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ORIGINAL ARTICLE

MicroRNA-155 Mediates Obesity-Induced Renal Inflammation and Dysfunction

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Abstract— Chronic inflammation is a major contributor to obesity-related renal damage. Recent studies have demonstrated that microRNA (miR)-155 is closely associated with hyperglycemia-induced nephropathy, but whether renal miR-155 participates in the inflammatory response and development of obesity-related nephropathy is unknown. In present study, we investigated the pathophysiological role of renal miR-155 in palmitic acid (PA)-treated endothelial cell and high-fat-diet (HFD)-fed mouse models by specific miR-155 sponge. Mice fed with HFD exhibited higher levels of renal miR-155, which positively correlated with urine microalbumin and blood urea nitrogen. *In vitro* study, mouse renal vascular endothelial cells stimulated with PA also showed higher miR-155 levels, accompanied with increased inflammatory response. Suppression of renal miR-155 effectively attenuated HFD-induced renal structural damages and dysfunction. MiR-155 sponge treatment also significantly decreased NF-κB signaling and downstream gene expression *in vitro* and *in vivo*. The obesity-increased macrophage infiltration and lipotoxicity was decreased in mouse kidney after miR-155 sponge treatment. Mechanistically, miR-155 directly targeted 3′-UTR of SHIP1/*INPP5D* and suppressed its expression *in vitro* and *in vivo*, whereas silence of SHIP1/*INPP5D* abolished the renal protective benefits of miR-155 sponge in obese mice. Taken together, present findings for the first time provided evidence for the potential role of miR-155 in obesity-related nephropathy and clarified that SHIP1/NF-κB signaling was a potential molecular mechanism.

KEY WORDS: obesity; nephropathy; miR-155; inflammation; SHIP1/*INPP5D*.

INTRODUCTION

The population of obesity is hugely increasing worldwide and is associated with a significant health burden. The obesity initiates and exaggerates the process of many metabolic complications, including microvascular disease, insulin resistance, and hyperglycemia [1, 2]. Obesity-related nephropathy is one of key microvascular complications, which is characterized by structural and functional damage in kidney [3, 4]. Different types of nephropathy share similarities in terms of increased glomerular filtration, microalbuminuria, blood urea nitrogen, and process of renal failure [3, 4]. To protect the renal function in the obesity-associated nephropathy, it is urgent need to explore the underlying molecular mechanism.

Therefore, more detailed understanding of the development for nephropathy is needed. Both inflammatory response and oxidative stress are main phenotypes of

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obesity-related nephropathy [5–7]. Excess nutrition, especially fatty acids, directly enters and damages the renal glomerular and tubular [8, 9]. Saturated free fatty acids can bind to toll-like receptors (TLRs) resulting in the activation of the nuclear factor (NF)-κB inflammatory signaling, which induce the synthesis and secretion of chemokines, leading to the infiltration of proinflammatory immune cells [10–12]. Meanwhile, an accumulation in inflammatory response can exaggerate the production of free radicals, which represent the oxidative stress [13, 14]. Further support for inflammation to contribute to renal disease comes from studies where immunosuppressive strategies reduce renal inflammatory response and attenuate the development of diabetic nephropathy [15–17]. For example, the SH-2 containing inositol 5′ polyphosphatase 1 (SHIP1), encoded by *INPP5D* gene, functions as a negative regulator of inflammatory response [18, 19]. Overexpression of SHIP is reported to inhibit inflammatory cytokine-induced molecular signaling, including TLR4 and NF-κB pathways [18, 19]. However, the mediators that participate in the development of renal inflammation are not fully disclosed in obese status. Therefore, exploring the regulator in lipotoxicity-mediating renal dysfunction is critical for understanding the pathophysiological process of obesity-related nephropathy.

MicroRNA (miRNA) is a group of ~22-nt length, double-stranded non-coding RNA, which was first discovered in the nematode *Caenorhabditis elegans* [20]. Through selectively binding to complementary target sites of the 3′-UTR of mRNAs, miRNA can cause translational repression and/or mRNA destabilization. Several miRNAs, such as miR-21, 192, and 377, have closely linked to renal injuries [21–23]. Among them, renal miR-155 mediated hyperglycemia-induced nephropathy in type 1 diabetic mice, and positively associated with histological damages in patients with immunoglobulin A nephropathy [24, 25]. Meanwhile, circulating miR-155 was also closely correlated with renal clinical parameters, including estimated glomerular filtration rate (eGFR) and proteinuria [26]. However, whether miR-155 participates the process of obesity-associated nephropathy or the underlying molecular mechanism is still unclear.

Present study aims to explore the potential role of miR-155 in obesity-associated nephropathy *via* cell culture and mouse model studies. Here, we found that not only a close correlation between renal miR-155 and nephropathy parameters but also suppression of miR-155 could effectively improve cellular inflammatory response and renal function in obese mice. Mechanistically, SHIP1/*INPP5D*, an inflammatory suppressor, was a potential direct target gene of miR-155 in mediating renal disorders. Present study comprehensively provides the pathophysiological role of renal miR-155 in obese-related nephropathy.

MATERIALS AND METHODS

Reagents

trichrome Stain (Masson) Kit, hematoxylin, and eosin solution were purchased from Sigma (Louis, MO). Microalbumin and blood urea nitrogen test kits were purchased from Wako (Richmond, VA). Anti-p-IκB, anti-IκB, anti-SHIP1, and anti-GAPDH antibodies were purchased from Cell Signaling (Danvers, MA). Anti-F4/80 and anti-HNE antibodies were purchased from R&D (Minneapolis, MN).

Mouse Endothelial Cell Experiment

The mouse renal microvascular endothelial cells (MECs) were gifted from Dr. Dong Wang (Wenzhou Medical University), and cultured in endothelial cell growth medium. The palmitic acid (PA) was dissolved in fatty acid-free bovine serum albumin (BSA) solution, and the final concentration was 100 mM for storage. For mRNA analysis, the MECs were stimulated with 100 μM PA for 24-h. For protein analysis, the cells were stimulated with 100 μM PA for 30-min.

Animal Experiment

Male C57BL/6J mice, with 6 weeks of age, freely consumed water and chow diet. To induce obesity, mice were fed with high-fat diet which 60% energy from fat (Research Diet, #D12492), while the control mice were fed with chow diet. After high-fat-diet treatment for 20 weeks, the mice were tail-vein injected with microbubbles, which contained 1×10^{10} IU viral particles of lentivirus encoding miR-155 sponge or control vector. For silencing of *INPP5D*, 1×10^{10} IU viral particles of lentivirus encoding *INPP5D* siRNA was tail-vein injected with microbubbles. Then the mice were received ultrasonic irradiation on the kidney area to release the lentivirus. After 4-week treatment, mice were housed in metabolic cages for collecting 24-h urine, then sacrificed under ether anesthesia. Renal tissues were frozen in liquid nitrogen for analyzing gene and protein expression and embedded in 4% paraformaldehyde for structural analysis. In addition, blood samples were collected, then serum was separated and stored in − 80 °C condition until analysis. Protocols used for all

animal studies were approved by the Wenzhou Medical University Animal Policy and Welfare Committee.

Histopathological Analysis

After dehydration, the 5-μm sections were stained with hematoxylin and eosin (H&E) for evaluating the histopathological damage. Besides, the sections were also stained with Masson trichrome for evaluating the fibrosis. For the immunohistological analysis of F4/80 and 4-HNE, dehydrated sections were incubated with 3% H_2O_2 for 30-min, 10% BSA solution for 1-h, primary antibody for overnight, and secondary antibody for 1-h. Then the sections were developed with DAB for 2–5 min. Each image of sections was obtained using a light microscope (Nikon, Japan). The glomerular size and fibrosis content were quantified by ImageJ software (NIH).

Biochemical Analysis

Urine microalbumin and blood urea nitrogen were measured with biochemical reagents according to the manufacturer's instructions.

Gene Analysis

Mouse endothelial cells and renal tissue RNA (30–50 mg) were extracted by using TRIzol (Invitrogen, Shanghai). DNA was synthesized by RNA reverse kit and quantified by RT-qPCR Kit (Invitrogen, Shanghai). The sequence of primers was listed as follows: TNF-α: F-GCCACCACGCTCTTCTGTCTA, R-GATGAGAG GGAGGCCATTTG; IL-6: F-TCCATCCAGTTGCC TTCTTG, R-GGTCTGTTGGGAGTGGTATC; Cox-2: F-CCCTTGGGTGTCAAAGGTAA, R-GCCCTCGC TTATGATCTGTC; iNOS: F-ATGTCCGAAGCAAA CATCAC, R-TAATGTCCAGGAAGTAGGTG; and GAPDH: F-AGGAGCGAGACCCCACTAAC, R-GATGACCCTTTTGGCTCCAC. The relative amount of each gene was normalized to the amount of GAPDH.

Protein Analysis

Mouse endothelial cells and renal tissue protein samples (50 μg) were subjected to 12% SDS-polyacrylamide gel electrophoresis (PAGE) gel, and transferred onto polyvinyldene fluoride membrane (PVDF) (Bio-Rad Laboratory, CA). After blocked in 10% milk blocking buffer for 1.5-h, membranes were incubated with anti-p-IκB, anti-IκB, anti-SHIP1, or anti-GAPDH antibody overnight at 4 °C. Then membranes were washed in TBST and incubated with secondary antibody (Cell Signaling Technology, CA) for 1-h at room temperature. The protein bands were then visualized using enhanced chemiluminescence reagents (Bio-Rad, CA). The band density was quantified using ImageJ.

INPP5D Gene 3′ UTR Luciferase Reporter Assay

To generate *INPP5D* gene 3′ UTR luciferase reporter constructs, the miR-155 binding sites were synthesized by annealing the oligos: *INPP5D* 3′ UTR forward: CTTGCACT GGGCTTCTTAAT GCTTTCACCCCTCCGAACAC ACACCGTTTGGATCCA and *INPP5D* 3′ UTR reverse: TAGTG GATCCTAATTGTGCAGGTACAGGAAT TGTTCCACCAGCATTAGGAACTTTAGCATA. The products were ligated into the pMIR-REPORT vector (Ambion). To create a mutant 3′ UTR, mutations were introduced at two miR-155-seeding sequence regions with the following sites: GC were changed to CG, and UUA were changed to GGC. HEK-293 T cells were transfected with one of the above plasmids using PEI (Polyplus) according to the manufacturer's instruction. Luciferase activity was measured using the Dual-Luciferase Reporter Assay (Promega). Data are presented as ratio of *Renilla* to firefly luciferase activity.

Statistical Analysis

Data were presented as mean ± SD. Student's *t* test was used for comparing two groups, and one-way ANOVA was used for comparing multiple groups. GraphPad Prism 5 (GraphPad, San Diego, CA) was used to analyze the statistical significance between sets of data. Differences were considered to be significant at $p < 0.05$.

RESULTS

Induction of Renal miR-155 Levels Are Positively Associated With High-Fat-Diet-Induced Renal Dysfunction

To determine whether renal miR-155 is involved in the process of obesity-associated nephropathy, we firstly investigated the change of miR-155 expression in kidney of mice fed with high-fat diet (HFD). After HFD treatment for 24 weeks, the miR-155 level was obviously upregulated to 4-fold than lean mice (Fig. 1a). Meanwhile, the linear correlation analysis showed that the renal miR-155 expression was closely correlated with the urine microalbumin ($r = 0.8557$, $p = 0.0004$) and blood urea nitrogen (BUN; $r = 0.8760$, $p = 0.0002$) (Fig. 1b). These results indicated that renal miR-155 might be involved in the process of diet-induced mouse renal dysfunction.

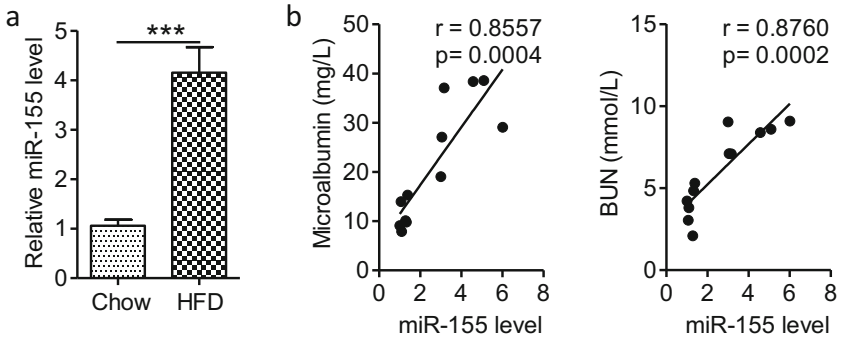

Fig. 1. Induction of renal miR-155 levels are positively associated with high-fat-diet-induced renal dysfunction. Male C57BL/6J mice were fed with high-fat diet (HFD) or chow diet for 24 weeks. **a** The expression of miR-155 in renal tissues; data are shown as mean ± SEM (***$p < 0.001$, $n = 6$ mice/group). **b** Analysis of the level of microalbumin in 24-h urine and blood urea nitrogen (BUN) and correlation with renal miR-155 level ($n = 12$).

Inhibition of miR-155 Decreases Palmitic Acid-Induced Inflammatory and Oxidative Response in MECs

Previous studies have already found that renal miR-155 was primarily localized in glomerular endothelial cells [24, 25], which controlled the renal metabolism. Therefore, we next tested the role of miR-155 in lipotoxicity-induced endothelial cell damages. At first, the renal MECs was stimulated with PA, which initiated the cellular damages, and RT-qPCR analysis showed that PA significantly increased miR-155 levels (Fig. 2a; $p < 0.01$). PA treatment also upregulated the expression of inflammatory (*TNF-α*,

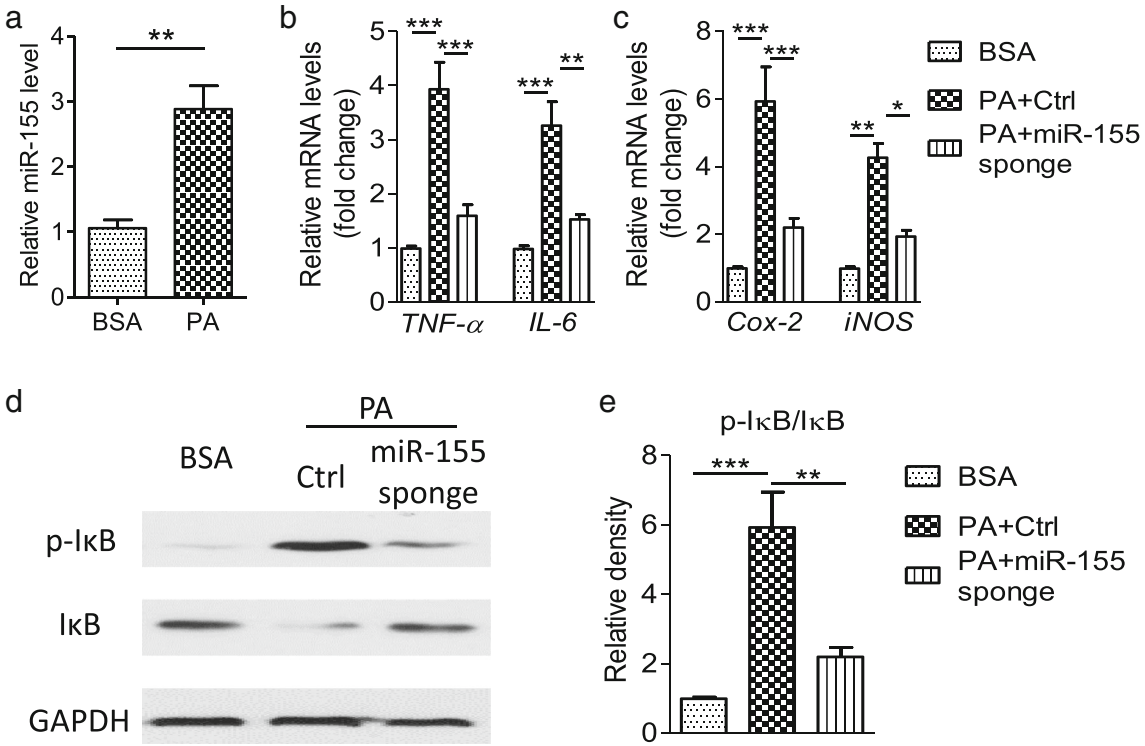

Fig. 2. Inhibition of miR-155 decreases palmitic acid-induced inflammatory and oxidative response in mouse renal microvascular endothelial cells (MECs). **a** The 1.2×10^6 mouse renal MECs were incubated with palmitic acid (PA; 100 μM) for 24-h, and RT-qPCR analysis of cellular miR-155 levels. **b**, **c** Pre-treatment with lentivirus encoding miR-155 sponge or control vector for 24-h, the MECs were incubated with PA for another 24-h. RT-qPCR analysis of cellular inflammatory cytokines, *TNF-α* and *IL-6* (**b**), and oxidative markers, *Cox-2*, and *iNOS* (**c**). **d**, **e** Western blot analysis of protein expression of phosphorylation (p)-IκB and IκB after PA treatment for 30-min, and quantitative analysis of p-IκB/IκB. The GAPDH acts as the loading control. Data are shown as mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 4$ individual experiments).

IL-6) and oxidative (*Cox-2*, *iNOS*) genes (Fig. 2b, c). However, pre-treatment with miR-155 sponge, a specific inhibitor of miR-155, remarkably inhibited the levels of these inflammatory or oxidative genes (Fig. 2b, c). The transcriptional factor, NF-κB, is a key regulator in cellular inflammation and oxidative damages [27]. PA increased the phosphorylation of IκB, further mediating the degradation of IκB, but miR-155 sponge effectively lowered the expression of p-IκB and restored IκB levels in endothelial cells (Fig. 2**d**, **e**; $p < 0.01$).

MiR-155 Sponge Improves High-Fat-Diet-Induced Mouse Renal Dysfunction and Inflammation

Although miR-155 deficiency attenuated hyperglycemia-induced renal damages in type 1 diabetic mice [24], but whether miR-155 inhibition improves obesity-associated nephropathy is unknown. Here, we treated obese mice with lentivirus encoding miR-155 sponge for 4 weeks. Results showed that miR-155 sponge efficiently decreased urine microalbumin (Fig. 3a; $p < 0.001$) and BUN (Fig. 3b; $p < 0.01$), as compared with control vector-treated obese mice. Furthermore, the H&E staining showed that miR-155 sponge attenuated high-fat-diet-induced glomerular enlargement and tubular damage (Fig. 3c, e; $p < 0.001$). The Masson trichrome staining further demonstrated that the fibrosis marker, collagen, was significantly decreased in miR-155 sponge-treated obese mice (Fig. 3d, f; $p < 0.01$). Meanwhile, the miR-155 inhibition in lean mice had no effect on renal function or structure.

Mechanistically, we investigated the inflammatory response after miR-155 sponge treatment in obese mice. As Fig. 4 shows, high-fat diet obviously increased the expression of inflammatory (*TNF-α*, *IL-6*) and oxidative (*Cox-2*, *iNOS*) genes (Fig. 4a, b), whereas miR-155 sponge inhibited these gene upregulation. Besides, miR-155 sponge also decreased the expression of p-IκB, but increased IκB level in renal tissues, as compared with control vector-treated obese mice (Fig. 4c, d; $p < 0.01$). Macrophage infiltration is an important character of nephropathy [28]. Similarly,

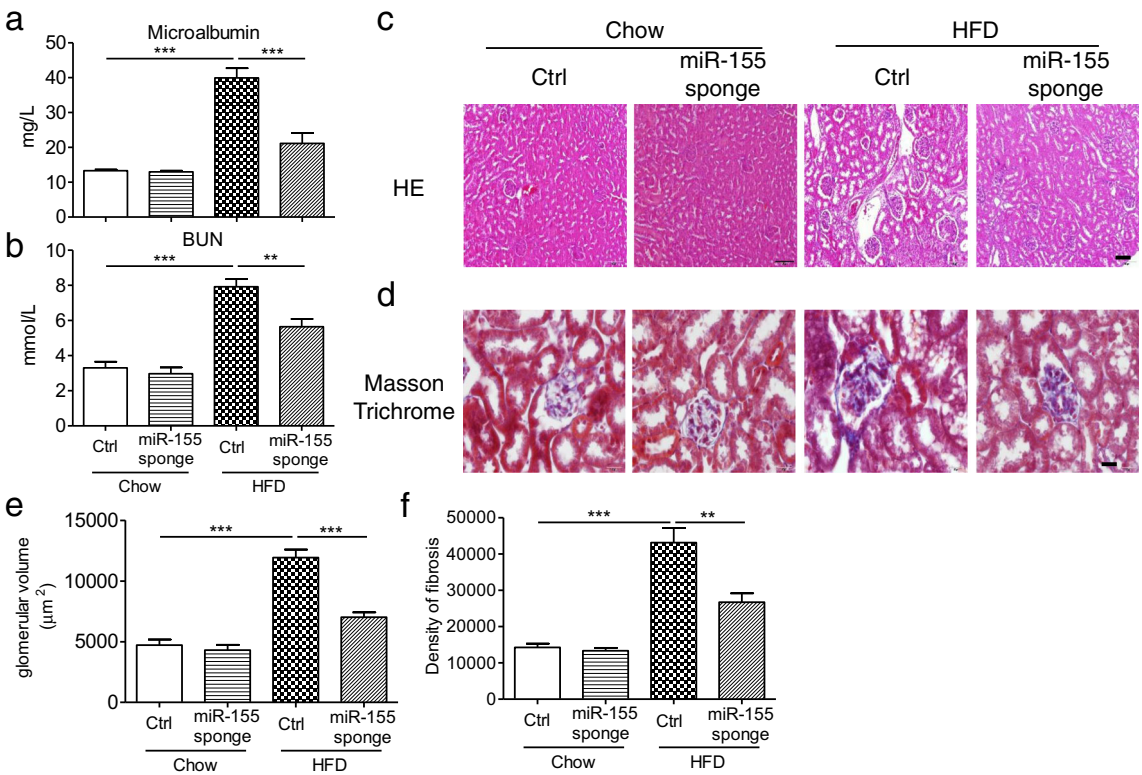

Fig. 3. Inhibition of miR-155 improves high-fat-diet-induced mouse renal dysfunction. Male C57BL/6J mice were fed with HFD or chow diet for 20 weeks, then tail-vein injection with 1×10^{10} IU lentivirus encoding miR-155 sponge or control vector for 4 weeks. Analysis of urine microalbumin (**a**) and BUN (**b**). **c**, **d** Representative images of H&E staining (scale bar = 100 μm) for histopathological analysis and Masson trichrome staining (scale bar = 20 μm) for collagen (blue color) in renal tissues. Quantitative analysis of glomerular volume (**e**) and collagen (**f**). Data are shown as mean ± SEM (**$p < 0.01$, ***$p < 0.001$; $n = 6$/group).

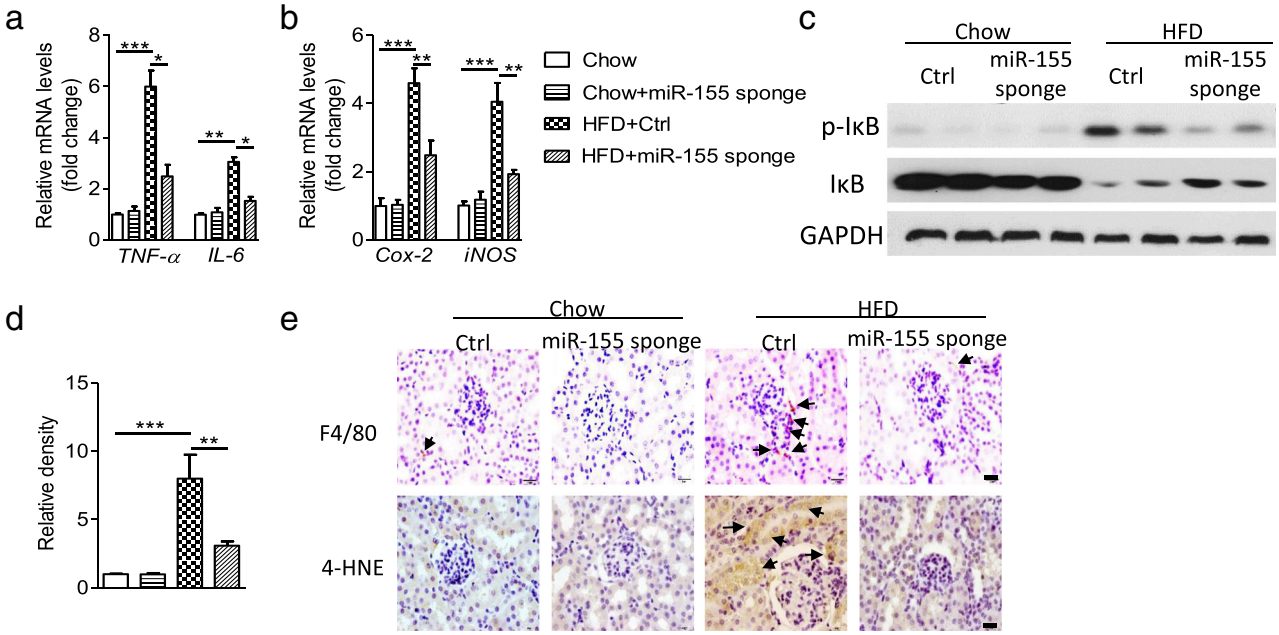

Fig. 4. Inhibition of miR-155 attenuates obesity-induced renal inflammatory response. RT-qPCR analysis of inflammatory cytokines, *TNF-α* and *IL-6* (**a**), and oxidative markers, *Cox-2* and *iNOS* (**b**) in renal tissues. **c**, **d** Western blot analysis of protein expression of phosphorylation (p)-IκB and IκB in renal tissues, and quantitative analysis of p-IκB/IκB. The GAPDH acts as the loading control. Data are shown as mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 6$/group). **e** Representative images of macrophage infiltration marker, F4/80, and lipid oxidative damage marker, 4-hydroxynonenal (HNE) (scale bar = 20 μm).

high-fat diet initiated the F4/80-positive macrophage infiltration into kidney, accompanied with lipid oxidative 4-hydroxynonenal accumulation (Fig. 4e). However, miR-155 inhibition effectively blocked macrophage and lipid metabolite deposit in obese mouse renal tissues (Fig. 4e).

MiR-155 Directly Suppresses the Expression of SHIP1/INPP5D

To investigate the posttranscriptional regulation whereby miR-155 exists in renal function, we focused on Src homology 2-containing inositol phosphatase-1 (SHIP-1/*INPP5D*) because it has been reported to be a possible target of miR-155 in murine myeloid cells [29, 30]. SHIP-1/*INPP5D* exhibited anti-inflammatory benefits, including suppressing NF-κB/Akt signaling, to protect cellular survival [31]. To further confirm the direct interaction between the miR-155 and SHIP-1/*INPP5D*, the dual-luciferase reporter assay was performed. The results showed that the overexpression of miR-155 significantly decreased the luciferase activity in the *INPP5D* 3′-UTR transfected cells, whereas no effect on the mutant *INPP5D* 3′-UTR (Fig. 5a, b; $p < 0.001$). To investigate the inhibition of miR-155 on *INPP5D* in renal MECs, cells were transfected with virus encoding miR-155 mimic or vehicle. The real-time PCR analysis showed that the gene level of *INPP5D* was significantly decreased by overexpression of miR-155 (Fig. 5c; $p < 0.001$). On the contrast, PA-induced *INPP5D* degradation was significantly reversed by miR-155 anti-sense (Fig. 5d; $p < 0.05$). Consistently, mouse treated with miR-155 sponge also prevented high-fat-diet-induced downregulation of renal SHIP1/*INPP5D* gene and protein expression (Fig. 5e–g; $p < 0.05$).

To elucidate the critical role of SHIP1/INPP5D in miR-155-mediated renal injuries in obese mice, we further treated the miR-155 sponge-injected obese mice with lentivirus encoding *INPP5D* siRNA. As shown in Fig. 6a, the upregulation of miR-155 sponge-mediated *INPP5D* mRNA was completely inhibited by treatment of *INPP5D* siRNA. The suppression of *INPP5D* had no effect on kidney weight (Fig. 6b), but increased the levels of urine microalbumin (Fig. 6c; $p < 0.05$) and BUN (Fig. 6d; $p < 0.05$), as compared with miR-155 sponge-treated obese mice. The suppression of *INPP5D* also damaged renal structure, as indicated by enlargement of glomerular size (Fig. 6e, f; $p < 0.01$). Furthermore, the renal inflammatory response and oxidative stress was upregulated in *INPP5D* siRNA-treated obese

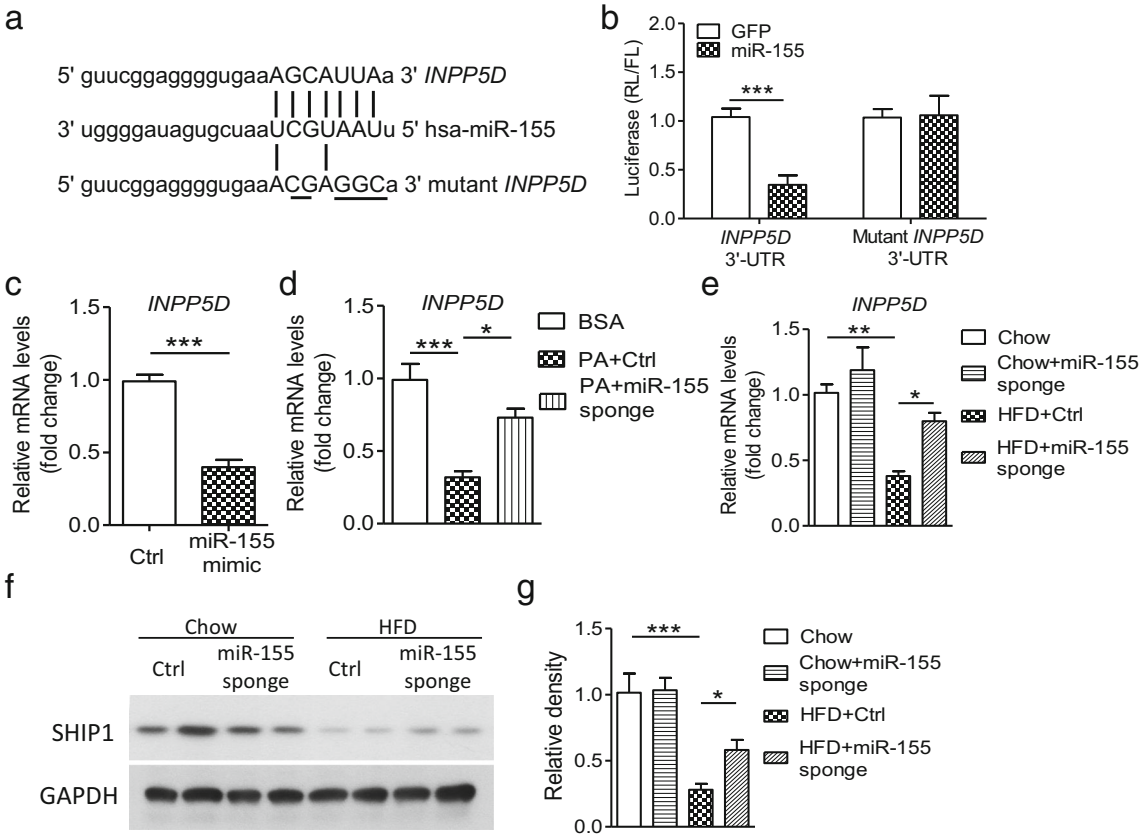

Fig. 5. MiR-155 directly suppresses the expression of SHIP1/*INPP5D*. **a** The potential binding sites in wild-type and mutant *INPP5D* 3′-UTR to miR-155. **b** Luciferase assay measured their binding activities. **c** The 1.2×10^6 mouse renal MECs were incubated with palmitic acid (PA; 100 μM) for 24-h, and RT-qPCR analysis of cellular *INPP5D* level. **d** Pre-treatment with lentivirus encoding miR-155 sponge or control vector for 24-h, the MECs were incubated with PA for another 24-h. RT-qPCR analysis of cellular *INPP5D* level. **e** RT-qPCR analysis of *INPP5D* level in mouse renal tissues. Western blot analysis of SHIP1 (**f**) and quantitative analysis of SHIP1 (**g**). The GAPDH acts as the loading control. Data are shown as mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 4$ individual experiments or 6 mice/group).

mice, as compared with miR-155 sponge-treated obese mice (Fig. 6g, h; $p < 0.05$). Therefore, these results demonstrated that the miR-155-mediated renal inflammatory response was largely through suppressing SHIP1/*INPP5D*.

DISCUSSION

This study supported the critical role of renal miR-155 in regulating inflammatory and oxidative damages in obesity-related nephropathy. The increasing renal miR-155 level was positively associated renal dysfunction. Suppression of renal miR-155 resulted in significant downregulation of palmitic acid-induced endothelial cellular injuries. More importantly, we found that the specific inhibition of renal miR-155 improved high-fat-diet-induced inflammation and dysfunction in mouse kidney. Mechanistically, miR-155 directly suppressed the expression of anti-inflammatory factor SHIP1/*INPP5D*, whereas silence of SHIP1/*INPP5D* abolished the renal protective benefits of miR-155 sponge in obese mice. Present study provided evidence that SHIP1/*INPP5D* was a potential therapeutic target in combating obesity-associated mouse nephropathy.

Nephropathy has traditionally been considered as a nonimmune disease; however, amounts of evidence showed a significant induction of macrophage infiltration in kidneys from obese individuals and experimental animal models [28, 32, 33]. Therefore, the inflammatory response plays a critical role in the development of obesity-related nephropathy. Meanwhile, the macrophage-sourced inflammatory cytokines, including TNF-α and IL-6, are strongly associated with the process of obesity and its complications [28, 32, 33]. Further evidence found that the

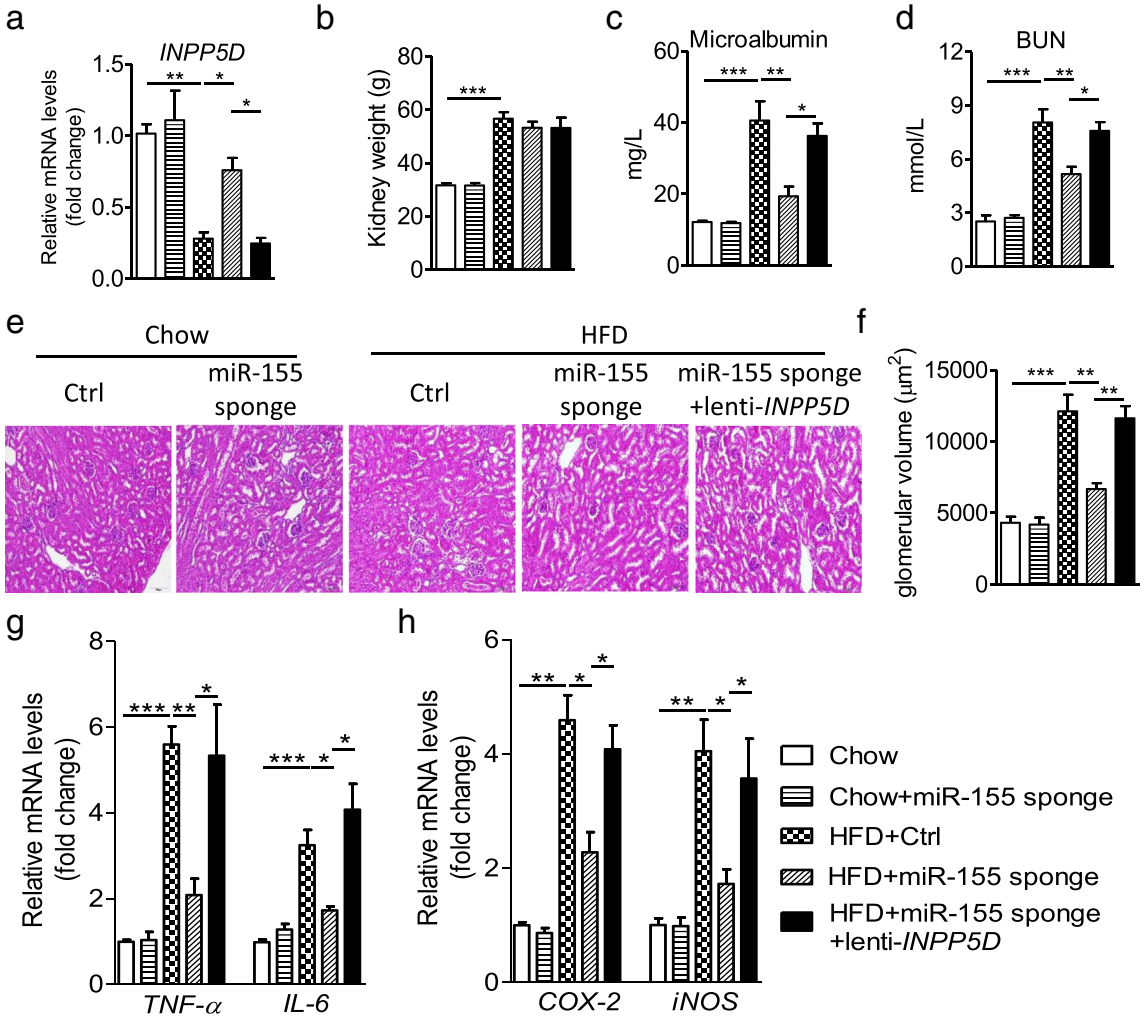

Fig. 6. Silence of *INPP5D* abolishes the renal protective benefits of miR-155 sponge in obese mice. Male C57BL/6J mice were fed with HFD or chow diet for 20 weeks, then tail-vein injection with 1×10^{10} IU lentivirus encoding miR-155 sponge, control, or co-administration with lentivirus encoding *INPP5D* siRNA for 4 weeks. **a** RT-qPCR analysis of *INPP5D* level in mouse renal tissues. The levels of kidney weight (**b**), urine microalbumin (**c**), and BUN (**d**). Representative images of H&E staining (scale bar = 100 μm) for histopathological analysis (**e**) and quantitative analysis of glomerular volume (**f**). **g**, **h** RT-qPCR analysis of gene levels in mouse renal tissues. Data are shown as mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 4$–5 mice/group).

immunosuppressive strategies of decreasing renal macrophage accumulation could effectively attenuate the development of obesity-related nephropathy [15–17]. Besides, increasing findings in both experimental and clinical studies implied that there was a close link between oxidative stress and obesity-related nephropathy [4, 5]. Huge upregulation of oxidative stress contributed to the pathogenesis of diet-induced nephropathy and its progression to end-stage renal disease. Present study showed that suppression of miR-155 effectively decreased palmitic acid- and high-fat-diet-induced macrophage infiltration and inflammation *in vitro* and *in vivo*. This renoprotective effect was consistent with recent report indicating that miR-155 deficiency significantly decreased the hyperglycemia-induced inflammatory IL-17A response [24]. Furthermore, present study firstly supported the critical role of renal miR-155 in inflammatory response and obesity-associated nephropathy.

Previous study demonstrated that miR-155 was obviously induced in primary macrophages after exposure to polyriboinosinic:polyribocytidylic acid or the cytokine IFN-β [34]. Pharmacological inhibition of the NF-κB/JNK blocked the induction of miR-155 in macrophage [34]. These findings provided the close links between miR-155 and transcriptional factors. Interestingly, our

present results showed that suppression of miR-155 could inhibit the phorsphorylation and degradation of IκB, then decreased downstream gene expression. It demonstrated a positive-feedback loop between miR-155 and NF-κB signaling. Previous studies have demonstrated that SHIP1/*INPP5D* was an important regulator of NF-κB/Akt activity [31]. SHIP1/*INPP5D* activator, AQX-1125, effectively inhibited NF-κB-mediated inflammation and oxidative stress [35]. Our luciferase assay and genetic intervention results showed that miR-155 could directly bind to SHIP1/*INPP5D* 3′-UTR, and induce the gene degradation. This finding firstly disclosed the interaction between miR-155 and SHIP1/*INPP5D* in renal endothelial cells. More importantly, silence of *INPP5D* abolished the renal protective benefits of miR-155 sponge in obese mice. Taken together, these results implied that miR-155 activated NF-κB signaling *via* suppressing SHIP1/*INPP5D* level in cell culture and mouse renal tissues.

Previous clinical studies demonstrated that miR-155 was a potential biomarker for immunoglobulin A nephropathy and hyperglycemia-induced renal disease [24, 25]. The expression level of miR-155 was upregulated more than 5-fold in the renal samples from the type 1 diabetic nephropathy patients compared with the controls, and the miR-155 expression was closely correlated with the serum creatinine levels [24, 25]. However, whether miR-155 also exhibited similar role in obesity-related renal dysfunction was still unknown. Present results showed that increasing renal dysfunctional markers, including microalbumin and blood urea nitrogen, were strongly correlated with renal miR-155 levels. On the contrast, specific suppression of renal miR-155 protected high-fat-diet-induced renal structural disorders and dysfunction, including decreasing glomerular enlargement, fibrosis, and the levels of urine microalbumin and blood urea nitrogen.

In summary, the overnutrition-induced renal miR-155 participated in the process of obesity-associated renal inflammation and dysfunction. Specific suppression of renal miR-155 improved diet-induced inflammation, abnormal structural modification, and dysfunction through inhibiting SHIP1/NF-κB signaling in kidney. Taken together, targeting on renal miR-155 is a potential therapeutic approach to combat obesity-associated nephropathy.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflicts of interest.

REFERENCES

1. Grundy, S.M. 2004. Obesity, metabolic syndrome, and cardiovascular disease. *The Journal of Clinical Endocrinology and Metabolism* 89: 2595–2600.
2. Seidell, J.C. 2000. Obesity, insulin resistance and diabetes–a worldwide epidemic. *The British Journal of Nutrition* 83 (Suppl 1): S5–S8.
3. Maric, C., and J.E. Hall. 2011. Obesity, metabolic syndrome and diabetic nephropathy. *Contributions to Nephrology* 170: 28–35.
4. Bayliss, G., L.A. Weinrauch, and J.A. D'Elia. 2012. Pathophysiology of obesity-related renal dysfunction contributes to diabetic nephropathy. *Current Diabetes Reports* 12: 440–446.
5. Elmarakby, A.A., and J.C. Sullivan. 2012. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. *Cardiovascular Therapeutics* 30: 49–59.
6. Wada, J., and H. Makino. 2013. Inflammation and the pathogenesis of diabetic nephropathy. *Clinical Science* 124: 139–152.
7. Lim, A.K., and G.H. Tesch. 2012. Inflammation in diabetic nephropathy. *Mediators of Inflammation* 2012: 146154.
8. Lennon, R., D. Pons, M.A. Sabin, C. Wei, J.P. Shield, R.J. Coward, J.M. Tavare, P.W. Mathieson, M.A. Saleem, and G.I. Welsh. 2009. Saturated fatty acids induce insulin resistance in human podocytes: Implications for diabetic nephropathy. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 24: 3288–3296.
9. de Vries, A.P., P. Ruggenenti, X.Z. Ruan, M. Praga, J.M. Cruzado, I.M. Bajema, V.D. D'Agati, H.J. Lamb, D. Pongrac Barlovic, R. Hojs, M. Abbate, R. Rodriquez, C.E. Mogensen, E. Porrini, and ERA-EDTA Working Group Diabesity. 2014. Fatty kidney: Emerging role of ectopic lipid in obesity-related renal disease. *The lancet Diabetes & endocrinology* 2: 417–426.
10. Zhang, M.Z., Y. Wang, P. Paueksakon, and R.C. Harris. 2014. Epidermal growth factor receptor inhibition slows progression of diabetic nephropathy in association with a decrease in endoplasmic reticulum stress and an increase in autophagy. *Diabetes* 63: 2063–2072.
11. Liu, G., Y. Sun, Z. Li, T. Song, H. Wang, Y. Zhang, and Z. Ge. 2008. Apoptosis induced by endoplasmic reticulum stress involved in diabetic kidney disease. *Biochemical and Biophysical Research Communications* 370: 651–656.

12. Lu, Y.C., W.C. Yeh, and P.S. Ohashi. 2008. LPS/TLR4 signal transduction pathway. *Cytokine* 42: 145–151.
13. Conner, E.M., and M.B. Grisham. 1996. Inflammation, free radicals, and antioxidants. *Nutrition* 12: 274–277.
14. Fernandez-Sanchez, A., E. Madrigal-Santillan, M. Bautista, J. Esquivel-Soto, A. Morales-Gonzalez, C. Esquivel-Chirino, et al. 2011. Inflammation, oxidative stress, and obesity. *International Journal of Molecular Sciences* 12: 3117–3132.
15. Utimura, R., C.K. Fujihara, A.L. Mattar, D.M. Malheiros, I.L. Noronha, and R. Zatz. 2003. Mycophenolate mofetil prevents the development of glomerular injury in experimental diabetes. *Kidney International* 63: 209–216.
16. Zhang, Y., B. Chen, X.H. Hou, G.J. Guan, G. Liu, H.Y. Liu, et al. 2007. Effects of mycophenolate mofetil, valsartan and their combined therapy on preventing podocyte loss in early stage of diabetic nephropathy in rats. *Chinese Medical Journal* 120: 988–995.
17. Rodriguez-Iturbe, B., Y. Quiroz, A. Shahkarami, Z. Li, and N.D. Vaziri. 2005. Mycophenolate mofetil ameliorates nephropathy in the obese Zucker rat. *Kidney International* 68: 1041–1047.
18. An, H., H. Xu, M. Zhang, J. Zhou, T. Feng, C. Qian, et al. 2005. Src homology 2 domain-containing inositol-5-phosphatase 1 (SHIP1) negatively regulates TLR4-mediated LPS response primarily through a phosphatase activity- and PI-3K-independent mechanism. *Blood* 105: 4685–4692.
19. Conde, C., X. Rambout, M. Lebrun, A. Lecat, E. Di Valentin, F. Dequiedt, et al. 2012. The inositol phosphatase SHIP-1 inhibits NOD2-induced NF-kappaB activation by disturbing the interaction of XIAP with RIP2. *PLoS One* 7: e41005.
20. Lau, N.C., L.P. Lim, E.G. Weinstein, and D.P. Bartel. 2001. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. *Science* 294: 858–862.
21. Zhang, Z., H. Peng, J. Chen, X. Chen, F. Han, X. Xu, X. He, and N. Yan. 2009. MicroRNA-21 protects from mesangial cell proliferation induced by diabetic nephropathy in db/db mice. *FEBS Letters* 583: 2009–2014.
22. Putta, S., L. Lanting, G. Sun, G. Lawson, M. Kato, and R. Natarajan. 2012. Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy. *Journal of the American Society of Nephrology : JASN* 23: 458–469.
23. Wang, Q., Y. Wang, A.W. Minto, J. Wang, Q. Shi, X. Li, and R.J. Quigg. 2008. MicroRNA-377 is up-regulated and can lead to increased fibronectin production in diabetic nephropathy. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* 22: 4126–4135.
24. Lin, X., Y. You, J. Wang, Y. Qin, P. Huang, and F. Yang. 2015. MicroRNA-155 deficiency promotes nephrin acetylation and attenuates renal damage in hyperglycemia-induced nephropathy. *Inflammation* 38: 546–554.
25. Wang, G., B.C. Kwan, F.M. Lai, K.M. Chow, P.K. Li, and C.C. Szeto. 2011. Elevated levels of miR-146a and miR-155 in kidney biopsy and urine from patients with IgA nephropathy. *Disease Markers* 30: 171–179.
26. Wang, H., W. Peng, X. Shen, Y. Huang, X. Ouyang, and Y. Dai. 2012. Circulating levels of inflammation-associated miR-155 and endothelial-enriched miR-126 in patients with end-stage renal disease. Brazilian journal of medical and biological research =. *Revista brasileira de pesquisas medicas e biologicas* 45: 1308–1314.
27. Baeuerle, P.A., and V.R. Baichwal. 1997. NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. *Advances in Immunology* 65: 111–137.
28. Nguyen, D., F. Ping, W. Mu, P. Hill, R.C. Atkins, and S.J. Chadban. 2006. Macrophage accumulation in human progressive diabetic nephropathy. *Nephrology* 11: 226–231.
29. O'Connell, R.M., A.A. Chaudhuri, D.S. Rao, and D. Baltimore. 2009. Inositol phosphatase SHIP1 is a primary target of miR-155. *Proceedings of the National Academy of Sciences of the United States of America* 106: 7113–7118.
30. Kurowska-Stolarska, M., S. Alivernini, L.E. Ballantine, D.L. Asquith, N.L. Millar, D.S. Gilchrist, J. Reilly, M. Ierna, A.R. Fraser, B. Stolarski, C. McSharry, A.J. Hueber, D. Baxter, J. Hunter, S. Gay, F.Y. Liew, and I.B. McInnes. 2011. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. *Proceedings of the National Academy of Sciences of the United States of America* 108: 11193–11198.
31. Jin, H.M., T.J. Kim, J.H. Choi, M.J. Kim, Y.N. Cho, K.I. Nam, S.J. Kee, J. Moon, S.Y. Choi, D.J. Park, S.S. Lee, and Y.W. Park. 2014. MicroRNA-155 as a proinflammatory regulator via SHIP-1 down-regulation in acute gouty arthritis. *Arthritis Research & Therapy* 16: R88.
32. Awad, A.S., H. You, T. Gao, T.K. Cooper, S.A. Nedospasov, J. Vacher, P.F. Wilkinson, F.X. Farrell, and W. Brian Reeves. 2015. Macrophage-derived tumor necrosis factor-alpha mediates diabetic renal injury. *Kidney International* 88: 722–733.
33. Klessens, C.Q.F., M. Zandbergen, R. Wolterbeek, J.A. Bruijn, T.J. Rabelink, I.M. Bajema, et al. 2017. Macrophages in diabetic nephropathy in patients with type 2 diabetes. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 32: 1322–1329.
34. O'Connell, R.M., K.D. Taganov, M.P. Boldin, G. Cheng, and D. Baltimore. 2007. MicroRNA-155 is induced during the macrophage inflammatory response. *Proceedings of the National Academy of Sciences of the United States of America* 104: 1604–1609.
35. Vyas, P., and D. Vohora. 2017. Phosphoinositide-3-kinases as the novel therapeutic targets for the inflammatory diseases: Current and future perspectives. *Current Drug Targets* 18: 1622–1640.