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Original Article

miR-216a-5p promotes mesangial cell proliferation by targeting FoxO1 in diabetic nephropathy

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Abstract: Background: Diabetic nephropathy (DN) is a leading cause of end-stage renal disease worldwide. microRNAs (miRNAs) have been reported to play essential roles in DN progression. However, the mechanism of miR-216a-5p on DN progression is still unclear. Methods: A DN model was established in human mesangial cells (HMC) by high glucose treatment. Cell proliferation was investigated using the cell counting kit-8 (CCK-8) assay. The cell cycle was measured through a propidium iodide (PI) cell cycle kit with flow cytometry. The interaction between miR-216a-5p and forkhead boxO1 (FoxO1) was probed by a bioinformatics analysis and luciferase activity assay. The expression of miR-216a-5p was detected using a quantitative real-time polymerase chain reaction (qRT-PCR). The abundances of FoxO1 and cell cycle-related cyclinD1, cyclin-dependent kinase 4 (CDK4), CDK6 and p27 were examined by qRT-PCR and Western blots (WB). Results: miR-216a-5p was up-regulated while FoxO1 was down-regulated in DN tissues. Moreover, miR-216a-5p promoted cell proliferation by regulating the cell cycle in high glucose-treated HMC cells. Notably, FoxO1 was a direct target and negatively correlated with miR-216a-5p. In addition, miR-216a induced cyclinD1, CDK4 and CDK6 but inhibited p27 expressions at the mRNA and protein levels. Furthermore, FoxO1 restoration reversed the regulatory effect of miR-216a on the cell cycle by regulating cyclinD1, CDK4, CDK6 and p27 abundances at the mRNA and protein levels. Conclusion: miR-216a-5p is ectopic in DN and it promotes cell proliferation through regulating the cell cycle by targeting FoxO1 in high glucose-stimulated HMC cells, indicating it may serve as a novel biomarker for DN treatment.

Keywords: Diabetic nephropathy, mesangial cells, miR-216a-5p, FoxO1, proliferation, cell cycle

Introduction

Diabetic nephropathy (DN) is a common complication faced by diabetes patients and is one of the main causes of end-stage renal disease throughout the world [1]. In recent years, a number of investigations have shown great promise in our understanding of the pathophysiology of DN progression, yet there are few novel and approved therapeutic options to support renal function in DN [2]. Emerging evidence indicates that the dysfunction of endothelial, tubulointerstitial cells, podocytes, and mesangial cells are associated the DN progression [3]. With the advances in the understanding of the signaling pathways in DN, preventative and effective strategies have been developed [4]. However, a novel biomarker driving DN progression is still needed.

microRNAs (miRNAs), a class of non-coding RNAs, are reported to be important regulators of glomerular function in several diseases, including DN [5]. The available evidence indicates that miRNAs have emerged as potential biomarkers for the risk assessment of DN [6]. Recently, miRNAs biomarkers are under development and have been used for early detection and therapy in DN progression [7]. For example, miR-455-3p inhibits renal fibrosis by regulating cell proliferation and the inflammatory response by targeting Rho-associated coiled coil-containing protein kinase 2 (ROCK2) in DN [8]. Moreover, miR-21 silencing has been reported to ameliorate the progression of DN by regulating cell division cycle 25a (CDC25a) and cyclin-dependent kinase 6 (CDK6) expressions in mice [9]. Also, miR-27a may induce mesangial

cell injury by mediating the peroxisome proliferator-activated receptor γ (PPARγ), the inhibition of which prevents DN progression in vivo [10]. As for miR-216a-5p, a novel miRNA, it has been suggested as a way to regulate cell proliferation and migration in prostate cancer cells [11]. Furthermore, mir-216a-5p contributes to tumorigenesis by regulating cell proliferation and migration in cervical cancer [12]. Moreover, miR-216a-5p has been regarded as an oncogene because it regulates cell proliferation and apoptosis in renal cell carcinoma [13]. Notably, miR-216a-5p has been shown to be expressed in the glomerulus and may participate in DN progression [14]. However, the mechanism that underlies miR-216a-5p's regulating of cell proliferation of mesangial cells remains unclear.

Forkhead box01 (FoxO1) has been reported to associated with high glucose-induced HK-2 cell injury in DN progression [15]. Moreover, FoxO1 is thought to be involved in mesangial cell proliferation and extracellular matrix accumulation in mice with DN [16]. Notably, FoxO1 has shown to negatively correlate with cell proliferation in rat mesangial cells under high glucose treatment [17]. Intriguingly, a bioinformatics analysis showed some promising binding sites of miR-216a-5p and FoxO1. Here we first measured the expressions of miR-216a-5p and FoxO1 in DN tissues and investigated the effect of miR-216a-5p on cell proliferation and the cell cycle in high glucose-treated human mesangial cells (HMC). In addition, we probed the link of miR-216a-5p with FoxO1 and further explored the effect of miR-216a-5p and FoxO1 on cell cycle-related biomarkers at the mRNA and protein levels.

Materials and methods

Tissue samples

Renal biopsies were collected from 32 DN patients and 32 normal controls that had renal carcinoma without diabetes or hypertension. Serums were obtained from the patients with or without DN (n = 10). This study was accepted by the Institutional Research Ethics Committee of Shenzhen Guangming New District People's Hospital and a written informed consent was obtained from every donor.

Cell culture and transfection

The HMC cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in normal glucose (5.5 mmol/l) DMEM (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (Gibco), 1% penicillin and streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C. To establish the DN model of HMC cells, a high glucose culture medium was used, supplemented with 25 mmol/l glucose in DMEM medium.

miR-216a-5p mimics, miR-216a-5p inhibitors (anti-miR-216a-5p), negative control (NC), FoxO1 overexpression vectors (FoxO1), and the vector alone were synthesized by Genepharma (Shanghai, China). Transfection was preformed into HMC cells using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol.

Cell proliferation

Cell proliferation was measured using the cell counting kit-8 (CCK-8, Sigma, St. Louis, MO, USA) assay. Cells were seeded into 96-well plates at a density of 1×10^4 cells per well for 24, 48 or 72 h. Then 10 μL of the CCK-8 solution was added to each well, interacting with the cells for 2 h at 37°C. The absorbance was measured at 450 nm with a microplate reader (Bio-Rad, Hercules, CA, USA).

Cell cycle analysis

The cell cycle was analyzed using a propidium iodide (PI) cell cycle kit (Abcam, Cambridge, UK) with flow cytometry. After transfecting for 72 h, HMC cells were washed with PBS, fixed with 70% ethanol at 4°C overnight, followed by incubation with RNase at 37°C and staining with PI before being measured with a flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

Luciferase activity assay

Putative sites of 3'-UTR of FoxO1 and miR-216a-5p were described by sequence alignment in NCBI. Wide type or mutant type 3'-UTR of FoxO1 were amplified and cloned into pmirGLO vectors (Promega, Madison, WI, USA) to obtain wt or mut luciferase report vectors, respectively. Then the luciferase reporter vectors were co-transfected with miR-216a-5p mimics or negative controls (miR-NC) in the HMC cells using Lipofectamine 3000 according to the manufacturer's protocols. Then cells were collected and analyzed with a Dual-Luciferase Assay Kit (Promega) after 48 h.

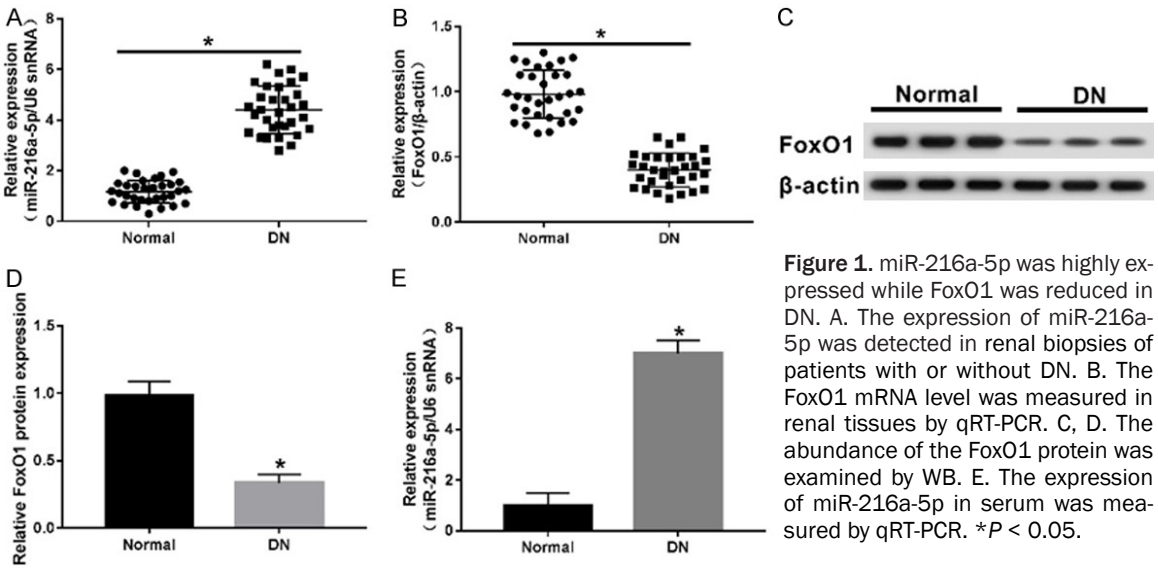


Figure 1. miR-216a-5p was highly expressed while FoxO1 was reduced in DN. A. The expression of miR-216a-5p was detected in renal biopsies of patients with or without DN. B. The FoxO1 mRNA level was measured in renal tissues by qRT-PCR. C, D. The abundance of the FoxO1 protein was examined by WB. E. The expression of miR-216a-5p in serum was measured by qRT-PCR. *$P < 0.05$.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from tissues or cells with the *mir*Vana™ miRNA isolation Kit (Thermo Fisher, Wilmington, DE, USA) following the manufacturer's instructions. The miRNA in the serum was prepared using a TaqMan miRNA ABC Purification Kit (Applied Biosystems, Foster City, CA, USA). The RNA was reverse transcribed to first strand cDNA using either a TaqMan mRNA or a microRNA Reverse Transcription Kit (Applied Biosystems). Then the mRNA or miRNA expressions were measured by qRT-PCR using SYBR Green, following the amplification instructions. U6 small RNA or β-actin was used to normalize the relative expressions according to the $2^{-\Delta\Delta CT}$ method. All the primers used are listed as follows: miR-216a-5p (Forward, 5'-GGGTAATCTCAGCTGGCAA-3'; Reverse, 5'-CAGTGCGTGTCGTGGAGT-3'), U6 (Forward, 5'-GCTTCGGCAGCACATATACTAAAAT-3'; Reverse, 5'-CGCTTCACGAATTTGCGTGTCAT-3'), FoxO1 (Forward, 5'-AAGAGCGTGCCCTACTTCAA-3'; Reverse, 5'-CTGTTGTTGTCC ATGGATGC-3'), CyclinD1 (Forward, 5'-CTGGCCATGAACTACCTGGA-3'; Reverse, 5'-GTCACACTTGATCACTCTGG-3'), CDK4 (Forward, 5'-GAAGAAG AAGCGGAGGAAGAGG-3'; Reverse, 5'-TTAGGTTAGTGCGGGAATGAAT-3'), CDK6 (Forward, 5'-CCAGATGGCTCTAACCTCAGT-3'; Reverse, 5'-AACTTCCA CGAAAAAGAGGCTT-3'), p27 (Forward, 5'-GTCAAACGTGCGAGTGTCTA-3'; Reverse, 5'-CATGTCTCTGCAGTGCTTCT-3'), β-actin (Forward, 5'-ATCACCATTG GCAATGAGCG-3'; Reverse, 5'-TTGAAGGTAGTTTCGTGGAT-3').

Western blots (WB)

Tissues or cells were washed with PBS and isolated in a lysis buffer with 1% protease inhibitor (Thermo Fisher). Then the proteins were quantified using a BCA assay kit (Thermo Fisher) according to the instructions, followed by being boiled at 98°C for 10 min. Subsequently, the denatured samples were separated using SDS-PAGE gel and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking in 5% BSA (Sigma) for 1 h at room temperature, the membranes were incubated with primary antibodies against FoxO1 (#2880, 1:1000 dilution, Cell Signaling Technology, Beverly, MA, USA), CyclinD1 (#2978, 1:1000 dilution, Cell Signaling Technology), p27 (#3686, 1:1000 dilution, Cell Signaling Technology), CDK4 (#12790, 1:1000 dilution, Cell Signaling Technology), CDK6 (#13331, 1:1000 dilution, Cell Signaling Technology) or β-actin (#4970, 1:1000 dilution, Cell Signaling Technology) at 4°C overnight, followed by interaction with HRP-conjugated IgG secondary antibodies (#93702, 1:1000 dilution, Cell Signaling Technology) for 2 h at room temperature. The protein bands were measured by an enhanced chemiluminescence (ECL) chromogenic substrate (Thermo Fisher).

Statistical analysis

Data were expressed as the mean ± standard deviation (SD) from three independent experiments. The statistical analysis was conducted using GraphPad Prism (GraphPad Software,

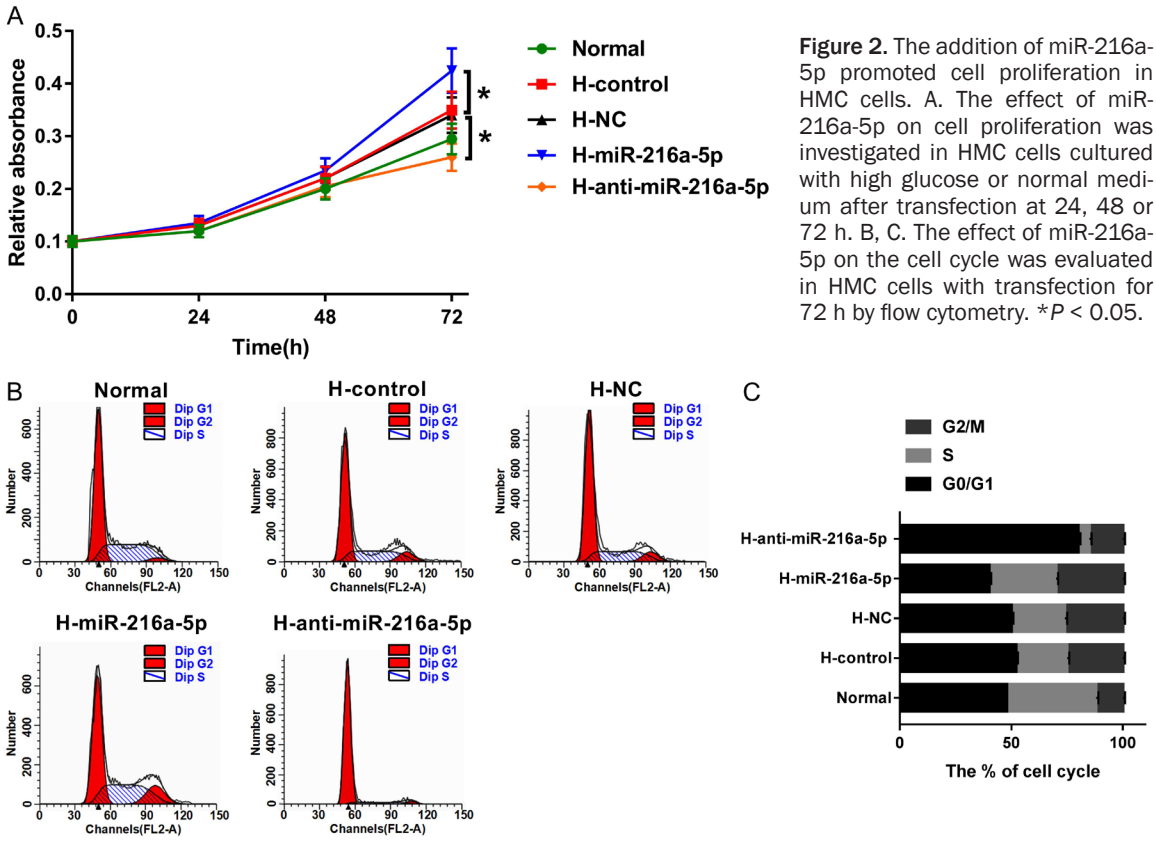


Figure 2. The addition of miR-216a-5p promoted cell proliferation in HMC cells. A. The effect of miR-216a-5p on cell proliferation was investigated in HMC cells cultured with high glucose or normal medium after transfection at 24, 48 or 72 h. B, C. The effect of miR-216a-5p on the cell cycle was evaluated in HMC cells with transfection for 72 h by flow cytometry. *$P < 0.05$.

San Diego, CA, USA). Student's *t* test was used to investigate significant differences and *$P < 0.05$ was regarded as being statistically significant.

Results

miR-216a-5p was enhanced and FoxO1 was impaired in DN

To investigate whether miR-216a-5p and FoxO1 were required for DN progression, the expressions of miR-216a-5p and FoxO1 were measured in the DN tissues. A great increase of the miR-216a-5p levels was observed in DN tissues compared with the normal group (n = 32) (**Figure 1A**). However, the mRNA expression of FoxO1 was obviously inhibited in the DN group (**Figure 1B**). Similarly, a strong reduction of FoxO1 was displayed in DN compared with the normal group at the protein level (**Figure 1C** and **1D**). Moreover, the miR-216a-5p abundance was also detected in the serum of patients with or without DN (n = 10). The results showed an abnormally elevated expression of miR-216a-5p in the DN group (**Figure 1E**). These findings showed highly expressed miR-216a-5p and impaired FoxO1 in DN, indicating that miR-216a-5p and FoxO1 were associated with DN progression.

miR-216a-5p promoted cell proliferation and arrested the cell cycle in high glucose-treated HMC cells

To further investigate the function of miR-216a-5p in DN progression, cell proliferation was measured in the high glucose-treated HMC cells by CCK-8. HMC cells were transfected with miR-26a-5p mimics or inhibitors and incubated with high glucose. The results showed that miR-216a-5p overexpression promoted cell proliferation compared with the NC group upon the treatment of high glucose, whereas the knockdown of miR-216a-5p impaired cell proliferation (**Figure 2A**). As the cell cycle was directly associated with cell proliferation, the effect of miR-216a-5p on the cell cycle was evaluated in HMC cells. Compared with the cells transfected with the NC group, the addition of miR-216a-5p inhibited the percentage of G1/G0 phase cells and enhanced the cells at the S phase (**Figure 2B** and **2C**). However, the

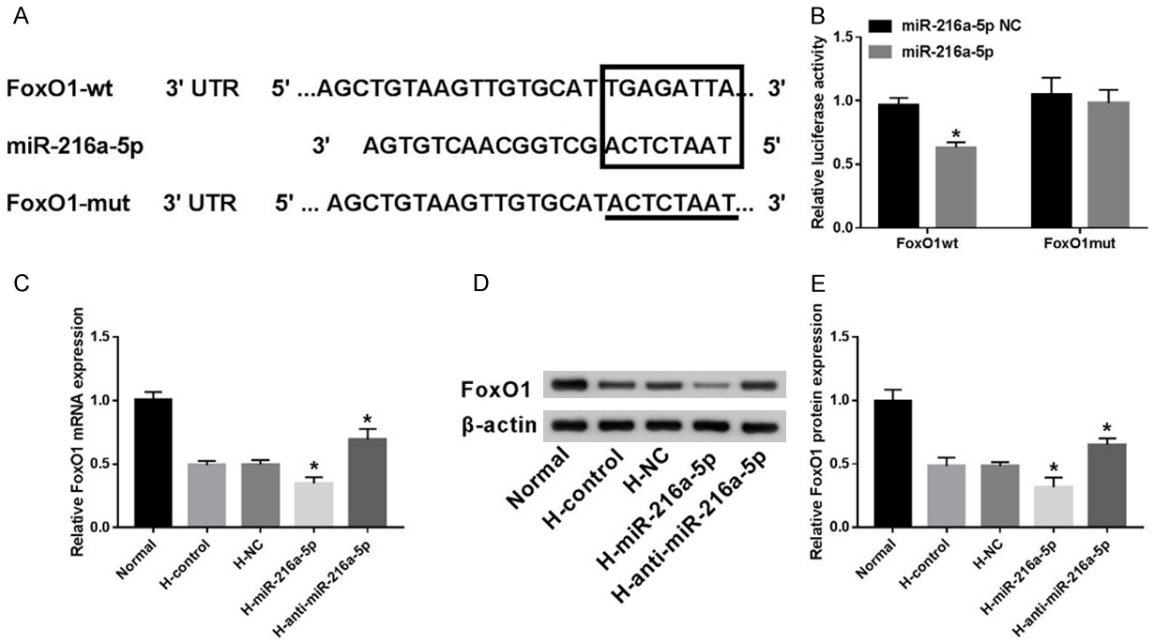


Figure 3. FoxO1 was a target of miR-216a-5p. A. The potential binding sites of FoxO1 and miR-216a-5p were predicted by sequence alignment. B. The luciferase activity was investigated in HMC cells co-transfected FoxO1-wt or FoxO1-mut with miR-216a-5p or miR-216a-5p NC. C. The effect of miR-216a-5p on FoxO1 mRNA expression was measured in HMC cells with both high and normal glucose treatment. D, E. The FoxO1 protein abundance was measured in the transfected HMC cells. *$P < 0.05$.

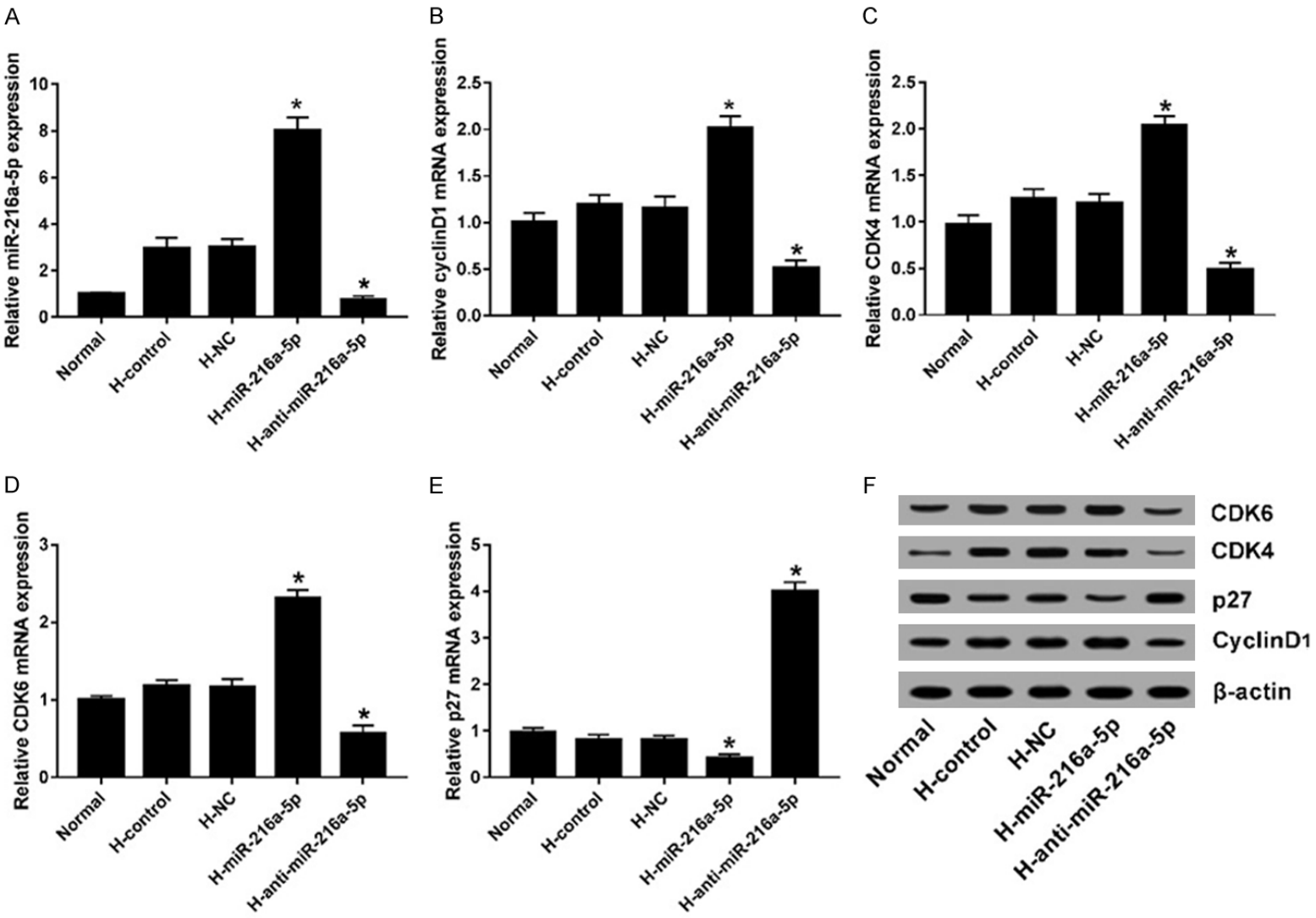
abrogation of miR-216a-5p arrested the cell cycle at the G1/G0 phase and induced an obvious loss of a percentage of the S phase cells (**Figure 2B** and **2C**). Taken together, these findings suggested that miR-216a-5p induced cell proliferation by regulating the cell cycle in high glucose-stimulated HMC cells.

FoxO1 was a target of miR-216a-5p

Since FoxO1 was inhibited in DN, we next investigated whether FoxO1 was directly inhibited by miR-216a-5p. Bioinformatics analysis predicted the potential binding sites of miR-216a-5p and FoxO1 by sequence alignment in NCBI (**Figure 3A**). To validate the prediction, luciferase activity was investigated in HMC cells transfected with luciferase report vectors and miR-216a-5p or NC. The results showed that the luciferase activity of the FoxO1-wt reporter gene was obviously inhibited in cells with miR-216a-5p transfection compared with the NC group, whereas there was little change by the miR-216a-5p mimics in response to the FoxO1-mut vectors (**Figure 3B**). Moreover, the effect of miR-216a-5p on FoxO1 expression was investigated in high glucose-induced HMC cells. As a result, high glucose treatment impaired FoxO1 mRNA expressions, and miR-216a-5p addition exacerbated the inhibitory effect on the FoxO1 mRNA level, while miR-216a-5p depletion protected the abundance of FoxO1 mRNA (**Figure 3C**). Furthermore, the abundance of the FoxO1 protein showed similar trends with respect to miR-216a-5p mimics or inhibitors upon high glucose treatment (**Figure 3D** and **3E**). These data indicated that FoxO1 might be a direct target of miR-216a-5p.

miR-216a-5p regulated cell cycle-related biomarkers in high glucose-treated HMC cells

Seeing that miR-216a-5p regulated the cell cycle, the related biomarkers were measured at the mRNA and protein levels in high glucose-treated HMC cells. The cells were transfected with miR-216a-5p or anti-miR-216a-5p. As a result, high glucose induced highly expressed miR-216a-5p and miR-216a-5p expression was enhanced by the transfection of miR-216a-5p mimics while being inhibited by anti-miR-216a-5p treatment (**Figure 4A**). Moreover, the accumulation of miR-216a-5p enhanced cyclinD1, CDK4 and CDK6 expressions while it


A
Relative miR-216a-5p expression
B
Relative cyclinD1 mRNA expression
C
Relative CDK4 mRNA expression
D
Relative CDK6 mRNA expression
E
Relative p27 mRNA expression
F
CDK6
CDK4
p27
CyclinD1
β-actin
Normal
H-control
H-NC
H-miR-216a-5p
H-anti-miR-216a-5p

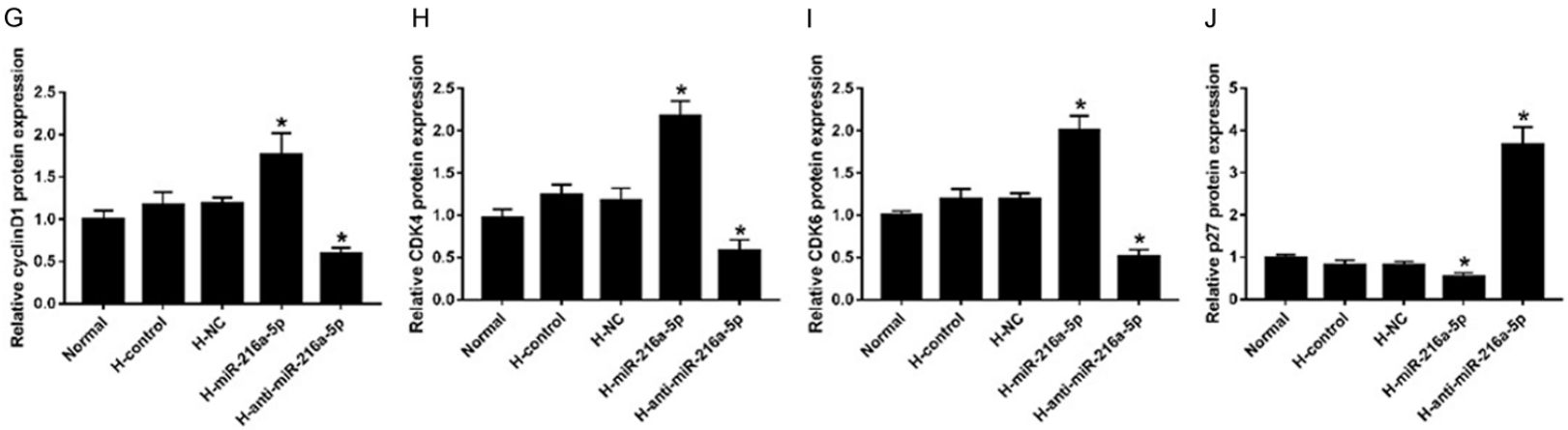


Figure 4. miR-216a-5p regulated the expression of the cell-cycle-related biomarkers in HMC cells. A. The expression of miR-216a-5p was measured in the HMC cells after transfection with miR-216a-5p or anti-miR-216a-5p. B-E. The mRNA expressions of cyclinD1, CDK4, CDK6 and p27 were detected in the HMC cells. F-J. The protein abundances of cyclinD1, CDK4, CDK6 and p27 were examined in HMC cells. $*P < 0.05$.

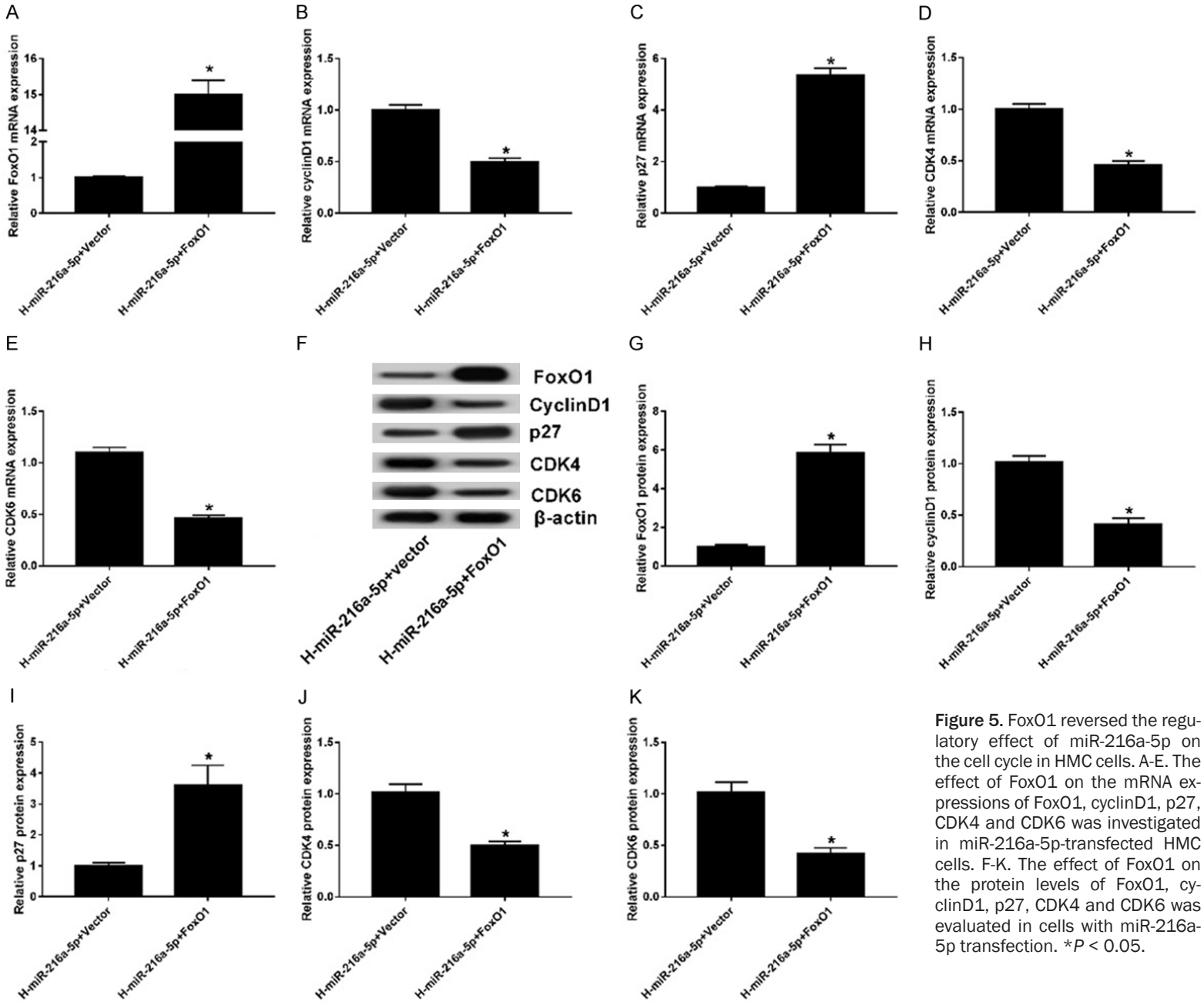


Figure 5. FoxO1 reversed the regulatory effect of miR-216a-5p on the cell cycle in HMC cells. A-E. The effect of FoxO1 on the mRNA expressions of FoxO1, cyclinD1, p27, CDK4 and CDK6 was investigated in miR-216a-5p-transfected HMC cells. F-K. The effect of FoxO1 on the protein levels of FoxO1, cyclinD1, p27, CDK4 and CDK6 was evaluated in cells with miR-216a-5p transfection. *$P < 0.05$.

inhibited the p27 level at the mRNA levels, but the absence of miR-216a-5p showed the opposite effect on these abundances (**Figure 4B-E**). In addition, the protein abundances of cyclinD1, CDK4, and CDK6 were enhanced while p27 was suppressed in high glucose-treated HMC cells transfected with miR-216a-5p compared with the NC group (**Figure 4F-J**). However, reduction of cyclinD1, CDK4, and CDK6 and elevated p27 were shown in the HMC cells by the anti-miR-216a-5p treatment (**Figure 4F-J**). Considered together, miR-216a-5p might regulate cell cycle-related biomarkers at the mRNA and protein levels in high glucose-treated HMC cells.

FoxO1 reversed miR-216a-5p-mediated effect on cell cycle-related biomarkers in high glucose-treated HMC cells

To investigate the effect of FoxO1 on the miR-216a-5p-mediated regulation of the cell cycle, FoxO1 overexpression vectors or vectors alone were introduced into high glucose-treatment HMC cells with miR-216a-5p transfection. As a result, the mRNA expression of FoxO1 was evidently enhanced with the transfection of FoxO1 overexpression vectors compared with vectors alone (**Figure 5A**). Moreover, the levels of cell cycle-related genes were measured after transfection. The results showed that the restoration of FoxO1 inhibited the expressions of cyclinD1, CDK4 and CDK6 while it induced p27 abundance at the mRNA level (**Figure 5B-E**). The regulatory effect was also investigated in HMC cells at the protein level. An obvious increase of FoxO1 protein abundance was evident in the high glucose-treated HMC cells transfected with miR-216a-5p and FoxO1 compared to the miR-216a-5p and vectors alone (**Figure 5F** and **5G**). In addition, the presence of FoxO1 resulted in a strong reduction of cyclinD1, CDK4 and CDK6, while it led to a specially enhanced p27 at protein level (**Figure 5F** and **5H-K**). Together, the results uncovered that FoxO1 restoration overturned the effect of miR-216a-5p on the cell cycle by regulating cyclinD1, CDK4, CDK6 and p27 abundances in high glucose-treated HMC cells.

Discussion

DN is a common lethal complication of diabetes which may result in end stage renal disease. Previous research suggests that miRNAs may play a vital role in DN progression and provide new therapeutic targets [18]. Here we first detected the expression of miR-216a-5p in DN tissues and our results show that miR-216a-5p is elevated in DN tissues. This is in agreement with previous studies that also suggested increased levels of miR-216a-5p in DN [14, 19]. These data indicated that miR-216a-5p might play an important role in DN progression.

HMC cells are the primary targets of glomerular diseases, including ND [20]. miR-216a-5p is reported as a novel miRNA involved in cell proliferation in cervical and prostate cancer [11, 12]. However, there is no direct evidence in support of miR-216a-5p being associated with cell proliferation in DN progression. Transforming growth factor beta 1 (TGF-β1) and high glucose are usually used to induce the model of DN in vitro [8]. Hence, we next investigated the effect of miR-216a-5p on cell proliferation in high glucose-treated HMC cells. We found that miR-216a-5p overexpression promoted cell proliferation while its knockdown suppressed cell proliferation in high-treated cells, which was also similar to that in renal cell carcinoma [13]. Moreover, the cell cycle was investigated because of the association with cell proliferation. The results showed that miR-216a-5p decreased the percentage of G1/G0 phase cells, whereas miR-216a-5p deficiency arrested cells at the G1/G0 phase. These findings revealed that miR-216a-5p promoted the cell proliferation of HMC under high glucose conditions. This is consistent with the functions of miRNAs in other efforts. miR-34a knockdown inhibited mesangial cell proliferation by regulating growth arrest-specific 1 (GAS1) expression in mice with early DN [21]. Moreover, up-regulated miR-27a contributed to the proliferation of high glucose-induced mesangial cells by targeting PPARγ, and its knockdown suppressed DN progression [10]. In contrast, there are some miRNAs having the opposite effect on mesangial cell proliferation. For example, the inhibition of miR-195 promoted cell proliferation and protected mesangial cells in mice with early DN from cell apoptosis [22]. Also, miR-451 overexpression inhibited mesangial cell proliferation in vitro and in vivo by regulating the p38 mitogen-activated protein kinases (MAPK) signaling pathway by targeting tyrosine3-monooxygenase/tryptophan5-monooxygenase activation protein zeta (YWHAZ) [23]. In addition, miR-21 also inhibited mesangial cell

proliferation by regulating the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway by targeting the phosphate and tension homology deleted on chromosome ten (PTEN) [24].

Functional miRNAs were realized by regulating target gene levels in many conditions. Therefore, we further expected to probe a promising target of miR-216a-5p in the HMC cells. A former study suggested that Hexokinase-2 (HK2) was a target of miR-216a-5p and regulated uveal melanoma growth by mediating Warburg effect [25]. Moreover, miR-216a-5p facilitated the tumorigenesis of cervical cancer cells by regulating cell proliferation and migration by targeting the zinc finger E-box-binding homeobox 1 (ZEB1) [12]. FoxO1 was reported to be associated with the proliferation of mesangial cells in DN [16]. Moreover, FoxO1 might inhibit mesangial cell proliferation by regulating G1 progression [26]. Having established that FoxO1 was inhibited in DN, we next expected to validate whether FoxO1 was a target of miR-216a-5p. A bioinformatics analysis showed the potential binding sites of miR-216a-5p and FoxO1 by sequence alignment and luciferase activity analysis and supported the prediction. Moreover, the results showed that FoxO1 was negatively correlated with miR-216a-5p in high glucose-treated HMC cells.

Cell cycle regulation involved complex events. Such events revealed that cell cycle related proteins provided a promising mechanism for the inhibition of growth [27, 28]. An earlier study suggested that the up-regulation of cyclinD1, CDK4 and CDK6 were involved in cell cycle progression and arrested cells at the G0/G1 phase [28]. P27 has been reported as an inhibitor of CDK protein with an anti-proliferative effect on mesangial cells [29]. In our study, we found that miR-216a increased the expressions of cyclinD1, CDK4 and CDK6 while it inhibited the p27 level at the mRNA and protein levels. However, the regulatory effect was abated by FoxO1 restoration in high glucose-treated HMC cells. This is consistent with a previous study indicating that FoxO1 was inhibited in DN and regulated the cell cycle in mesangial cells treated with high glucose [17]. Accordingly, in the study, the results also showed that miR-216a-5p inhibited the abundances of cyclinD1, CDK4, and CDK6. Hence, we expect to study the interaction between miR-216a-5p and cyclinD1, CDK4 and CDK6 in the future. Moreover, we just evaluated the effect of miR-216a on DN progression in vitro. A mouse model of DN is needed for a better understanding the mechanism in vivo.

Conclusion

In conclusion, miR-216a-5p was increased and FoxO1 was impaired in DN tissues. miR-216a-5p contributed to cell proliferation by regulating the cell cycle in high glucose-treated HMC cells. Notably, FoxO1 was directly bound and inhibited by miR-216a-5p. Moreover, miR-216a-5p enhanced cyclinD1, CDK4 and CDK6 abundances but reduced the p27 level at the mRNA and protein levels, but the regulatory effect was reversed by FoxO1 restoration. Collectively, miR-216a-5p promoted cell proliferation by regulating the cell cycle by targeting FoxO1 in high glucose-stimulated HMC cells, providing a promising therapeutic biomarker for DN treatment.

Acknowledgements

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Disclosure of conflict of interest

None.

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