhibitor) successfully abolished the protective effect of Cordycepin on renal fibrosis through inducing apoptosis and renal fibrosis. The above protective effects of Cordycepin were exhibited in a dose-dependent manner.

Conclusion Cordycepin participated in the modulation of cell apoptosis, fibrosis, and autophagy induction in DN. Our study for the first time revealed that Cordycepin had a certain therapeutic effect on DN in rats through autophagy induction.

Keywords Diabetic nephropathy · Cordycepin · Autophagy · Apoptosis

Abbreviations

DN	Diabetic nephropathy
DM	Diabetes mellitus
3-MA	3-Methyladenine
HGF	Hepatocyte growth factor
T2D	Type 2 diabetes
TG	Tripterygium glycoside
STZ	Streptozotocin

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Introduction

Diabetic nephropathy (DN) is one of the most serious chronic complications of diabetes mellitus (DM), and the clinical manifestations of DN are proteinuria and renal dysfunction, finally developing into end-stage renal disease [1]. The pathological features of early DN include the aggregation of mesangial matrix and the thickening glomerular basement membrane, which can easily lead to renal failure. Besides, mesenchymal transdifferentiation of renal tubular epithelial cells is the major cause of renal fibrosis and diabetic nephropathy [2]. The incidence of DN is increasing gradually with the rapid development of economy and the improvement of living standard, especially the acceleration of population aging, so it is vital to take measures to prevent and delay the occurrence and development of DN [3].

Podocytes locate outside the basement membrane of the glomerular capillaries and act as the key structures of the glomerular filtration barrier through preventing leakage of plasma proteins. Studies have shown that the damage and loss of podocytes can severely damage the integrity of the glomerular filtration barrier and contribute to glomerular sclerosis, gradually developing to the end stage of renal failure [4]. Under some stress factors, such as diabetes mellitus, a large number of damaged proteins and organelles accumulate in podocytes and produce toxic effects on podocytes, inducing the apoptosis or necrosis of podocytes [5]. Therefore, it is of great significance to decrease organelle injury and maintain intracellular homeostasis to inhibit the formation and development of DN.

Autophagy, an intrinsic physiological function of eukaryotic cells, through which cells can effectively remove and degrade damaged or harmful substances, and transfer the degraded products to cytoplasm for reuse, plays essential role in maintaining the cellular environment and cell survival [6]. Previous studies showed high levels of autophagy in physiological state of podocyte, suggesting that autophagy may be an important regulator for podocytes to maintain homeostasis [7].

Cordycepin is one of the main active components in Cordyceps and *Cordyceps militaris*, which are similar to adenosine analogs [8]. Cordycepin has been successfully applied to the treatment of many types of cancers through regulating cell proliferation, migration, apoptosis, and lysosomal degradation [9]. Besides, Cordycepin has the ability to inhibit the activation of renal interstitial myofibroblast by elevating the expression of hepatocyte growth factor *n*, thus preventing renal interstitial fibrosis-induced deterioration and eventual loss of renal function [10]. In addition, Cordycepin was reported to improve some metabolic syndrome symptoms by regulating glucose absorption during diabetes in vivo and thus was regarded as a therapeutic agent for the treatment of diabetes and related complications [11]. Moreover, *Cordyceps militaris* was demonstrated to exert protective effect in Type 2 DN mice through regulating glucose utilization and insulin sensitivity [12]. However, whether autophagy is a regulation path of Cordycepin in DN has not been identified before.

In our present study, we aimed to explore the effect of Cordycepin in DN mice model and in human renal glomerular endothelial cells (HRGECs) treated with high glucose. We found that Cordycepin had a certain therapeutic effect on DN through balancing the abnormal physiological indexes and suppressing inflammatory process. Further investigation showed that Cordycepin suppressed cell apoptosis and induced autophagy, providing novel insights into the therapeutic potential of Cordycepin on DN.

Materials and methods

Ethics statement

All animal experiments were carried out in accordance with the Declaration of Helsinki (2008) of the World Medical Association, and were performed with approval from the animal ethics committee of the Shenzhen Second People's Hospital. A total of 60 male SPF Wistar Rats (age: 6–8 weeks; weighing: 180–200 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China).

Cell culture and construction of in vitro model

Human renal glomerular endothelial cells (HRGECs) were obtained from ScienCell Research Laboratories (Carlsbad, CA, USA) and were cultured in endothelial cell medium (ECM, ScienCell) containing with 5% fetal bovine serum (ScienCell), 100 IU/ml penicillin (Sigma-Aldrich, St. Louis, MO, USA), and 100 μg/ml penicillin/streptomycin (Sigma-Aldrich) at 37 °C in a humidified 5% CO_2 incubator.

The cells in DN model group were treated with high glucose (HG, 33 mM/L) for 24 h and the cells in control group were cultured in normal glucose (NG, 5.5 mM/L).

Autophagy inhibitor 3-methyladenine (3-MA, Sigma-Aldrich, USA) was used to treat cells at the concentration of 5 mM/L.

DN model construction

All rats were fed with a normal diet and free access to drinking water, and were allowed to adapt for 1 week before the study (20–24 °C and 40–60% relative humidity). Then, the rats were randomly divided into model group (50 rats) and control groups (10 rats). The rats in the model group were injected with a single dose (150 mg/kg) of freshly prepared Streptozotocin (STZ, Sigma, No. 101764603) administered via the intraperitoneal cavity. The rats in control group received sodium citrate buffer simultaneously. Three days later, blood was collected from the tail vein of rats, and rats with a blood glucose levels between 16.7 and 27.0 mmol/L were defined as diabetes [13]. One week later, rats with a urinary protein level higher than 30 mg/24 h continuously were defined as DN controls (continuous monitoring was conducted throughout the trial to ensure the establishment of DN model).

According to the relevant literature search and the preliminary experiment of the research group, the low, medium, and high doses of Cordycepin (purity ≥ 98%,

Table 1 24 h urine protein (UP) levels (mg/ml) in the experimental groups prior to intervention

UP level	Group				
	Control group	DN model group	Cordycepin low-dose group (10 mg/kg)	Cordycepin medium-dose group (100 mg/kg)	Cordycepin high-dose group (500 mg/kg)
	0.0037	0.1226	0.1896	0.1357	0.1632
	0.0028	0.1137	0.1643	0.1828	0.1741
	0.0031	0.2138	0.1621	0.1239	0.1326
	0.0042	0.0976	0.2132	0.1941	0.0973
	0.0046	0.1457	0.2378	0.0823	0.0864
	0.0033	0.0861	0.1408	0.0994	0.2147
	0.0027	0.2217	0.0994	0.1524	0.2368
	0.0048	0.2386	0.1217	0.1346	0.1946
	0.0037	0.2141	0.1572	0.2543	0.1857
	0.0046	0.1706	0.1348	0.2357	0.1543
Mean value	0.0038	0.1625	0.1621	0.1595	0.1640

Urine protein (UP) level is a typical clinical characteristic of diabetic nephropathy

Sigma) group were set to 10 mg/kg, 100 mg/kg, and 500 mg/kg, respectively [11] (Table 1).

Then, the rats in the model group were randomly divided into DN controls, Cordycepin low-dose group (10 mg/kg), Cordycepin medium-dose group (100 mg/kg), and Cordycepin high-dose group (500 mg/kg). The rats in the experimental group were administrated by means of intragastric administration with indicated dose of Cordycepin at the 10th week after the DN model was established. The rats in control group and the DN controls were given the same dose of normal saline for gavage.

At 30th week, the level of cholesterol, blood glucose, triglyceride, urinary protein, and creatinine was detected. Then, the rats were put to death by cervical vertebra dislocation with both kidneys were removed immediately.

Physiological index detection

Rats were put into the metabolic cage after modeling. Cholesterol, blood glucose, triglyceride, serum creatinine, and urinary protein were detected by automatic biochemical analyzer in our biochemical department.

Enzyme-linked immunosorbent assay

ELISA kits (Wuhan Boster Biological Technology., Ltd) were employed to detect the levels of three cytokines IL-1β, IL-6, and IL-18 in the serum of control group, DN controls, and experimental group. Each sample was repeated for three times and the average value was obtained.

Immunohistochemistry analysis

The expression of Beclin1 and Bax in renal tissue was examined by IHC staining. Briefly, the tissues were sliced into 5-μm sections and deparaffinized in xylene, and then were rehydrated gradually. Subsequently, the sections were incubated in 30% H_2O_2 for 30 min and treated with heated 10 mM citrate buffer for 10 min for antigen retrieval. Then, the tissues were immune-stained with primary antibody against Beclin1 and Bax, respectively (Abcam, Cambridge, UK) at 4 °C overnight. After washing three times with PBS, all sections were incubated with goat anti-rabbit IgG for 1 h at room temperature and stained with the color reagent 3,3′-diaminobenzidine (DAB). In the end, sections were viewed under a light microscopy and the expression of Beclin1 and Bax was quantitative analyzed by Image-pro-plus (IPP) software (Media Cybernetics, Washington, USA).

Hematoxylin and eosin (HE) staining

Hematoxylin and eosin staining was used for semi-quantitative evaluation of glomerular sclerosis. Slices of renal tissues were dewaxed in xylene twice for 5 min, dehydrated with gradient alcohol, and washed with distilled water for 5 min. Then, slices were stained with hematoxylin stain for 5 min and differentiated with 1% hydrochloric acid for 30 s, followed by 1% eosin-alcohol dyeing for 5 min, which could be observed under a microscope after regular gradient alcohol dehydration and mounting.

Glomerular sclerosis was quantified per glomerulus according to the following criteria: score 1, no change; score 2, focal lesions: increase in mesangial matrix mainly occurs in the hilar area of glomerulus or focal/multifocal proliferation of segmental mesangial; score 3, diffuse lesions: increase in mesangial matrix mainly occurs in the hilar area in the glomerulus or global mesangial proliferation [14]. ImageJ 1.44P (Wayne Rasband, National Institutes of Health, USA) software was used in our study to value the percentage of PAS-positive area in the glomerular tuft. The mesangial and glomerular crosssectional areas were assessed by pixel counts on a minimum of ten glomeruli per section in a blinded manner (×400) (Olympus Corporation, Tokyo, Japan) [15].

Western blotting analysis

Proteins extracted from tissues were subjected to 10% sodium dodecyl sulfate–polyacrylamide gel and transferred to polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% non-fat milk for 2 h at room temperature, and incubated with primary antibody against α-SMA, t-TG,TIMP-1(Abcam, USA), cleaved-caspase3, Bax, Bcl-2 (Proteintech, USA), Beclin1, LC3)I/II, p62 (Abcam, USA), and GAPDH (CST) at 4 °C overnight. After washes for several times next day, the members were incubated with corresponding HRP-conjugated secondary antibodies, followed by detection and visualization using a ChemiDoc XRS imaging system and analysis software (Bio-Rad, San Francisco, California, USA).

Statistical analyses

All analyses were performed using SPSS v. 20.0. Quantitative data were presented as the mean ± standard deviation (SD) of at least three assays performed independently. The *t* test was adopted for comparison between two groups. Differences among groups were carried out by the one-way analysis of variance followed by a Student–Newman–Keuls post hoc test. Significance was set at $p < 0.05$.

Results

Cordycepin has a certain therapeutic effect on DN in the rat model

The physiological and histological changes were presented after the rats in indicated groups were treated with different doses of Cordycepin. Compared with the control group, the DN rats had a significantly higher cholesterol, blood sugar, triglyceride, creatinine, and urine protein level. At the same time, we could see that elevated physiological index was reduced by Cordycepin in a dose-dependent manner (Fig. 1a–e). Besides that, elevated level of inflammatory factors IL-1β, IL-6, and IL-18 in DN group was also suppressed by Cordycepin in a dose-dependent manner (Fig. 1f–h). In addition, HE staining was conducted here to examine histological changes. In the control group, the structure of renal interstitium, glomerulus, and tubule was basically normal. However, obvious vacuolar degeneration, deformation, abscission, and partial necrosis were showed in renal tubular epithelial cells in the DN model group. Besides, renal tubules showed obvious lumen dilation, fibrosis, or atrophy accompanied by edema and infiltration of inflammatory cells. Moreover, the glomerular basement membrane was thickened, the glomerulus was partially sclerotic, and the renal interstitium was markedly hyperplasia of fibrous tissue. Damaged tissue structure and unbalanced physiological index of DN model rats were much recovered by Cordycepin in dose-dependent manner (Fig. 1i, j). These results suggest that Cordycepin successfully has a certain therapeutic effect on DN in the rat model.

Cordycepin suppresses renal fibrosis and apoptosis

Here, western blot was performed to detect the effect of Cordycepin on renal fibrosis in DN model. As shown in Fig. 2, the level of fibrosis markers α-SMA, t-TG, and TIMP-1 was up-regulated in DN model group compared with the control group. Just as shown in the HE staining, the expression of α-SMA, t-TG, and TIMP-1 was suppressed by Cordycepin in a dose-dependent manner (Fig. 2a–d). Besides that, the results of western blot showed that the expression of cleaved-caspase3 and pro-apoptotic protein Bax was both elevated, and the level of anti-apoptotic protein Bcl-2 was inhibited in the model group compared with the control group. Then, the changes of apoptosis-related proteins in DN were reversed by Cordycepin through decreasing the level of cleaved-caspase3 and Bax and increasing the level of Bcl-2 in a dose-dependent manner (Fig. 3e–h). At the same time, IHC was employed to detect the effect of Cordycepin on cell apoptosis in DN. As shown in Fig. 3i, j, Bax-positive area in DN model group was strongly increased compared with the control group. As expected, the degree of apoptosis gradually decreased with increasing concentration of Cordycepin treatment. The results above suggested that Cordycepin could successfully block renal fibrosis and suppress apoptosis in DN rat model.

Cordycepin induces autophagy in the DN model

We have known that high level of autophagy was essential for podocytes to maintain homeostasis and autophagy is new direction of the treatment and research of DN, and thus, we also investigated that whether Cordycepin exerted protective effect in DN through autophagy induction. The results of western blot showed that autophagy was indeed suppressed in the model group compared with the control group. Cordycepin treatment induced obvious autophagy

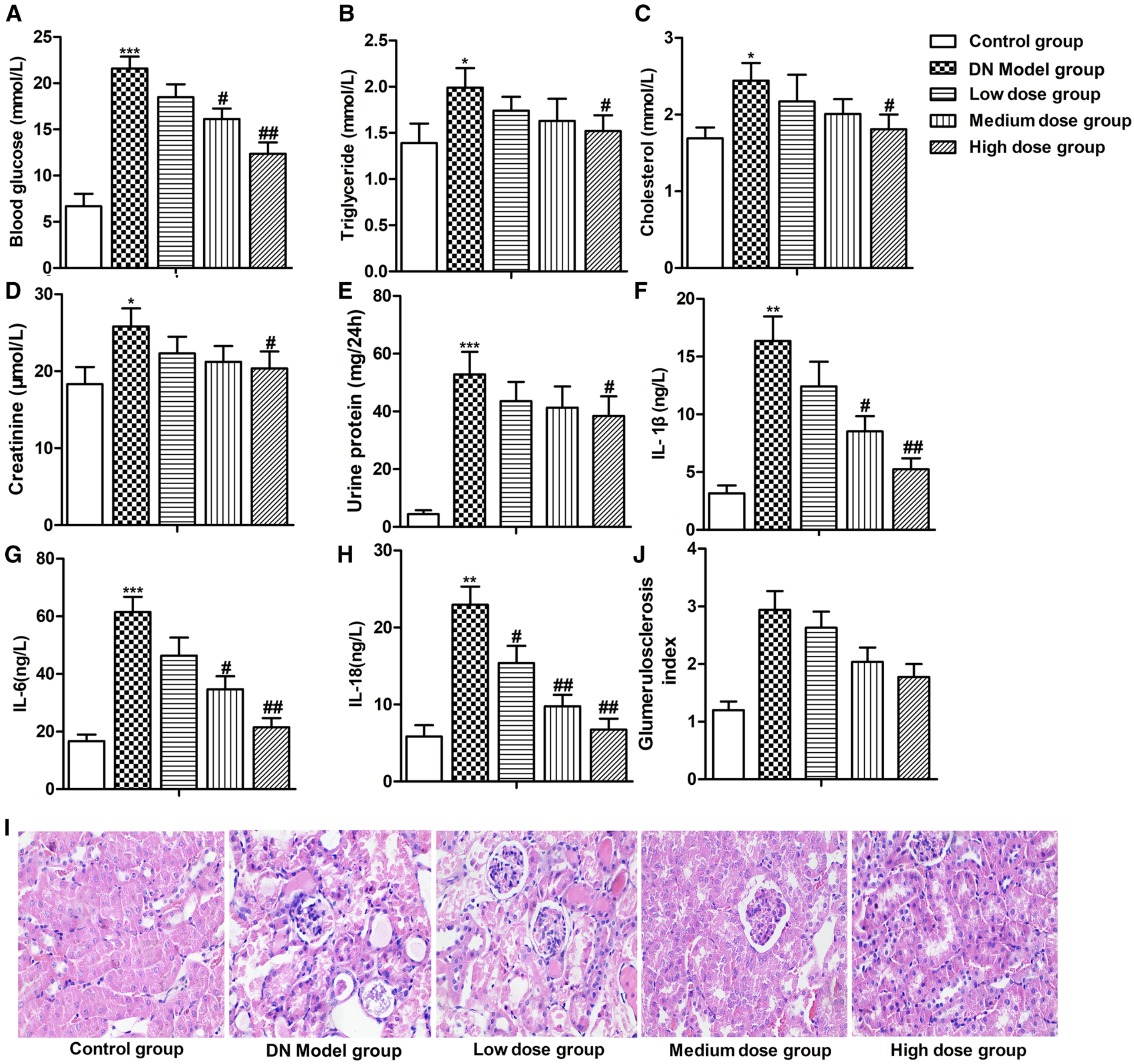

Fig. 1 Cordycepin has a certain therapeutic effect on diabetic nephropathy in the rat model. **a–e** The level of blood glucose (**a**), triglyceride (**b**), cholesterol (**c**), creatinine (**d**), and urine protein (**e**) was examined through an automatic biochemistry analyser. **f–h** The level of IL-1β (**f**), IL-6 (**g**), and IL-18 (**h**) was detected through Elisa using corresponding ELISA Kit. **i**, **j** HE staining was used to examine histological changes. Images are representatives of five mice in each group (**i**) and data analysis was also shown (**j**). Data were expressed as the mean ± SD. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$ versus the model group

through elevating the level of Beclin1, promoting the transformation of LC3I into LC3II, and suppressing the level of p62 (Fig. 3a–d). The result of IHC also showed that the suppressed percentage of Beclin1-positive cells was gradually rescued by Cordycepin in a dose-dependent manner (Fig. 3e, f). These results above indicated that Cordycepin exerted protective effect in DN through inducing autophagy.

Cordycepin ameliorates DN through inducing autophagy in HRGECs

Having investigated the effect of Cordycepin in the DN rat model, corresponding in vitro effect of Cordycepin in HRGECs was further explored. As shown in Fig. 4a, b, HG greatly suppressed cell autophagy compared with the control group. Then, the treatment of Cordycepin gradually

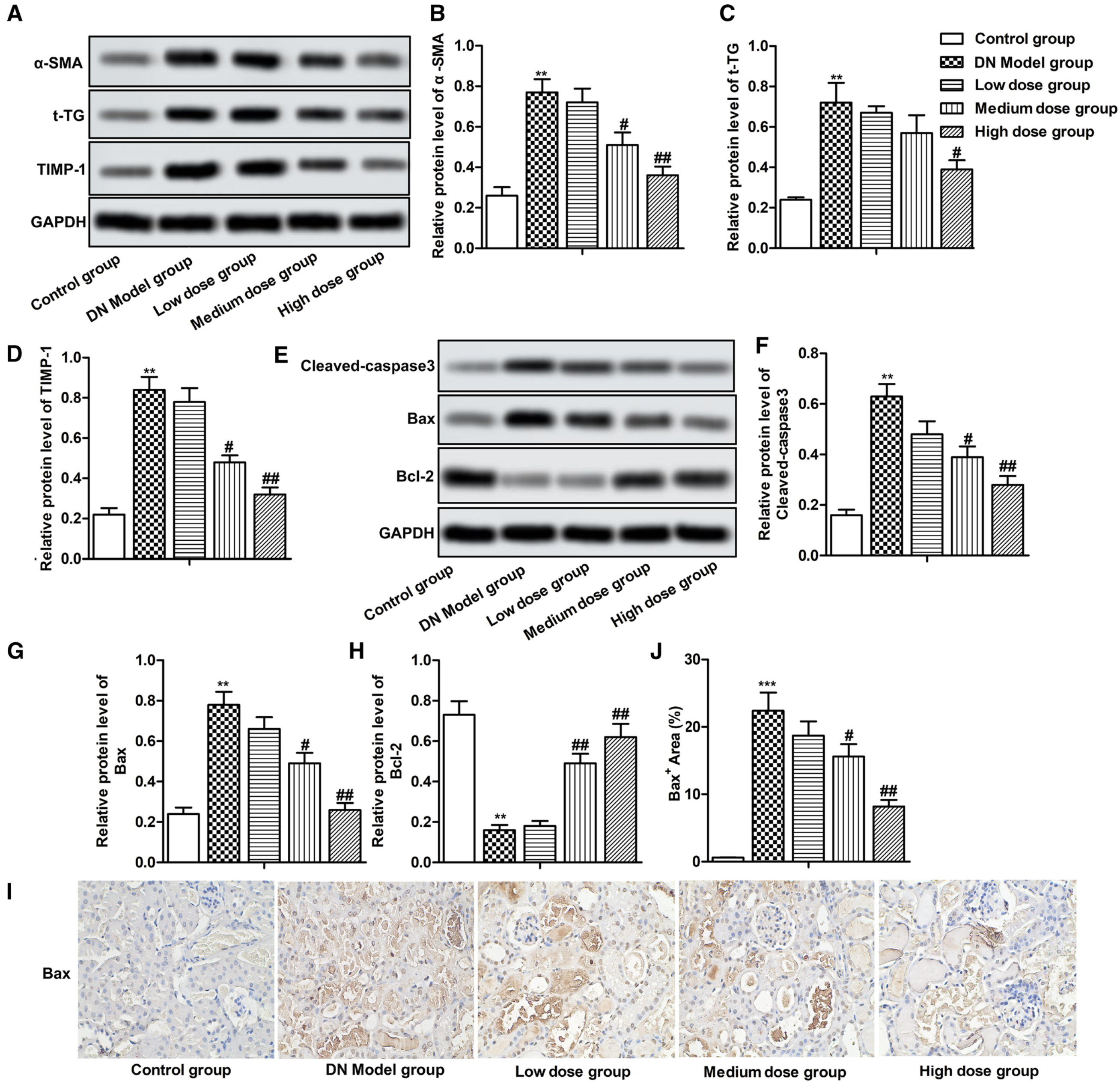

Fig. 2 Cordycepin suppresses renal fibrosis and apoptosis. **a** The expressions of α-SMA, t-TG, and TIMP-1 were detected by Western blot. GAPDH was used as an internal reference. **b–d** Quantitative analysis of α-SMA (**b**), t-TG (**c**), and TIMP-1 (**d**). **e** The expressions of cleaved-caspase3, Bax, and Bcl-2 were detected by Western blot. GAPDH was used as an internal reference. **f–h** Quantitative analysis of cleaved-caspase3 (**f**), Bax (**g**) and Bcl-2 (**h**). **i** The expression of Bax in renal tissues was detected through IHC. **j** Quantitative analysis of the area of Bax-positive cells. Data were expressed as the mean ± SD. $^{**}P<0.01$, $^{***}P<0.001$ versus control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$ versus the model group

promoted autophagy in a dose-dependent manner. At the same time, 3-MA, an autophagy inhibitor, was used in our following experiments to suppress autophagy as a control. Then, we could find that the expression of renal fibrosis indexes α-SMA and TIMP-1 were up-regulated by HG and were then suppressed by the treatment of Cordycepin in a dose-dependent manner. What is interesting, the combination of 3-MA elevated the level of α-SMA and TIMP-1 compared with the usage of Cordycepin solely, identifying that Cordycepin suppressed renal fibrosis through inducing cell autophagy (Fig. 4c, d). In addition, cell apoptosis was also suppressed by Cordycepin through suppressing the level of Bax and elevating the level of Bcl-2 as shown in Fig. 4e, f. Similarly, the addition of 3-MA abolished the inhibiting

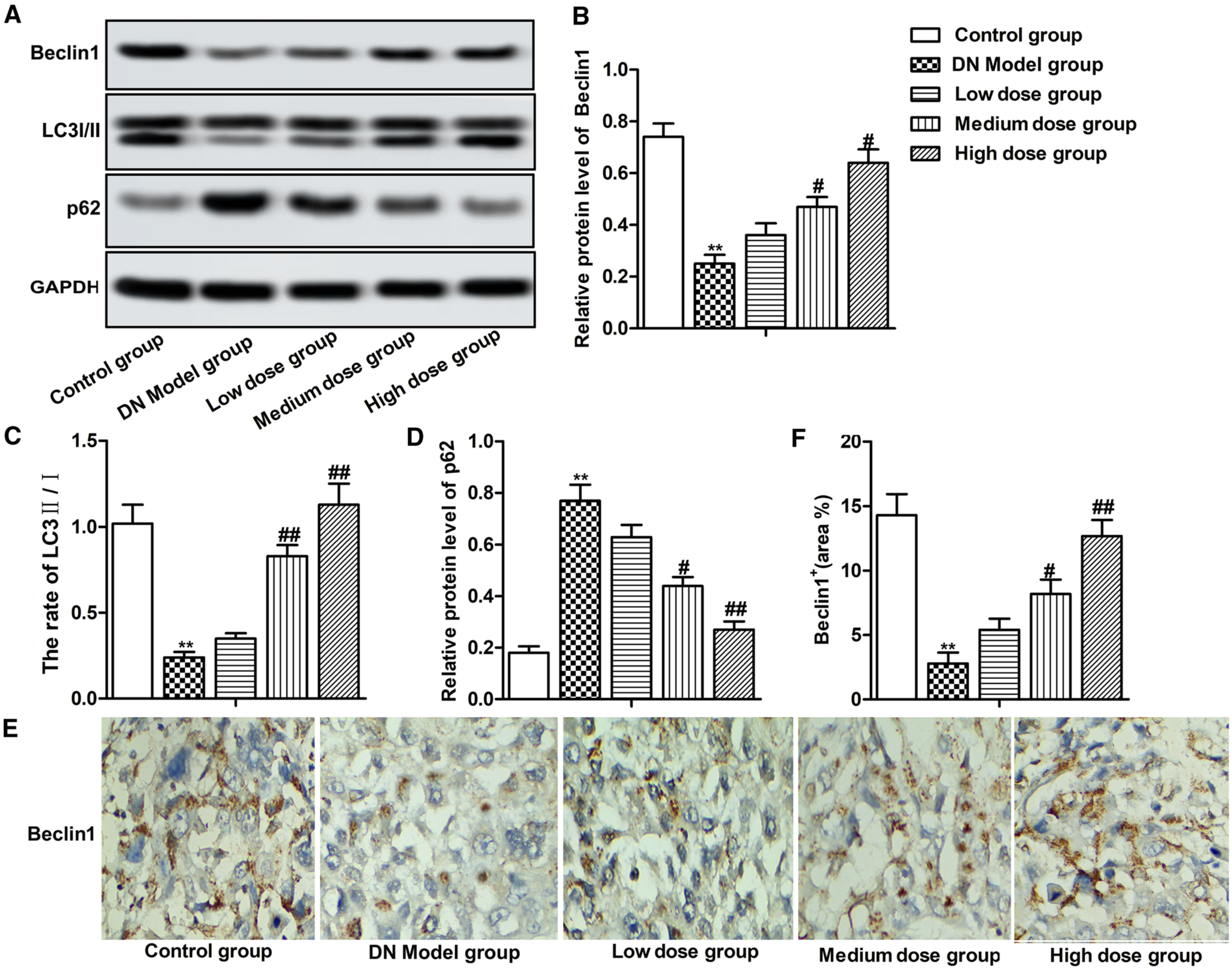

Fig. 3 Cordycepin induces autophagy in the DN model. **a** The expressions of Beclin1, LC3I/II, and p62 were detected by Western blot. **b**–**d** Quantitative analysis of Beclin1 (**b**), LC3I/II (**c**), and p62 (**d**). E. Expression of Beclin1 in renal tissues was detected through IHC. **f** Quantitative analysis of the percentage of Beclin1-positive cells. Data were expressed as the mean ± SD. $^{**}P < 0.01$ versus control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus the model group

effect of Cordycepin on cell apoptosis, identifying that Cordycepin suppressed apoptosis in renal fibrosis through inducing cell autophagy. These results above indicated that Cordycepin relieved DN through inducing autophagy in HRGECs.

Discussion

Diabetic nephropathy is one of the most serious complications of diabetes mellitus and the most common cause of end-stage nephropathy. More and more studies demonstrate that podocytes play an important role in maintaining the stability of glomerular filtration membrane. However, podocyte is a kind of terminal cell with limited proliferative and differentiative ability. Under the action of stimulating factors such as diabetes mellitus, a large number of damaged proteins and organelles will accumulate in podocytes and produce toxic effects on podocytes. Without timely clearance, these toxic substances would induce the apoptosis or necrosis of podocytes. The reduction and destruction of podocytes lead to proteinuria in DN, which further lead to renal dysfunction and even glomerulosclerosis [16]. DN patients show a rapid increasing trend every year, but up to now, there are still no effective prevention and treatment measures [17]. Therefore, it is very important to explore the pathogenesis of diabetic nephropathy and find new therapeutic targets. In our present study, we revealed that Cordycepin, the main active components in Cordyceps and *Cordyceps militaris*, has a good effect on diabetic nephropathy in rats.

In recent years, the role of traditional Chinese medicine in clinical treatment is becoming more and more important.

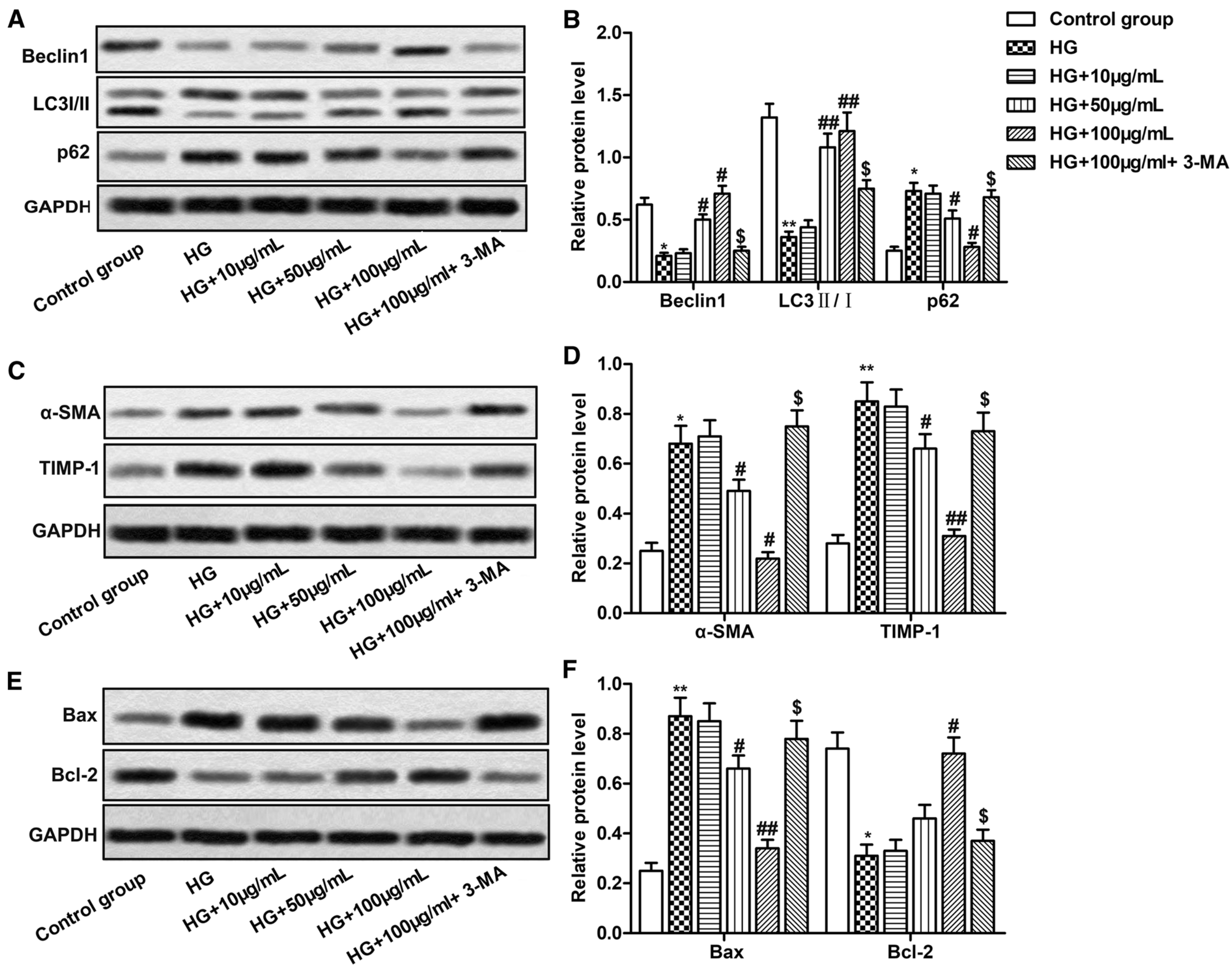

Fig. 4 Cordycepin ameliorates DN through inducing autophagy in HRGECs. **a** The expressions of Beclin1, LC3I/II, and p62 in HRGECs were detected by Western blot. **b** Quantitative analysis of Beclin1, LC3I/II, and p62. **c** Expression of α-SMA and TIMP-1 in HRGECs was detected through western blot. **d** Quantitative analysis of α-SMA and TIMP-1. **e** Expression of cell apoptosis-related proteins Bax and Bcl-2 in HRGECs was detected through western blot. **d** Quantitative analysis of Bax and Bcl-2. GAPDH was used as an internal reference. Data were expressed as the mean ± SD. *$P<0.05$, **$P<0.01$ versus control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$ versus the HG-induced group. $^{\$}P<0.01$ versus the HG + 100 μg/ml Cordycepin group

Cordycepin (cordycepin) is a nucleoside analogs isolated from *Cordyceps militaris*. Various biological activities including immunomodulation, antibacterial, anti-hyperuricemic, anti-inflammatory, and anti-tumor activities have been explored in Cordycepin [18–20]. Sun et al. have demonstrated that Cordycepin treatment reduced the levels of urinal protein, blood urea nitrogen, and creatinine, and improved lipid profile and redox capacity in chronic kidney disease patients, exerting obvious renal protection effect [21]. Besides that, Cordycepin was reported to protect podocyte from C5b-9-induced injury through inhibiting the activation of p38/JNK signaling pathway [22]. In addition, renal interstitial fibrosis is the common end point of progressive renal diseases and is also one of the main features of DN. Cordycepin was reported to inhibit renal interstitial myofibroblast activation by up-regulating the level of hepatocyte growth factor (HGF), which has anti-fibrotic effect [10]. At the same time, Cordycepin was demonstrated to attenuate type 2 diabetes (T2D) by suppressing the expression of diabetes regulating genes [23]. However, the effect of Cordycepin in DN has never been explored. In our present study, we found that Cordycepin obviously balanced the changed physiological index and suppressed inflammation in the DN rat model. Moreover, the protective effect of Cordycepin is more significant with the increase of concentration in a certain range.

As previously described, damaged proteins and organelles induced by various stimulating factors may lead to the formation of DN. Under normal conditions, high autophagic activity in podocytes can clear away these damaged proteins and organelles, thereby preventing damage. Studies have also shown that podocyte-specific autophagy-deficient mice developed podocyte loss and massive proteinuria in a high-fat diet-induced diabetic model for inducing minimal proteinuria [24]. Besides, microRNA-22 was able to promote renal tubulointerstitial fibrosis by targeting PTEN and suppressing autophagy in diabetic nephropathy, announcing the relationship between autophagy and renal fibrosis in DN [25, 26]. These previous studies suggest that autophagy plays a pivotal role in protecting podocytes and preventing the formation of DN, indicating that autophagy may be a new therapeutic target for advanced diabetic nephropathy. In our present study, we found that Cordycepin not only effectively inhibited the occurrence of renal fibrosis, but also could reduce cell apoptosis. In our further study, we found that there was a low autophagy level in DN model group, in accordance with previous studies. However, Cordycepin successfully induced autophagy in a dose-dependent manner. Thus, we concluded that Cordycepin could effectively rescue DN through autophagy induction. To further confirm the protective effect of Cordycepin was associated with autophagy induction, the in vitro experiments were conducted in HRGECs. Then, we could find that obvious autophagy was induced by Cordycepin in HRGECs. At the same time, the inhibiting effect of Cordycepin on renal fibrosis and cell apoptosis was abolished by the combination of 3-MA, indicating that the protective effect of Cordycepin on renal fibrosis was exerted through autophagy induction.

Collectively, the current study explored that Cordycepin participated in the modulation of cell apoptosis, fibrosis, and autophagy induction in DN, which were all closely correlated with the development of DN. Our study for the first time revealed that Cordycepin may ameliorate DN in rats though autophagy induction, providing novel insights into the therapeutic potential of Cordycepin in DN-targeted therapy.

Compliance with ethical standards

Conflict of interest The authors have declared that no competing interest exists.

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