Involvement of the IP3R-Grp75-VDAC1-MCU calcium axis in proteinuria in adriamycin-induced nephropathy rats

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Abstract

Background Podocyte injury plays a key role in the development of proteinuria. We previously found that the intracellular inositol 1, 4, 5-trisphosphate receptor (IP3R)- glucose-regulated protein 75 (Grp75)- voltage dependent anion channel 1 (VDAC1)- mitochondrial calcium uniporter (MCU) calcium axis contributes to podocyte injury in cultured mouse podocytes.

Objective This study investigated whether the IP3R-Grp75-VDAC1-MCU calcium axis is involved in the development and improvement of proteinuria in nephropathy rats.

Methods The expression of members of the IP3R-Grp75-VDAC1-MCU calcium axis in the renal cortex of a previously established adriamycin (ADR)-induced nephropathy rat model and cultured mouse podocytes was investigated by western blot analysis and immunohistochemical staining. The effects of ruthenium red (RR), an MCU inhibitor, oninteractions in the IP3R-Grp75-VDAC1-MCU calcium axis were investigated by in vitro co-immunoprecipitation assays.

Results The overexpression and inhibition of members of the glomerular IP3R-Grp75-VDAC1-MCU calcium axis were accompanied by the development and improvement of proteinuria, respectively, in nephropathy rats. RR inhibited the upregulation of members of the IP3R-Grp75-VDAC1-MCU calcium axis induced by ADR and their interactions.

Conclusions The IP3R-Grp75-VDAC1-MCU calcium axis is involved in proteinuria in ADR-induced nephropathy and can be inhibited by RR.

Background

Nephrotic syndrome is a common renal disease in children characterized by heavy proteinuria, hypoalbuminemia, oedema and hypercholesterolemia. Podocytes have been confirmed to be the key cell type that maintains the function of the glomerular filtration barrier. Podocyte injury is the core mechanism that contributes to the development of proteinuria. However, as the mechanism of podocyte injury has not been fully elucidated and clinical drugs used to control proteinuria remain limited, further investigation is needed [1].

Previously, we investigated the mechanism of podocyte injury with a focus on the intracellular calcium axis [2]. The endoplasmic reticulum (ER) and mitochondria are the two main and most important calcium reservoirs in podocytes. Charged calcium is transferred from the ER to the mitochondrial matrix in a process called mitochondrial-endoplasmic reticulum coupling. The inositol 1, 4, 5-trisphosphate receptor (IP_3R)-glucose-regulated protein 75 (Grp75)-voltage dependent anion channel 1 (VDAC1)-mitochondrial calcium uniporter (MCU) axis is the molecular basis by which Ca^{2+} transfer from the ER to mitochondria is regulated in mitochondrial-endoplasmic reticulum coupling. In the calcium axis, IP_3R is a calcium release channel located at the ER membrane, and VDAC1 is located at the outer membrane of mitochondria. MCU, which is located at the inner membrane of mitochondria, is the final channel through

which calcium enters the mitochondrial matrix. Grp 75 is a bridging protein that interacts with both IP_3R and VDAC1 to form a complex [3, 4]. Proper calcium transfer from the ER to the mitochondrial matrix is key to maintaining ATP production and normal mitochondrial function [5]. We previously confirmed that the upregulation of members in the IP_3R-Grp75-VDAC1-MCU calcium axis contributes to mitochondrial calcium overload and podocyte apoptosis induced by adriamycin (ADR) and angiotensin II, respectively. Antagonists of this calcium axis were shown to prevent podocyte apoptosis [2]. We also found that the MCU inhibitor ruthenium red (RR) significantly improved proteinuria and podocyte foot process effacement in ADR-induced nephropathy rats [2]. Based on the above findings, we hypothesized that the IP_3R-Grp75-VDAC1-MCU calcium axis is involved in the development of proteinuria. To clarify its involvement, this study investigated changes in the expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis during the development and improvement of proteinuria and podocyte injury in ADR-induced nephropathy rats and cultured mouse podocytes.

Materials And Methods

1. ADR nephropathy rat model development and MCU inhibitor treatment

All protocols were approved by the Institutional Animal Care and Use Committee of Peking University First Hospital (number: 11400700229305). This study used a previously reported model [2]. ADR (D1515, Sigma, Santa Clara, California, USA) was successfully used to induce a nephropathy model in Sprague-Dawley rats (Beijing Vital River Laboratory Animal Technology Co. Ltd.) as previously reported. After the injection of ADR, 24 h urinary protein excretion in the rats increased significantly after 6 weeks. We previously confirmed that the injection of RR (R2751, Sigma), an MCU inhibitor, significantly improves proteinuria induced by ADR after 6 weeks [2]. As reported, the Sprague-Dawley rats were divided into four groups: a normal saline control group (Ctl, n = 6), an RR control group (RR, n = 6), an ADR group (ADR, n = 10), and an ADR plus RR group (ADR + RR, n = 6). All rats were sacrificed and harvested at the 6-week time point [2]. Stored renal tissues were used for this study.

2. Western blot analysis

Western blot analysis was performed to detect target molecules in the renal cortex or cultured mouse podocytes. RIPA lysis buffer (89901, Thermo Scientific) containing protease inhibitor was used to extract total cellular proteins, and the proteins were boiled at 100℃ for 10 min After SDS-PAGE on 6%-15% gels, the proteins were transferred to Immuno-Bot PVDF membranes (Bio-Rad). The membranes were blocked with 5% BSA for 1 h and then incubated overnight at 4℃ with the primary antibodies anti-IP_3R diluted 1:1000 (ab5804, Abcam), anti-Grp75 diluted 1:1000 (#3593, Cell Signalling Technology), anti-VDAC1 diluted 1:1000 (ab14734, Abcam), anti-MCU diluted 1:1000 (D2Z3B, #14997, Cell Signalling Technology) and anti-GAPDH diluted 1:3000 (C1312, Applygen). Blots were then incubated with HRP-labelled secondary antibody diluted 1:3000 (C2247, C1309, Applygen). ImageJ software was used to semi-

quantitate the ratio of the target protein/GAPDH. The specific details of these methods were the same as those that we reported previously [2].

3. Immunohistochemical analysis showing glomerular expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis

Paraffin-embedded sections (4 μM) from ADR-induced nephropathy rat's kidneys were used. After dewaxing with xylene and hydration using a gradient series of ethanol, the renal slides underwent antigen retrieval with a sodium citrate solution (pH 6.0) in an autoclave for 20 min, and endogenous peroxidase was then blocked with 3% hydrogen peroxide for 5 min. Afterwards, the sections were sealed with normal goat serum for 30 min at 37°C and then incubated with anti-IP_3R (1:1000, ab5804, Abcam), anti-Grp75 (1:1000, ab2799, Abcam), anti-VDAC1 (1:4000, ab14734, Abcam) and anti-MCU (1:200, ab121499, Abcam) antibodies overnight at 4℃. After washing with phosphate-buffered saline (PBS), the sections were incubated with HRP-conjugated anti-rabbit secondary antibody (PV-9000, ZSGB-BIO) for 20 min at room temperature. Then, the sections were sequentially stained with diaminobenzidine (DAB) and haematoxylin. Finally, the sections were sealed with resin. Fifteen images of randomly glomeruli from each rat were captured with a 40× objective under a light microscope (Olympus, Tokyo, Japan). Ratios of the glomerular area showing positive staining for the target molecule to the total glomerular area were analysed using Image-Pro Plus 6.0 software and used to define the glomerular expression of each molecule [6, 7].

4. Immunofluorescence staining showing glomerular expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis

Frozen sections (4 μM) from ADR-induced nephropathy rat kidneys were used for immunofluorescence staining. Sections were fixed with pre-cooled acetone for 10 min, permeabilized with 0.5% Triton X-100 for 10 min at room temperature and blocked with 5% PBS-BSA for 60 min at room temperature to decrease non-specific protein binding. Then, the renal slides were incubated with anti-IP_3R (1:100, ab5804, Abcam) and anti-synaptopodin (1:200, ab224491, Abcam), anti-Grp75 (1:100, ab2799, Abcam) and anti-podocin (1:100, ab50339, Abcam), anti-VDAC1 (1:100 ab14734, Abcam) and anti-podocin, or anti-MCU (1:200, ab121499, Abcam) and anti-synaptopodin antibodies overnight at 4℃. After washing with PBS, sections were incubated with Alexa 488-conjugated goat anti-mouse IgG and Alexa 594-conjugated goat anti-rabbit IgG (Invitrogen) for 1 h each. Nuclei were stained for 2 min with 4'6-diamidino-2-phenylindol dihydrochloride (DAPI). Images were taken with a laser scanning confocal microscope (Olympus FluoView FV1000) equipped with a 100× oil immersion objective lens [8].

5. Real-time quantitative- polymerase chain reaction (PCR)

Total RNA was extracted from podocytes using Trizol (Invitrogen, USA) according to the protocol. The cDNA was synthesized using TransScript First-Strand cDNA Synthesis SuperMix kit (TransGene, China) following the instructions of the kit, then the cDNA was used for real-time quantitative-PCR analysis using PowerUp SYBR Green Master Mix (Applied Biosystems, USA) and ABI Prism 7500 (Applied Biosystems, USA) according to the protocols provided by the kit [9]. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression of target genes to GAPDH [10]. The primers used were as followed: GAPDH: 5'-TCCTCGTCCCGTAGACAAAATG-3' and 5'-CGCCCAATACGGCCAAA-3'; IP_3R1(Itrp1): 5'-CCTTAACAATCCACCCAAGAAATT and 5'-TGCGGAGTATCGATTCATAGGA-3'; Grp75 (Hspa9): 5'-AGAGATTATGCATCAGAAGCAATCA-3' and 5'-TCCATAACAGCCACACAGGAGTT-3'; VDAC1: 5'-AGCTCACCTTTGATTCGTCATTC-3' and 5'-CCCTGTCTTGATTTTAGCATTTTTTT-3'; MCU: 5'-ACCACGTACGGCCACCAA-3' and 5'-CAGGGTCTTCACGTCGTTCA-3'.

6. Mouse podocyte culture

Mouse podocytes (Endlich mouse podocytes, a generous gift from Prof. Hong Hui Wang from Hunan University, China) were cultured as we previously reported [2]. The podocytes were cultured at 33°C in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin/streptomycin, and 10 U/ml recombinant mouse interferon-γ (IFN-γ) to induce proliferation and transferred to culture media lacking IFN-γ at 37°C to differentiate for 10–14 days [2]. The podocytes were pre-treated with the MCU inhibitor RR for 60 min before the addition of 0.5 μg/ml ADR for 24 h [2].

7. Co-immunoprecipitation assay

Co-immunoprecipitation assay was used to detect the effect of RR on the interactions between members of the IP_3R-Grp75- VDAC1-MCU complex in cultured mouse podocytes, as we previously reported [2]. Podocyte lysates were collected and centrifuged, following which protein concentrations were determined. Co-immunoprecipitation was performed using a Thermo Scientific Pierce Co-IP Kit (26149, Thermo Fisher Scientific) according to the manufacturer's protocols. Anti-Grp75 antibody (D13H4, #3593, Cell Signalling Technology) was used to capture proteins coupled in mitochondria/the ER, and normal rabbit IgG without antigenicity was used as a negative control. After co-immunoprecipitation, proteins pulled down by the anti-Grp75 antibody were analysed by western blotting. Lysates from both Ctl and ADR-treated podocytes without immunoprecipitation were used as positive controls (input).

8. Statistical analysis

Data are presented as the means ± SDs and were analysed using one-way AVONA and Student's t-tests. Differences for which $P<0.05$ were considered statistically significant.

Results

1. Upregulated renal cortical expression of molecules in the IP_3R-Grp75-VDAC1-MCU calcium axis in ADR nephropathy rats

Western blot analysis showed that, compared with the Ctl and RR groups, renal cortical expression of IP_3R, Grp75, VDAC1 and MCU was significantly increased in rats in the ADR group at 6 weeks after the injection of ADR (Fig. 1) when proteinuria peaked.

2. Upregulated glomerular expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis in ADR nephropathy rats

The glomerular expression of molecules in the IP_3R-Grp75-VDAC1-MCU calcium axis was analysed by immunohistochemical analysis. The ratio of the glomerular staining area to the total glomerular area was used to reflect the glomerular expression level of each molecule. In the Ctl group, glomerular staining for members of the IP_3R-Grp75-VDAC1-MCU calcium axis was weak. No significant differences in the glomerular expression levels of each molecule were found between the Ctl and RR groups. Compared with the Ctl group, the glomerular expression of IP_3R, Grp75, VDAC1 and MCU was significantly increased in the ADR group (Fig. 2). Similar findings were found by immunofluorescence staining for the glomerular expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis (Fig. 3). In the ADR group, IP_3R, Grp75, VDAC1 and MCU expression in the glomerulus co-localized with podocyte-specific podocin and synaptopodin molecules.

3. RR inhibited the increased glomerular expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis in ADR rats

Compared with the ADR group, the renal cortical expression of IP_3R, Grp75, VDAC1 and MCU was decreased significantly in the ADR+RR group at the 6-week time point, as shown by western blot analysis (Fig. 1). By immunohistochemical analysis, the glomerular expression of IP_3R, Grp75, VDAC1 and MCU was shown to be significantly decreased in rats in the ADR+RR group compared with the ADR group (Fig. 2).

4. RR inhibited the increased expression of members in the IP_3R-Grp75-VDAC1-MCU calcium axis in cultured mouse podocytes

As shown by western blot analysis, compared with the Ctl group of cultured mouse podocytes, the expression of IP_3R (P=0.036, n=6), Grp75 (P=0.027, n=6), VDAC1 (P=0.033, n=6) and MCU (P=0.024, n=6) was significantly increased in ADR-treated cultured mouse podocytes, (Fig. 4A). Compared with ADR-treated podocytes, the expression of IP_3R (P=0.025, n=6), Grp75 (P=0.009, n=6), VDAC1 (P=0.045, n=6) and MCU (P=0.028, n=6) was significantly decreased in ADR+RR-treated podocytes (Fig. 4A).

As shown by RT quantitative PCR, compared with the Ctl group of cultured mouse podocytes, the expression of IP_3R, Grp75, VDAC1 and MCU was significantly increased in the ADR-treated cultured

mouse podocytes. Compared with the ADR-treated podocytes, the expression of IP_3R, Grp75, VDAC1 and MCU was significantly decreased in the ADR+RR-treated podocytes (Fig. 5).

5. RR reduced the interactions among members of the IP_3R-Grp75-VDAC1-MCU complex in cultured mouse podocytes

Co-immunoprecipitation experiments showed that ADR increased the interactions among members of the IP_3R-Grp75-VDAC1 complex. Compared with the Ctl group, the quantities of IP_3R (P=0.006, n=3), Grp75 (P=0.003, n=3) and VDAC1 (P=0.006, n=3) pulled down by anti-Grp75 antibody were significantly increased in the ADR group (Fig. 4B). Compared with the ADR group, the quantities of IP_3R (P=0.006, n=3), Grp75 (P=0.012, n=3), and VDAC1 (P=0.023, n=3) pulled down by anti-Grp75 antibody in the ADR+RR group were significantly decreased (Fig. 4B).

Discussion

Podocytes play a key role in maintaining the glomerular filtration barrier and preventing the development of proteinuria. Different mechanisms of podocyte injury have been reported [11]. We previously investigated the mechanism of podocyte injury with a focus on intracellular calcium regulation by the IP_3R-Grp75-VDAC1-MCU calcium axis, which is involved in mitochondrial-ER coupling. The IP_3R-Grp75-VDAC1-MCU calcium axis regulates calcium transfer from the ER to the mitochondrial matrix. Proper calcium transfer into the mitochondrial matrix is a prerequisite for mitochondrial ATP production [12]. We previously found that the upregulation of members of the IP_3R-Grp 75-VDAC1-MCU axis in cultured mouse podocytes contributed to podocyte injury induced by ADR and that antagonists to members of this axis inhibited mitochondrial calcium overload and podocyte apoptosis. We also found that the inhibition of MCU by RR significantly improved podocyte injury and proteinuria in ADR-induced nephropathy rats [2]. To the best of our knowledge, whether the IP_3R-Grp75-VDAC1-MCU axis is involved in the development and improvement of proteinuria is unclear. Considering the crucial role played by podocytes in the development of proteinuria, we hypothesized that the abnormal expression of IP_3R-Grp75-VDAC1-MCU axis members is involved in the development of proteinuria. In this study, we used tissues from a previously established ADR nephropathy rat model to investigate this hypothesis, which revealed several findings.

First, we found that the upregulation of members of the IP_3R-Grp75-VDAC1-MCU calcium axis was involved in the development of proteinuria in ADR-induced nephropathy rats. At 6 weeks after ADR injection, when urinary protein peaked, as reported previously [2], the renal cortical expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis in ADR-induced nephropathy rats was increased significantly. By immunofluorescence staining with the podocyte marker molecules podocin and synaptopodin, we found that glomerular staining for members of the IP_3R-Grp75-VDAC1-MCU calcium axis was very weak in normal control rats but obvious in ADR nephropathy rats. Glomerular staining for IP_3R-Grp75-VDAC1-MCU axis molecules showed their expression in podocytes. The glomerular expression of molecules in

the IP_3R-Grp75-VDAC1-MCU calcium axis was further semi-quantitated by immunohistochemical staining, which showed a significant increase in glomerular staining for IP_3R-Grp75-VDAC1-MCU calcium axis molecules in ADR-induced nephropathy rats. This phenomenon has not been reported and suggests that the increased expression of members of the IP_3R-Grp75-VDAC1-MCU axis is involved in the mechanism of proteinuria. This result is consistent with our previous finding that the increased expression of members of this axis is involved in the mechanism of podocyte injury in cultured mouse podocytes.

Second, based on our previous finding that the MCU inhibitor RR significantly decreased proteinuria and foot process effacement in ADR-induced nephropathy rats [2], we investigated the relationship between the expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis and improved proteinuria in the same rat model. We found that the glomerular expression levels of members of the IP_3R-Grp75-VDAC1-MCU calcium axis decreased significantly with improvements in proteinuria in rats in the ADR + RR group. The results of this study support the involvement of molecules in the IP_3R-Grp75-VDAC1-MCU calcium axis in proteinuria. In addition, RR was found to regulate members of the IP_3R-Grp75-VDAC1-MCU calcium axis. We further explored the effect of RR on these molecules in cultured mouse podocytes and found that RR significantly downregulated their expression both in protein and mRNA levels. In addition, co-immunoprecipitation experiments revealed that RR decreased interactions between members of the IP_3R-Grp75-VDAC1 complex. The exact molecular mechanism by which RR regulates the expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis is unclear. RR is an MCU inhibitor that can inhibit calcium influx into the mitochondrial matrix [13], which may have a negative regulatory effect on the IP_3R-Grp75-VDAC1-MCU calcium axis.

In conclusion, this study clearly reveals that changes in the expression of members of the IP_3R-Grp75-VDAC1-MCU calcium axis are involved in the development and improvement of proteinuria in ADR-induced nephropathy rats. This finding further supports the previously identified role of the IP_3R-Grp75-VDAC1-MCU calcium axis in podocyte injury in cultured mouse podocytes and suggests that the IP_3R-Grp75-VDAC1-MCU calcium axis is a new pathway with which to protect podocytes.

Abbreviations

ADR: Adriamycin; Ang II: Angiotensin II; Co-IP: Co-immunoprecipitation; Ctl: Control; ER: Endoplasmic reticulum; Grp75: Glucose-regulated protein 75; IP3: Inositol 1,4,5-triphosphate tripotassium salt; IP_3R: Inositol 1,4,5- triphosphate receptor; MCU: Mitochondrial calcium uniporter; RR: Ruthenium red; VDAC1: Voltage-dependent anion channel 1; RT-PCR: Real-time quantitative- polymerase chain reaction.

Declarations

Ethics approval and consent to participate

The rats used in this study were bought from Beijing Vital River Laboratory Animal Technology Co., Ltd. All protocols were approved by the Institutional Animal Care and Use Committee of Peking University First Hospital (Number: 11400700229305).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Na Guan designed the investigation, revised the manuscript, approved the final version to be submitted and is responsible for all aspects of the work. Sisi Wang, Han Xu, Na Guan, Qijiao Wei, Yinghong Tao, Guosheng Yang and Yali Ren carried out the experiments. Sisi Wang and Han Xu analysed the data and made the figures. Sisi Wang drafted and revised the paper.

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Figures

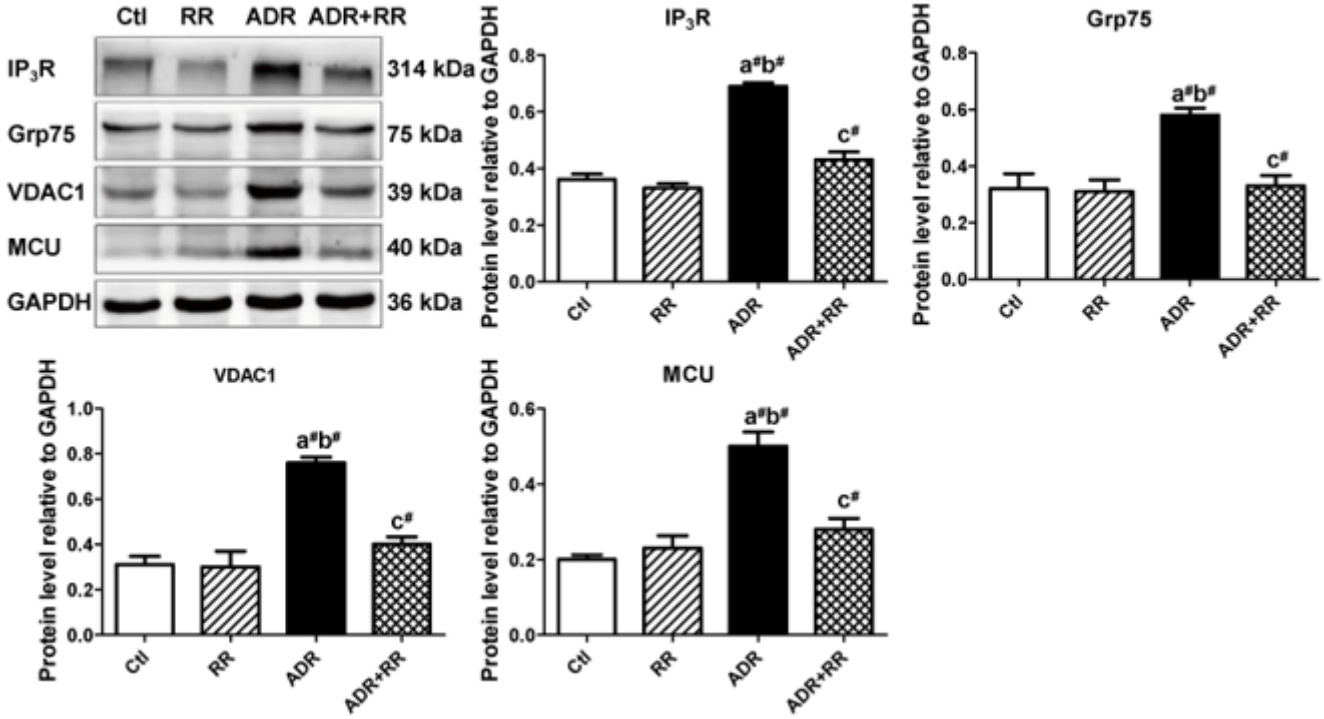

Figure 1

Upregulation of the renal cortical expression of molecules in the IP3R-Grp75-VDAC1-MCU calcium axis and the inhibition of this effect by ruthenium red in adriamycin-induced nephropathy rats. IP3R, inositol 1,4,5-triphosphate receptor; Grp75, glucose-regulated protein 75; VDAC1, voltage dependent anion channel 1; MCU, mitochondrial calcium uniporter; Ctl, the control group (n=6); RR, the ruthenium red group (n=6); ADR, the adriamycin group (n=10); ADR+RR, the ADR plus RR group (n=6). a, compared with the Ctl group; b, compared with the RR group; c, compared with the ADR group. *, P<0.05; #, P<0.01. As shown by western blot analysis, the renal cortical expression of IP3R (P=0.000), Grp75 (P=0.001), VDAC1 (P=0.000) and MCU (P=0.005) was significantly increased in nephropathy rats in the ADR group compared to the Ctl group. Compared with the ADR group, the renal cortical expression of IP3R (P=0.000), Grp75 (P=0.001), VDAC1 (P=0.000) and MCU (P=0.036) was significantly decreased in the ADR+RR group.

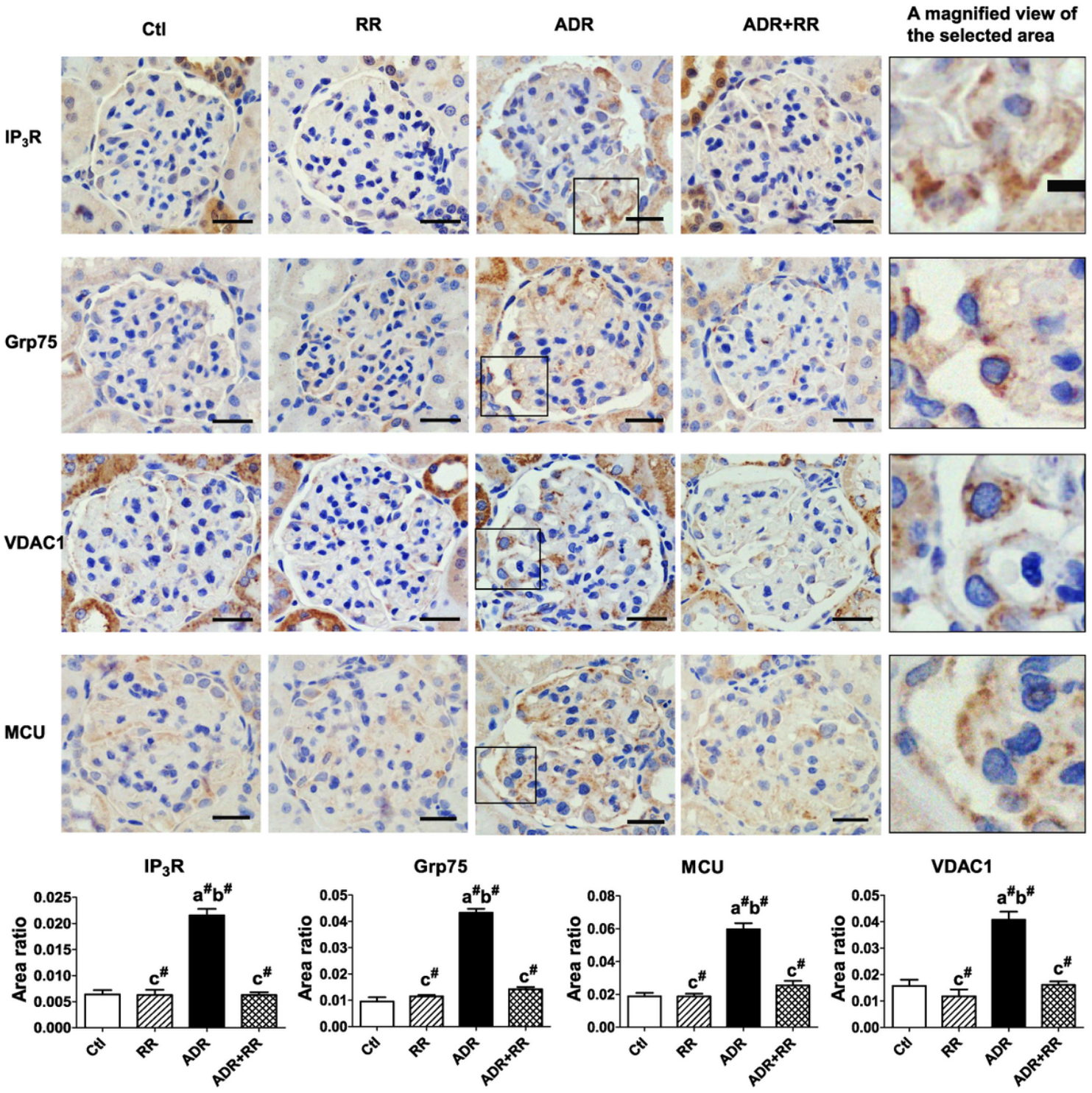

Figure 2

Upregulation of the glomerular expression of molecules in the IP3R-Grp75-VDAC1-MCU axis, which was inhibited by ruthenium red in adriamycin-induced nephropathy rats. Ctl, the control group (n=6); RR, the ruthenium red group (n=6); ADR, the adriamycin group (n=10); ADR+RR, the ADR plus RR group (n=6). a, compared with the Ctl group; b, compared with the RR group; c, compared with the ADR group. *, P<0.05; #, P<0.01. Immunohistochemical staining was performed in paraffin-embedded rat renal sections, followed by image analysis. The ratio of the positive staining area for each molecule to the total

glomerular area was used to reflect its glomerular expression level. Compared with the Ctl group, the glomerular expression of IP3R (P=0.000), Grp75 (P=0.000), VDAC1 (P=0.000) and MCU (P=0.000) was significantly increased in rats in the ADR group. Compared with the ADR group, the glomerular expression of IP3R (P=0.000), Grp75 (P=0.000), VDAC1 (P=0.000) and MCU (P=0.000) was significantly decreased in the ADR+RR group. 400×, bar=40 μm.

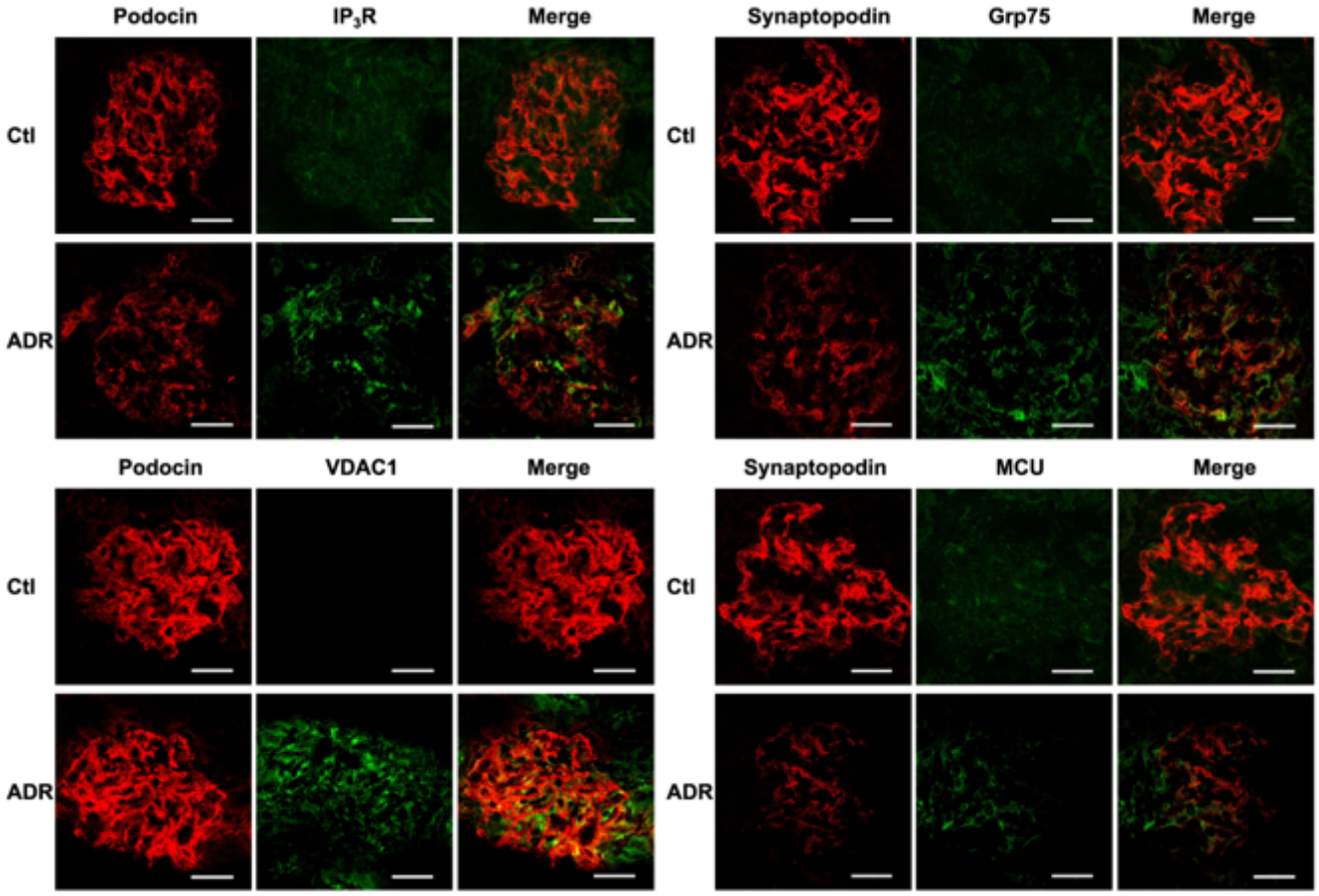

Figure 3

Immunofluorescence staining showing the glomerular expression of molecules in the IP3R-Grp75-VDAC1-MCU calcium axis in control and adriamycin-induced nephropathy rats. Ctl, the control group; ADR, the adriamycin group. Immunofluorescence double staining for IP3R/Grp75/VDAC1/MCU and podocin/synaptopodin displayed their co-localization. Unlike that in the Ctl group, glomerular staining for molecules in the calcium axis was obvious in rats in the ADR group. Bar =50 μm.

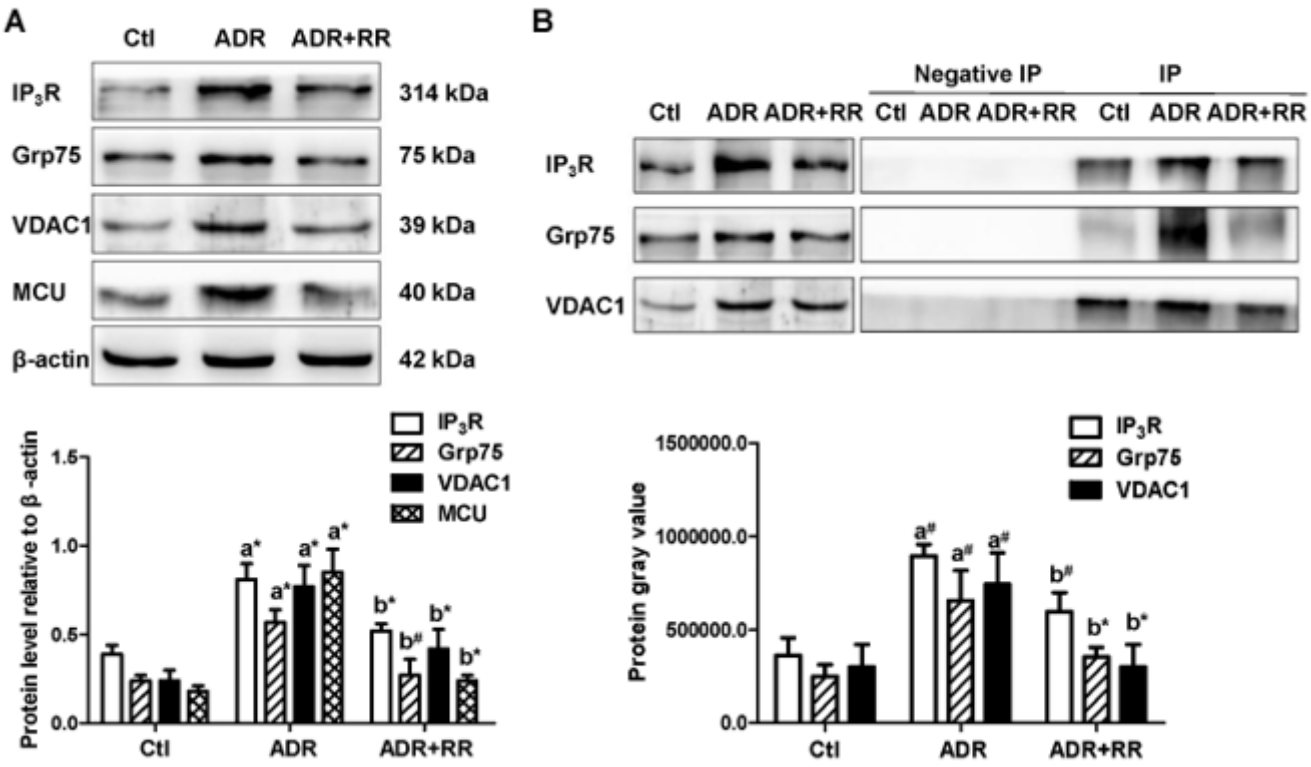

Figure 4

The expression of members of the IP3R-Grp75-VDAC1-MCU calcium axis in cultured mouse podocytes was upregulated by adriamycin and inhibited by ruthenium red, as analysed by western blotting and co-immunoprecipitation experiments.in cultured mouse podocytes. Ctl, the control group; ADR, the adriamycin group; ADR+RR, the ADR plus RR group. a, compared with the Ctl group; b, compared with the ADR group; *, P<0.05; #, P<0.01. A, Western blot analysis of molecules in the IP3R-Grp75-VDAC1-MCU calcium axis. Compared with the Ctl group (n=6), the expression levels of IP3R, Grp75, VDAC1 and MCU were significantly increased in ADR-treated podocytes. Compared with ADR-treated podocytes (n=6), the expression of molecules in the IP3R-Grp75-VDAC1-MCU calcium axis was inhibited in ADR+RR-treated podocytes (n=6). B, Co-immunoprecipitation analysis of interactions between members of the IP3R-Grp75-VDAC1 calcium axis. Compared with podocytes in the Ctl group, the amount of IP3R, Grp75, and VDAC1 pulled down from samples of ADR-treated podocytes (n=3) was significantly increased. Compared with the ADR group, there was a significant decrease in the amount of IP3R, Grp75 and VDAC1 pulled down from the ADR+RR group by anti-Grp75 antibody (n=3).

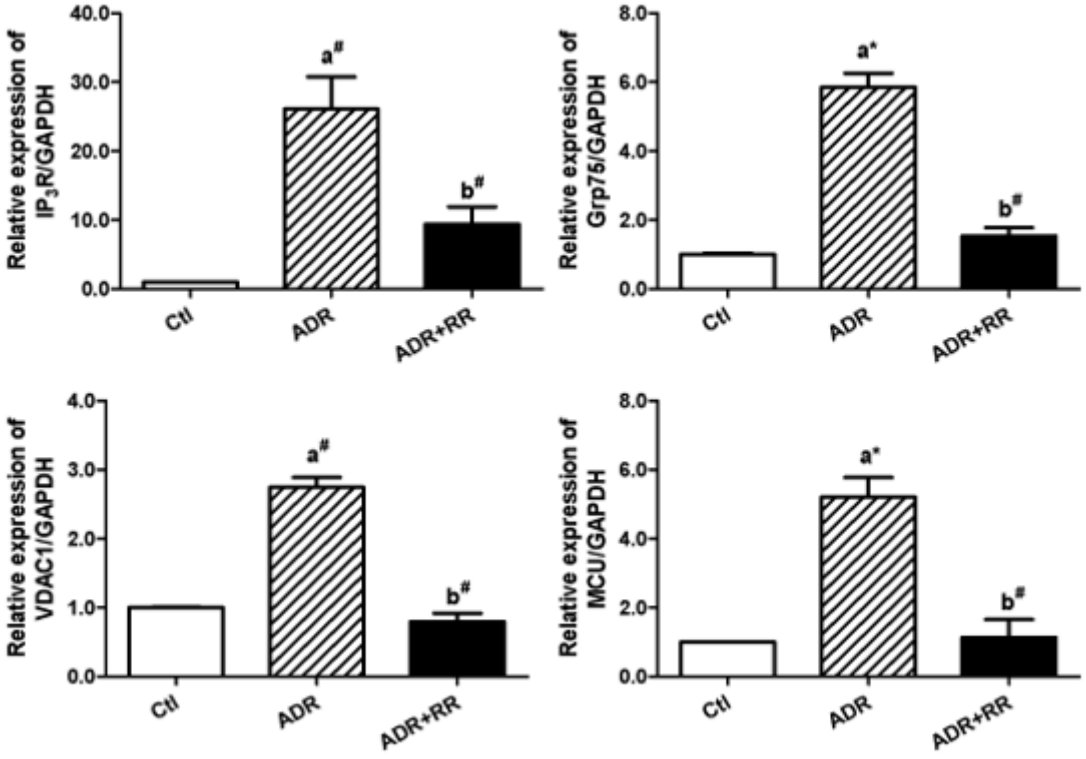

Figure 5

The mRNA expression of members of the IP3R-Grp75-VDAC1-MCU calcium axis in cultured mouse podocytes was upregulated by adriamycin and inhibited by ruthenium red, as analysed by real time quantitative PCR. Ctl, the control group; ADR, the adriamycin group; ADR+RR, the ADR plus RR group. a, compared with the Ctl group; b, compared with the ADR group; *, $P<0.05$; #, $P<0.01$. Compared with the Ctl group, the mRNA expression of IP3R (n=4, P=0.000), Grp75 (n=3, P=0.012), VDAC1 (n=4, P=0.003) and MCU (n=4, P=0.010) was significantly increased in the ADR-treated podocytes. Compared with the ADR-treated podocytes, the mRNA expression of IP3R (n=4, P=0.004), Grp75 (n=3, P=0.004), VDAC1 (n=4, P=0.000) and MCU (n=4, P=0.004) were significantly decreased in the ADR+RR-treated podocytes.