Original Paper

Rapamycin Induces Autophagy and Reduces the Apoptosis of Podocytes Under a Stimulated Condition of Immunoglobulin A Nephropathy

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Key Words

Rapamycin • Autophagy • Podocyte • Immunoglobulin A nephropathy • Light chain 3

Abstract

Background/Aims: The aim of this study was to investigate the potential renoprotective effect of rapamycin on the autophagy of podocytes treated with the supernatant of mesangial cells cultured with aggregated IgA1 (aIgA1) from immunoglobulin A nephropathy (IgAN) patients. **Methods:** Monomeric IgA1 (mIgA1) was isolated from the serum of IgAN patients or healthy volunteers, and then transformed to aIgA1 by heating. Subsequently, the aIgA1-mesangial cell supernatant was prepared by collecting the medium of mouse mesangial cells (MSC1097) cultured with aIgA1 (100 mg/L) from different IgAN patients or healthy volunteers for 48 h. Subsequently mouse podocytes (MPC5) were exposed to the supernatant of the aIgA1-mesangial cells for 24 h, using 100 mg/L aIgA1 from healthy volunteers as the control group or 100 mg/L aIgA1 from IgAN patients as the IgANs group, in RPMI 1640 medium. The MPC5 cells in the IgANs+Rap group were cultured with rapamycin (10 nmol/L) and the supernatant of MSC-1097 cells cultured with aIgA1 from IgAN patients in RPMI 1640 medium. Autophagy was assessed by western blot analysis (LC3, p62), electron microscopy, and immunofluorescence staining (LC3, p62, and CD63). The apoptosis of podocytes was evaluated by flow cytometry, and the expression of apoptosis-associated proteins cleaved-caspase-3 and caspase-3 were determined by western blot analysis. **Results:** Deficient autophagy, which was evident by decreased LC3-II and CD63 levels, caused accumulation of p62, and fewer autophagosomes were observed in the MPC5 cells cultured with the IgAN supernatant, along with stronger expression of cleaved caspase-3 and a higher apoptosis rate. Inhibition of autophagy was alleviated in the IgANs+Rap group. The LC3-II/LC3-I ratio increased by almost 30%, the accumulated p62 amount was reduced by 50%, and the number of autophagosomes per podocyte increased to about 7 times that of the IgAN groups. These results were confirmed

by immunofluorescence staining. In addition, the apoptosis rate of MPC5 cells decreased from 19.88% in the IgAN group to 16.78% in the IgANs+Rap group, which was accompanied by a weaker expression level of cleaved caspase-3. **Conclusions:** Rapamycin can reduce the apoptosis of podocytes by inducing autophagy in IgAN.

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Introduction

Rapamycin, also known as sirolimus, belongs to a novel class of immunosuppressive drugs that inhibit mammalian target of rapamycin (mTOR), which plays a central role in the regulation of cell proliferation, growth, differentiation, migration, and survival. Recently, rapamycin has been widely utilized to induce autophagy in several types of cells *in vitro*, such as leukemia cells [1], neurons [2], and podocytes [3].

Autophagy is a highly conserved lysosomal pathway, which plays a role in the recycling of the cytosol and removal of superfluous or damaged organelles. In fact, autophagy regulates the survival, differentiation, development, and homeostasis of many types of cells. Accordingly, dysregulated autophagy is associated with the pathogenesis of many diseases, including cancer, neurodegeneration, heart disease, and certain kidney diseases [4]. Therefore, autophagy is important for quiescent and terminally differentiated cells such as neurons and glomerular epithelial cells [5]. Podocytes, also called visceral glomerular epithelial cells, are a type of terminally differentiated cell, and their homeostasis is dependent on functional autophagy. Thus, maintenance of podocyte health and function is important for the glomerular filtration in the kidney. Any significant damage to the podocytes such as apoptosis [6] and rheological influence [7] may induce irreversible glomerular injury [8]. A previous study [9] using podocyte-specific transgenic mice with deletion of the *Atg5* gene eloquently demonstrated the importance of autophagy to podocytes. Under pathophysiological conditions, the loss of autophagy in podocytes appears to result in a dramatically increased susceptibility to glomerular disease, thereby highlighting the particular importance of autophagy as a critical homeostatic mechanism for maintaining podocyte integrity, not only under physiological conditions but also under stressful conditions.

Several lines of evidence have revealed that podocyte injury plays an important role during the progression of immunoglobulin A nephropathy (IgAN). IgAN accounts for the vast majority of primary glomerulonephritis cases and manifests with extremely variable clinical signs and histopathological features. IgAN is mainly characterized by the presence of a mesangioproliferative lesion in the glomeruli with IgA-containing immune deposits in the mesangium. The glomerular IgAs from biopsies of IgAN patients almost always belong to the IgA1 subclass and are often polymeric. Intriguingly, they are poorly glycosylated, and exhibit a defect of galactose molecules, which are usually linked to O-glycans in the hinge region. Moreover, the blood levels of defective glycosylated IgA1 molecules are higher in IgAN patients than in healthy subjects [10]. Lai et al [11] demonstrated the important role of tumour necrosis factor- α and transforming growth factor- β (TGF- β) secreted from the mesangial cells to activate podocytes exposed to the supernatant of mesangial cells cultured with aggregated IgA1 (algA1) from IgAN patients. Similarly, a platelet-activating factor receptor antagonist could prevent the nephrin loss in podocytes [12]. Podocyte detachment from the glomerular basal membrane has been reported in IgAN patients [13]. Indeed, podocytes could be detected in the urine of IgAN patients, and the level of podocytopenia is tightly associated with the pathological severity of the associated glomerular lesions [14]. Another study [15] showed that IgA1-IC in IgAN patients might up-regulate the production of chemokine (C-X-C motif) ligand 1 (CXCL1) and TGF- β from the mesangial cells. This suggests that CXCL1 and TGF- β might exert a synergistic effect on podocytes, inducing their dysfunction and death.

The *Fas* mRNA expression level and the apoptosis of podocytes, exposed to the supernatant of mesangial cells cultured with algA1 from IgAN patients, were reported to

significantly increase when compared to those of control cells [16]. A previous study found that both aIgA1 from IgAN patients and the supernatant of mesangial cells incubated with aIgA1 from IgAN patients suppressed the nephrin expression of podocytes [17].

However, the characteristics of podocyte autophagy in IgAN have not been clarified in detail, and little is known about the effect of rapamycin on the autophagy of podocytes in IgAN. Rapamycin is a well-known stimulator of autophagy. Therefore, we hypothesized that rapamycin may improve the inhibition of podocyte autophagy in IgAN. To test this hypothesis, we prepared aIgA1 by heating monomeric IgA1s (mIgA1s) obtained from IgAN patients, and utilized them to culture mouse mesangial cells (MSC1097), and finally harvested the supernatant of the MSC1097 culture, which was used to incubate mouse podocytes (MPC5), alone or combined with rapamycin. We further observed the effect of rapamycin on podocyte autophagy under the experimental conditions. These results should provide new insights into the renoprotective effect of rapamycin on the autophagy of podocytes *in vitro*, and suggest new treatment options to prevent or slow the progression of IgAN.

Materials and Methods

aIgA1 purification

This study was approved by the local ethics committee and all participants provided informed consent. Pooled serum from three healthy volunteers or 20 IgAN patients was directly separated from the whole blood (20 mL per individual), double-diluted with phosphate-buffered saline (PBS) (pH = 7.4), filtered with a 0.2- μ m syringe-driven filter, and subjected to Jacalin Agarose affinity chromatography. The eluent was collected, concentrated, and subjected to Sephacryl S-200 molecular sieve chromatography. Three peaks were evident, which were attributed to the following proteins: the first peak was aIgA1, the second peak was mIgA1, and the last one was a non-IgA1 Jacalin-binding protein such as α -HS glycoprotein. Subsequently, these fractions were pooled separately, concentrated and filtered. Because the amount of aIgA1 recovered from the purification process was not sufficient for further analyses, mIgA1 was transformed to aIgA1 by heating. First, mIgA1 was kept at 63°C for 150 min and then centrifuged at 4°C at 12,000 rpm for 5 min. The supernatant was added to a Sephacryl S-200 molecular sieve column and washed slowly by a buffer solution containing 0.15 mol/L NaCl and 0.05 mol/L Na₃PO₄. Finally, the purified aIgA1 was confirmed by western blotting and stored at –70°C for further analysis.

Cell culture

Mouse podocytes (MPC5, Tong Pai Bio-tech Co., Ltd, Shanghai, China) were maintained in RPMI 1640 medium (HyClone, Pittsburgh, PA, USA) containing 10% heat-inactivated fetal calf serum (Gibco, Waltham, MA, USA), 100 U/mL penicillin (Gibco), and 100 μ g/mL streptomycin (Gibco) in a humidified atmosphere with 5% CO₂. MPC5 cells were cultured and expanded by growth in a medium containing 50 U/mL mouse interferon-gamma (IFN- γ , Peprotech, Rocky Hill, NJ, USA) at 33°C with 100% relative humidity and 5% CO₂, and were induced to differentiate by culture at 37°C in a medium without IFN- γ for 10–14 days. All experiments were performed on podocytes differentiated for 10–14 passages in the present study. Then, the MPC5 cells were exposed to the supernatant of mouse mesangial cells (MSC1097, Tong Pai Bio-tech Co., Ltd.) that had been cultured for 24 h with 100 mg/L aIgA1 from healthy volunteers as the control, or with 100 mg/L aIgA1 from IgAN patients as the IgANs group, in RPMI 1640 medium containing 0.5% heat-inactivated fetal calf serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin, in a humidified atmosphere with 5% CO₂. The MPC5 cells in the IgANs+Rap group were cultured with rapamycin (10 nmol/L) and the supernatant of MSC-1097 cells of the IgANs group, in RPMI 1640 medium. After co-culture for 24 h, the podocytes were harvested for further experiments.

Immunofluorescence staining

To investigate the autophagy activity of podocytes exposed to the supernatant of cultured mesangial cells with aIgA1 from IgAN patients, we examined the changes in the abundances of autophagy-related proteins in podocytes. Microtubule-associated protein light chain 3 (LC3), a soluble protein in mammalian cells, is found in two forms: LC3-I and LC3-II. As the conjugated product of LC3-I and phosphatidyl ethanolamine, LC3-II is a critical protein for the process of autophagy. Therefore, an elevated expression

level of LC3-II or the LC3-II /LC3-I ratio is considered as the typical and standard marker for detecting the activation of autophagy. In general, LC3-II binds to the autophagosomal membrane and produces puncta when an autophagosome is generated. Thus, immunofluorescence staining can be performed to evaluate the LC3-II distribution and monitor the autophagy of a cell. CD63, also known as lysosomal-associated membrane protein-3, localizes on the granule membranes of basophils, mast cells, platelets, and so on. Most importantly, CD63 is considered to be a widely used marker of secretory lysosomes and correlates well with the formation of autolysosomes. Therefore, we also examined the expression of CD63 by immunofluorescence staining. Cells grown on glass cover slips were fixed with 4% paraformaldehyde for 10 min at room temperature and permeabilised with PBS containing 1% Triton X-100. After washing twice with PBS, the cells were blocked with 3% bovine serum albumin in PBS for 30 min and incubated with a primary antibody (1:200, Abcam, Cambridge, MA, USA) in blocking buffer overnight at 4°C. Next, the cells were stained with the Alexa Fluor 488-conjugated Affinipure anti-goat secondary antibody (1:500, San Ying Bio-tech Co., Ltd, Wuhan, China). Finally, after counterstaining with 4,6-diamidino-2-phenylindole (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 10 min, the cells were observed and imaged using a laser-scanning confocal microscope (Perkin Elmer&Olympus, Waltham, MA, USA). All experiments were performed a minimum of three times.

Western blot analysis

The levels of LC3-I, LC3-II, p62, cleaved-caspase-3, and caspase-3 of MPC5 cells in the different treatment groups were determined by western blot analysis to evaluate the autophagy and apoptosis status and confirm the results of immunofluorescence. Cell proteins were extracted by the addition of a lysis buffer at 4°C. The suspension was centrifuged at 12,000 rpm for 5 min, and media containing cellular proteins were collected. For western blot, a 6–15% sodium dodecyl sulphate polyacrylamide gel was run under standard electrophoresis conditions, with 30µg of total protein loaded in each lane. The gel was placed in transfer buffer for 15 min and set up for transfer to a nitrocellulose membrane (Millipore, Temecula, CA, USA) at 200 mA for 2 h. The membrane was rinsed in Tris-buffered saline followed by rinsing in blocking buffer (5% lipid-free milk powder) for 2 h. The membrane was then incubated with primary antibodies (LC3, p62, rabbit polyclonal antibody, dilution 1:1000; cleaved-caspase-3, caspase-3, and GAPDH, rabbit polyclonal antibody, dilution 1:5000; San Ying Bio-tech Co., Ltd.) at 4°C overnight. After rinsing in wash buffer, the membrane was incubated in horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit, dilution 1:3000; San Ying Bio-tech Co., Ltd.) for 1 h. After a final wash, the membrane was developed using ECL Chemiluminescence Reagent (Millipore). The levels of the target protein relative to that of the control GAPDH were analysed by densitometric scanning using Gel-Pro Application.

Transmission electron microscopy investigation

We also observed the presence of autophagosomes in the podocytes of the different treatment groups under a transmission electron microscope. Podocytes were trypsinised and collected into centrifuge tubes after washing with PBS. Then, the cells were fixed in 3% glutaraldehyde at 4°C for 48 h, and dehydrated by dimethylketone. After embedment in Epon-812, the samples were cut into ultrathin sections (1µm) and dyed with uranium acetate and plumbum citrate. The samples were observed with transmission JEM-1400 plus electron microscopy. The autophagosomes were counted in approximately 10 randomly selected podocytes for each group to obtain the average number of autophagosomes per podocyte.

Apoptosis assays by flow cytometry

Autophagy is suggested to play a cytoprotective role against podocyte apoptosis. To verify this hypothesis and investigate the potential mechanism, we also examined the apoptosis rate of podocytes in the different groups. The percentages of apoptotic cells in the different groups were determined using an FITC-Annexin V/PI Apoptosis Detection Kit, according to the manufacturer instructions (Shanghai BeiBo Biotech, Shanghai, China). Podocytes were trypsinised and resuspended in binding buffer. Annexin V staining was applied for 10 min at room temperature and 5 µl of propidium iodide was added before analysis. The analysis was performed with the FAC Scan flow cytometer (BD Biosciences, Franklin Lanes, NJ, USA).

Statistical analysis

Western blotting, immunofluorescence staining, and flow cytometry were all repeated at least three times independently. The statistical analyses were performed using the SPSS 19.0 statistical software

Fig. 1. Immunofluorescence of autophagy markers including LC3-II, p62 and CD63. A Immunofluorescence analysis of autophagy markers. B Average optical density of autophagy markers in Immunofluorescence. This figure shows that autophagy was down-regulated in MPC5 cells cultured with the supernatant of MSC1097 treated with algA1 from IgAN patients. The staining of LC3-II and CD63 decreased but p62 was enhanced in cells, compared with the CON. This phenomenon was alleviated in IgANs+Rap (Magnification = $\times 1000$). * $P < 0.05$ versus the CON, # $P < 0.05$ versus the IgANs.

program. Data are expressed as means $\pm$ SEM unless otherwise stated. Data were analysed by repeated-measures analysis of variance followed by the Student t-test. P -values < 0.05 were considered statistically significant.

Results

Podocytes in the IgANs group exhibited autophagy dysfunction.

As shown in Fig. 1, obviously bright puncta were observed in the cytoplasm of MPC5 cells in the control group, which were much more scarce in the IgANs group. Similar to LC3-II, the average optical density of CD63 in the IgANs group was decreased in comparison with that of the control. Concurrently, obvious accumulation of p62 was observed in podocytes of the IgANs group. To verify the deficient autophagy detected by immunofluorescence, we also performed western blotting of LC3-II and p62 (Fig. 2). The results showed that the LC3-II / LC3-I ratio of MPC5 cells exposed to the supernatant of MSC1097 cells cultured with algA1 from IgAN patients was decreased significantly, with about half of the level observed in the control ($P < 0.05$). By contrast, the MPC5 cells in the IgANs group showed a significantly increased expression level of p62, which was almost 7-times higher than that of the control. Therefore, the statistical results from western blotting for LC3 were in agreement with the findings of immunofluorescence staining. Evidently, all of the data revealed attenuated autophagy activity in MPC5 cells of the IgANs group. This conclusion was further supported by the transmission electron microscopy observations. As shown in Fig. 3, the autophagosome was detected as a condensed ribosome present with limited membranes and some lipid droplets. There were significantly fewer autophagosomes in podocytes treated with the supernatant of mesangial cells cultured with algA1 from IgAN patients, along with swollen mitochondria (white arrow in Fig. 3), compared to the control cells ($P = 0.02$).

Podocytes of the IgANs group showed an elevated percentage of apoptosis.

Figure 4 demonstrates that the percentage of apoptotic cells was increased from 14.47% in the control to 19.88% in IgANs group ($P < 0.05$). Simultaneously, the expression level of cleaved caspase-3 in the IgANs group, determined by western blot, was significantly increased in comparison with that in the other groups (Fig. 5). Collectively, these two lines of evidence suggested that impaired autophagy resulted in the apoptosis of podocytes *in vitro*.

Fig. 2. Western blot of autophagy-related protein including LC3 and p62. A Western blot analysis of autophagy-related protein. B Densitometry of these protein signals in western blots. Deficient autophagy was exhibited in IgANs, which was featured with decreased LC3-II/I and accumulated p62. Autophagy of MPC5 was induced by rapamycin in IgANs+Rap in comparison with the IgANs. * $P < 0.05$ versus the CON, # $P < 0.05$ versus the IgANs.

Fig. 3. Transmission Electronic microscopy investigation. The result shows that there were significantly fewer autophagosomes (black arrows) along with swelling mitochondria (white arrow) in podocytes treated with supernatant of mesangial cells cultured with aIgA1 from IgAN patients, compared to the Con. However, podocyte in IgANs+Rap exhibited more autophagosomes than that in IgANs. * $P < 0.05$ versus the CON, # $P < 0.05$ versus the IgANs. Magnification = $\times 20000$.

Fig. 4. Measurement of apoptosis by flow cytometry. A Flow cytometry Annexin V/PI staining for measuring apoptosis after 24 h exposure of MPC-5 cells to the supernatant of mesangial cell. B The corresponding linear diagram of flow cytometry. The IgANs presented a significant increase in the rate of podocytes apoptosis when compared to the CON. In addition, rapamycin reduced the apoptosis of MPC5 cells. * $P < 0.05$ versus the CON, # $P < 0.05$ versus the IgANs.

Rapamycin, an mTOR inhibitor, induced autophagy and alleviated the apoptosis of podocytes

As expected, after being co-cultured with rapamycin and the mesangial supernatant of the IgAN patients, the podocytes exhibited enhanced induction of autophagy, which was indicated by a markedly elevated LC3-II/LC3-I ratio ($P = 0.011$ vs. the IgANs group, Figs. 1 and 2), and a significantly mitigated expression level of p62 ($P = 1.486E-7$ vs. the IgANs group). Podocytes from the Rap+IgANs group exhibited more autophagosomes in comparison with those of the IgANs group ($P = 0.001$), further indicating that rapamycin promotes the autophagy of podocytes (Fig.3). Moreover, as shown in Fig. 4, the increase in the apoptosis of IgAN podocytes was alleviated from 19.88% to 16.78%. Accordingly, the ratio of cleaved caspase-3 to caspase-3 also decreased significantly in the Rap+IgANs group in comparison with the IgANs group (Fig. 5, $P < 0.05$).

Discussion

IgAN is usually a persistent and progressive disease [18]. However, 20–40% of IgAN patients progress to end-stage renal disease within 20 years, and an additional 20% show progressive kidney damage [19]. Therefore, it should not be regarded as a benign disorder.

The functional role of autophagy in the kidneys has drawn heated debate among nephrologists. Autophagy was reported to be renoprotective in several animal models such as aging and acute kidney injury models [20]. Recently, autophagy was revealed to be particularly important for the maintenance of podocytes, which have only a limited capacity for regeneration [21]. For example, aging mice, with podocyte-specific deletion of autophagy-

Fig. 5. Western blot measurement of caspase-3 and cleaved caspase-3. A Western blot analysis of caspase-3 and cleaved caspase-3. B Densitometry of caspase-3 and cleaved caspase-3 signals in western blots. Compared to the CON, an elevated expression level of cleaved caspase-3 was observed in the IgANs group. Similar to the change of apoptosis, the level of cleaved caspase-3 was decreased in the IgANs+Rap group. * $P < 0.05$ versus the CON, # $P < 0.05$ versus the IgANs.

related genes, exhibited proteinuria and glomerulopathy [9]. Angiotensin II-induced autophagy in podocyte could be attenuated by the plant alkaloid sinomenine through the regulation of reactive oxygen species generation [22].

Among the typical proteins used to monitor the autophagy pathway, LC3 is considered as one of the most important molecules involved in forming the autophagosome. During autophagosome formation, LC3-I is processed into a lipidated form, LC3-II. Thus an elevated LC3-II level is regarded as a marker for the activation of autophagy. In addition, the level of p62 is negatively correlated with autophagic activity. Indeed, the characteristic features and pathological mechanism of podocyte autophagy in IgAN remain largely unknown. In this study we found that podocytes cultured with alGA1 from IgAN patients presented dysfunction of autophagy, which was characterized by decreased LC3-II and accumulated p62 levels. Concurrently, in parallel with the result of a previous study [23], in which the supernatant of cultured mesangial cells with IgA1 from IgAN patients induced the expression of *Fas* mRNA and the apoptosis of podocytes, we also found that podocytes in the IgANs group showed a higher apoptosis rate, along with an increased level of cleaved-caspase-3.

It was reported that the early application of the low-dose mTOR inhibitor rapamycin, at 1 mg·kg⁻¹·day⁻¹ for 6 weeks, may slow the progression of IgAN-induced renal injury in rats [24]. The underlying mechanism for the beneficial effect of rapamycin on IgAN is likely to be cell cycle-dependent, because rapamycin inhibited mesangial cell proliferation and arrested the cell cycle in the G1 phase, without affecting the expression of cyclin E and cyclin-dependent kinase [25]. In fact, the same authors found that the Akt/mTOR/p70S6K pathway was activated in the renal cortex of rats with IgAN. Moreover, immunohistochemical analysis showed increased phosphorylation of S6 only in the mesangial region of the glomeruli. However, their study did not consider the relationship between rapamycin and the autophagy of podocytes in IgAN in detail [26]. Autophagy has been shown to engage in a complex interplay with apoptosis. The two processes may be triggered by common upstream signals, resulting in combined autophagy and apoptosis. In some cellular settings, autophagy can serve as a cell survival pathway, suppressing apoptosis [27]. Indeed, in the present study, rapamycin was found to decrease the apoptosis and concurrently improved the autophagy of podocytes induced by IgAN. Therefore we conclude that rapamycin has a partly protective role for podocytes in IgAN. These results are in line with previous studies of podocytes in other diseases. For example, rapamycin attenuated the podocyte injury caused by C5b-9 attack by enhancing autophagy [28].

Inhibition of mTOR has recently emerged as a novel mode of slowing down the progressive course in some experimental chronic kidney disorders models. For example, rapamycin prolonged survival in murine lupus nephritis, with maintained normal renal function, restored nephrin and podocin levels, and ameliorated histological lesions [29]. Similar phenomena were observed in an experimental passive Heymann nephritis (PHN) model of membranous nephropathy, in which rapamycin monotherapy significantly reduced proteinuria and alleviated pathological lesions [30]. This beneficial effect may be mediated by inhibition of the allo-immune response during the autologous phase of PHN and by normalizing the expression of nephrin and podocin in podocytes. However, in some cases, rapamycin has proven to be harmful. Rapamycin-induced renal injury in healthy mice, which was accompanied by an increased mean podocyte foot process width, and reduced the levels of nephrin and podocin proteins [31]. The contrasting actions of rapamycin on experimental renal disease have also been observed in patients. Preventive medication of rapamycin in renal transplant recipients was proven to protect renal function and histology [32]. However, the conversion from calcineurin inhibitor to sirolimus in later stages of chronic allograft nephropathy is often accompanied by adverse effects, including an elevated level of proteinuria and consequent deterioration of kidney function [33]. The reason for this discrepancy may be attributed to the action of mTOR, an important protein involved in many physical and pathological processes.

As a principal component of a complex signalling network, mTOR is highly evolutionarily conserved and is expressed ubiquitously throughout the cells of the body. Inhibitors of mTOR, such as rapamycin or everolimus, bind to an intracellular cytoplasmic receptor, the FK506-binding protein-12, and further exhibit multiple effects, including anti-proliferation, anti-inflammation, anti-fibrosis, and anti-tumour activities. Therefore, the final result, beneficial or harmful, depends on their interaction, types of diseases and cells, stages of the disease and many other unknown factors.

Rapamycin has been reported to play a protective role in progressive IgAN. IgAN patients with a poor prognosis, defined as a glomerular filtration rate within 30–60 mL/min and/or proteinuria >1 g/day, administered low-dose sirolimus (initial dose of 1 mg/day followed by an adjustment within 4–8 ng/mL during blood level monitoring) combined with an angiotensin-converting enzyme inhibitor and statin presented stable renal function and relieved glomerular proliferation [34]. This indicates that the timing, degree of pre-existing disease, and dosage seem to be critical for the outcome of rapamycin treatment. Combining our present *in vitro* results with the findings of this clinical trial, we can speculate, that rapamycin is definitely beneficial, at least in part, in the prevention of the progression of IgAN. Therefore, more and larger clinical studies are needed to further clarify the underlying mechanism, appropriate opportunity, dosage, and period of medication in treating IgAN with rapamycin.

Of course, there are still some shortcomings in this study owing to the limits of time, energy, and the research resource, such as the lack of the dose-dependency curve and the use of murine cells and not human cells. It would be better to use human cell culture systems to extrapolate the results on the human podocyte function. However, the use of murine cells could facilitate the subsequent animal studies in this field. This inadequacy will be improved in our follow-up research.

Conclusion

Our study demonstrated that rapamycin can reduce the apoptosis of podocytes by inducing autophagy in IgAN, and rapamycin is helpful, at least partly, in the prevention of the progression of IgAN.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

Acknowledgments

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