Biochemical and Biophysical Research Communications 444 (2014) 455–460

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Inhibition of miRNA-21 prevents fibrogenic activation in podocytes and tubular cells in IgA nephropathy

Hao Bao, Shuai Hu, Changming Zhang, Shaolin Shi, Weisong Qin, Caihong Zeng, Ke Zen, Zhihong Liu *

National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China

ARTICLE INFO

Article history:
Received 13 January 2014
Available online 24 January 2014

Keywords:
IgA nephropathy
miRNA-21
Fibrogenic activation

ABSTRACT

Podocytopathy and tubular interstitial fibrosis impact on renal outcomes of IgA nephropathy (IgAN). We found that level of miR-21 was up regulated in both glomerular and tubular–interstitial tissues of patients with IgAN. Enhanced expression of miR-21 mainly located in podocytes and tubular cells. Mesangial cell derived cytokines contributed to the increase of miR-21 in podocytes and HK2 cells. IgA-HMC medium prepared with pIgA from IgAN, lead to obvious fibrogenic activation, evidenced by the loss of Podocin and CD2AP in podocytes, loss of E-cadherin and Megalin in HK2 cells and increase of FN and Col I in both cells. miR-21 targeted PTEN in these cells. Expression of PTEN was decreased and phosphorylation of Akt was increased in podocytes and HK2 cells exposed to the medium prepared with pIgA from IgAN. Inhibition of miR-21 preserved the expression of PTEN, prevented the activation of Akt and inhibited the fibrogenic activation in podocytes and HK2 cells exposed to the IgA-HMC medium prepared with pIgA from IgAN. In conclusion, our study suggests that inhibition of miR-21 prevents fibrogenic activation in podocytes and tubular cells by preventing PTEN/Akt pathway activation in IgAN.

1. Introduction

IgA nephropathy (IgAN) is characterized by deposition of the IgA antibody in the glomerulus, which is the most common glomerulonephritis in China [1]. A series of pathological features exist in IgAN, ranging from segmental cell proliferation to glomerular sclerosis. Oxford classification has suggested that, tubular atrophy and interstitial fibrosis impact on renal outcomes of IgAN [2,3]. Recent studies also show that, podocytopathy similar to that in primary FSGS occurs frequently in IgAN, and is associated with worse renal survival of patients [4].

miRNAs are small non-coding single stranded RNAs of about 21–23 nucleotides, which negatively modulate protein expression by targeting mRNA transcripts and triggering either translation repression or RNA degradation [5]. Insights in pathogenesis reveal that miRNAs play crucial roles in major renal diseases [6]. The role of miRNAs in the renal damages of IgAN remains poorly understood [7]. We performed a miRNA expression profile scanning of microdisected glomerular and tubular–interstitial tissues of IgAN, and found that miR-21 was significant increased in both the glomerular and tubular–interstitial tissues of patients with IgAN. In the present study, we looked into the role of miR-21 in the injuries of podocyte and tubular cells of IgAN.

2. Materials and methods

2.1. Enrollment of patients

The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional ethics committee for studies in human. All subjects (patients and controls) gave their written informed consent for sample collection. Twenty patients with clinical and renal immunopathological diagnosis of primary IgAN were studied [8]. Control renal tissues were obtained from the intact pole of kidneys removed for single circumscribed tumor in ten subjects.

2.2. Laser capture microdissection

Laser capture microdissection (LCM) was performed on a Leica AS LMD System (Leica Microsystems AG, Wetzlar, Germany) according to the manufacturer's manual [9]. Briefly, the tissues were embedded in OCT and immediately frozen in liquid nitrogen. Sections (8 μm thick) were cut and mounted on glass slides. The sections were fixed in ethanol:acetic acid (19:1), rinsed with RNase-free water, stained with hematoxylin and eosin, rinsed with RNase-free water, and

* Corresponding author. Address: National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, 305 East Zhong Shan Road, Nanjing 210002, China. Fax: +86 25 84801990.
E-mail address: Zhihong-liu@hotmail.com (Z. Liu).

http://dx.doi.org/10.1016/j.bbrc.2014.01.065
0006-291X/

completely dried. Approximately 50 glomerular and tubular–interstitial sections were collected by LCM for each specimen.

2.3. miRNA PCR array analysis

miRNA of microdissected tissues was converted to cDNA using the miRNA first-strand kit. miRNAs were detected using the Human miFinder miRNA PCR Array (MIHS-001Z; SABiosciences, Frederick, MD) that profile the expression of the 84 most abundantly expressed and best characterized miRNA sequences[10].

2.4. RT-PCR analysis

Total RNA from tissues or cells was extracted using mirVana™ miRNA Isolation Kit. Template cDNA was prepared using reverse transcriptase, and miR-21 expression was quantified by the Taqman miRNA assay (Applied Biosystems, Foster City, CA) with small nuclear RNA U6 as an endogenous control [11]. RT-PCR analysis of Podocin, CD2AP, E-cadherin, Megalin, FN and Col I was performed using PrimeScript 1st Strand cDNA Synthesis Kit and SYBR® Premix Ex Taq™ II kit (TaKaRa, Dalin, China).

2.5. In situ hybridization analysis of miR-21

MiR-21 *in situ* hybridization was performed on routinely fixed paraffin-embedded tissue sections [12]. Paraffin tissue sections were deparaffinized with xylene, rehydrated with ethanol dilution series and treated with 15 μg/ml proteinase K (Roche) at 37 °C for 15 min. After a washing step with 0.2% Glycine in PBS, slides were fixed with 4% formaldehyde and washed with phosphate buffered saline (PBS), and 2× SSC. Then slides were hybridized with 20 nM DIG-labeled miR-21 probe (Exiqon, Copenhagen, Denmark) diluted in hybridization buffer at 53 °C for 3 h. After washing, slides were treated with blocking buffer for 30 min. Slides were then incubated with anti-DIG-AP in blocking buffer for 1 h. miR-21 was visualized in a staining reaction with NBT/BCIP solution. In all experiments a negative control, i.e. staining without miR-21 probe, was included.

2.6. Purification of pIgA and preparation of IgA-HMC conditioned medium

IgA purified using a jacalin agarose affinity column was fractionated as described previously [13]. Growth arrested human mesangial cells (HMC) were cultured with culture medium containing 0.5% FBS and pIgA (final concentration 500 μg/ml) prepared from IgAN or controls for 48 h. The conditioned medium (IgA-HMC medium) after culture were collected and kept frozen at −70 °C until used.

2.7. Human cytokine array

A biotin label-based human antibody array kit was purchased from RayBiotech Inc. (AAH-CYT-G6, Norcross, GA, USA) [14]. 100 μl of conditioned media was added and incubated at room temperature for 2 h. The membranes were washed, and 70 μl of primary biotin-conjugated antibody was added and incubated at room temperature for 2 h. The membranes were incubated with 70 μl of fluorescent dye-conjugated streptavidin at room temperature for 2 h. Then the glass chips were scanned and analyzed by Axon GenePix.

2.8. ELISA analysis

Quantification of human TGF-β1 and TNF-α were measured by commercially available ELISA systems (R&D systems, Minneapolis, MN, USA).

2.9. Cell culture

Human podocytes and human tubule epithelial (HK2) cells were grown in RPMI-1640 and DMEM/F12 medium that contained 10% fetal bovine serum. For examining the effect of IgA-HMC medium, different preparations of IgA-HMC medium were diluted 8-fold with medium containing 0.5% FBS. In order to study the role of cytokines in the induction of miR-21, cells were treated with TGF-β1(0.5 ng/ml) or TNF-α(80 pg/ml) that were comparable with the concentration of cytokines in the diluted conditioned medium, or were incubated with antibodies against TGF-β1 (10 μg/ml) and TNF-α(0.1 μg/ml) 1 h before stimulating with different medium.

2.10. Cell transfection

Transfection of miR-21 ASO, miR-21 sponge and PTEN siRNA was carried out by using the Lipofectamine 2000 according to the instruction [15]. Immunofluorescent staining revealed that the transfection efficiency was more than 50%.

2.11. Western blot analysis

Western blot analysis was performed as described previously [11]. The antibodies against PTEN, Akt and p-Akt were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.12. Immunohistochemistry staining of PTEN

Paraffin-embedded sections (3 μm) were deparaffinized, rehydrated and stained using routine laboratory protocols [12]. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in phosphate buffered saline (PBS) for 30 min. The sections were incubated for 1 h at room temperature with rabbit anti-PTEN antibody diluted in 1% BSA in PBS. The staining was visualized with Polyvalent HRP/DAB detection kit (Abcam). Negative controls were obtained by omission of the primary antibody from the staining procedure.

2.13. Statistical analyses

Data are expressed as mean ± SD or percentage. Statistical analysis of the data from multiple groups was performed by one-way ANOVA followed by Student–Newman–Kuels test. Data from two groups were compared by t test. $p < 0.05$ was considered significant.

3. Results

3.1. Enhanced expression of miR-21 is associated with podocyte and tubular cell injuries in patients with IgAN

The demographic and baseline clinical data of the study subjects are summarized in Table 1. To look into the change of miRNAs in the glomerular and tubular–interstitial tissues separately, the glomerular and tubular–interstitial tissues were collected by laser capture microdissection and analyzed for the expression of miRNAs using PCR arrays (SABiosciences) that profile 84 most abundantly expressed and best characterized miRNA sequences. As the result shown, tissues from patients show different expression file of miRNAs from normal controls, with significant increase of miR-21 in both glomerular and tubular–interstitial tissues of patients with IgAN. Quantification of all the enrolled patients confirmed the change of miR-21 in the glomerular and tubular–interstitial tissues from IgAN patients (Fig. 1).

Table 1
Clinical and pathological features of patients with IgAN.

	IgAN (n = 20)
Age, mean ± SD (years)	31.2 ± 9.9
Male/female	10/10
Scr, mean ± SD (mg/dl)	0.94 ± 0.30
Proteinuria, mean ± SD (g/24 h)	0.95 ± 0.49
Urine RBCs count, range (10^4/ml)	126.0(42.0–255.0)
Frequency of glomerular sclerosis >10%	8 (40.0%)
Frequency of moderate or severe mesangial proliferation	10 (50.0%)
Frequency of moderate or severe interstitial fibrosis	4 (20.0%)
Frequency of moderate or severe interstitial inflammation	4 (20.0%)

Scr, serum creatinine; RBCs, red blood cells.

Relationship between the level of renal miR-21 and clinical–pathological parameters was analyzed. Level of glomerular miR-21 was positive correlated with the amount of urine protein and the percentage of glomerular sclerosis, while the value of tubular–interstitial miR-21 was positive correlated with the amount of urine protein, the level of serum creatinine and the score of interstitial fibrosis. MiR-21 was found to be mainly up expressed in glomerular podocytes and tubular cells in the tissues of patients with IgAN (Fig. 1).

3.2. Mesangial-derived cytokines up regulate miR-21 in podocytes and HK2 cells in vitro

Cultured human mesangial cells (HMC) were stimulated with pIgA prepared from normal control or patients with IgAN (500 μg/ml) for 48 h, and then human podocytes and HK2 cells was cultured and exposed to IgA-HMC conditioned medium *in vitro*. As the results shown, IgA-HMC medium prepared with pIgA from IgAN lead to a significant increase of miR-21 in both podocytes and HK2 cells (Fig. 2).

Change of cytokines in the IgA-HMC conditioned medium was analyzed using the AAH-CYT-G6 cytokine array. TGF-β1 and TNF-α was found to be significantly increased in the HMC culture medium stimulated with pIgA from patients with IgAN, and the findings were further confirmed by ELISA analysis. Cytokines, at concentration comparable to that in 8-fold dilution of the IgA-HMC conditioned medium, significantly increased miR-21 in podocytes and HK2 cells.

We then looked into the effect of antagonizing antibody on the induction of miR-21. Both anti-TGF-β1 and anti-TNF-α antibody blunted, and their combine treatment prevented the increase of miR-21 in podocytes and HK2 cells treated with IgA-HMC conditioned medium prepared with pIgA from IgAN.

3.3. Inhibition of miR-21 prevents fibrogenic activation in podocytes and tubular cells in IgAN

Expression of Podocin and CD2AP was significantly decreased, while the expression of FN and Col I was increased in podocytes when exposed to the IgA-HMC medium prepared with pIgA from IgAN. The loss of Podocin and CD2AP, and the expression of FN and Col I was inhibited by anti-miR-21 treatment in podocytes exposed to IgA-HMC medium prepared with pIgA from IgAN (Fig. 3).

Expression of E-cadherin and Megalin was significantly suppressed, and the expression of FN and Col I was increased in HK2 cells when exposed to the IgA-HMC medium prepared with pIgA from IgAN. The loss of normal epithelial adhesion was evident in the fluoresce analysis of β-catenin. The loss of E-cadherin, Megalin and β-catenin, and the increase of FN and Col I was significantly inhibited by anti-miR-21 treatment in HK2 cells exposed to IgA-HMC medium prepared with pIgA from IgAN (Fig. 3).

3.4. Inhibition of miR-21 prevents PTEN/Akt pathway activation in podocytes and tubular cells in IgAN

PTEN was one of the validated miR-21 target mRNAs, which has been reported to be involved in various diseases. We looked into

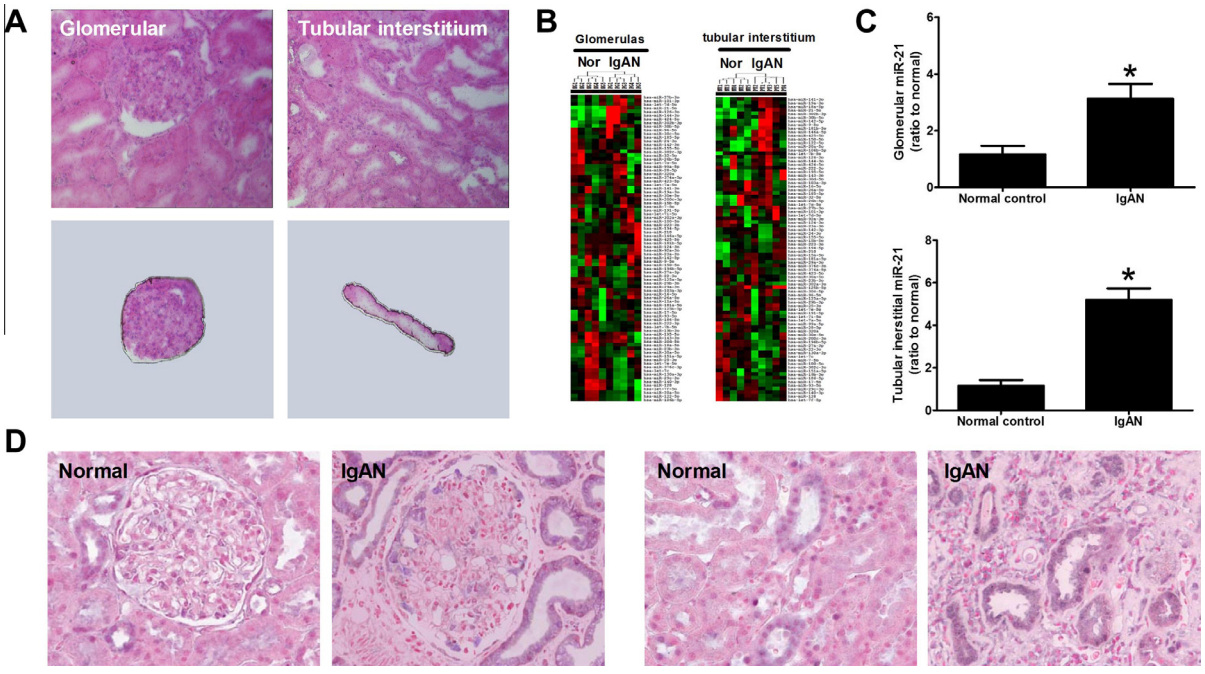

Fig. 1. Change of miRNAs in renal tissues of patients with IgAN. (A) Isolation of glomerular and tubular–interstitial tissues by laser capture microdissection; (B) result list of RT-PCR analysis of miRNAs in the glomerular and tubular–interstitial tissues; and (C) change of miR-21 in glomerular and tubular–interstitial tissues. (D) ISH analysis of miR-21 in renal tissues of normal control and IgAN patients (400×). *$p < 0.05$, *versus* normal control.

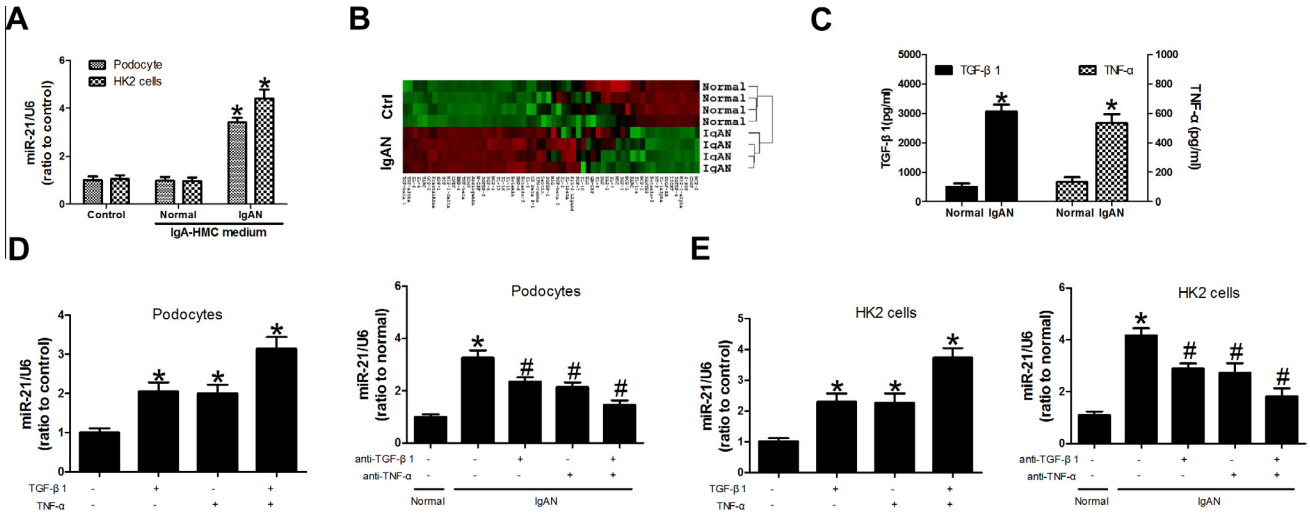

Fig. 2. Mesangial-derived cytokines up-regulate miR-21 in podocytes and HK2 cells. (A) Change of miR-21 after expoure with IgA-HMC conditioned medium prepared with pIgA from IgAN patients and normal controls; (B) cytokine array analysis of IgA-HMC conditioned medium prepared with pIgA from IgAN patients and normal controls; (C) ELISA analysis of IgA-HMC conditioned medium; (D) change of miR-21 in podocytes treated with TGF-β1 and TNF-α, and change of miR-21 in podocytes treated with IgA-HMC conditioned medium and cytokine antagonizing antibodies; and (E) change of miR-21 in HK2 cells treated with TGF-β1 and TNF-α, and change of miR-21 in HK2 cells treated with IgA-HMC conditioned medium and antagonizing antibodies. $^{*}p < 0.05$, *versus* control; $^{\#}p < 0.05$, *versus* cells exposed with IgA-HMC conditioned medium prepared with pIgA from IgAN.

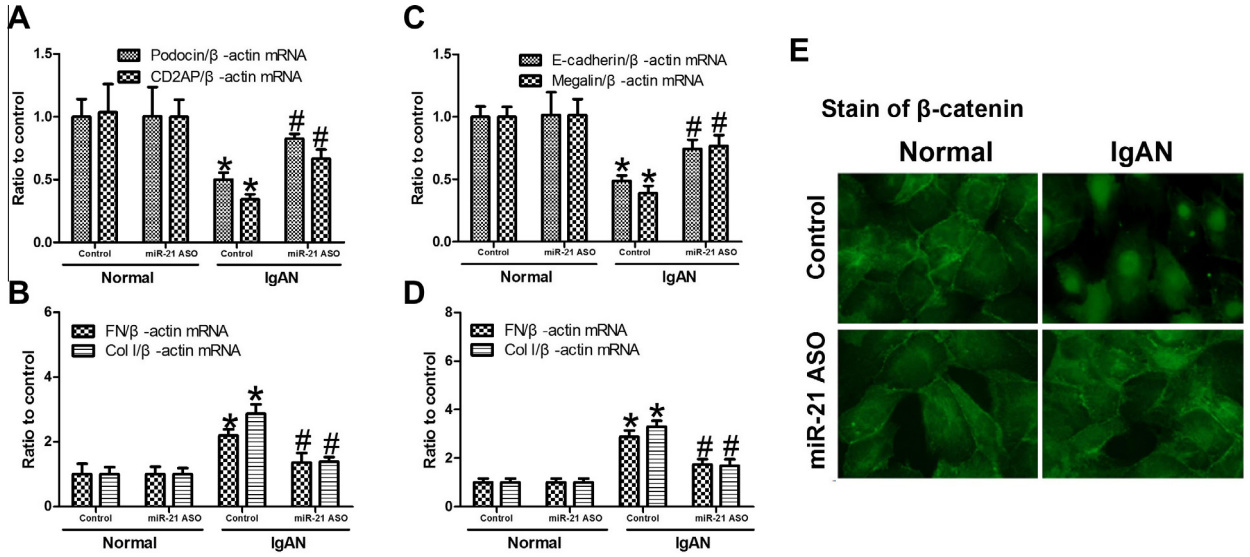

Fig. 3. Inhibition of miR-21 prevent fibrogenic activation in podocytes and HK2 cells. (A) Change of Podocin and CD2AP mRNA in podocytes after expoure with IgA-HMC conditioned medium with or without miR-21 inhibitor; (B) change of FN and Col I mRNA in podocytes after expoure with IgA-HMC conditioned medium with or without miR-21 inhibitor; (C) change of E-cadherin and Megalin mRNA in HK2 cells after expoure with IgA-HMC conditioned medium with or without miR-21 inhibitor; (D) change of FN and Col I mRNA in HK2 cells after expoure with IgA-HMC conditioned medium with or without miR-21 inhibitor; and (E) immunofluorence analysis of β-catenin in HK2 cells after expoure with IgA-HMC conditioned medium with or without miR-21 inhibitor. $^{*}p < 0.05$, *versus* normal control; $^{\#}p < 0.05$, *versus* cells exposed with IgA-HMC conditioned medium prepared with pIgA from IgAN.

the change of PTEN in IgAN. As the result shown, PTEN was significantly suppressed in the glomerulus and tubular interstitium of patients with IgAN. The level of PTEN was also decreased *in vitro*, with the activation of Akt in podocytes and HK2 cells cultured with IgA-HMC medium prepared with pIgA from IgAN patients. PTEN over expression significantly suppressed the phosphorylation of Akt, preservered the expression of Podocin, CD2AP, E-cadherin and Megalin, and suppressed the expression of FN and Col I in podocytes and HK2 cells cultured with IgA-HMC medium prepared with pIgA from IgAN (Fig. 4).miR-21 mimics obviously suppressed the level of PTEN in podocytes and HK2 cells. Transfection of miR-21 sponge prevented the loss of PTEN, suppressed the activation of Akt, preserved the expression of Podocin, CD2AP, E-cadherin and Megalin, and suppressed the expression of FN and Col I in podocytes and HK2 cells cultured with IgA-HMC medium prepared with pIgA from IgAN. These protective effects were abolished by PTEN siRNA treatment.

4. Discussion

Primary IgAN is characterized by deposition of the IgA antibody in the glomerulus, which is the most common glomerulonephritis throughout the world [1]. Recent studies imply that tubular interstitial chronic lesions and glomerular podocytopathy have signifi-

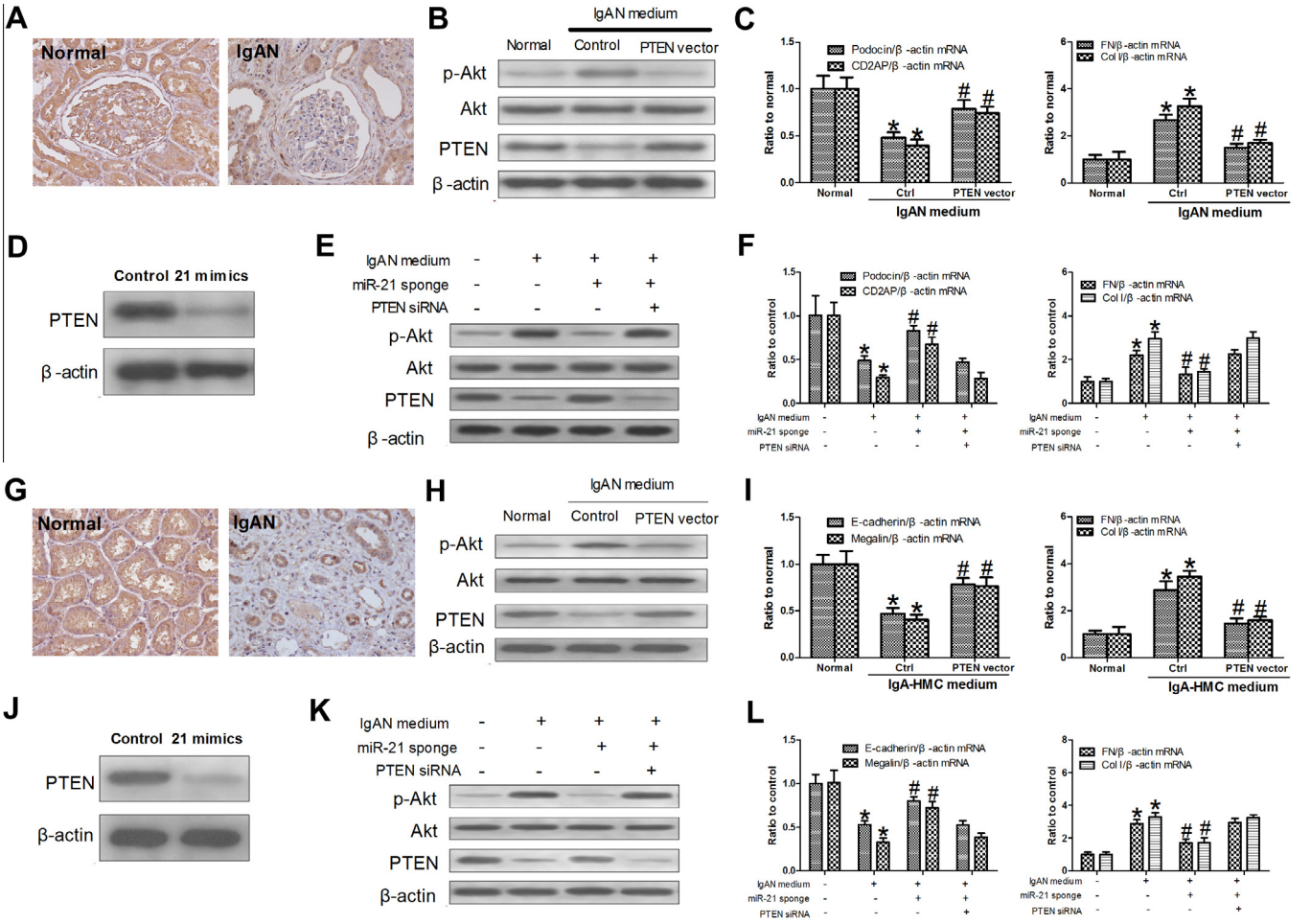

Fig. 4. Inhibition of miR-21 prevent PTEN/Akt pathway activation in podocytes and tubular cells. (A and G) IHC staining analysis of PTEN in the glomerulus and tubular interstitium; (B and H) change of PTEN/Akt in podocytes and HK2 cells exposed to IgA-HMC medium prepared with pIgA from IgAN with or without PTEN expressing vector; (C and I) change of Podocin, CD2AP, E-cadherin, Megalin, FN and Col I in podocytes and HK2 cells exposed to IgA-HMC medium prepared with pIgA from IgAN with or without PTEN expressing vector; (D and J) Western blot analysis of PTEN in podocytes and HK2 cells transfected with miR-21 mimics; (E and K) Western blot analysis of PTEN/Akt in podocytes and HK2 cells exposed to IgA-HMC medium prepared with pIgA from IgAN with or without miR-21 sponge and PTEN siRNA; and (F and L) change of Podocin, CD2AP, E-cadherin, Megalin, FN and Col I in podocytes and HK2 cells exposed to IgA-HMC medium prepared with pIgA from IgAN with or without miR-21 sponge and PTEN siRNA. $^{*}p < 0.05$, *versus* Normal control; $^{\#}p < 0.05$, *versus* cells exposed with IgA-HMC conditioned medium prepared with pIgA from IgAN.

cant influence on renal outcomes [2,3]. Although IgAN is intensively studied, the pathogenesis underling the lesions is still poorly understood [7]. miRNAs play crucial roles in major renal diseases [6]. In the present study, we performed a miRNA expression profile scanning of the microdisected glomerular and tubular–interstitial tissues of IgAN, and found that miR-21 was significant increased in both glomerular and tubular–interstitial tissues of patients with IgAN.

miR-21 is one of the prominent miRNAs that regulate a plethora of target proteins involved in cellular survival, apoptosis and cell invasiveness [16]. We found a significant correlation between the up expression of miR-21 in tissues and level of serum creatinine, percentage of glomerular sclerosis, and score of interstitial fibrosis, which are the marks of renal chronic lesions. In line with our results, Zhong et al. and Zarjou et al. reported that, miR-21 demonstrated significant increase in UUO kidneys, and blocking miR-21 attenuated UUO induced renal fibrosis [11,17]. We further performed localization studies and found that the enhanced expression of miR-21 was mainly located on tubular cells and glomerular podocytes of IgAN. The relationship between miR-21 and podocytopathy is inspired by the clinical study conducted by Kong et al., which found that the actual level of urinary albumin excretion correlated with the urinary level of miR-21 [18].

Renal injury in IgAN is initiated by the deposition of IgA in the mesangial area. Previous studies found that IgA-HMC medium prepared with pIgA from IgAN patients, lead to an enhanced proliferation and increased synthesis of TNF-α, MIF, and sICAM-1 in proximal tubular epithelial cells, and cause a significant down-regulation of specific proteins in podocytes [19,20]. We examined the cells exposed to the IgA-HMC conditioned medium, and found that miR-21 was induced in both podocytes and tubular cells after the treatment. The crosstalk between mesangial cell and podocytes or tubular cells may involve in the induction of miR-21, which leads to the injuries of podocytes and tubular cells in IgAN. Cytokine array analysis reveal that various cytokines significantly increase in the IgA-HMC medium prepared with pIgA from IgAN, such as TGF-β1 and TNF-α. Treatment with the cytokine antagonistic antibodies blunts the up-regulation of miR-21.

Podocyte dedifferentiation and mesenchymal transition is a potential pathway leading to their dysfunction, which playing an important role in the genesis of proteinuria [21]. IgA-HMC conditioned medium prepared with pIgA from IgAN, suppress the expression of Podocin and CD2AP, and induce the expression of FN and Col I in podocytes, which indicate a fibrogenic activation. This is in consistent with the study conducted by Wang et al., which reported that TGF-β1 treatment increased Col I, III, and IV

mRNA levels, as well as α-SMA and vimentin in podocytes [22]. Tubular cells also actively participate in the mechanisms of renal fibrosis through epithelial–mesenchymal transition [23]. Independent studies underlined the relationship between miR-21 up expression and fibrosis response of tubular cells [11,17]. We found that miR-21 inhibition prevent the loss of E-cadherin and Megalin, and reversed the level of FN and Col I in cells exposed to IgA-HMC medium prepared with pIgA from IgAN.

Phosphatase and tensin homolog (PTEN) is a phosphati-dylinositol-3,4,5-trisphosphate 3(PIP3)-phosphatase that inhibits phosphoinositide-3-kinase pathway by dephosphorylating PIP3 and thus prevents Akt activation [24]. Bowen et al. reported that PTEN loss involved in the epithelial–mesenchymal transition in human colon cancer cells [25]. PTEN is one of the identified direct targets of miR-21 [26]. We detected obvious decrease of PTEN with the activation of Akt in both podocytes and tubular cells treated with IgA-HMC medium prepared with pIgA from IgAN. PTEN over expression prevented the activation of Akt, and rescued fibrogenic activation in both podocytes and tubular cells that exposed to the IgA-HMC medium prepared with pIgA from IgAN. In consistent with our findings, Yao et al. reported that PTEN suppression was followed by the activation of Akt and the expression of vimentin in tubular cells treated with TGF-β1 [27]. More recently, Jamie Lin et al. created the podocyte-specific PTEN KO (PPKO) mice, and found about a 2-fold increase in albuminuria over control. Pathological examination detects early glomerulosclerosis, interrupted nephrin immunostaining and extensive foot process effacement in PPKO mice [28].

In conclusion, miR-21 is up expressed in both podocytes and tubular cells of patients with IgAN. Inhibition of miR-21 prevents fibrogenic activation in podocytes and tubular cells by preventing PTEN/Akt pathway activation in IgAN, thus may represent a new therapeutic approach for IgAN.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

This work was supported by the grants from the National Basic Research Program of China 973 Program No. 2012CB517600 (No. 2012CB517606), National Natural Science Foundation of China (Nos. 81020108016 and 81200516) and the Natural Science Foundation of Jiangsu Province (No. BK2012372). This work has been orally presented and awarded "best abstract prize" at the 13th Annual Symposium on IgA Nephropathy in Nanjing, 2013.

References

[1] G. D'Amico, The commonest glomerulonephritis in the world: IgA nephropathy, Q. J. Med. 64 (1987) 709–727.
[2] H. Lee, S.H. Yi, M.S. Seo, J.N. Hyun, J.S. Jeon, H. Noh, D.C. Han, S.D. Hwang, S.Y. Jin, S.H. Kwon, Validation of the Oxford classification of IgA nephropathy: a single-center study in Korean adults, Korean J. Intern. Med. 27 (2012) 293–300.
[3] C.H. Zeng, W. Le, Z. Ni, M. Zhang, L. Miao, P. Luo, R. Wang, Z. Lv, J. Chen, J. Tian, N. Chen, X. Pan, P. Fu, Z. Hu, L. Wang, Q. Fan, H. Zheng, D. Zhang, Y. Wang, Y. Huo, H. Lin, S. Chen, S. Sun, Y. Wang, Z. Liu, D. Liu, L. Ma, T. Pan, A. Zhang, X. Jiang, C. Xing, B. Sun, Q. Zhou, W. Tang, F. Liu, Y. Liu, S. Liang, F. Xu, Q. Huang, H. Shen, J. Wang, Y. Shyr, S. Phillips, S. Trojanov, A. Fogo, Z.H. Liu, A multicenter application and evaluation of the oxford classification of IgA nephropathy in adult Chinese patients, Am. J. Kidney Dis. 60 (2012) 812–820.
[4] K.K. El, G.S. Hill, A. Karras, L. Moulonguet, V. Caudwell, A. Loupy, P. Bruneval, C. Jacquot, D. Nochy, Focal segmental glomerulosclerosis plays a major role in the progression of IgA nephropathy. II. Light microscopic and clinical studies, Kidney Int. 79 (2011) 643–654.
[5] M. Lagos-Quintana, R. Rauhut, W. Lendeckel, T. Tuschl, Identification of novel genes coding for small expressed RNAs, Science 294 (2001) 853–858.
[6] K. Bhatt, Q.S. Mi, Z. Dong, MicroRNAs in kidneys: biogenesis, regulation, and pathophysiological roles, Am. J. Physiol. Renal Physiol. 300 (2011) F602–F610.
[7] W. Le, S. Liang, Y. Hu, K. Deng, H. Bao, C. Zeng, Z. Liu, Long-term renal survival and related risk factors in patients with IgA nephropathy: results from a cohort of 1155 cases in a Chinese adult population, Nephrol. Dial. Transplant. 27 (2012) 1479–1485.
[8] G. Wang, B.C. Kwan, F.M. Lai, K.M. Chow, L.P. Kam-Tao, C.C. Szeto, Expression of microRNAs in the urinary sediment of patients with IgA nephropathy, Dis. Markers 28 (2010) 79–86.
[9] T. Kobayashi, S. Uehara, T. Ikeda, H. Itadani, H. Kotani, Vitamin D3 up-regulated protein-1 regulates collagen expression in mesangial cells, Kidney Int. 64 (2003) 1632–1642.
[10] A.A. Divekar, S. Dubey, P.R. Gangalum, R.R. Singh, Dicer insufficiency and microRNA-155 overexpression in lupus regulatory T cells: an apparent paradox in the setting of an inflammatory milieu, J. Immunol. 186 (2011) 924–930.
[11] X. Zhong, A.C. Chung, H.Y. Chen, X.M. Meng, H.Y. Lan, Smad3-mediated upregulation of miR-21 promotes renal fibrosis, J. Am. Soc. Nephrol. 22 (2011) 1668–1681.
[12] L. Qi, J. Bart, L.P. Tan, I. Platteel, T. Sluis, S. Huitema, G. Harms, L. Fu, H. Hollema, A. Berg, Expression of miR-21 and its targets (PTEN, PDCD4, TM1) in flat epithelial atypia of the breast in relation to ductal carcinoma in situ and invasive carcinoma, BMC Cancer 9 (2009) 163.
[13] J.C. Leung, L.Y. Chan, S.C. Tang, M.F. Lam, C.W. Chow, A.I. Lim, K.N. Lai, Oxidative damages in tubular epithelial cells in IgA nephropathy: role of crosstalk between angiotensin II and aldosterone, J. Transl. Med. 9 (2011) 169.
[14] Y. Xu, J. Kulkosky, E. Acheampong, G. Nunnari, J. Sullivan, R.J. Pomerantz, HIV-1-mediated apoptosis of neuronal cells: proximal molecular mechanisms of HIV-1-induced encephalopathy, Proc. Natl. Acad. Sci. USA 101 (2004) 7070–7075.
[15] R. Gong, A. Rifai, Y. Ge, S. Chen, L.D. Dworkin, Hepatocyte growth factor suppresses proinflammatory NFkappaB activation through GSK3beta inactivation in renal tubular epithelial cells, J. Biol. Chem. 283 (2008) 7401–7410.
[16] V. Jazbutyte, T. Thum, MicroRNA-21: from cancer to cardiovascular disease, Curr. Drug Targets 11 (2010) 926–935.
[17] A. Zarjou, S. Yang, E. Abraham, A. Agarwal, G. Liu, Identification of a microRNA signature in renal fibrosis: role of miR-21, Am. J. Physiol. Renal Physiol. 301 (2011) F793–F801.
[18] A.P. Kong, K. Xiao, K.C. Choi, G. Wang, M.H. Chan, C.S. Ho, I. Chan, C.K. Wong, J.C. Chan, C.C. Szeto, Associations between microRNA (miR-21, 126, 155 and 221), albuminuria and heavy metals in Hong Kong Chinese adolescents, Clin. Chim. Acta. 413 (2012) 1053–1057.
[19] L.Y. Chan, J.C. Leung, A.W. Tsang, S.C. Tang, K.N. Lai, Activation of tubular epithelial cells by mesangial-derived TNF-alpha: glomerulotubular communication in IgA nephropathy, Kidney Int. 67 (2005) 602–612.
[20] K.N. Lai, J.C. Leung, L.Y. Chan, M.A. Saleem, P.W. Mathieson, F.M. Lai, S.C. Tang, Activation of podocytes by mesangial-derived TNF-alpha: glomerulo-podocytic communication in IgA nephropathy, Am. J. Physiol. Renal Physiol. 294 (2008) F945–F955.
[21] Y. Li, Y.S. Kang, C. Dai, L.P. Kiss, X. Wen, Y. Liu, Epithelial-to-mesenchymal transition is a potential pathway leading to podocyte dysfunction and proteinuria, Am. J. Pathol. 172 (2008) 299–308.
[22] B. Wang, R. Komers, R. Carew, C.E. Winbanks, B. Xu, M. Herman-Edelstein, P. Koh, M. Thomas, K. Jandeleit-Dahm, P. Gregorevic, M.E. Cooper, P. Kantharidis, Suppression of microRNA-29 expression by TGF-beta1 promotes collagen expression and renal fibrosis, J. Am. Soc. Nephrol. 23 (2012) 252–265.
[23] Y. Liu, New insights into epithelial–mesenchymal transition in kidney fibrosis, J. Am. Soc. Nephrol. 21 (2010) 212–222.
[24] B.H. Jiang, L.Z. Liu, PI3K/PTEN signaling in angiogenesis and tumorigenesis, Adv. Cancer Res. 102 (2009) 19–65.
[25] K.A. Bowen, H.Q. Doan, B.P. Zhou, Q. Wang, Y. Zhou, P.G. Rychahou, B.M. Evers, PTEN loss induces epithelial–mesenchymal transition in human colon cancer cells, Anticancer Res. 29 (2009) 4439–4449.
[26] F. Meng, R. Henson, M. Lang, H. Wehbe, S. Maheshwari, J.T. Mendell, J. Jiang, T.D. Schmittgen, T. Patel, Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines, Gastroenterology 130 (2006) 2113–2129.
[27] Y. Yao, H. Wei, L. Liu, L. Liu, S. Bai, C. Li, Y. Luo, R. Zeng, M. Han, S. Ge, G. Xu, Upregulated DJ-1 promotes renal tubular EMT by suppressing cytoplasmic PTEN expression and Akt activation, J. Huazhong Univ. Sci. Technol. Med. Sci. 31 (2011) 469–475.
[28] M.D. Jamie Lin, M.D. Jing Xu, W.E.M. PhD, F. MD, M.D. Zhaoyong Hu. PTEN Deletion in Podocytes: A Potential General Mechanism Causing Foot Processes Effacement and Proteinuria, ASN, FR-PO688, 2012.