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Celastrol modulates damage of renal tissues in Immunoglobulin A nephropathy via targeting TGase-2/HMGB1 signaling pathway

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Abstract

Background Persistent controversies surround the treatment of Immunoglobulin A nephropathy (IgAN), necessitating the exploration of safer and more effective therapeutic agents. Tripterygium wilfordii, a traditional Chinese medicine, demonstrates promising benefits in reducing proteinuria and enhancing renal function in IgAN. This study aims to elucidate the therapeutic mechanisms of celastrol, a principal medicinal component of Tripterygium wilfordii, in IgAN treatment.

Methods An IgAN rat model was induced using lipopolysaccharide, carbon tetrachloride, and bovine serum albumin. HMCs cells were stimulated by algA1 to establish IgAN model in vitro. Immunofluorescence, immunohistochemistry and HE staining were performed using renal tissues. Western Blot and RT-PCR were utilized to measure the protein and mRNA expressions of TGase-2, HMGB1, TLR4, and MYD88 in vivo and *in vitro*.

Results Celastrol treatment exhibited reduced levels of proteinuria, diminished pathological kidney damage, and decreased expression of TGase-2 and HMGB1 in the renal tissue of IgAN rats. Furthermore, celastrol treatment or TGase-2 knockdown decreased the expression of TGase-2, HMGB1, TLR4, and MYD88 in both proteins and mRNA levels in, and the contents of HMGB1, TNF-α, IL-6, and FN in the in algA1 stimulated HMCs.

Conclusion Our study findings provide evidence supporting the efficacy of celastrol in treating IgAN. The potential underlying mechanisms involve the reduction of cell proliferation and inflammatory response by inhibiting the expression of the TGase-2/HMGB1 pathway.

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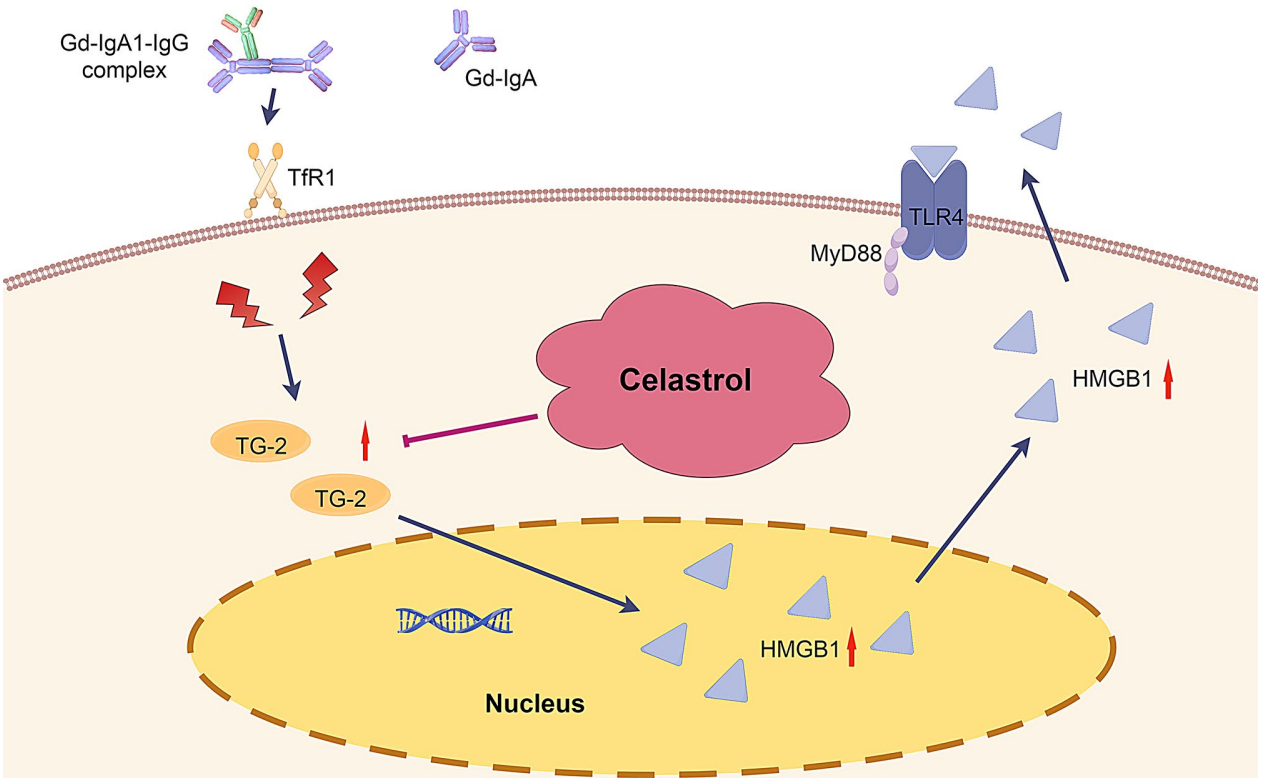
Graphical Abstract



Keywords IgA nephropathy, Transglutaminase-2, High mobility group protein B1, Celastrol

Introduction

Primary IgA nephropathy (IgAN) stands as the most prevalent primary glomerular disease globally, encompassing 37.8–45.3% of primary glomerular diseases within China [1–3]. This condition typically exhibits a chronic, progressive nature, with an alarming statistic indicating that 20–40% of afflicted individuals advance to end-stage renal disease, becoming a leading cause necessitating dialysis or kidney transplantation [4]. Despite numerous efforts, the complete pathogenesis of IgAN remains elusive. Conventional Western medicine often recommends cytotoxic drugs and T cell inhibitors; however, their efficacy and associated risks remain subjects of contention. Thus, there exists a critical need for deeper exploration into the underlying mechanisms of IgAN and the formulation of Traditional Chinese Medicine (TCM) treatment modalities. Clinical investigations have corroborated the efficacy of Tripterygium wilfordii in IgA nephropathy treatment, attributed to its properties of "dispelling wind and removing dampness, promoting blood circulation, and detoxifying" [5]. However, the precise mechanism through which it operates necessitates further elucidation.

Transglutaminase-2 (TGase-2), also recognized as tissue transglutaminase, represents a member within the transglutaminase family. Previous investigations have revealed an augmented expression of TGase-2 in the renal tissue of IgA nephropathy (IgAN) patients, demonstrating a correlation with the extent of glomerular pathological injury. High Mobility Group Protein B1 (HMGB1), a non-histone nuclear DNA-binding protein, has been found in glomeruli, renal tubules, interstitium, and urine of individuals with IgAN. Studies [6] have indicated that the severity of lesions in IgAN is linked to the scope and degree of HMGB1 expression. Notably, in vitro studies utilizing cross-linking site localization and mass spectrometry have unveiled HMGB1 as the active substrate of TGase-2. Under TGase-2 catalysis, HMGB1 undergoes cross-linking, leading to the formation of HMGB1 complexes that bind to its receptor, thereby triggering inflammatory activation [7]. Consequently, this study endeavors to explore the therapeutic impact and underlying mechanism of Tripterygium wilfordii on IgAN. This investigation aims to discern the effect of Tripterygium wilfordii (specifically Celastrol, CL) on the

expression of TGase-2/HMGB1 within the kidney tissue of rats afflicted with IgA nephropathy.

Materials and methods

Reagents and drugs

Celastrol (purity > 98%, Dalian Meilun Biotechnology Co., LTD., Lot: MB6739), 20 μg/ml suspension prepared with distilled water; Methylprednisolone tablets (Pfizer, USA, 4 mg/ tablet, Lot: EP9715), prepared as a suspension with a concentration of 0.5 mg/ml in distilled water; Bovine serum albumin (BSA, Beijing Boaotuoda Technology Co., LTD.,Lot: A6020) was prepared in distilled water at a concentration of 100 mg/ml; Castor oil for CCL4 (Shanghai McLean Biochemical Technology Co., LTD, Lot: C805332-100, C805201-100), mixed at 1:5; Lipopolysaccharide (LPS, Dalian Meilen Biotechnology Co., LTD., Lot: MB5198-1) was diluted in normal saline to a concentration of 0.25 mg/ml.

MIgA1 was isolated from serum of IgA nephropathy patients by Jacalin-agarose affinity chromatography combined with molecular sieve chromatography, and then heat mIgA1 to aIgA1 for cell treatment. TGase-2 antibody, HMGB1 antibody, IgA antibody (Lot: ab2386, ab79823, ab102198, Abcam Company, UK); TLR4 antibody, MYD88 antibody (Lot:19811-1-AP, 67969-1-Ig, Proteintech, Wuhan, China); HMGB1, IL-6, tumor necrosis factor-α (TNF-α) and FN ELISA kits (Lot: MM-13713H1, MM-0049H2, MM-0122H2, MM-0043H2, respectively; China Jiangsu Enzyme Immunoassay Biotechnology Co., LTD.); DEME medium and Fetal bovine serum (Gibco, Thermo Fisher Scientfic), BCA protein concentration determination kit (enhanced) (Beyotime, Shanghai, China). Anti-rabbit EnVision + System-HRP Labelled Polymer (Lot: K4003; DAKO). DAB tablet (Lot: 045-22833; FujiFilmWako Pure Chemical Corporation). RIPA lysis buffer (Lot: P0013B; Beyotime, Shanghai, China).The primers for TGase-2, HMGB1, TLR4, MYD88 and GAPDH were synthesized by Beijing Qingke Biotechnology Co., LTD.

Instruments experimental apparatus and instruments

Sysmex Chemix-180 automatic biochemistry analyzer (Sysmex Company, Japan); Multifunctional microplate reader Flexstation3 (Molecular Devices, USA); ULTRA TURRAX T25 tissue homogenizer (IKA, Germany); HPIAS-1000 high definition medical color image analysis system (Qianscreen Imaging Company); Alphalmager mini Gel Imaging Analysis System (Thermo Fisher Scientific, USA); Steponetm Real-time PCR instrument (Life technologies, USA); Super clean Table (1300 series A2; Thermo Corporation, USA); Inverted phase contrast microscope IX51(OLYMPUS Corporation, Japan); CO2 incubator MCO-15AC(SANYO Company, Japan); STS-2 decolorization shaker (Shanghai St Analytical Instrument Co., LTD.); Cryogenic ultracentrifuge (Eppendorf, Germany), etc.

Animal experiments

Fifty male Sprague-Dawley rats of clean grade, weighing (180 ± 10) g, were procured from the Animal Experiment Center of Hubei University of Chinese Medicine. These rats were housed in a controlled barrier environment with good ventilation, following a 12-hour light-dark circadian rhythm, at an ambient temperature of 20–25 ℃, and relative humidity between 40 and 70%. They were accommodated in closed isolation with free access to food and water. The laboratory animal license number for this study is SYXK(Hubei)2017-0067. All experimental procedures involving animals were approved by the Laboratory Animal and Ethics Committee of Hubei University of Chinese Medicine (Animal Ethics approval number: HUCMS202204005). Animal handling and experimentation strictly adhered to the regulations for the Management of Experimental Animals.

After an acclimation period of 14 days, male rats were randomly assigned to one of the five experimental groups, each consisting of 10 rats: control, model, high-dose celastrol (CL-high), low-dose celastrol (CL-low), and methylprednisolone (MP) group.

Rats in the control group received oral gavage of distilled water at a dosage of 600 mg/kg once every other day throughout the experimental duration. Additionally, they were subjected to once-weekly injections of saline (0.2 mL/rat) via the tail vein during the 6th, 8th, and 10th weeks.

The remaining rats were utilized to induce an IgA nephropathy (IgAN) model through the administration of BSA + LPS + CCl4. Specifically, BSA (600 mg/kg) was orally administered every other day, while subcutaneous injections of 0.1 mL of CCl4 and 0.5 mL of castor oil were given weekly until the 12th week. Furthermore, during the 6th, 8th, and 10th weeks, the rats received once-weekly injections of 0.05 mg of LPS.

In the 8th week, rats in the control and model groups were orally administered sterile saline solution (2 mL/100 g), while rats in the CL-low and CL-high groups received 0.5 and 1.0 mg/kg/day of celastrol, respectively. Rats in the methylprednisolone group were provided with a 4 mg/kg/day methylprednisolone suspension.

Sample collection

At the culmination of the 15th week, the rats underwent a 12-hour fasting period during which they had access to water. Subsequently, urine samples were collected over a 24-hour period using metabolism cages. Blood samples obtained from the rats were centrifuged at 3000×g for 10 min at 4 °C, and the resulting supernatant was utilized for detecting serum alanine aminotransferase (ALT),

albumin (ALB), serum creatinine (Scr), and blood urea nitrogen (BUN).

Rats were euthanized by intraperitoneal injection of 160 mg/kg of 2% pentobarbital sodium. Following sacrifice, the rats underwent perfusion with 0.9% saline (250 mL) via the left ventricles. The left kidneys were then fixed overnight at 4 °C using 4% PFA for subsequent pathological examination. Samples from the right kidneys were promptly frozen in liquid nitrogen and stored at – 80 °C for further analyses and validation.

Biochemical parameter and histological analyses

The serum biochemical parameters (serum alanine aminotransferase (ALT), albumin (ALB), serum creatinine (Scr), and blood urea nitrogen (BUN)) were assessed using an automatic biochemistry analyzer. The urine protein content was determined based on the 24-hour volume and Ponceau S staining method.

For kidney samples, they were fixed in 4% neutral PFA, embedded in paraffin, and then sectioned into 2 μm slices. These sections underwent deparaffinization with xylene, dehydration with gradient ethanol, staining with Schiff reagent, washing with water, nuclear staining with hematoxylin solution, further water washing, differentiation using a hydrochloric acid alcohol solution, blue reversion with ammonia water, dehydration, clarification, sealing with neutral gadhesive, and finally, observation under a light microscope for histopathological characterization.

Additionally, frozen rat kidney samples embedded in OCT were sectioned into 3 μm slices for subsequent immunofluorescence analysis. After rinsing with a PBS solution, the sections were treated with FITC labeled anti-rat IgA antibody and placed in a humid chamber. Incubation took place at 37 ℃ for 45 min, followed by PBS rinsing and sealing with mounting solution containing an anti-fluorescence quenching agent. The immunofluorescence analysis involved detecting FITC-tagged IgA, imaging with a fluorescence microscope, and assessing intensity using ImageJ software.

Immunohistochemistry

Paraffin sections (3 μm) of rat kidney tissue were prepared for subsequent immunohistochemical detection by subjecting them to antigen retrieval using a citric acid antigen-retrieval buffering solution. They were then boiled for 15 min in a pressure cooker to aid in restoration. Following this, the sections were treated with hydrogen peroxide at 37 ℃ in the dark for 20 min.

After the hydrogen peroxide incubation, the sections were incubated with TGase-2 antibody (dilution 1:200) at 4 ℃ overnight. Subsequently, they were incubated with an anti-rabbit secondary antibody at room temperature for 60 min. Finally, color development was achieved using DAB tablets, and the stained sections were viewed under a light microscope for analysis.

Cell cultures and treatment

Cells Human renal mesangial cells (HMCs) were purchased from Fuheng Biotechnology Co., LTD (Shanghai, China). The human mesangial cells (HMCs) were cultured in DEME medium supplemented with 10% fetal bovine serum (FBS), 100U/mL penicillin, and 100ug/mL streptomycin. These cells were then stimulated with or without aIgA1 (100ug/mL; Isolated from serum of patients with IgA nephropathy) for a duration of 24 h.

In order to investigate the inhibitory effect of celastrol on the stimulation of HMCs with aIgA1, several approaches were undertaken. HMCs were either silenced to express TGase-2 protein or pre-treated with celastrol (0.3 μm) or 0.1% DMSO for 24 h prior to the addition of aIgA1. Each study consisted of a minimum of three independent experiments to ensure reliable results and reproducibility.

Western blot analysis

The total protein from rat kidney tissues and HMCs cells was extracted using RIPA lysis buffer. Subsequently, these proteins were subjected to electrophoresis on a 10–12% SDS-PAGE gel and subsequently transferred to a Nitrocellulose Transfer Membrane. Following transfer, the membrane was blocked to prevent nonspecific binding and then incubated with primary antibodies at 4 ℃ overnight.

After the primary antibody incubation, the membranes were washed and incubated with a secondary antibody. The expression levels of the proteins were visualized using appropriate detection methods (such as chemiluminescence) and subsequently captured. Finally, the intensity of the protein bands was quantitatively analyzed using ImageJ software for precise determination of protein expression levels.

Real-time PCR assay

The total RNA extracted from rat kidneys and HMCs cells was obtained using TRIzol reagent according to the manufacturer's instructions. Real-time quantitative PCR was conducted using the QuantStudio 6 and 7 Flex Real-time PCR systems (4489826; Thermo Fisher Scientific) and SYBR Green Supermix (1725122; Bio-Rad).

The primers utilized in this study are detailed in Table 1. These primers were employed to amplify specific target sequences during the quantitative PCR analysis.

ELISA assay

The determination of protein concentration in the cell supernatant was carried out in accordance with the

Table 1 RT-PCR primer sequences

Gene	Primer sequences(5′-3′)	Length (bp)
Rat GAPDH	F: ACAGCAACAGGGTGGTGGAC	253
	R: TTTGAGGGTGCAGCGAACTT	
Rat TGase-2	F: AAGTATGATGCGTCCTTCGTG	259
	R: CCCCTGTCTCCTCTTTCTCTG	
Rat HMGB1	F: CTTATCCATTGGTGATGTTGC	213
	R: TCGTCTTCCTCTTCCTTCTTT	
Homo GAPDH	F: TCAAGAAGGTGGTGAAGCAGG	115 bp
	R: TCAAAGGTGGAGGAGTGGGT	
Homo TGase-2	F: CACAAATCCATCAACCGTTCCC	183 bp
	R: CATCCCTGTCTCCTCCTTCTCG	
Homo HMGB1	F: TGCGAAGAAACTGGGAGAGA	227 bp
	R: CCTCCTCCTCCTCATCCTCT	
Homo TLR4	F: ACCTGTCCCTGAACCCTAT	135 bp
	R: CTAAACCAGCCAGACCTTG	
Homo MYD88	F: ACTTGGAGATCCGGCAACTG	169 bp
	R: CATCCGGCGGCACCAATG	

instructions provided by the specific protein assay kit used for this purpose.

Statistical analyses

All data are expressed as mean ± SEM (standard error of the mean). Statistical analyses were conducted utilizing one-way ANOVA to compare multiple groups, employing Prism 9.0 software from GraphPad Software, based in San Diego, CA. A significance level of $p < 0.05$ was considered to indicate statistical significance.

Results

Celastrol treatment decreased the urinary protein in renal tissue of IgAN rats

Compared with the control group, the level of urinary protein in the model group was significantly increased (Fig. 1A); Compared with the model group, the levels of urinary protein in CL-H, CL-L and MP groups were significantly decreased (Fig. 1A). There were no significant differences in the serum levels of ALT (Fig. 1B), Scr (Fig. 1C), BUN (Fig. 1D) and ALB (Fig. 1E) among the groups.

Celastrol treatment decreased the IgA deposition in renal tissue of IgAN rats

The immunofluorescence staining results showed that compared with the control group, the IgA fluorescence staining of renal tissues in the model group and all the drug groups were positive, and the immune complexes were deposited in the mesangial area of glomeruli in clump-like and granular forms. According to the analysis of the average fluorescence intensity of each group, the IgA deposition intensity of the model group was significantly increased, and the high and low concentration

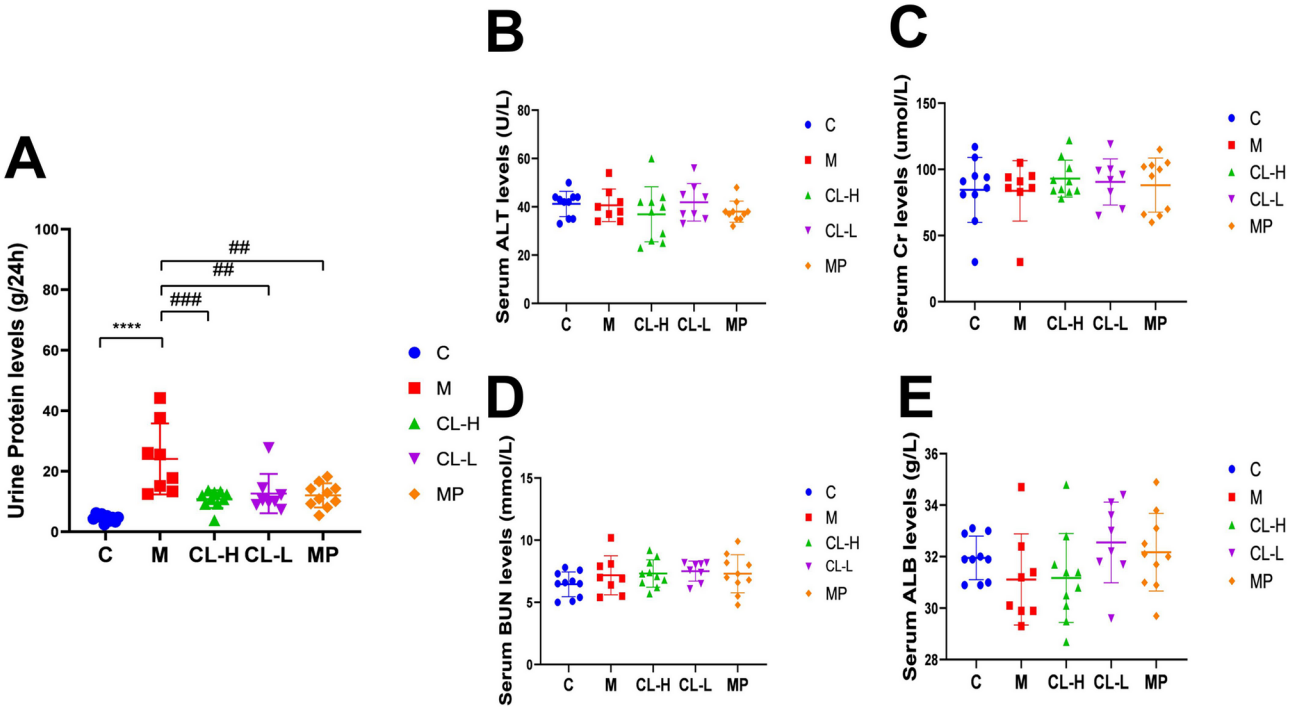


Fig. 1 Celastrol treatment decreased the urinary protein in renal tissue of IgAN rats. The 24-hour urinary protein quantification (**A**), serum ALT (**B**), serum albumin (**C**) and serum urea nitrogen (**D**) levels in the IgAN rats. C, control group; M, model group; CL-H, celastrol high dose group; CL-L, celastrol low dose group; MP, methylprednisolone group. Data are expressed as the means ± SD. ***$p < 0.001$ vs. the control group; ###$p < 0.001$, ##$p < 0.01$ vs. the model group

groups of celastrol could significantly reduce IgA deposition (Fig. 2A).

Celastrol decreased the TGase-2 in the renal tissue of IgAN rats

Immunohistochemical staining showed that brown particles appeared in glomeruli, renal tubules and interstitium as positive expression. The expression of TGase-2 in glomeruli and tubules of normal rats was low. In the model group, the expression was significantly increased in the glomerular and renal tubular cells; TGase-2 was mainly detected in the glomerular mesangial area and the cytoplasm of renal tubular epithelial cells. The expression of TGase-2 in CL-H, CL-L and MP groups was lower than that in the model group, but still more than the control group (Fig. 2B).

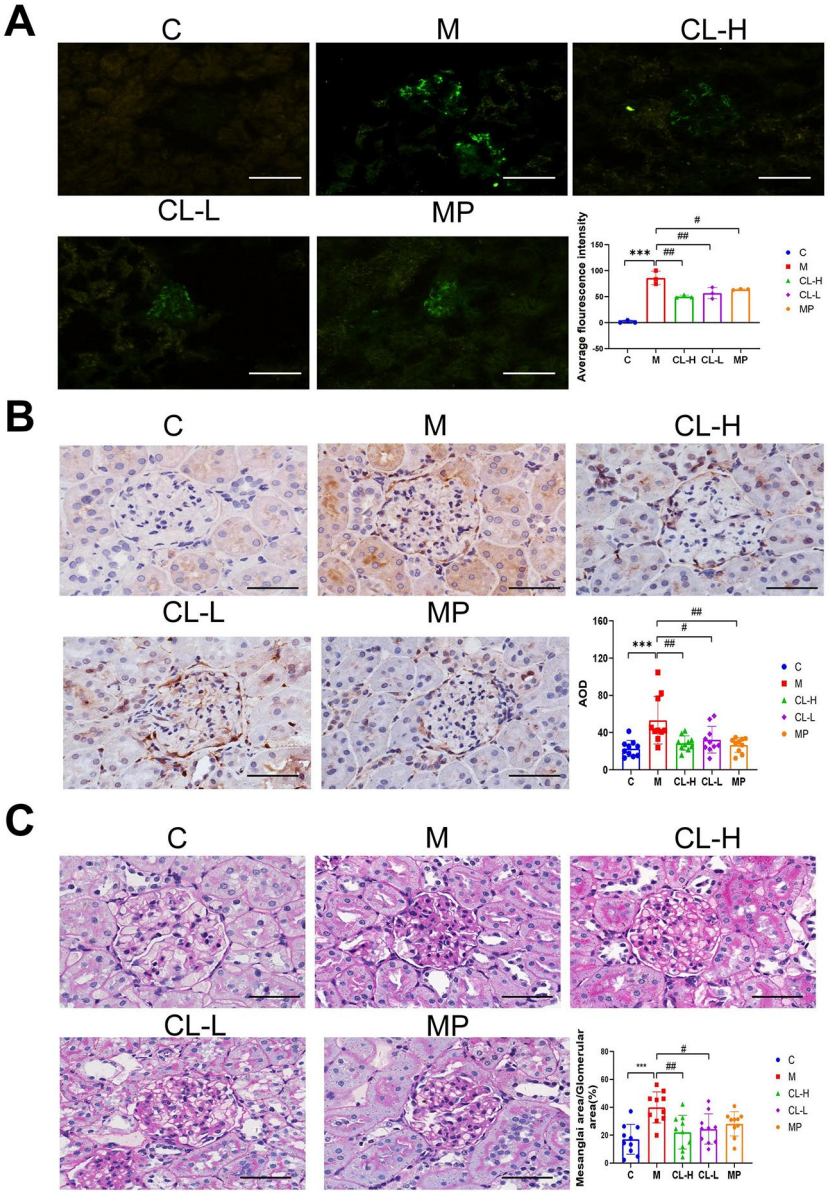


Fig. 2 Renal tissue staining results of celastrol treated IgAN rats. (**A**) Immunofluorescence staining (×200) of IgA in renal tissues of rats in C group, M group, CL-H group, CL-L group and MP group. The mean fluorescence intensity of each group was analyzed. (**B**) Immunohistochemical detection (×400) of TGase-2 in renal tissues of rats in C group, M group, CL-H group, CL-L group and MP group. IOD value of each group was analyzed. (**C**) PAS staining (×400) of renal tissues of rats in C group, M group, CL-H group, CL-L group and MP group. Mesangial area ratio of each group (%). C, control group; M, model group; CL-H, celastrol high dose group; CL-L, celastrol low dose group; MP, methylprednisolone group. Data are expressed as the means ± SD. ***$p < 0.001$ vs.the control group; ###$p < 0.001$, ##$p < 0.01$, #$p < 0.05$ vs. the model group

Celastrol alleviated the pathological structure of renal tissue in IgAN rats

The structure and morphology of renal tissue in the control group were basically normal; In the model group, the glomerular mesangial area was diffusely widened, mesangial cells and mesangial matrix increased, and some capillaries were occluded. Compared with the model group, the proliferation of mesangial cells and mesangial matrix was reduced in CL-H, CL-L and MP groups (Fig. 2C).

Celastrol decreased the TGase-2, HMGB1, TLR4 and MYD88 protein levels in the renal tissue of IgAN rats

The expressions of TGase-2, HMGB1, TLR4 and MYD88 proteins in renal tissues of rats in each group are shown in Fig. 3. Compared with the control group, the protein expression levels of TGase-2, HMGB1, TLR4 and MYD88 in the kidney tissue of the model group were significantly increased; Compared with the model group, the expression of TGase-2, TLR4 and MYD88 protein in the kidney tissue of the CL-H, CL-L and MP groups was significantly decreased, while the expression of HMGB1 decreased significantly in CL-H group (Fig. 3A).

Celastrol decreased the TGase-2, HMGB1, TLR4 and MYD88 mRNA levels in the renal tissue of IgAN rats

Compared with the control group, the expression levels of TGase-2, HMGB1, TLR4 and MYD88 mRNA in the kidney tissue of the model group were significantly increased; Compared with the model group, the expression levels of TGase-2, HMGB1, TLR4 and MYD88 mRNA in the kidney tissues of the CL-H, CL-L and MP groups were significantly decreased (Fig. 3B).

Celastrol treatment and TGASe-2 knockdown decreased the protein expressions of TGase-2, HMGB1, TLR4 and MYD88 in the aIgA1 stimulated HMCs

Compared with the control cells, the protein expressions of TGase-2, HMGB1, TLR4 and MYD88 in HMCs stimulated by aIgA1 or transfected with NC-siRNA were significantly increased. Celastrol treatment significantly reduced the protein expression of TGase-2, HMGB1, TLR4 and MYD88 in the aIgA1 stimulated HMCs. Besides, the protein levels of TGase-2, HMGB1, TLR4 and MYD88 were significantly decreased in the aIgA1 stimulated HMCs after TGASe-2 knockdown (Fig. 4A).

Celastrol treatment and TGASe-2 knockdown decreased the mRNA levels of TGase-2, HMGB1, TLR4 and MYD88 in the aIgA1 stimulated HMCs

Compared with the control group, the mRNA expressions of TGase-2, HMGB1, TLR4 and MYD88 in HMCs stimulated by aIgA1 or transfected with NC-siRNA were significantly increased. Celastrol treatment significantly reduced the mRNA expression of TGase-2, HMGB1, TLR4 and MYD88 in HMCs stimulated by aIgA1. Besides, the mRNA levels of TGase-2, HMGB1, TLR4 and MYD88 were significantly decreased in the aIgA1 stimulated HMCs after TGASe-2 knockdown (Fig. 4B).

Celastrol treatment and TGASe-2 knockdown decreased the contents of HMGB1, FN, TNF-α and IL-6 in the aIgA1 stimulated HMCs

The levels of HMGB1, FN, TNF-α and IL-6 in the cell supernatant of each group after aIgA1 stimulation are shown in Fig. 5. Compared with the control group, the expressions of HMGB1 (Fig. 5A), TNF-α (Fig. 5B), IL-6 (Fig. 5C) and FN (Fig. 5D) in the supernatant of HMCs stimulated by aIgA1 and NC-siRNA were significantly increased. Celastrol treatment significantly reduced the contents of HMGB1 (Fig. 5A), TNF-α (Fig. 5B), IL-6 (Fig. 5C) and FN (Fig. 5D) in the supernatant of HMCs stimulated by aIgA1. Besides, the contents of HMGB1 (Fig. 5A), TNF-α (Fig. 5B), IL-6 (Fig. 5C) and FN (Fig. 5D) were significantly decreased in the aIgA1 stimulated HMCs after TGASe-2 knockdown.

Discussion

The understanding of IgA nephropathy in Traditional Chinese Medicine (TCM) categorizes it under kidney wind, hematuria, turbidity, and deficiency. In recent years, multiple studies have found that traditional Chinese medicine has excellent effects on the treatment of IgA [8, 9]. In patients with IgA nephropathy, excessive production of low-galactosylated IgA1 (Gd-IgA1) due to previous infections contributes to IgA deposition via binding to specific receptors on glomerular mesangial cells [10]. TGase-2 has been identified as one of these receptors for Gd-IgA1 deposition in the kidney, promoting Gd-IgA1 complex deposition and continuous activation of mesangial cells [11]. In vitro studies have revealed TGase-2's role in catalyzing the cross-linking of HMGB1 with antigens, forming complexes that translocate from the nucleus to the cell surface, interacting with receptors such as RAGE, TLR2, and TLR4, prompting inflammatory cell recruitment and pro-inflammatory factor release [7]. In this study, elevated protein and mRNA expressions of TGase-2 and HMGB1 were observed in the renal tissue of IgAN rats, correlating with renal tissue damage. This suggests that TGase-2/HMGB1-mediated inflammatory activation plays a crucial role in IgAN pathogenesis (Graphical abstract).

Tripterygium wilfordii Hook F (TwHF) is a TCM plant known for its attributes of dispelling wind, removing dampness, promoting blood circulation, and reducing swelling and pain. Previous research in patients with rheumatic internal disturbance IgAN indicated that TwHF combined with RAS blockers improved renal function and reduced proteinuria [5]. Celastrol, an active

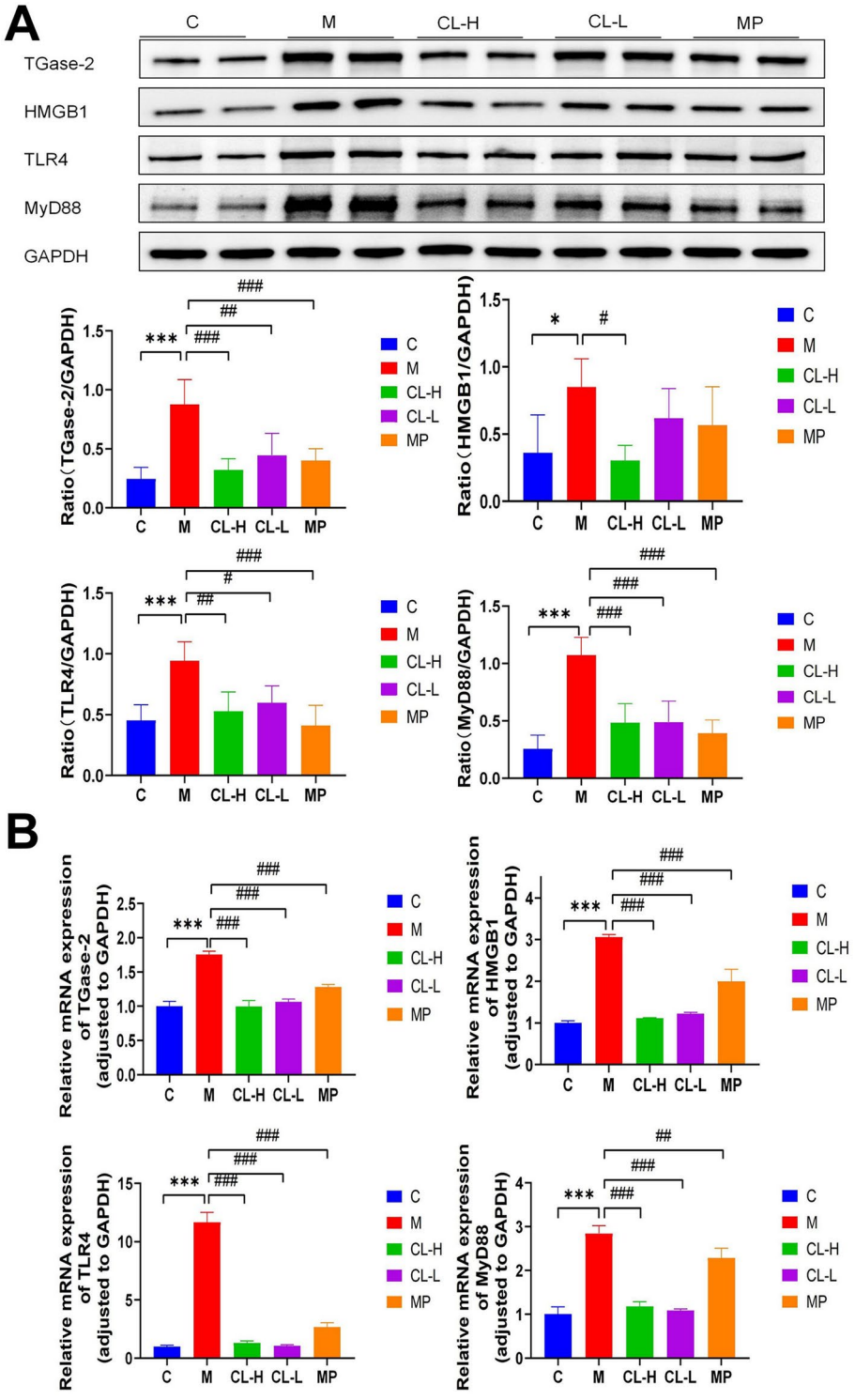


Fig. 3 Celastrol decreased the TGase-2, HMGB1, TLR4 and MYD88 levels in the renal tissue of IgAN rats. (**A**) The protein levels of TGase-2, HMGB1, TLR4 and MYD8 in the renal tissue of IgAN rats were detected by western blots and quantization. (**B**) The mRNA levels of TGase-2, HMGB1, TLR4 and MYD8 in the renal tissue of IgAN rats were detected by RT-qPCR. C, control group; M, model group; CL-H, celastrol high dose group; CL-L, celastrol low dose group; MP, methylprednisolone group. Data are expressed as the means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs.the control group; ###$p < 0.001$, ##$p < 0.01$ vs. the model group

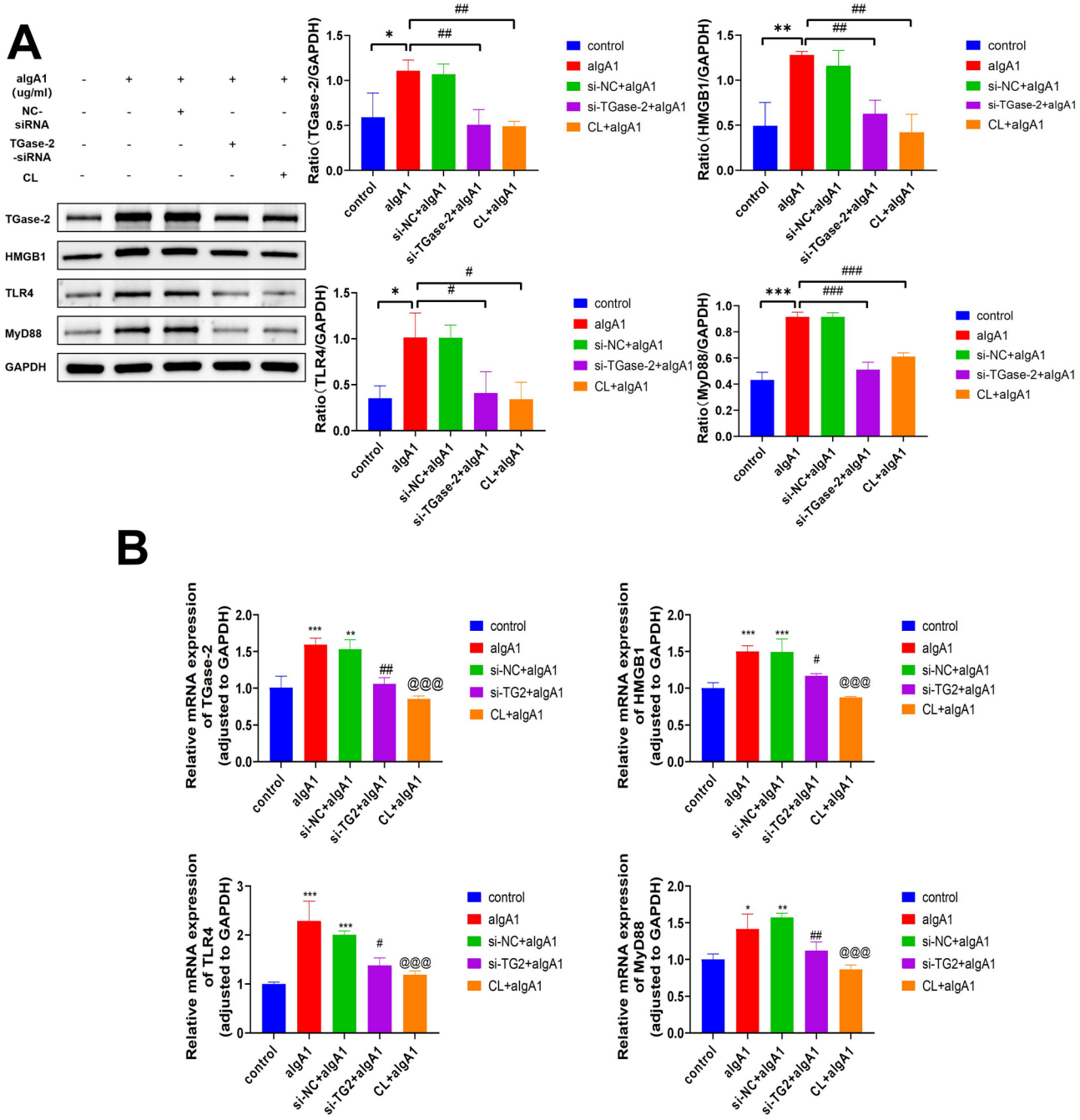


Fig. 4 Celastrol treatment and TGASe-2 knockdown decreased the TGase-2, HMGB1, TLR4 and MYD88 levels in the algA1 stimulated HMCs. (**A**) The protein expressions of TGase-2, HMGB1, TLR4 and MYD8 in the algA1 stimulated HMCs were detected by western blots and quantization after Celastrol treatment and TGASe-2 knockdown. (**B**) The protein expressions of TGase-2, HMGB1, TLR4 and MYD8 in the algA1 stimulated HMCs were detected by RT-qPCR after Celastrol treatment and TGASe-2 knockdown. Data are expressed as the means ± SD. **P* < 0.05, ***P* < 0.01 vs. the control group; @*P* < 0.05, @@*P* < 0.01, @@@*P* < 0.001 vs. the model group; #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001 vs. cells transfected with NC-siRNA

compound in Tripterygium wilfordii, exhibits immunosuppressive, anti-inflammatory, and anti-tumor properties [12]. Studies indicate that low-dose celastrol can ameliorate renal function and structural changes via renal metabolism and anti-inflammatory effects [13]. Furthermore, celastrol alleviated IgAN renal injury through elevateing the expression of PTEN and inhibiting PCNA and Cyclin D1 levels [14]. In the present study, celastrol reduced urinary protein, curbed glomerular mesangial cell proliferation, and ameliorated renal pathological injury in IgAN rats. Furthermore, celastrol inhibited the protein and mRNA expression of TGase-2 and

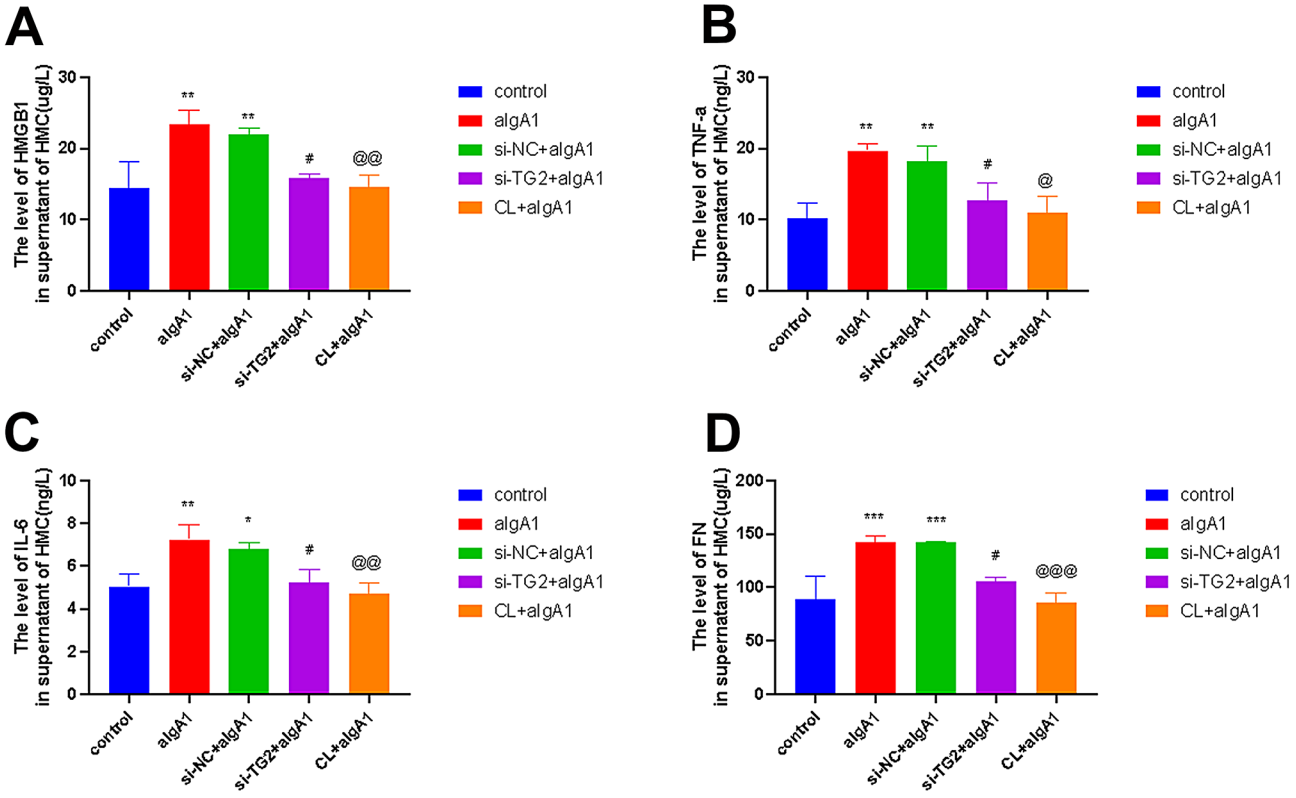


Fig. 5 Celastrol treatment and TGASe-2 knockdown decreased the contents of HMGB1, FN, TNF-α and IL-6 in the algA1 stimulated HMCs. The contents of HMGB1 (**A**), TNF-α (**B**), IL-6 (**C**) and FN (**D**) in the cell supernatants of the algA1 stimulated HMCs after Celastrol treatment and TGASe-2 knockdown were detected by ELISA kits. Data are expressed as the means ± SD. *$p < 0.05$ **$P < 0.01$ ***$p < 0.001$ vs. the control group; @$p < 0.05$ @@$p < 0.01$ @@@$p < 0.001$ vs. the model group; #$P < 0.05$ vs. cells transfected with NC-siRNA

HMGB1 in IgAN rat renal tissue. Therefore, the suppression of TGAase-2/HMGB1 expression, reduction in IgA deposition, and improvement in pathological indicators may constitute mechanisms underlying celastrol's therapeutic action in IgAN.

In conclusion, this study demonstrated that celastrol treatment inhibited the TGase-2/HMGB1 signaling pathway in the IgAN rats and aIgA1 stimulated HMCs. Celastrol might relieve the renal tissue injury through targeting TGase-2/HMGB1 signaling pathway. This study provides a new method for the treatment of IgAN and a solid theoretical basis for the promotion and application of celastrol in future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12882-025-04139-7.

Supplementary Material 1

Author contributions

XW conceived the study; YW and RC conducted the experiments; DD and WW analyzed the data; YW and RC ware a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Data availability

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

Declarations

Ethical approval

All experimental procedures involving animals were approved by the Laboratory Animal and Ethics Committee of Hubei University of Chinese Medicine (Animal Ethics approval number: HUCMS202204005). Animal handling and experimentation strictly adhered to the regulations for the Management of Experimental Animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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