OXFORD Toxicology Research

Toxicology Research, 2021, **10**, 551–560

doi: 10.1093/toxres/tfab009
Advance Access Publication Date: 17 May 2021
Paper

PAPER

Galangin attenuates oxidative stress-mediated apoptosis in high glucose-induced renal tubular epithelial cells through modulating renin–angiotensin system and PI3K/AKT/mTOR pathway

Jie Liao,[1] Bo Liu,[1] Ke Chen,[1] Sheng Hu,[1] Zheng-Yu Liu,[1] Yu-Xin Li,[1] Zhi-Ming Yang,[2] Meng Zhang[1] and Xiong Chen[1,2,3,]

[1]Department of Geriatric Medicine, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, P.R. China, [2]International Medical Center, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, P.R. China and [3]National Clinical Research Center for Geriatric Disorders, Changsha 410008, Hunan Province, P.R. China

Correspondence address. Department of Geriatric Medicine, Xiangya Hospital, Central South University & International Medical Center, Xiangya Hospital, Central South University & National Clinical Research Center for Geriatric Disorders, No.78, Xiangya Road, Changsha 410008, Hunan Province, P.R. China. Tel: +86-13574187264; E-mail: chenxiong@csu.edu.cn

Abstract

This study was to evaluate the regulatory network among Galangin (Gal), oxidative stress, and renin–angiotensin system (RAS) in diabetic nephropathy (DN) *in vitro*. A cell model of DN was set up by exposing HK-2 cells to high glucose (HG, 30 mM) for 48 h and Gal was applied at 10 μM when needed. mRNA expression was analyzed by qPCR and protein level was detected by western blot. Malondialdehyde level and superoxide dismutase activity were evaluated by commercial kits. We analyzed cell viability by CCK8 assay and apoptosis by flow cytometry. DCFH-DA staining was conveyed for reactive oxygen species detection. HG induced RAS activation, oxidative stress, while inhibited cell viability. Gal suppressed oxidative stress-mediated apoptosis of HK-2 cells under the stimulation of HG via inhibiting RAS activation. Moreover, overexpression of AT1R, a RAS gene, could restrain the mitigative effect of Gal on cell injury. Furthermore, repression of RAS induced by AT1R knockdown partially reversed HG-induced PI3K/AKT/mTOR activation and oxidative stress in HK-2 cells. Also, AKT activation could antagonize Gal's functional roles in renal cell damage. Collectively, Gal alleviates HG-induced oxidative stress injury of renal tubular epithelial cells through PI3K/AKT/mTOR signal via modulating RAS activation. This finding would help to better understand mechanism of DN development and support future studies.

Key words: diabetic nephropathy, Galangin, renin–angiotensin system, PI3K/AKT/mTOR signal, oxidative stress

Received: 25 September 2020; **Revised:** 10 January 2021; **Accepted:** 29 January 2021

Introduction

Diabetic nephropathy (DN) is a chronic microvascular complication caused by diabetes mellitus, ranking second in risk factors of end-stage renal disease. According to early studies, there were 425 million diabetes mellitus cases worldwide by 2017, and 100 million DN patients, and 25–40% of the type 1 diabetes (T1D) patients and 5–40% type 2 diabetes (T2D) patients would eventually develop diabetic kidney disease [1, 2]. Upon diagnosis, it was found that 20% of T2D patients had already developed DN and up to 40% would develop DN within 10 years [3]. Although there are several clinical methods to delay the onset, there is still a lack of specific therapies to prevent the progress of the disease. This was largely due to the poor understanding of the complex nature and mechanism of DN. It is necessary to elucidate the molecular mechanism during DN development.

Renal damage in DN is closely related to oxidative stress [4]. Several inflammatory cytokines including tumor necrosis factor (TNF), NF-κB, and interleukins are also involved in DN progress [5]. Galangin (Gal) is a kind of flavonoids, which mainly exists in the dried rhizomes of *Alpinia officinarum Hance*. It has a wide spectrum of pharmacological activities, including anti-inflammatory, antioxidation, antifibrosis, and hypoglycemia [6–9]. It has been reported that Gal can reverse the changes of oxidative stress markers induced by ischemia–reperfusion through PI3K/AKT signaling pathway and alleviate liver injury [10, 11]. In addition, Gal could regulate signal pathways of inflammatory cytokines and enhance the sensitivity and antioxidant capacity of the body to insulin, presumably due to modulating TNF, NF-κB, and IL-6 pathways [12, 13] or act as dipeptidyl peptidase-4 (DPP-4, therapeutic drug target proven to reduce blood glucose levels in diabetes mellitus) inhibitor [12], and alleviate the renal damage induced by sugar [14].

Renin–angiotensin system (RAS) is a powerful regulatory system in homeostasis and blood pressure, coordinating cardiovascular system and kidney. RAS could process angiotensinogen into angiotensin I and II (the major biologically active peptides of RAS), which could exert their functions through AT1R and AT2R (Angiotensin II type 1 and type 2 receptor) [15]. RAS is involved in blood pressure regulation, inflammation, oxidative stress, and insulin resistance. In recent years, it has been found that RAS is closely related to DN. The efficacy of blocking RAS in the treatment of DN has also been confirmed, and RAS inhibitors were currently advocated as first-line therapy for diabetes patients exerting signs of renal damage [16, 17]. However, the association between Gal and RAS in DN has not been reported.

Therefore, this study explores the molecular mechanism through which Gal regulated RAS, modulating PI3K/AKT/mTOR signaling pathway, mediating oxidative stress inhibition, and alleviating the damage of renal tubular epithelial cells under high glucose (HG) condition.

Materials and Methods

Cell culture and treatment

Human renal tubular epithelial cells HK-2 were purchased from ATCC and cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal bovine serum at 37°C with 5% CO_2. For DN *in vitro* model, cells were cultured under 15, 30, 45 mM glucose for 48 h. Normal glucose (5.5 mM) or osmotic control (OS; 5.5 mM glucose and 24.5 mM mannitol) was set as control for HG. Gal was purchased from Shanghai Yuan Ye Biological Technology Co., Ltd. (Shanghai, China) and application of 10 μM concentration (obtained from our previous experiments, not shown) in later experiments.

Cell transfection

siRNA of AT1R (CUAAAGAAGGCUUAUGAAATTUUUCAUAAGCCUUCUUUAGTT) and scrambled control (NC, UUCUCCGAACGUGUCACGUTTACGUGACACGUUCGGAGAATT) were provided by GenePharma (Shanghai, China). For overexpression of AT1R, plasmid containing cDNA of AT1R was constructed using pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). HG-treated cells were transfected using lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. Subsequent experiment was conveyed 24 h post transfection, unless indicated elsewhere.

Cell viability assay

Cell viability was analyzed by Enhanced Cell Counting Kit-8 (CCK-8; Beyotime Biotechnology, Shanghai, China) following manufacturer's instruction. Briefly, 2000 cells were seeded into each well of a 96-well plate with 100 μl medium and 10 μl CCK-8 solution. After 4 h of incubation, absorbance at 450 nm was detected by a microplate reader.

Flow cytometry for cell apoptosis analysis

After treatment, cells were collected, washed with phosphate-buffered saline (PBS), and dissolved with staining buffer (PBS, 0.01% NaN_3). The concentration was adjusted to 1×10^7 cells/ml. Propidium iodide (PI) and Annexin-V-FITC (Sigma-Aldrich, USA) were mixed with 100 μl of cells, and incubated at dark place for 15 min following manufacturer's instruction. Then, cells were collected by centrifugation again, and washed with staining buffer. Signal was detected by a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA).

Western blot

Cells were collected and lysed by RIPA lysis buffer (Sigma-Aldrich, Massachusett, USA). Briefly, cells were collected by centrifugation at 1000 rpm for 4 min, and every 10^7 cells were lysed with 1 ml RIPA buffer on ice for 20 min. Samples were centrifuged again at 12 000 rpm for 15 min, and supernatants were protein solution. Proteins were subjected to 10% SDS-PAGE. After separation, proteins were transferred to nitrocellulose membranes and blocked by 5% BSA in TBST buffer for 1 h at room temperature followed by incubation with primary antibodies at 4°C overnight. After wash, membranes were incubated for 1 h with secondary antibodies at room temperature. Membranes were then washed three times by TBST and signals were detected by enhanced chemiluminescence (ThermoFisher, Waltham, MA, USA). Antibodies of Bcl-2 (1:1000), Bax (1:1000), cleaved caspase3 (c-caspase3, 1:1000), AKT (1:1000), phosphorylated AKT (p-AKT, 1:1000), ERK (1:1000), p-ERK (1:2000), P38 MAPK (1:1000), p-P38 MAPK (1:2000), JNK (1:1000), p-JNK (1:2000), and GAPDH (1:1000) were all from Cell Signaling Technology (CST, Danvers, MA, USA). Renin (1:1000), mTOR (1:2000), p-mTOR (1:1000), angiotensin-converting enzyme (ACE, 1:1000), and AT1R (1:1000) antibodies were obtained from Abcam (Cambridge, UK).

Quantitative real-time polymerase chain reaction (qPCR)

Collected cells were lysed with TRIzol (Invitrogen, CA, USA) according to the manufacturer's instruction and extracted

cells total RNA. RNAs were stored at −80°C. We next synthesized first strand cDNA using PrimeScript RT reagent Kit (Takara, China) according to instruction. The qPCR reaction was performed on Applied Biosystems 7500 Real Time PCR System (ThermoFisher, Waltham, MA, USA) with 2× SYBR Green master mix kit (ThermoFisher, Waltham, MA, USA). Relative expression of AT1R was analyzed using the $2^{-\Delta\Delta Ct}$ method and internal control was GAPDH. Primers of AT1R were synthesized and the sequences were (Sequence 5′ > 3′): AT1R-Forward: GGAAACAGCTTGGTGGTGAT, AT1R-Reverse: CCAGCGGTATTCCATAGCTG; GAPDH-Forward: CCAGGTGGTCTCCTCTGA, GAPDH-Reverse: GCTGTAGCCAAATCGTTGT.

Reactive oxygen species (ROS) detection

Dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay was performed as previously described [18]. Briefly, cells in a 6-well plate were treated with PBS containing 5 μM DCFH-DA. After 30 min incubation, wash and digest cells and make a cell suspension at a concentration of 10^7 cells/ml. Signal intensity was then observed by flow cytometry.

Malondialdehyde (MDA) and superoxide dismutase (SOD) analysis

MDA levels in treated HK-2 cells were determined by using Micro MDA Assay Kit (Solarbio, Beijing, China) and SOD activity was measured through SOD assay kit (Solarbio, Beijing, China) following manufacturer's instructions.

Statistical analysis

Statistical analysis was performed by using Prism 6.0 (GraphPad Software, USA). All experiments were done in triplicates at least. Student's *t* test or one-way analysis of variance (ANOVA) was performed between two groups or among multiple groups. For multiple comparison, if necessary, Tukey *post hoc* test was subsequently applied. The results were represented as mean ± standard derivation (SD). $P < 0.05$ was considered statistically significant (* indicates $P < 0.05$; ** indicates $P < 0.01$).

Results

HG induces oxidative stress injury and RAS activation in renal cells

In order to investigate whether DN-induced renal injury is related to oxidative stress and RAS activation, we treated HK-2 cells with HG to establish an *in vitro* DN model, and detected changes of related markers.

Viability of these cells was detected by MTT assay. Results indicated that compared with NG (normal glucose, 5.5 mM), HG decreased cell viability in a dose-dependent manner (Fig. 1A). Apoptosis was also significantly increased by HG (30 mM) compared with OS (osmotic control) when analyzed by flow cytometry (Fig. 1B). To confirm this finding, we detected protein level of apoptosis related Bcl-2, Bax and c-caspase3 by western blot. HG significantly upregulated the expression of Bax and c-caspase3 and downregulated the expression of Bcl-2 (Fig. 1C). We also detected ROS level, MDA level, and SOD activity in this DN model to assess oxidative stress and found that HG induced ROS production, increased MDA level, and reduced SOD activity (Fig. 1D and E). To analyze RAS activation, we evaluated expression of Renin, ACE, and AT1R by both qPCR and western blot. The results showed that HG treatment upregulated the expression of Renin, ACE, and AT1R (Fig. 1F). These results made clear that oxidative stress and RAS activation are involved in the development of DN.

Gal alleviates HG-induced oxidative stress and RAS activation

In order to investigate whether Gal could regulate oxidative stress, activate RAS and alleviate renal cell injury, cells were treated with Gal in the absence or presence of HG (30 mM) for 48 h. Changes of apoptosis, oxidative stress, and RAS-related indicators were then analyzed.

As shown, Gal significantly inhibited the decrease of cell viability induced by HG (Fig. 2A), and suppressed the expression of Renin, ACE, and AT1R (Fig. 2B). Gal also remarkably suppressed HG-induced apoptosis, downregulated expression of Bax and c-Caspase3 and upregulated expression of Bcl-2, as reflected by FACS and western blot results (Fig. 2C and D). As for assessment of oxidative stress, Gal evidently restrained HG-induced ROS production, MDA increase, and the SOD activity inhibition (Fig. 2E and F). All these results indicated that Gal may relieve HG-induced renal cell damage by inhibiting oxidative stress and RAS activation.

Gal improves oxidative stress injury of HG-induced kidney cells by inhibiting RAS activation

For further exploration whether Gal regulates oxidative stress and alleviates kidney cell injury by inhibiting RAS activation, we transferred AT1R overexpression plasmid into HG-induced cells.

Firstly, we detected AT1R expression by qPCR, and confirmed that pcDNA3.1-AT1R significantly upregulated AT1R expression (Fig. 3A). The expression level of AT1R which was downregulated by Gal under HG stimulation was also reversed by AT1R overexpression (Fig. 3B). Flow cytometry results showed that overexpression of AT1R significantly abolished the inhibitory effect of Gal on cell apoptosis (Fig. 3C). We then detected the protein levels of apoptosis-related proteins and results showed that Gal could significantly inhibit the expression of Bax and c-Caspase3 and upregulate the expression of Bcl-2. Transfection of pcDNA3.1-AT1R reversed these effects, which was consistent with the results of flow cytometry (Fig. 3D). In Fig. 3E and F, we found that Gal could significantly inhibit ROS production and MDA levels, and enhance SOD activity. Overexpression of AT1R under Gal treatment reversed these changes. These results may provide stronger evidence that Gal could regulate oxidative stress by inhibiting RAS activation, thereby alleviating renal cell injury.

RAS modulates oxidative stress injury of HG-treated renal cells by activating PI3K/AKT/mTOR signaling pathway

Next, we investigated the expression of related signaling pathways and the changes of renal injury indicators by knocking down AT1R with si-AT1R. Downregulation of AT1R was verified in Fig. 4A. Then, expression of AT1R, p-AKT, and p-mTOR was detected by western blot. It was found that HG increased the expression of AT1R, p-AKT, and p-mTOR, while AT1R knockdown markedly decreased the expression of these proteins (Fig. 4B).

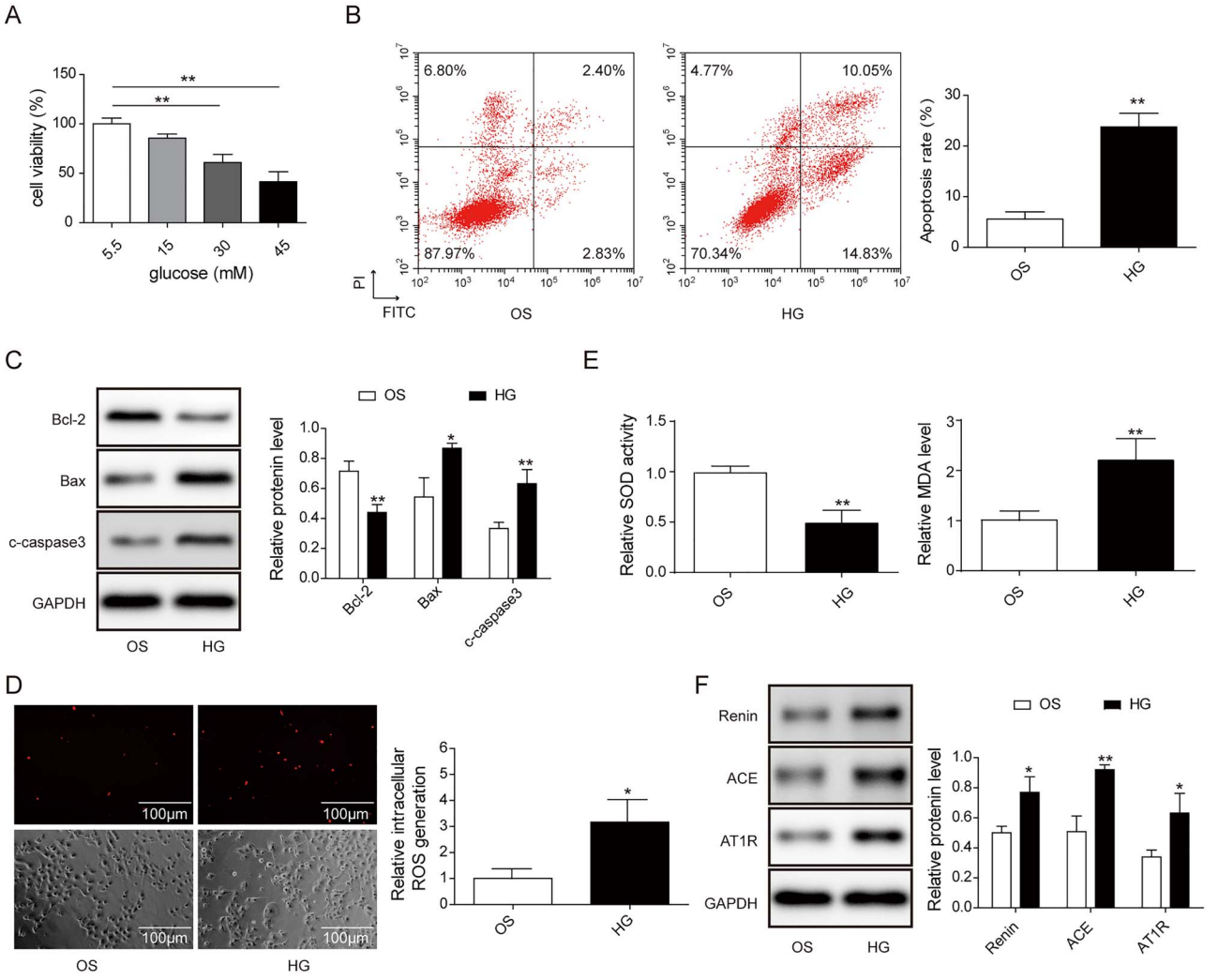

Figure 1: High glucose induces oxidative stress injury and RAS activation in renal cells. (A) HK-2 cells were cultured under increasing glucose concentrations as indicated and detected for viability by CCK8 assay. (B) Apoptosis of HK-2 cells under osmotic control or high glucose condition were detected by FACS. (C) Protein levels of Bcl-2, Bax, and cleaved caspase3 were detected by western blot. (D) DCFH-DA staining was allowed to determine ROS level. (E) The kits were used to test MDA level and SOD activity. (F) The protein levels of Renin, ACE, and AT1R in HK-2 cells were detected by western blot. The data are shown as the mean ± SD. * indicates $P < 0.05$; ** indicates $P < 0.01$.

Moreover, silencing AT1R could suppress HG-induced apoptosis (Fig. 4C) and inhibit expression of Bax and c-caspase3, while promote expression of Bcl-2 (Fig. 4D). We then examined the production of ROS, MDA level, and SOD activity. The results showed that si-AT1R remarkably inhibited the production of ROS, the increase of MDA level and the decrease of SOD activity induced by HG (Fig. 4E and F). These results suggested that RAS activation may modulate oxidative stress injury of renal cells through PI3K/AKT/mTOR signaling pathway.

Gal represses HG-induced oxidative stress in renal cells via PI3K/AKT/mTOR signaling by regulating RAS activation

Finally, we attempted to further verify whether Gal can regulate PI3K/AKT/mTOR signaling pathway by affecting RAS, and ultimately mediate oxidative stress inhibition to alleviate HG-induced renal tubular epithelial cell injury. Compared with Control group (HG-induced cells), Gal inhibited the expression of p-AKT and p-mTOR, while administration of AKT activator SC79 (200 nM, Sigma) resisted the effect of Gal (Fig. 5A). We next assessed apoptosis by flow cytometry and found that Gal could inhibit HG-induced apoptosis, while SC79 reversed such inhibition (Fig. 5B). The changes of apoptosis-related proteins were consistent with this finding. Gal significantly decreased the expression of Bax and c-Caspase3 and upregulated Bcl-2, while SC79 partially reversed these changes (Fig. 5C). Meanwhile, SC79 significantly inhibited the effects of Gal on HG-induced ROS production, MDA level, and SOD activity (Fig. 5D and E). These results suggested that Gal may suppress oxidative stress injury of renal cells induced by HG through RAS and PI3K/AKT/mTOR signaling.

Discussion

DN is a serious complication of diabetes mellitus which usually lead to terminal renal failure. Meanwhile, DN could also increase morbidity and mortality of cardiovascular diseases [19]. Controversial with the clinical pathological features, management or prevention of DN remained a puzzle for clinicians [20]. Therefore,

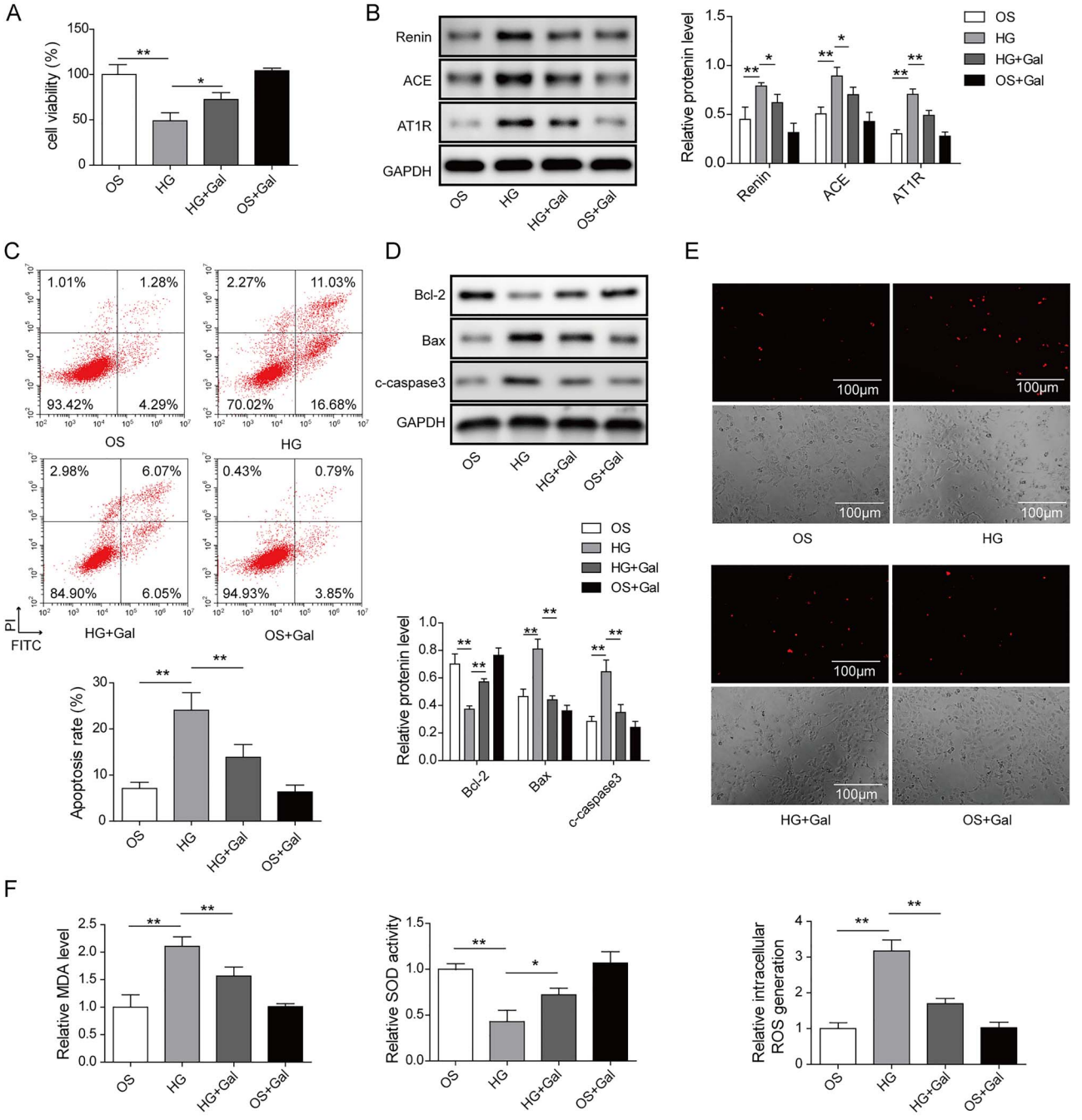

Figure 2: Galangin alleviates high glucose-induced oxidative stress and RAS activation. HK-2 cells were treated with high glucose and galangin as indicated. (A) Cell viability was measured by CCK8 assay. (B) The protein levels of Renin, ACE, and AT1R were detected by western blot. (C, D) Cell apoptosis were detected by FACS and protein levels of Bcl-2, Bax, and cleaved caspase3 were measured by western blot. (E, F) The ROS and MDA levels, SOD activity were determined by DCFH-DA staining and commercial kits. The data are shown as the mean ± SD. * indicates $P < 0.05$; ** indicates $P < 0.01$.

investigating on the molecular mechanisms by which DN was regulated was an unmet need. In this study, we revealed that Gal could alleviate HG-induced renal cell oxidative stress injury, which is the case of renal damage in DN patients, via regulating RAS activation and PI3K/AKT/mTOR signal axis.

We observed increased apoptosis and apoptotic proteins, together with increased ROS, MDA, and decreased SOD activity under HG condition, indicating that HG induced oxidative stress injury in renal cells, proving that we successfully induced *in vitro* DN model. Elevated Renin, ACE, and AT1R expression demonstrated that DN was accompanied with RAS activation. This was in agreement with previous studies that RAS activation was usually observed in DN [21, 22], and supported our hypothesis that RAS was involved in DN progression. Interfering RAS activation therefore could suppress DN development. This notion was proved by our results, when RAS activation was resisted by Gal. The consequence of RAS inhibition was consistent with previous report that DN could be prevented when RAS was repressed by neprilysin or myeloid differentiation 2 blockade [23, 24]. The association between Gal and RAS, however,

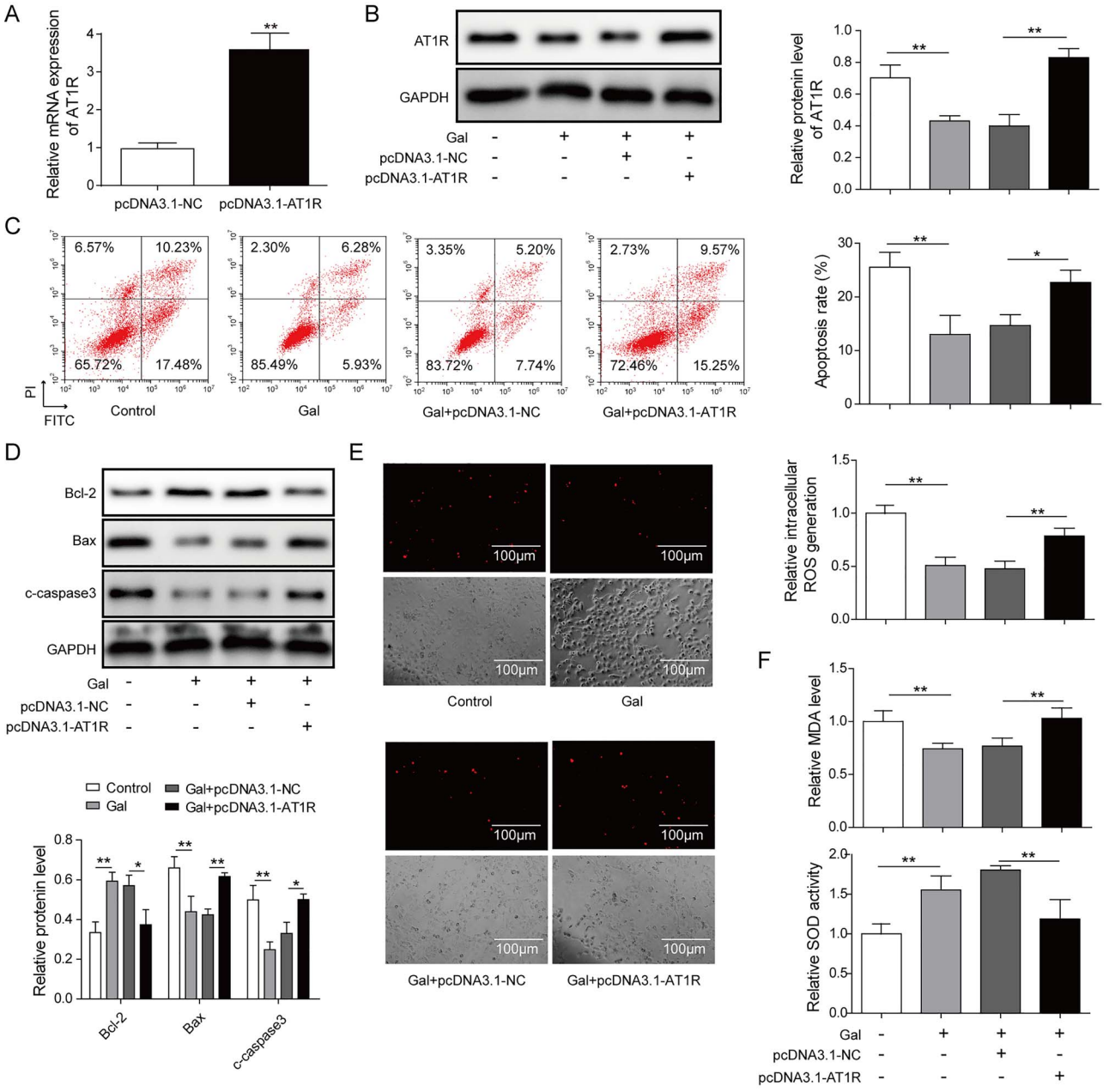

Figure 3: Galangin improves high glucose-induced oxidative stress injury of kidney cells by inhibiting RAS activation. HK-2 cells induced by high glucose were treated with pcDNA3.1-AT1R and/or galangin as indicated. (A) The expression level of AT1R in pcDNA3.1-AT1R transfected cells was detected by qPCR. (B) The protein level of AT1R was measured via western blot. (C) Apoptosis of cells were analyzed by FACS. (D) Protein levels of Bcl-2, Bax, and cleaved caspase3 were assessed by western blot. (E, F) DCFH-DA staining and commercial kits were used to assess ROS, MDA levels, and SOD activity. The data are shown as the mean ± SD. * indicates $P < 0.05$; ** indicates $P < 0.01$.

was firstly revealed by our study. Although Gal and RAS were indecently proven critical in DN regulation, our results firstly uncovered the inhibitory effect of Gal on RAS activation under HG condition. It was reported that flavonoids could suppress RAS cascade that mediated oxidative stress [25]. Consistently, we also found Gal repressed HG-induced oxidative damage in HK-2 cells via blocking RAS activation.

Gal has various biological functions besides RAS inhibition, such as PTEN activation [26] or inhibition of NF-κB and NLRP3 [27]. It was not clear whether Gal inhibited DN progression (as indicated by alleviated apoptosis and oxidative stress) through RAS inhibition. The AKT pathway activation is closely related to cell injury induced by oxidative stress, and its downstream mTOR could affect cell proliferation. RAS mainly regulated cell damage induced by HG via angiotensin II-AT1R signal through PI3K/AKT/mTOR pathway. Previous study also found that mTOR pathway was involved in DN [28]. When AT1R was knocked down or cells were treated with AKT activator SC79, we could both observe antagonizing effects to Gal. However, the effects of PI3K/AKT in DN were controversial. For instance, chronic activation of the AKT/mTOR signaling contributed to oxidative stress and cell apoptosis [15], which was consistent with our results.

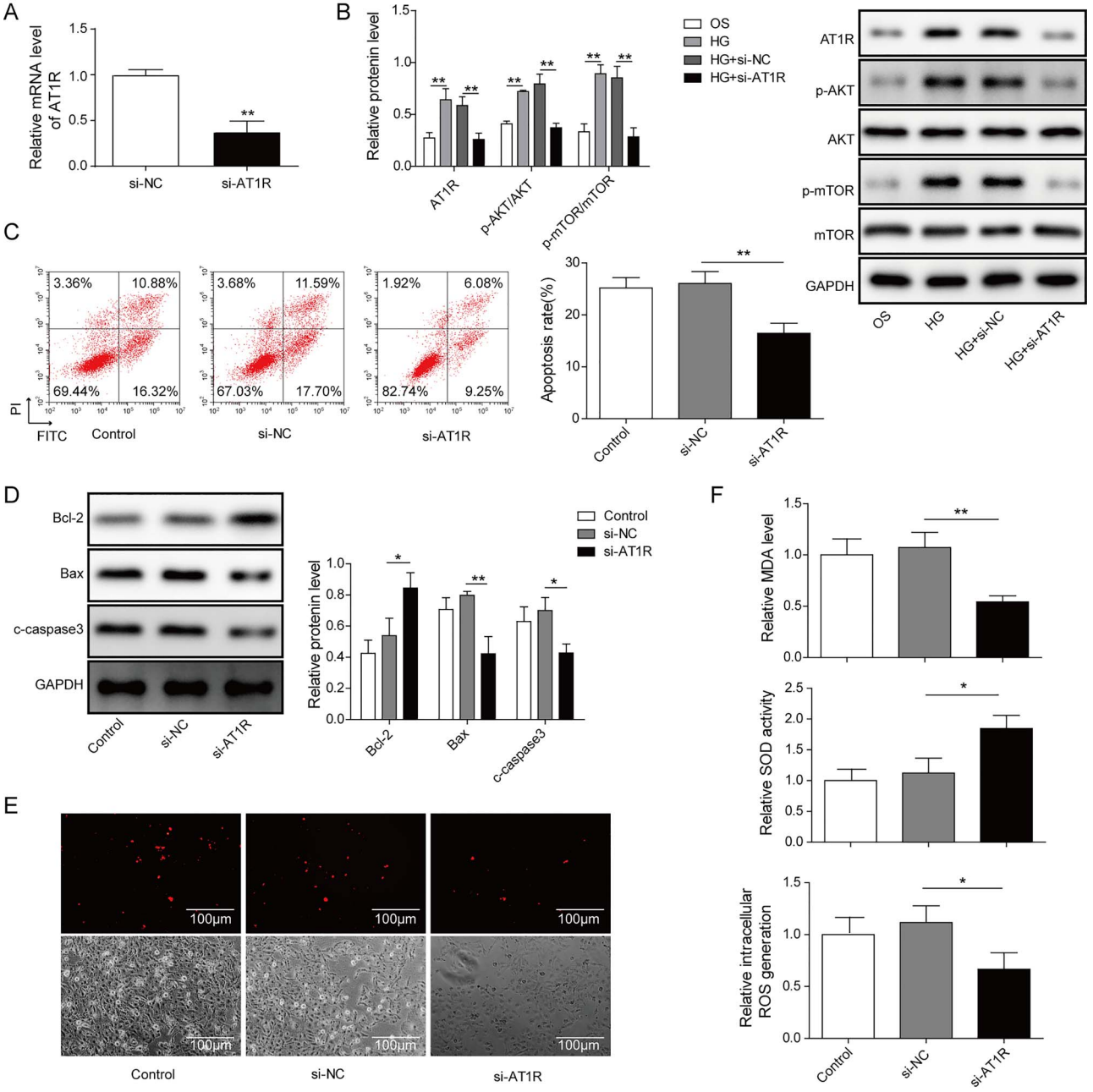

Figure 4: RAS modulates oxidative stress injury of HG-treated renal cells by activating PI3K/AKT/mTOR signaling pathway. HK-2 cells were treated with si-AT1R and high glucose as indicated. (A) The expression level of AT1R in si-AT1R transfected cells was detected by qPCR. (B) Protein levels of AT1R, p-AKT, and p-mTOR were analysed by western blot. Then, high glucose-treated cells were transfected with si-NC or si-AT1R. (C, D) Apoptosis and protein levels of Bcl-2, Bax, and cleaved caspase3 were detected by FACS and western blot, respectively. (E, F) ROS, MDA levels, and SOD activity were detected by DCFH-DA staining and commercial kits. The data are shown as the mean ± SD. * indicates $P < 0.05$; ** indicates $P < 0.01$.

Meanwhile, other papers indicated that activation of PI3K/AKT signal could increase cell viability and inhibit apoptosis under HG condition [29]. This might be caused by different regulatory mechanism and signal networks triggered by various conditions the authors used in their researches. Regardless of this unsolved question, these findings could suggest that RAS repressed by Gal activated PI3K/AKT/mTOR was most important in HG-induced oxidative stress injury of renal cells. However, since Gal is a natural flavonoid instead of artificial compound, it might affect a complexed signal network. Therefore, Gal might regulate oxidative stress partially via suppressing AT1R. Besides, AT1R could also affect MAPK signal pathway, which was also involved in oxidative stress regulation [30, 31]. We also examined whether Gal could affect AT1R/MAPK signal. It turned out that Gal could also regulate apoptosis and oxidative stress via affecting MAPK signal (Fig. S1). Hence, the whole signal network still remains to be studied. In addition, the functional roles and mechanisms of Gal in DN model *in vivo* deserve further study in the future. It is also unknown the mechanisms by which Gal or HG regulates RAS activation.

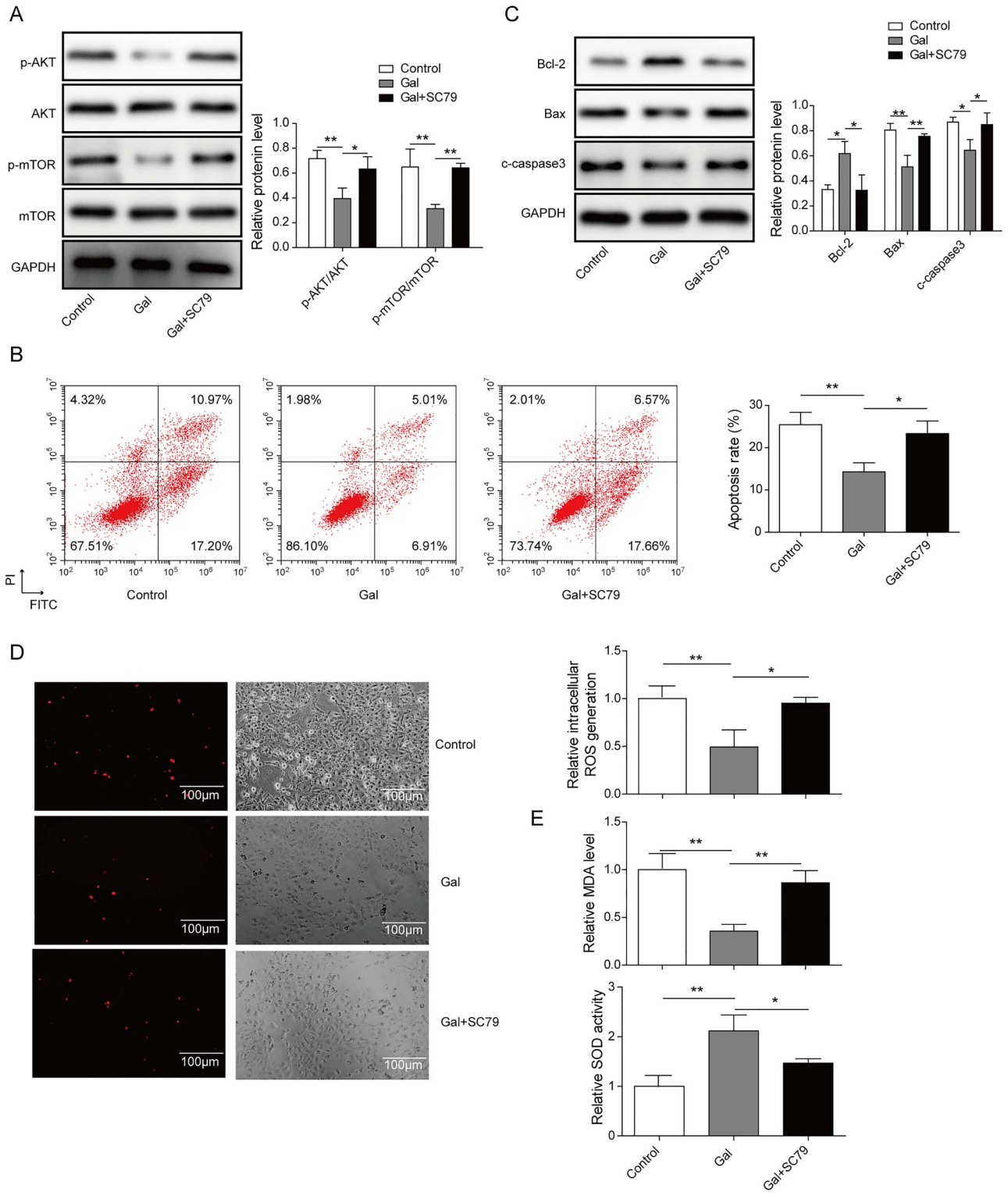

Figure 5: Galangin represses high glucose-induced oxidative stress in renal cells by regulating RAS/PI3K/AKT/mTOR signaling. High glucose-induced HK-2 cells were treated with SC79 and/or galangin as indicated. (A) The protein levels of AKT, p-AKT, p-mTOR, and mTOR were detected by western blot. (B, C) Cell apoptosis and protein levels of Bcl-2, Bax, and c-caspase3 were determined by FACS and western blot. (D, E) DCFH-DA staining and commercial kits were allowed to measure ROS and MDA levels, and SOD activity. The data are shown as the mean ± SD. * indicates $P < 0.05$; ** indicates $P < 0.01$.

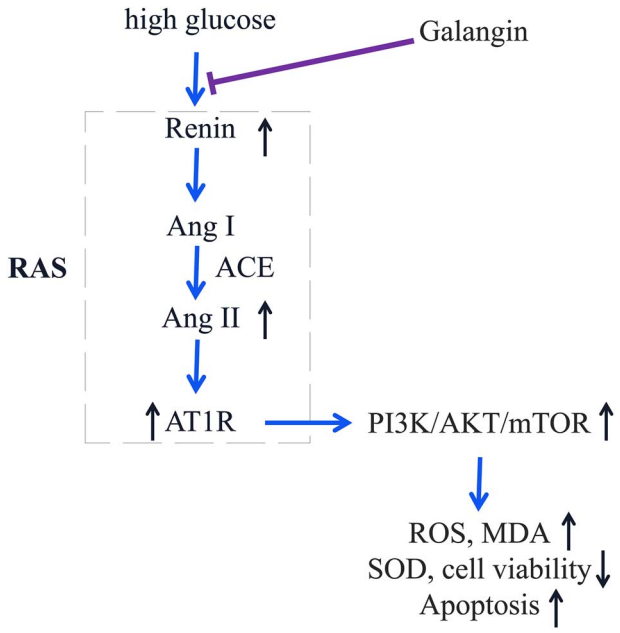

Figure 6: The schematic diagram of such a regulatory process in this study. Galangin alleviates HG-induced oxidative stress injury of HK-2 cells through PI3K/AKT/mTOR signal via modulating RAS activation.

Conclusion

In this study, we demonstrated that Gal could inhibit oxidative stress and alleviate HG-induced renal cell apoptosis. We also revealed that Gal exerted such functions through inhibiting RAS activation. Of all signal pathways involved in DN regulation, Gal may inhibit DN progression mainly by inhibiting RAS activation and PI3K/AKT/mTOR signal pathway. This was the first description of the inhibitory function of Gal in DN progression *in vitro*, and was the first description of its detailed mechanism (Fig. 6). Our research would provide powerful support for future studies about DN regulation, and may provide potential targets for development of new therapeutic methods.

Supplementary data

Supplementary data is available at *TOXRES* Journal online.

Authors' Contribution

Conception and study design: J.L. and B.L.; Data acquisition: K.C. and S.H.; Data analysis: Z.-Y.L., Y.-X.L. and Z.-M.Y.; Manuscript drafting: M.Z.; Manuscript revising: X.C.

Acknowledgements

This work was supported by Natural Science Foundation of Hunan Province (2019JJ40506). We would like to give our sincere gratitude to the reviewers for their constructive comments.

Conflict of Interests

The authors declare that they have no conflict of interests.

Declarations

Ethical Approval

Not Applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for Publication

Not Applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Availability of Data and Material

All data generated or analyzed during this study are included in this article [and its supplementary information files]. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Papadopoulou-Marketou N, Chrousos GP, Kanaka-Gantenbein C. Diabetic nephropathy in type 1 diabetes: a review of early natural history, pathogenesis, and diagnosis. *Diabetes Metab Res Rev* 2017;**33**.
2. Flyvbjerg A. The role of the complement system in diabetic nephropathy. *Nat Rev Nephrol* 2017;**13**:311–8.
3. Parving HH, Hommel E, Mathiesen E et al. Prevalence of microalbuminuria, arterial hypertension, retinopathy and neuropathy in patients with insulin dependent diabetes. *Br Med J (Clin Res Ed)* 1988;**296**:156–60.
4. Zhang J, Yang S, Li H et al. Naringin ameliorates diabetic nephropathy by inhibiting NADPH oxidase 4. *Eur J Pharmacol* 2017;**804**:1–6.
5. Donate-Correa J, Martin-Nunez E, Muros-de-Fuentes M et al. Inflammatory cytokines in diabetic nephropathy. *J Diabetes Res* 2015;**2015**:948417.
6. Aloud AA, Chinnadurai V, Govindasamy C et al. Galangin, a dietary flavonoid, ameliorates hyperglycaemia and lipid abnormalities in rats with streptozotocin-induced hyperglycaemia. *Pharm Biol* 2018;**56**:302–8.
7. Aloud AA, Veeramani C, Govindasamy C et al. Galangin, a dietary flavonoid, improves antioxidant status and reduces hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats. *Redox Rep* 2017;**22**:290–300.
8. Wen SY, Chen JY, Weng YS et al. Galangin suppresses H2 O2 -induced aging in human dermal fibroblasts. *Environ Toxicol* 2017;**32**:2419–27.
9. Choi MJ, Lee EJ, Park JS et al. Anti-inflammatory mechanism of galangin in lipopolysaccharide-stimulated microglia: critical role of PPAR-gamma signaling pathway. *Biochem Pharmacol* 2017;**144**:120–31.
10. Wang HB, Huang SH, Xu M et al. Galangin ameliorates cardiac remodeling via the MEK1/2-ERK1/2 and PI3K-AKT pathways. *J Cell Physiol* 2019;**234**:15654–67.
11. Li Y, Tong L, Zhang J et al. Galangin alleviates liver ischemia-reperfusion injury in a rat model by mediating the PI3K/AKT pathway. *Cell Physiol Biochem* 2018;**51**:1354–63.
12. Kalhotra P, Chittepu V, Osorio-Revilla G et al. Discovery of Galangin as a potential DPP-4 inhibitor that improves insulin-stimulated skeletal muscle glucose uptake: a combinational therapy for diabetes. *Int J Mol Sci* 2019;**20**:1228.

13. Sivakumar AS, Anuradha CV. Effect of galangin supplementation on oxidative damage and inflammatory changes in fructose-fed rat liver. *Chem Biol Interact* 2011;**193**:141–8.
14. Sivakumar AS, Viswanathan P, Anuradha CV. Dose-dependent effect of galangin on fructose-mediated insulin resistance and oxidative events in rat kidney. *Redox Rep* 2010;**15**:224–32.
15. Sparks MA, Crowley SD, Gurley SB et al. Classical renin-angiotensin system in kidney physiology. *Compr Physiol* 2014;**4**:1201–28.
16. Dounousi E, Duni A, Leivaditis K et al. Improvements in the Management of Diabetic Nephropathy. *Rev Diabet Stud* 2015;**12**:119–33.
17. Bolignano D, Pisano A, Coppolino G. The dark side of blocking RAS in diabetic patients with incipient or manifested nephropathy. *Exp Clin Endocrinol Diabetes* 2016;**124**:350–60.
18. Aranda A, Sequedo L, Tolosa L et al. Dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay: a quantitative method for oxidative stress assessment of nanoparticle-treated cells. *Toxicol In Vitro* 2013;**27**:954–63.
19. Rossi L, Gesualdo L. Diabetic nephropathy and cardiovascular risk. *G Ital Nefrol* 2017;**34**:104–18.
20. Bose M, Almas S, Prabhakar S. Wnt signaling and podocyte dysfunction in diabetic nephropathy. *J Invest Med* 2017;**65**:1093–101.
21. Leite APO, Aragao DS, Nogueira MD et al. Modulation of renin angiotensin system components by high glucose levels in the culture of collecting duct cells. *J Cell Physiol* 2019;**234**:22809–18.
22. Rahimi Z. The role of renin angiotensin aldosterone system genes in diabetic nephropathy. *Can J Diabetes* 2016;**40**:178–83.
23. Malek V, Sharma N, Sankrityayan H et al. Concurrent neprilysin inhibition and renin-angiotensin system modulations prevented diabetic nephropathy. *Life Sci* 2019;**221**:159–67.
24. Wang Y, Fang Q, Jin Y et al. Blockade of myeloid differentiation 2 attenuates diabetic nephropathy by reducing activation of the renin-angiotensin system in mouse kidneys. *Br J Pharmacol* 2019;**176**:2642–57.
25. Wunpathe C, Potue P, Maneesai P et al. Hesperidin suppresses renin-angiotensin system mediated NOX2 overexpression and Sympathoexcitation in 2K-1C hypertensive rats. *Am J Chin Med* 2018;**46**:751–67.
26. Zou WW. Xu SP. Galangin inhibits the cell progression and induces cell apoptosis through activating PTEN and Caspase-3 pathways in retinoblastoma. *Biomed Pharmacother* 2018;**97**:851–63.
27. Lu H, Yao H, Zou R et al. Galangin suppresses renal inflammation via the inhibition of NF-kappaB, PI3K/AKT and NLRP3 in uric acid treated NRK-52E tubular epithelial cells. *Biomed Res Int* 2019;**2019**:3018357.
28. Huang C, Zhang Y, Kelly DJ et al. Thioredoxin interacting protein (TXNIP) regulates tubular autophagy and mitophagy in diabetic nephropathy through the mTOR signaling pathway. *Sci Rep* 2016;**6**:29196.
29. Chen F, Sun Z, Zhu X et al. Astilbin inhibits high glucose-induced autophagy and apoptosis through the PI3K/Akt pathway in human proximal tubular epithelial cells. *Biomed Pharmacother* 2018;**106**:1175–81.
30. Gao Y, Kang L, Li C et al. Resveratrol ameliorates diabetes-induced cardiac dysfunction through AT1R-ERK/p38 MAPK Signaling pathway. *Cardiovasc Toxicol* 2016;**16**:130–7.
31. Xie X, Deng T, Duan J et al. Exposure to polystyrene microplastics causes reproductive toxicity through oxidative stress and activation of the p38 MAPK signaling pathway. *Ecotoxicol Environ Saf* 2020;**190**:110133.