

Celastrol Alleviates Rosiglitazone-induced Fluid Retention Through the miR-27/ITSN2/ α -ENaC Signaling Axis

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Abstract:

This study aimed to investigate whether celastrol (Cel) alleviates rosiglitazone-induced renal sodium reabsorption and fluid retention via regulating the miR-27/ITSN2/ α -ENaC signaling axis and clarify the underlying mechanisms. Twenty type 2 diabetes mellitus patients receiving rosiglitazone treatment were divided into edema and non-edema groups, with serum miR-27 levels measured. In vitro experiments were performed using mouse inner medullary collecting duct cells, including quantitative real-time PCR, Western blotting, flow cytometry, and function assays. A high-fat/high-sucrose diet-induced diabetic mouse model was also established to evaluate in vivo effects. The results showed that rosiglitazone significantly upregulated miR-27 expression, decreased ITSN2 levels, and enhanced α -ENaC/SGK1 expression and sodium accumulation both in vitro and in vivo, while Cel treatment remarkably reversed these effects. Functional studies confirmed that miR-27 inhibition mimicked Cel's protective effects, whereas miR-27 overexpression abolished them. In conclusion, celastrol attenuates rosiglitazone-induced renal sodium and fluid retention by suppressing miR-27 and restoring ITSN2-mediated α -ENaC regulation, providing a potential therapeutic strategy for PPAR γ agonist-associated edema.

Keywords:

Celastrol, Rosiglitazone, miR-27, Intersectin-2 (ITSN2), Epithelial Sodium Channel (α -ENaC)

1. Introduction

Rosiglitazone (ROS), a peroxisome proliferator-activated receptor gamma (PPAR γ) agonist, is widely used in the treatment of type 2 diabetes mellitus (T2DM) because of its ability to improve insulin sensitivity and effectively reduce blood glucose levels [1,2,3]. However, its clinical application is limited by adverse effects such as sodium and water retention, peripheral edema, weight gain, and an increased risk of heart failure [4,5]. Previous studies have demonstrated that ROS-induced fluid retention is

closely associated with enhanced sodium reabsorption mediated by the epithelial sodium channel (α -ENaC) in the renal collecting duct [6,7,8]. Nevertheless, the upstream regulatory mechanisms underlying this process remain incompletely understood. Early identification of patients who are susceptible to edema and clarification of the underlying molecular mechanisms would facilitate precision medicine and improve the management of ROS-related adverse effects in patients with T2DM.

MicroRNA-27 (miR-27) is an important regulator of lipid metabolism and sodium-water homeostasis. It has been reported to modulate α -ENaC-mediated sodium transport by targeting intersectin-2 (ITSN2) [9,10]. Celastrol (Cel), a bioactive triterpenoid extracted from *Tripterygium wilfordii*, possesses a wide range of pharmacological activities, including anti-inflammatory, antioxidant, and metabolic regulatory effects [11,12,13]. However, whether Cel can alleviate ROS-induced fluid retention remains unclear. In the present study, ROS-treated IMCD3 cells and T2DM mice were used as experimental models to investigate the effects of Cel on the miR-27/ITSN2/ α -ENaC signaling axis and renal sodium reabsorption. In addition, patients with T2DM receiving ROS therapy were enrolled to evaluate the association between circulating miR-27 levels and the development of edema. These findings may provide new insights into the prevention and treatment of ROS-associated fluid retention.

2. Materials and Methods

2.1. Reagents and Materials

The mouse inner medullary collecting duct epithelial cell line IMCD3 was purchased from Stem Cell Bank (STEMCELL Technologies, Canada). Cells were cultured in F-12 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μ g/mL streptomycin at 37°C in a humidified incubator containing 5% CO₂.

Celastrol (Cat. No. C0869) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against ITSN2 (PHF6709S) and SGK1 (TA001M) were obtained from Abmart (Shanghai, China). The α -ENaC antibody (HY-P81204) was purchased from MedChemExpress (MCE, USA). GAPDH antibody (13937-1) was obtained from Proteintech (Wuhan, China). Anti-miR-27, miR-27 mimic oligonucleotides, and siRNA-Mate transfection reagent were purchased from GenePharma (Shanghai, China). ReverTra Ace qPCR RT Master Mix was obtained from Toyobo (Osaka, Japan). Western blotting reagents were purchased from Beyotime Biotechnology (Shanghai, China).

2.2. Clinical Data Collection and Patient Grouping

Twenty patients with type 2 diabetes mellitus (T2DM) who received rosiglitazone (ROS) treatment at the Department of Endocrinology, Shanghai Pudong Gongli Hospital, Shanghai University of Medicine & Health Sciences, between January 2024 and December 2024 were enrolled.

Inclusion criteria were as follows: (1) diagnosis of T2DM according to the Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes Mellitus; (2) age between 18 and 75 years; (3) first-time ROS treatment for at least 12 weeks; and (4) complete clinical records. Exclusion criteria included: (1) severe cardiovascular diseases such as heart failure or coronary artery disease; (2) chronic renal

insufficiency or severe hepatic dysfunction; (3) acute infection, autoimmune disease, or malignancy; (4) pregnancy or lactation; and (5) recent use of glucocorticoids or other medications affecting sodium and water metabolism.

Patients received ROS treatment for 12 weeks. Fasting blood glucose (FBG) levels were measured before and after treatment. At the end of treatment, patients were classified into edema and non-edema groups according to physical examination findings, including pitting edema of the lower extremities, ankle edema, and other signs of fluid retention. Peripheral venous blood samples (5 mL) were collected under fasting conditions, centrifuged at 3000 rpm for 10 min at 4°C, and serum samples were stored at -80°C until analysis. Serum miR-27 expression was determined by quantitative real-time PCR (qRT-PCR).

All participants provided written informed consent. The study was approved by the Ethics Committee of Shanghai Pudong Gongli Hospital (Approval No. GLYY1s2024-012) and conducted in accordance with the Declaration of Helsinki.

2.3. Establishment of a T2DM Mouse Model

Male C57BL/6J mice (6 weeks old, 18–22 g) were purchased from Shanghai Model Organisms Center, Inc. (Shanghai, China). All animal procedures were approved by the Institutional Animal Care and Use Committee (Approval No. P0820240921A).

After one week of acclimatization, mice were randomly assigned to either a control group or a high-fat/high-sucrose diet group. Control mice received a standard chow diet, whereas the experimental group was fed a high-fat diet (60% kcal from fat) for 8 weeks to induce insulin resistance. Subsequently, mice received a low-dose streptozotocin (STZ, 25 mg/kg) injection via the tail vein to induce hyperglycemia.

Body weight and fasting blood glucose levels were monitored weekly. Mice with fasting blood glucose levels ≥ 11.1 mmol/L on two consecutive measurements were considered successfully diabetic. Diabetic mice were then randomly divided into three groups: T2DM, ROS, and ROS+Cel. Mice in the ROS group received rosiglitazone (10 mg/kg/day) by oral gavage, while mice in the ROS+Cel group additionally received celastrol (1 mg/kg/day) by intraperitoneal injection for 2 weeks. Control and T2DM groups received an equal volume of saline. At the end of treatment, body weight, fasting blood glucose, circulating miR-27 expression, 24-h urinary sodium excretion, and renal tissue parameters were evaluated.

2.4. Determination of Serum miR-27 Expression

Total RNA was extracted from serum samples using a commercial RNA extraction kit (Feijie Biotechnology, Shanghai, China) according to the manufacturer's instructions. Two micrograms of total RNA were reverse-transcribed into cDNA. miR-27 primers were synthesized by GenePharma (Shanghai, China). U6 small nuclear RNA was used as the internal control. Relative miR-27 expression levels were quantified by qRT-PCR.

2.5. Measurement of Blood Glucose

Fasting plasma glucose levels were measured using the glucose oxidase-peroxidase method according to the manufacturer's instructions.

2.6. Western Blot Analysis

After treatment, IMCD3 cells were lysed using RIPA buffer, and total protein concentrations were determined using a BCA protein assay kit. Equal amounts of protein (30 μ g) were separated by 10% SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked with 5% non-fat milk for 2 h at room temperature and incubated overnight at 4°C with primary antibodies against ITSN2, α -ENaC, SGK1, and GAPDH (1:1000 dilution). After washing with TBST, membranes were incubated with HRP-conjugated secondary antibodies (1:5000 dilution) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system and quantified using ImageJ software. Relative protein expression levels were normalized to GAPDH.

2.7. Quantitative Real-Time PCR

Total RNA was extracted from cells and kidney tissues using TRIzol reagent. Reverse transcription was performed according to the manufacturer's instructions. Quantitative PCR was conducted using SYBR Green Master Mix on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA). U6 was used as the endogenous control for miR-27. Relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method. All samples were analyzed in triplicate and repeated three times.

2.8. Determination of Intracellular Sodium Levels

Following treatment, IMCD3 cells were harvested and adjusted to a concentration of 1×10^6 cells/mL. Cells were incubated with the sodium-sensitive fluorescent probe CoroNa Green AM (5 μ mol/L) at 37°C for 30 min in the dark. After washing three times with PBS, cells were resuspended in 500 μ L PBS and analyzed by flow cytometry. Fluorescence signals were collected in the FITC channel using a 488-nm excitation laser. At least 10,000 cells were analyzed per sample. Mean fluorescence intensity (MFI) was calculated using FlowJo software and used to represent intracellular sodium levels.

2.9. Hematoxylin and Eosin (H&E) Staining

Kidney tissues were fixed in 10% neutral buffered formalin for 24–48 h, embedded in paraffin, and sectioned at a thickness of 4 μ m. Sections were stained with hematoxylin, differentiated in acid alcohol, blued, counterstained with eosin, dehydrated through graded ethanol, cleared in xylene, and mounted with neutral resin.

2.10. Construction of miR-27 Knockdown and Overexpression Cell Models

IMCD3 cells in the logarithmic growth phase were seeded into 6-well plates. When cell confluence reached 60–70%, transfection was performed using siRNA-Mate reagent according to the manufacturer's instructions. Cells were transfected with anti-miR-27, miR-27 mimic, or corresponding negative controls.

The anti-miR-27 sense strand sequence was 5'-UUCACAGUGGCUAAGUCCGctt-3', and the antisense strand sequence was 5'-GCGGAAGTtagccactgtgaAtt-3'. The miR-27 mimic primer sequences were: forward, TGCGGTTCACAGTGGCTAAG; reverse, CTCAACTGGTGTCTGTGA.

After 6 h of transfection, the medium was replaced with fresh complete medium, and cells were cultured for an additional 24–48 h. Transfection efficiency was verified by qRT-PCR before subsequent Western blot and intracellular sodium assays.

2.11. Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.0 software. Data are presented as mean $\pm$ standard deviation (SD). Comparisons between two groups were performed using Student's t-test, whereas comparisons among multiple groups were analyzed by one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Elevated Serum miR-27 Expression in Patients with Rosiglitazone-Associated Edema

To investigate the relationship between miR-27 and rosiglitazone (ROS)-associated edema, changes in blood glucose levels and serum miR-27 expression were evaluated in 20 patients with T2DM before and after ROS treatment. The results showed that fasting blood glucose levels significantly decreased from 8.285 ± 0.753 mmol/L before treatment to 6.375 ± 0.402 mmol/L after 12 weeks of ROS therapy ($P < 0.05$), indicating the effective glucose-lowering activity of ROS.

After 12 weeks of treatment, patients were further classified into edema and non-edema groups based on clinical physical examination findings. qRT-PCR analysis demonstrated that serum miR-27 expression was significantly higher in the edema group than in the non-edema group, with an approximately 2.94-fold increase in relative expression ($P < 0.05$). These findings suggest that elevated serum miR-27 levels are associated with the development of ROS-related edema and may contribute to the pathogenesis of ROS-induced fluid retention.

3.2. Rosiglitazone Induced miR-27 Upregulation and Activation of the ITSN2/ α -ENaC Signaling Pathway in IMCD3 Cells

To investigate the effects of ROS on sodium transport-related signaling pathways in renal collecting duct cells, an in vitro model was established using IMCD3 cells. qRT-PCR analysis showed that ROS treatment significantly increased miR-27 expression compared with the control group ($P < 0.05$), reaching approximately 1.5-fold of the control level (Figure 1A).

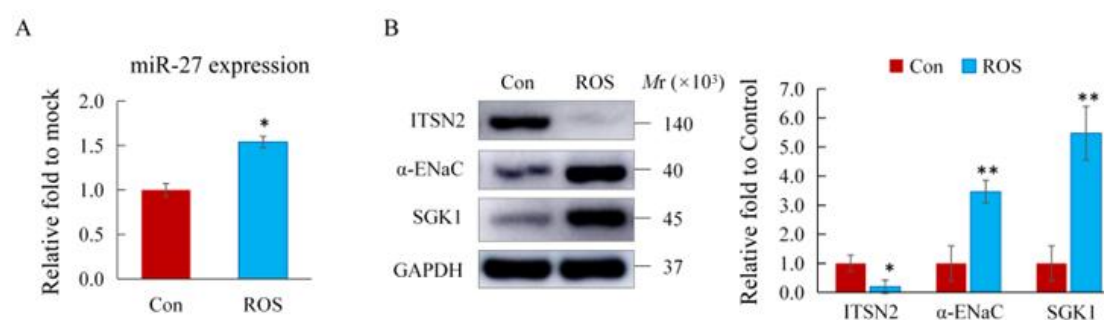


Figure 1. Rosiglitazone induced miR-27 expression and activated the ITSN2/ α -ENaC signaling pathway in IMCD3 cells.

A: Relative expression of miR-27 determined by qRT-PCR; B: Representative Western blot images and quantitative analysis of ITSN2, α -ENaC and SGK1 protein expression. Con, control group; ROS, rosiglitazone-treated group. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$. $n=3$, $\bar{x} \pm s$.

Western blot analysis was subsequently performed to determine the expression levels of ITSN2, α -ENaC, and SGK1. Compared with the control group, ROS treatment significantly decreased ITSN2 protein expression ($P < 0.05$), whereas the expression levels of α -ENaC and SGK1 were markedly increased (both $P < 0.01$) (Fig. 1B). Densitometric analysis revealed that α -ENaC and SGK1 protein expression increased by approximately 3.5-fold and 5.4-fold, respectively, while ITSN2 expression was substantially reduced.

Collectively, these results indicate that ROS markedly upregulates miR-27 expression in IMCD3 cells, accompanied by downregulation of ITSN2 and upregulation of α -ENaC and SGK1. These findings suggest that activation of the miR-27/ITSN2/ α -ENaC signaling axis may contribute to ROS-induced enhancement of sodium reabsorption in renal collecting duct cells.

3.3. Celastrol Suppressed Rosiglitazone-Induced miR-27 Upregulation and Intracellular Sodium Retention

To evaluate the effects of celastrol (Cel) on ROS-induced sodium retention, intervention experiments were performed in IMCD3 cells. qRT-PCR analysis showed that miR-27 expression was significantly increased following ROS treatment compared with the control group ($P < 0.05$). However, Cel treatment markedly reduced miR-27 expression ($P < 0.05$), indicating that Cel effectively inhibited ROS-induced miR-27 upregulation (Figure 2A).

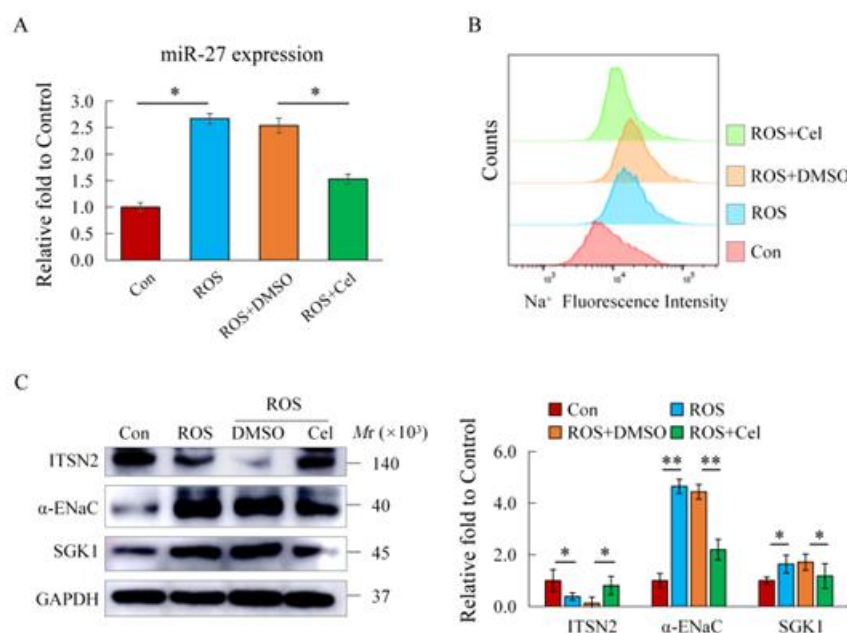


Figure 2. Celastrol attenuated rosiglitazone-induced miR-27 upregulation, intracellular sodium accumulation, and activation of the ITSN2/ α -ENaC signaling pathway in IMCD3 cells.

A: Relative expression of miR-27 determined by qRT-PCR in IMCD3 cells; B: Representative flow cytometric histograms showing intracellular Na⁺ levels measured using a sodium-sensitive fluorescent probe; C: Representative Western blot images and quantitative analysis of ITSN2, α -ENaC, and SGK1 protein expression. Con, control group; ROS, rosiglitazone-treated group; ROS+DMSO, vehicle control group; ROS+Cel, celastrol-treated group. Data are presented as mean $\pm$ SD from three independent experiments. * $P < 0.05$, ** $P < 0.01$. $n = 3$, $\bar{x} \pm s$.

Intracellular sodium levels were further assessed using a Na⁺-sensitive fluorescent probe combined with flow cytometry. The results demonstrated that ROS treatment

significantly increased Na⁺ fluorescence intensity, indicating enhanced intracellular sodium accumulation. In contrast, Cel treatment markedly decreased Na⁺ fluorescence intensity, suggesting that Cel alleviated ROS-induced sodium retention in IMCD3 cells (Figure 2B).

Western blot analysis revealed that ROS treatment significantly decreased ITSN2 protein expression while markedly increasing the expression levels of α -ENaC and SGK1 compared with the control group ($P < 0.05$). No significant differences were observed between the ROS and ROS+DMSO groups ($P > 0.05$). Notably, Cel treatment significantly restored ITSN2 expression and simultaneously reduced α -ENaC and SGK1 protein levels ($P < 0.05$) (Figure 2C).

Taken together, these findings indicate that celastrol reverses ROS-induced miR-27 upregulation and abnormal activation of the ITSN2/ α -ENaC signaling pathway, thereby attenuating sodium retention in renal collecting duct cells.

3.4. miR-27 Mediates the Regulatory Effects of Celastrol on the ITSN2/ α -ENaC Signaling Pathway

To determine whether miR-27 is involved in the protective effects of celastrol (Cel) against ROS-induced sodium retention, miR-27 knockdown and overexpression cell models were established. qRT-PCR analysis demonstrated that transfection with anti-miR-27 significantly reduced intracellular miR-27 expression compared with the Mock group ($P < 0.01$), whereas transfection with miR-27 mimic markedly increased miR-27 expression ($P < 0.001$), indicating successful transfection efficiency (Figure 3A, B).

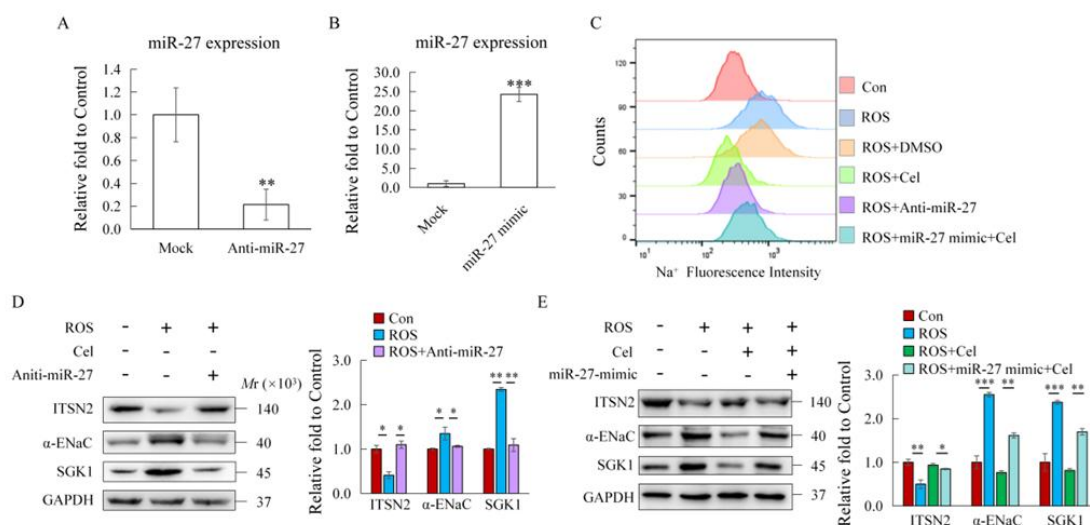


Figure 3. miR-27 mediated the protective effects of celastrol on rosiglitazone-induced sodium retention through regulation of the ITSN2/ α -ENaC signaling pathway.

Intracellular sodium levels were subsequently assessed using a Na⁺-sensitive fluorescent probe combined with flow cytometry. The results showed that ROS treatment significantly increased intracellular Na⁺ levels. Notably, anti-miR-27 transfection markedly attenuated ROS-induced Na⁺ accumulation. In contrast, overexpression of miR-27 in ROS+Cel-treated cells substantially weakened the inhibitory effect of Cel on intracellular Na⁺ accumulation (Figure 3C).

Western blot analysis further demonstrated that, compared with the ROS group, anti-miR-27 significantly restored ITSN2 protein expression ($P < 0.05$) and

suppressed the upregulation of α -ENaC ($P < 0.01$) and SGK1 ($P < 0.01$) proteins (Fig. 3D). Conversely, overexpression of miR-27 in the ROS+Cel group resulted in a significant reduction in ITSN2 expression ($P < 0.01$), accompanied by renewed increases in α -ENaC and SGK1 protein levels (both $P < 0.001$), thereby partially abolishing the protective effects of Cel (Figure 3E).

Taken together, these findings indicate that miR-27 is a critical mediator of Cel-mediated regulation of the ITSN2/ α -ENaC signaling pathway and plays an essential role in the attenuation of ROS-induced sodium retention.

A: Validation of miR-27 knockdown efficiency by qRT-PCR following transfection with anti-miR-27; B: Validation of miR-27 overexpression efficiency by qRT-PCR following transfection with miR-27 mimic; C: Representative flow cytometric histograms showing intracellular Na^+ levels measured using a sodium-sensitive fluorescent probe; D: Representative Western blot images and quantitative analysis of ITSN2, α -ENaC, and SGK1 protein expression in IMCD3 cells treated with ROS in the presence or absence of anti-miR-27; E: Representative Western blot images and quantitative analysis of ITSN2, α -ENaC, and SGK1 protein expression in IMCD3 cells treated with ROS+Cel in the presence or absence of miR-27 mimic.

Con, control group; ROS, rosiglitazone-treated group; ROS+Cel, rosiglitazone plus celastrol group; ROS+Anti-miR-27, rosiglitazone plus anti-miR-27 group; ROS+miR-27 mimic+Cel, rosiglitazone plus celastrol and miR-27 mimic group. Data are presented as mean $\pm$ SD from three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. $n=3$, $\bar{x} \pm s$.

3.5. Celastrol Ameliorated Rosiglitazone-Induced Sodium and Water Retention in Diabetic Mice

To further verify the protective effects of celastrol (Cel) against ROS-induced fluid retention in vivo, a high-fat/high-sucrose diet-induced T2DM mouse model was established. qRT-PCR analysis demonstrated that circulating miR-27 expression was significantly elevated in the ROS group compared with the control group ($P < 0.05$). No significant difference was observed between the ROS and ROS+DMSO groups ($P > 0.05$). However, Cel treatment significantly reduced miR-27 expression levels ($P < 0.05$) (Figure 4A).

Twenty-four-hour urinary sodium excretion was subsequently measured. Compared with the control group, mice treated with ROS exhibited a significant reduction in 24-h urinary sodium excretion ($P < 0.05$), indicating enhanced renal sodium reabsorption and sodium-water retention. No significant difference was detected between the ROS and ROS+DMSO groups ($P > 0.05$). In contrast, Cel administration markedly increased urinary sodium excretion ($P < 0.05$), restoring it to a level close to that of the control group (Figure 4B).

Histopathological examination by hematoxylin and eosin (H&E) staining revealed normal renal architecture in the control group, with intact glomerular and tubular structures. In the ROS and ROS+DMSO groups, renal tissues exhibited disorganized tubular arrangement, mild interstitial expansion, and focal tissue injury. These pathological alterations were substantially alleviated following Cel treatment (Figure 4C).

Immunohistochemical analysis further showed that renal α -ENaC expression was significantly increased in the ROS group compared with the control group ($P < 0.05$),

whereas no significant difference was observed between the ROS and ROS+DMSO groups ($P > 0.05$). Cel treatment significantly reduced α -ENaC expression in renal tissues ($P < 0.05$) (Figure 4D).

Collectively, these findings indicate that ROS promotes miR-27 upregulation, enhances α -ENaC expression, and decreases urinary sodium excretion in vivo. Celastrol effectively suppresses miR-27 expression, inhibits aberrant α -ENaC activation, increases urinary sodium excretion, and alleviates renal pathological injury, thereby attenuating ROS-associated sodium and water retention.

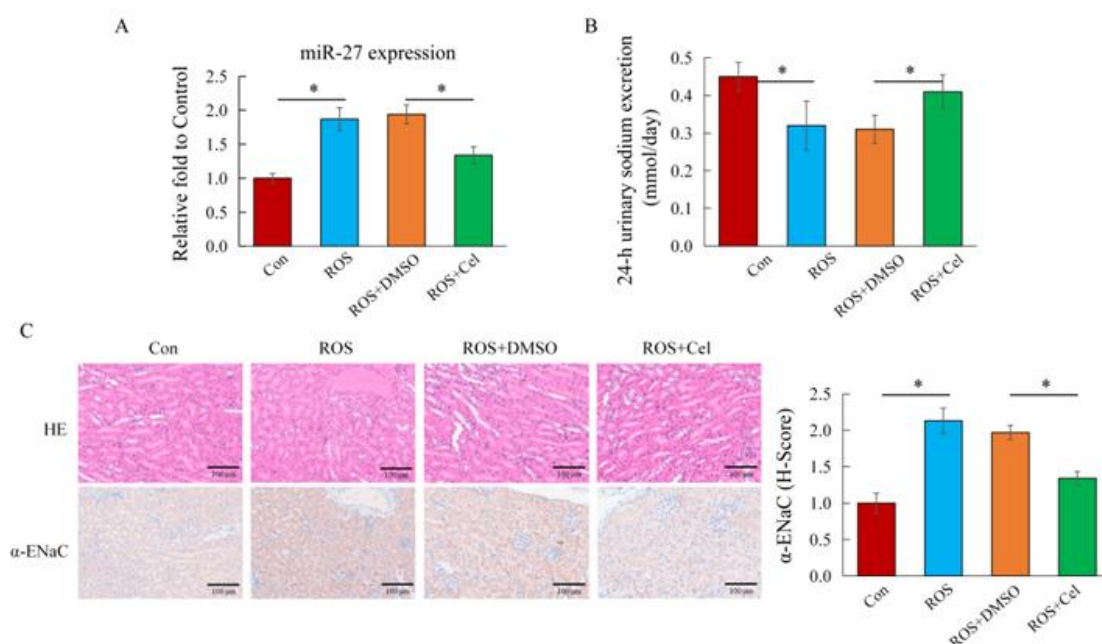


Figure 4. Celastrol attenuated rosiglitazone-induced sodium retention and renal α -ENaC overexpression in diabetic mice.

*A: Relative expression of circulating miR-27 in peripheral blood determined by qRT-PCR; B: Twenty-four-hour urinary sodium excretion in different groups of mice; C: Representative hematoxylin-eosin (H&E) staining and immunohistochemical staining of α -ENaC in renal tissues, together with quantitative analysis of α -ENaC expression (H-score). Scale bar = 100 μ m. Con, control group; ROS, rosiglitazone-treated group; ROS+DMSO, vehicle control group; ROS+Cel, rosiglitazone plus celastrol-treated group. Data are presented as mean $\pm$ SD. * $P < 0.05$. $n=3$, $\bar{x} \pm s$.*

4. Discussion

Rosiglitazone (ROS), a peroxisome proliferator-activated receptor gamma (PPAR γ) agonist, is an important therapeutic agent for the treatment of type 2 diabetes mellitus (T2DM) because of its ability to improve insulin resistance and effectively reduce blood glucose levels [13]. However, fluid retention and peripheral edema are among its most common adverse effects, and in severe cases may even induce or exacerbate heart failure, thereby limiting its clinical application [14,15]. Previous studies have suggested that ROS-induced sodium and water retention is primarily associated with enhanced sodium reabsorption mediated by activation of the epithelial sodium channel (α -ENaC) in renal collecting duct cells; however, the upstream regulatory mechanisms remain incompletely understood [5]. In the present study, we first evaluated 20 patients with T2DM receiving ROS therapy and found that fasting blood glucose levels were significantly reduced after 12 weeks of treatment, confirming the glucose-lowering efficacy of ROS. Meanwhile, serum miR-27 expression in patients

who developed edema was 2.94-fold higher than that in patients without edema. These findings suggest that miR-27 may be involved in the development of ROS-associated fluid retention and may serve as a potential biomarker for predicting the risk of ROS-induced edema.

In recent years, increasing attention has been paid to the role of microRNAs in the regulation of renal ion transport. Previous studies have demonstrated that miR-27 not only participates in lipid metabolism and insulin sensitivity regulation but also modulates sodium reabsorption by targeting intersectin-2 (ITSN2) and subsequently affecting α -ENaC membrane trafficking and activity in renal collecting duct cells [16]. In the present study, ROS treatment markedly increased miR-27 expression in IMCD3 cells, accompanied by decreased ITSN2 expression and increased α -ENaC and SGK1 protein levels. SGK1 is a critical downstream effector of aldosterone signaling and is known to enhance the membrane stability and activity of α -ENaC, thereby promoting sodium reabsorption. Furthermore, flow cytometric analysis revealed significant intracellular Na⁺ accumulation following ROS treatment, indicating enhanced sodium uptake by collecting duct cells. These findings suggest that ROS-induced enhancement of sodium reabsorption may be mediated by miR-27 upregulation, which suppresses ITSN2 expression and subsequently activates the α -ENaC/SGK1 signaling pathway. This mechanism provides a novel molecular explanation for ROS-associated sodium and water retention.

Celastrol, the major bioactive component isolated from *Tripterygium wilfordii*, possesses a wide range of pharmacological activities, including anti-inflammatory, antioxidant, and metabolic regulatory effects [17,18]. Emerging evidence has further demonstrated that Cel can exert therapeutic effects in metabolic diseases through the regulation of multiple microRNAs [19,20]. In the present study, Cel significantly inhibited ROS-induced miR-27 upregulation, reduced intracellular Na⁺ accumulation, restored ITSN2 expression, and suppressed α -ENaC and SGK1 expression. To further elucidate the role of miR-27, miR-27 knockdown and overexpression models were established. Anti-miR-27 treatment mimicked the protective effects of Cel by restoring ITSN2 expression and reducing α -ENaC, SGK1, and intracellular Na⁺ levels. In contrast, overexpression of miR-27 partially abolished the beneficial effects of Cel. These gain- and loss-of-function experiments collectively demonstrate that miR-27 is a critical target mediating the protective effects of Cel. Cel appears to alleviate ROS-induced sodium retention primarily through regulation of the miR-27/ITSN2 signaling axis and subsequent inhibition of abnormal α -ENaC activation.

The *in vivo* findings further validated the proposed mechanism. In high-fat/high-sucrose diet-induced T2DM mice, ROS treatment significantly increased circulating miR-27 levels, reduced 24-hour urinary sodium excretion, and enhanced renal α -ENaC expression, indicating increased sodium reabsorption *in vivo*. Cel intervention markedly decreased miR-27 expression, restored urinary sodium excretion, reduced α -ENaC expression, and improved renal histopathological alterations. These observations were highly consistent with the *in vitro* results and further support the notion that Cel attenuates ROS-associated sodium and water retention through modulation of the miR-27/ITSN2/ α -ENaC signaling axis. Nevertheless, several limitations should be acknowledged. The clinical sample size was relatively small, and renin–angiotensin–aldosterone system (RAAS)-related parameters, such as aldosterone and renin levels, were not evaluated. In addition, ITSN2-deficient animal models were not employed to further validate the causal role of ITSN2 *in vivo*. Future

studies involving larger clinical cohorts and gene-editing approaches are warranted to further elucidate the underlying molecular mechanisms.

5. Conclusions

In conclusion, the present study demonstrates for the first time that elevated miR-27 expression is closely associated with ROS-related edema. More importantly, celastrol alleviates ROS-induced sodium reabsorption and fluid retention through modulation of the miR-27/ITSN2/ α -ENaC signaling axis. These findings provide novel mechanistic insights and a potential therapeutic strategy for the prevention and management of adverse effects associated with PPAR γ agonist therapy.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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