RETRACTED ARTICLE: Silence of lncRNA GAS5 alleviates high glucose toxicity to human renal tubular epithelial HK-2 cells through regulation of miR-27a

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ABSTRACT
Renal tubular damage caused by persistent high glucose environment has been found to contribute to diabetic nephropathy. This study explored the effects of IncRNA growth arrest-specific 5 (GAS5) on high glucose-stimulated human renal tubular epithelial HK-2 damage, as well as the possible internal molecular mechanism. Viability and apoptosis of HK-2 cells were assessed with the help of CCK-8 assay and Annexin V-FITC/PI staining, respectively. Cell transfection was used to change the expression of GAS5, miR-27a and BNIP3. We found that high glucose stimulation suppressed HK-2 cell viability but induced cell apoptosis. The expression of GAS5 was increased in HK-2 cells under high glucose environment. Silence of GAS5 mitigated the high glucose-caused HK-2 cell viability reduction and apoptosis. Overexpression of miR-27a reversed the effects of GAS5 on high glucose-stimulated HK-2 cells. Overexpression of BNIP3 aggravated the high glucose-caused HK-2 cell viability reduction and apoptosis and activation of JNK pathway. Knockdown of BNIP3 had opposite effects. In conclusion, this research further confirmed the pro-apoptotic roles of GAS5 in renal tubular epithelial cells under high glucose environment. Silence of GAS5 alleviated high glucose toxicity to human renal tubular epithelial HK-2 cells might be via down-regulating miR-27a and BNIP3, and then inactivating JNK pathway.

HIGHLIGHTS
1. HG suppresses HK-2 cell viability, but promotes cell apoptosis;
2. HG enhances the expression of GAS5 in HK-2 cells;
3. Silence of GAS5 alleviates the HG-caused HK-2 cell toxicity;
4. miR-27a participates in the effects of GAS5 silencing on HG-stimulated HK-2 cells;
5. BNIP3 is regulated by miR-27a and related to the HG toxicity to HK-2 cells.

Introduction
Diabetic nephropathy is one of the most major complications of patients with type I or type II diabetes [1]. Approximately 30–40% of patients with either type I or type II diabetes will develop nephropathy ultimately and this renal damage has become the leading cause of end-stage renal failure-related death all over the world [2,3]. With the increasing prevalence of diabetic, diabetic nephropathy has become a serious challenge for government and researchers [4,5]. Nephron is the structural and functional unit of the kidney and renal tubular is one of the two basic components of the nephron with reabsorption and excretion features [6]. Pathology research found that persistent high glucose environment-caused renal tubular damage contributes to the occurrence and development of diabetic nephropathy [7,8]. Therefore, it is worthy believed that a more clear understanding of renal tubular damage caused by high glucose stimulation will be helpful for diabetic nephropathy prevention, diagnosis and treatment.

Long non-coding RNAs (lncRNAs) are defined as RNA transcripts longer than 200 nucleotides (nt) and no apparent protein-coding function [9]. The importance of lncRNAs in the occurrence and development of multiple human diseases, including diabetic nephropathy, has been uncovered in recent years [10,11]. lncRNA growth arrest-specific 5 (GASS) is a recently discovered lncRNA that play crucial regulatory roles in cell growth, proliferation, survival and apoptosis [12,13]. Carter et al. reported that GASS was correlated to the prevalence of type II diabetes [14]. Geng et al. indicated that GASS participated in the regulation of renal tubular epithelial cell apoptosis [15]. More experimental studies are still needed to further explore the effects of GASS on high glucose toxicity to renal tubular epithelial cells.

MicroRNAs (miRNAs) are another type of non-coding RNA transcripts with 20–24 nucleotides (nt) [16]. A number of
IncRNAs, including GAS5, have been found to be implicated in the occurrence and development of human diseases via modulating miRNAs expression [17,18]. miRNAs are able to regulate the expression of multiple genes at transcription and post-transcription levels [19]. miRNA-27a (miR-27a) up-regulation has been demonstrated to contribute to renal tubulointerstitial fibrosis and podocyte injury in diabetic nephropathy [20,21].

In the present study, human renal tubular epithelial HK-2 cells were exposed to high glucose stimulation. We focused our investigation on the potential regulatory effects of GAS5 on HK-2 cell viability loss and apoptosis caused by high glucose treatment. Moreover, the possible internal molecular mechanism related to miR-27a and Bcl-2 Nineteen kilodalton Interacting Protein 3 (BNIP3) were also analyzed. Our findings will provide experimental evidence for understanding the critical effects of GAS5 on renal tubular damage in diabetic nephropathy.

Materials and methods

Cell culture and treatment

Human renal tubular epithelial cell line HK-2 was provided by the Stem Cell Bank, Chinese Academy of Science (Shanghai, China). Cells were cultured in keratinocyte serum free medium (K-SFM, Invitrogen, Carlsbad, CA, USA) containing with 1% (v/v) gentamicin/amphotericin solution (Gibco, Life Technologies, Carlsbad, CA, USA) in 75 cm² flask according to the manufacturer’s instruction. Flask was placed in a humidity incubator (Sanyo, Jencons, United Kingdom) at 37°C with 5% CO₂.

D-glucose was purchased from Sigma-Aldrich (St Louis, MO, USA, catalogue number: G8270). For high glucose treatment, HK-2 cells were treated by 45 mM D-glucose for 48 h in this research. Cells cultured in normal medium served as control.

Cell transfection

si-GAS5, si-negative control (si-NC), miR-27a mimic and NC mimic were all designed and synthesized by GenePharma Corporation (Shanghai, China). The full-length BNIP3 sequence was constructed in pEX-2 plasmid (GenePharma Corporation), which was referred as pEX-BNIP3. The empty pEX-2 plasmid was used as NC. Short-hairpin RNA directed against BNIP3 was constructed in U6/GFP/Neo plasmid (GenePharma Corporation), which was referred as sh-BNIP3. The U6/GFP/Neo plasmid carrying a non-targeting sequence was constructed in pEX-2 plasmid (GenePharma Corporation (Shanghai, China). The full-length BNIP3 mimic were all designed and synthesized by GenePharma Corporation (Shanghai, China). Cells were isolated with the help of TRIzol™ Plus RNA Purification kit (Invitrogen). TaqMan™ Non-coding RNA Assay was conducted to measure the expression level of GAS5. β-Actin acted as internal control. For miR-27a, total miRNAs in HK-2 cells was isolated with the help of mirVana™ miRNA Isolation kit (Invitrogen). mirVana™ qRT-PCR miRNA Detection kit was performed to measure the expression level of miR-27a. U6 snRNA acted as internal control. Data were analyzed by 2^−ΔΔCt method [22].

Cell viability assay

Viability of HK-2 cells after different treatment or transfection was detected with the help of cell counting kit-8 (CCK-8) assay. Briefly, transfected or non-transfected HK-2 cells were seeded into 96-well plate with 5 × 10³ cells per well with or without 45 mM D-glucose treatment for 48 h. Then, 10 µl CCK-8 kit solution was added into the culture medium of each well and the 96-well plate was placed at 37°C for 1 h in incubator. Subsequently, the absorbance of each well at 450 nm was recorded using Micro-plate Reader (ELX800, BioTek Instruments, Winoski, VT, USA). Data were expressed as a percentage of control.

Cell apoptosis assay

Apoptosis of HK-2 cells after different treatment or transfection was evaluated with the help of Annexin V-FITC/PI apoptosis detection kit (BD Bioscience, Franklin Lakes, NJ, USA). Briefly, transfected or non-transfected HK-2 cells were seeded into 6-well plate with 1 × 10⁵ cells per well with or without 45 mM D-glucose treatment for 48 h. Then, cells in each group were harvested, washed with phosphate buffered saline (PBS) for twice and re-suspended in 200 µl binding buffer. After mixed with 5 µl Annexin V-FITC solution and 5 µl PI solution, the cells were incubated at room temperature for 15 min in the dark. Subsequently, cells were washed with PBS for twice, re-suspended in 400 µl binding buffer and subjected to flow cytometer (Beckman Coulter, Fullerton, CA, USA) analysis. Data were analyzed using FCS Express V3 software (De Novo Software, Los Angeles, CA, USA).

Western blotting

The protein used for western blotting was extracted using RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with protease inhibitors (Roche, Basel, Switzerland). The BCA Protein assay (Thermo Fisher Scientific) was used for measure the concentration of protein. Western blotting was done as previously described [23]. All antibodies were purchased from Abcam Biotechnology (Cambridge, MA, USA). The catalogue information was as follows: p53 (ab131442), Bcl-2 (ab196495), Pro-Caspase 3 (ab32150), Cleaved-Caspase 3 (ab2302), BNIP3
(ab38621), c-Jun N-terminal kinase (JNK, ab179461), p-JNK (ab124956), β-actin (ab8226), Goat Anti-Rabbit IgG H&L (HRP) (ab205718) and Goat Anti-Mouse IgG H&L (HRP) (ab205719). The signals of proteins were recorded with the help of Bio-Rad ChemiDoc™ XRS system (Bio-Rad Laboratories, Hercules, CA, USA). The intensities of bands were analyzed by Image Lab™ software (Bio-Rad Laboratories).

**Statistical analysis**

All experiments were repeated three times in triplicate. Results of multiple experiments were presented as mean ± standard deviation (SD). Graphpad 6.0 software was used for statistical analysis. p values between two groups were calculated using Student’s t-test, and p values between more than three groups were calculated using one-way analysis of variance (ANOVA). p < .05 was considered to indicate a statistically significant result.

**Results**

**High glucose stimulation suppressed HK-2 cell viability and promoted cell apoptosis**

First, we detected the viability and apoptosis of HK-2 cells after 45 mM D-glucose treatment. Results showed that 45 mM D-glucose treatment significantly reduced the viability of HK-2 cells (Figure 1(A), p < .01), while remarkably induced HK-2 cell apoptosis (Figure 1(B), p < .01). Moreover, compared to control group, the protein expression level (rate) of p-53 and C/P-Caspase 3 in HK-2 cells were both increased (Figure 1(C), p < .01), as well as the protein expression level of Bcl-2 was decreased (p < .05) in 45 mM D-glucose treatment group. These above results suggested that high glucose stimulation could suppress the viability of human renal tubular epithelial HK-2 cells and promote cell apoptosis.

**Silence of GAS5 mitigated the high glucose-caused HK-2 cell viability loss and apoptosis**

Next, we explored the effects of GAS5 on high glucose stimulation-caused HK-2 cell viability loss and apoptosis. Figure 2(A) displayed that 45 mM D-glucose treatment dramatically up-regulated the expression of GAS5 in HK-2 cells (p < .01). si-GAS5 transfection notably reduced the expression of GAS5 in HK-2 cells (Figure 2(B), p < .01). In addition, si-GAS5 transfection noticeably mitigated the 45 mM D-glucose treatment-caused cell viability reduction (Figure 2(C), p < .05) and apoptosis enhancement (Figure 2(D), p < .05). Besides, the protein expression level (rate) of p-53 and C/P-Caspase 3 in HK-2 cells were both decreased (Figure 2(E), p < .05 or p < .01), as well as the protein expression level of Bcl-2 was increased (p < .05) in 45 mM D-glucose treatment + si-GAS5 transfection group, relative to single 45 mM D-glucose treatment group. These above finding implied that up-regulation of GAS5 was related to the effects of high glucose on HK-2 cells and indicated that silence of GAS5 could mitigate the high glucose-caused HK-2 cell viability loss and apoptosis.

**Silence of GAS5 reversed the high glucose-caused up-regulation of miR-27a expression in HK-2 cells**

The expression of miR-27a in HK-2 cells after 45 mM D-glucose treatment and/or si-GAS5 transfection was measured.
As presented in Figure 3, 45 mM D-glucose treatment significantly up-regulated the expression of miR-27a in HK-2 cells ($p < .01$), while si-GAS5 transfection distinctly reversed the 45 mM D-glucose treatment-caused up-regulation of miR-27a expression in HK-2 cells ($p < .05$). This finding implied that down-regulation of miR-27a might participate in the effects of GAS5 silencing on high glucose-stimulated HK-2 cells.

**Down-regulation of miR-27a participated in the effects of GAS5 silencing on high glucose-stimulated HK-2 cells**

miR-27a mimic was transfected into HK-2 cells to up-regulate the expression of miR-27a (Figure 4(A), $p < .01$). Compared to high glucose + si-GAS5 + NC mimic group, the viability of HK-2 cells was decreased (Figure 4(B), $p < .05$) and the rate of apoptotic cells was increased (Figure 4(C), $p < .05$) in high glucose + si-GAS5 + miR-27a mimic group. Furthermore, the protein expression level (rate) of p53 and Bcl-2, Pro-Caspase 3 and Cleaved-Caspase 3 in HK-2 cells were both increased (Figure 4(D), $p < .05$ or $p < .01$), as well as the protein expression level of Bcl-2 was decreased ($p < .05$) in high glucose + si-GAS5 + miR-27a mimic group, relative to high glucose + si-GAS5 + NC mimic group. These above results indicated that silence of GAS5 mitigated the high glucose stimulation-caused HK-2 cell viability loss and apoptosis might be via down-regulating miR-27a.

**miR-27a regulated the expression of BNIP3 in HK-2 cells**

Then, we measured the expression of BNIP3 in HK-2 cells after 45 mM D-glucose treatment and/or si-GAS5 or miR-27a mimic transfection. Results displayed that 45 mM D-glucose
treatment remarkably enhanced the expression of BNIP3 in HK-2 cells (Figure 5, \( p < .01 \)). si-GAS5 transfection notably alleviated the 45 mM D-glucose treatment-caused enhancement of BNIP3 expression in HK-2 cells (\( p < .05 \)). More importantly, miR-27a mimic transfection significantly attenuated the effect of si-GAS5 transfection on BNIP3 expression in 45 mM D-glucose-treated HK-2 cells (\( p < .05 \)). This finding suggested

![Figure 3. Silence of GAS5 reversed the high glucose-caused up-regulation of miR-27a expression in HK-2 cells. After 45 mM D-glucose treatment and/or si-GAS5 transfection, the expression level of miR-27a in HK-2 cells was measured using qRT-PCR. HG: High glucose; GAS5: Long non-coding RNA growth arrest-specific 5; miR-27a: MicroRNA-27a; NC: Negative control. \( N = 3 \). \( *p < .05; **p < .01. \)](image)

![Figure 4. Overexpression of miR-27a reversed the effects of GAS5 silencing on high glucose-stimulated HK-2 cells. (A) After NC mimic or miR-27a mimic transfection, the expression level of miR-27a in HK-2 cells was measured using qRT-PCR. After 45 mM D-glucose treatment and/or si-GAS5 or miR-27a mimic transfection, the viability of HK-2 cells, (B) the apoptosis of HK-2 cells, and (D) the protein expression levels of p53, Bcl-2, Pro-Caspase 3 and Cleaved-Caspase 3 in HK-2 cells were assessed with the help of cell counting kit-8 (CCK-8) assay, Annexin V-FITC/PI apoptosis detection kit and western blotting, respectively. HG: High glucose; GAS5: Long non-coding RNA growth arrest-specific 5; miR-27a: MicroRNA-27a; NC: Negative control. \( N = 3 \). \( *p < .05; **p < .01. \)](image)

![Figure 5. miR-27a regulated the expression of BNIP3 in HK-2 cells. After 45 mM D-glucose treatment and/or si-GAS5 or miR-27a mimic transfection, the protein expression level of BNIP3 in HK-2 cells was evaluated using western blotting. HG: High glucose; GAS5: Long non-coding RNA growth arrest-specific 5; miR-27a: MicroRNA-27a; BNIP3: Bcl-2 Nineteen kilodalton Interacting Protein 3; NC: Negative control. \( N = 3 \). \( *p < .05; **p < .01. \)](image)
that miR-27a could regulate the expression of BNIP3 in HK-2 cells.

Overexpression of BNIP3 aggravated the high glucose-caused HK-2 cell viability loss and apoptosis while knockdown of BNIP3 had opposite effects.

To investigate the effects of BNIP3 on high glucose stimulation-caused HK-2 cell viability reduction and apoptosis, pEX-BNIP3 and sh-BNIP3 were transfected into HK-2 cells, respectively. Results presented that pEX-BNIP3 transfection remarkably enhanced the protein level of BNIP3 in HK-2 cells (Figure 6(A), p < .01) while sh-BNIP3 transfection notably reduced the protein level of BNIP3 in HK-2 cells (p < .01). pEX-BNIP3 transfection significantly aggravated the 45 mM D-glucose treatment-caused HK-2 cell viability reduction (Figure 6(B), p < .05) and apoptosis enhancement (Figure 6(C), p < .05). On the contrary, sh-BNIP3 transfection noticeably weakened the 45 mM D-glucose treatment-caused HK-2 cell viability reduction (p < .05) and apoptosis enhancement (p < .05). Moreover, compared to single 45 mM D-glucose treatment group, the protein expression level (rate) of p53 and C/P-Caspase 3 in HK-2 cells were both increased in 45 mM D-glucose treatment + pEX-BNIP3 transfection group (Figure 6(D), p < .05) and decreased in 45 mM D-glucose treatment + sh-BNIP3 transfection group (p < .05 or p < .01). The protein expression level of Bcl-2 was decreased in 45 mM D-glucose treatment + pEX-BNIP3 transfection group (p < .05) and increased in 45 mM D-glucose treatment + sh-BNIP3 transfection group (p < .05), relative to single 45 mM D-glucose treatment group. These above findings indicated that overexpression of BNIP3 could aggravate the high glucose-caused HK-2 cell viability reduction and apoptosis while knockdown of BNIP3 had opposite effects.

BNIP3 was involved in the high glucose-caused JNK pathway activation in HK-2 cells

Finally, we analyzed the activation of JNK pathway in HK-2 cells after 45 mM D-glucose treatment and/or pEX-BNIP3 (or sh-BNIP3) transfection. Results showed that 45 mM D-glucose treatment significantly activated JNK pathway in HK-2 cells via up-regulating the protein expression rate of p-JNK/JNK (Figure 7, p < .01). pEX-BNIP3 transfection aggravated the 45 mM D-glucose treatment-caused activation of JNK pathway in HK-2 cells by enhancing the protein expression rate of p-JNK/JNK (p < .05). On the contrary, sh-BNIP3 transfection weakened the 45 mM D-glucose treatment-caused activation of JNK pathway in HK-2 cells by reducing the protein expression rate of p-JNK/JNK (p < .05). This result suggested that BNIP3 was also involved in the high glucose-caused JNK pathway activation in HK-2 cells.

Discussion

Diabetic nephropathy is a common kidney disease caused by type I or type II diabetes [1,24]. Renal tubular damage is evident in patients with diabetic nephropathy [25]. Here, human
renal tubular epithelial HK-2 cells were stimulated by high glucose environment to cause damage and we found that the expression of GAS5 was increased in HK-2 cells. Moreover, we revealed that silence of GAS5 could mitigate the high glucose-caused HK-2 cell viability reduction and apoptosis by down-regulating the expression of miR-27a. Besides, miR-27a could regulate the expression of BNIP3 in HK-2 cells. Overexpression of BNIP3 could aggravate the high glucose-caused HK-2 cell viability reduction and apoptosis might be via promoting the activation of JNK pathway. Knockdown of BNIP3 had opposite effects.

As a critical class of regulatory RNAs in cells, lncRNAs have been demonstrated to participate in the modulation of multiple cellular biological processes, such as cell proliferation, autophagy and apoptosis [26]. Aberrant lncRNAs expression and its association with the occurrence and development of diseases have been uncovered in a number of human diseases, including diabetic nephropathy [10,27]. Sathishkumar et al. reported that GAS5 was up-regulated in peripheral blood mononuclear cells of patients with type II diabetes [28]. Geng et al. proved that up-regulation of GAS5 promoted the apoptosis of HK-2 cells while suppression of GAS5 inhibited the apoptosis of HK-2 cells [15]. In the current research, we revealed that high glucose stimulation significantly suppressed HK-2 cell viability and promoted cell apoptosis, as well as up-regulated the expression of GAS5, which suggested that GAS5 might exert pro-apoptotic effects on renal tubular epithelial cells in diabetic nephropathy. More importantly, we found that silence of GAS5 notably mitigated the HK-2 cell viability reduction and apoptosis caused by high glucose stimulation, which indicated that silence of GAS5 could exert anti-apoptotic effects on high glucose-stimulated renal tubular epithelial cells.

miRNAs are usually regulated by lncRNAs and related to the gene expression regulatory activities of lncRNAs [29]. Up-regulation of miR-27a has been proved to be associated with the renal tubulointerstitial fibrosis and podocyte injury in diabetic nephropathy [20,21]. Song et al. reported that omentin-1, a novel identified adipokine, could protect renal function of mice with type II diabetic nephropathy via down-regulating miR-27a [30]. In this study, we found that high glucose stimulation significantly enhanced the expression of miR-27a in HK-2 cells, while the silence of GAS5 notably reversed the up-regulation of miR-27a in HK-2 cells caused by high glucose stimulation. Besides, overexpression of miR-27a dramatically reversed the effects of GAS5 silencing on high glucose stimulation-caused HK-2 cell viability reduction and apoptosis. These results indicated that silence of GAS5 exerts anti-apoptotic effects on high glucose-stimulated renal tubular epithelial cells at least partially via down-regulating miR-27a.

BNIP3 is a member of the Bcl-2 protein family, which can induce cell apoptosis by interacting with viral and cellular anti-apoptotic proteins [31]. Huang et al. reported that high glucose stimulation could up-regulate the expression of BNIP3 in proximal tubular cells [32]. In consistent with the previous study, we found that high glucose stimulation also notably enhanced the expression of BNIP3 in HK-2 cells. A number of miRNAs have been found to participate in the regulation of BNIP3 expression in cells [33,34]. Here, we revealed that silence of GAS5 alleviated the high glucose stimulation-caused up-regulation of BNIP3 in HK-2 cells. Overexpression of miR-27a attenuated the effect of GAS5 silencing. Moreover, we discovered that overexpression of BNIP3 aggravated the high glucose stimulation-caused HK-2 cell viability reduction and apoptosis, while knockdown of BNIP3 had opposite effects. These above findings suggested that BNIP3 participated in the anti-apoptotic effects of GAS5 silencing on high glucose-stimulated renal tubular epithelial cells.

It is well known that JNK pathway plays critical roles in the promotion of cell apoptosis under high glucose environment [35]. BNIP3 has also been reported to be associated with the regulation of JNK pathway in cells [36,37]. Here, we found that high glucose stimulation activated the JNK pathway in HK-2 cells. Overexpression of BNIP3 intensified the high glucose stimulation-caused activation of JNK pathway while knockdown of BNIP3 had opposite effect. These findings indicated that BNIP3 also be related to the activation of JNK pathway in HK-2 cells under high glucose environment.

To sum up, this research further confirmed the pro-apoptotic roles of GAS5 in renal tubular epithelial cells after high glucose stimulation. Silence of GAS5 alleviated high glucose...
toxicity to human renal tubular epithelial HK-2 cells might be via down-regulating miR-27a and BNIP3, and then inactivating JNK pathway. We propose that GAS5, miR-27a and BNIP3 may be used as diagnostic and therapeutic targets for diabetic nephropathy in spite of further in vivo and clinical experiments are still needed.

Disclosure statement
No potential conflict of interest was reported by the authors.

References

[1] Boner G, Cooper ME. Diabetic nephropathy. Diabetes Technol Ther. 1999;1:489–496.

[2] Karar T, Elfaki EM, Qureshi S. Determination of the serum levels of troponin I and creatinine among Sudanese type 2 diabetes mellitus patients. J Nat Sci Biol Med. 2015;6:580–54.

[3] Tesch GH. Diabetic nephropathy – is this an immune disorder? Clin Sci. 2017;131:2183–2199.

[4] Alebiosu CO, Ayodele OE. The increasing prevalence of diabetic nephropathy in spite of further experiments are still needed.

[5] Lu Z, Liu N. Epigenetic regulations in diabetic nephropathy. J Nephrol Nurs J. 2003;30(2):185–190.

[6] Takao T, Horino T, Kagawa T, et al. Possible involvement of intracellular angiotensin II receptor in high-glucose-induced damage in renal proximal tubular cells. JN. 2011;24:218–224.

[7] Magee C, Grieve DJ, Watson CJ, et al. Diabetic nephropathy: a tangled web to weave. Cardiovasc Drugs Ther. 2017;31:579–592.

[8] Chmielewski C. Renal anatomy and overview of nephron function. Nephrol Nurs J. 2003;30(2):185–190.

[9] Takao T, Horino T, Kagawa T, et al. Possible involvement of intracellular angiotensin II receptor in high-glucose-induced damage in renal proximal tubular cells. JN. 2011;24:218–224.

[10] Mesarosova L, Ochodnicky P, Leemans JC, et al. High glucose induces HGF-independent activation of Met receptor in human renal tubular epithelium. J Recept Signal Transduction. 2017;37:535–542.

[11] Deng F, Zhang X, Wang W, et al. Identification of Gossypium hirsutum long non-coding RNAs (lncRNAs) under salt stress. BMC Plant Biol. 2018;18(1):23.

[12] Delas MJ, Hannon GJ. IncRNAs in development and disease: from functions to mechanisms. Open Biol. 2017;7:170121.

[13] Lu Z, Liu N. Epigenetic regulations in diabetic nephropathy. J Diabetes Res. 2017;2017:790508.

[14] Luo G, Liu D, Huang C, et al. LncRNA GASS inhibits cellular proliferation by targeting P27(Kip1). Mol Cancer Res. 2017;15:789–799.

[15] Wang X, Zhang W, Zhang J, et al. Long noncoding RNA-GASS: a novel regulator of hyperthermia-induced vascular remodeling. Hypertension (Dallas, Tex: 1979). 2016;68:736–748.

[16] Carter G, Miladnovic B, Patel AA, et al. Circulating long noncoding RNA GASS levels are correlated to prevalence of type 2 diabetes mellitus. BBA Clinical. 2015;4:102–107.

[17] Geng X, Xu X, Fang Y, et al. The effect of long noncoding RNA GAS5 on apoptosis in renal ischemia/reperfusion injury. Nephrology (Carlton, VIC). 2019;24:405–413.

[18] Hammond SM. An overview of microRNAs. Adv Drug Deliv Rev. 2015;87:3–14.

[19] Tao H, Zhang JG, Qin RH, et al. LncRNA GAS5 controls cardiac fibroblast activation and fibrosis by targeting miR-21 via PTEN/MMP-2 signaling pathway. Toxicology. 2017;386:11–18.

[20] Adams BD, Parsons C, Walker L, et al. Targeting noncoding RNAs in disease. J Clin Invest. 2017;127:761–771.

[21] Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: an overview of nuclear functions. Int J Mol Sci. 2016;17:21712.

[22] Bai X, Geng J, Li X, et al. Long noncoding RNA LINCO1619 regulates microRNA-27a/Forkhead box protein O1 and endoplasmic reticulum stress-mediated podocyte injury in diabetic nephropathy. Antioxid Redox Signal. 2018;29:355–376.

[23] Hou X, Tian J, Geng J, et al. MicroRNA-27a promotes renal tubulointerstitial fibrosis via suppressing PPARgamma pathway in diabetic nephropathy. Oncotarget. 2016;7:47760–47776.

[24] Ish-Shalom S, Lichter A. Analysis of fungal gene expression by Real Time quantitative PCR. Methods Mol Biol. 2010;638:103–114.

[25] Li R, Yin F, Guo YY, et al. Knockdown of ANRIL aggravates H2O2-induced injury in PC-12 cells by targeting microRNA-125a. Biomed Pharmacother. 2017;92:952–961.

[26] Balasescu E, Ion DA, Cioplea M, et al. Caspases, cell death and diabetic nephropathy. Rom J Intern Med. 2015;53:306–330.

[27] Ishii T, Fujita H, Narita T, et al. Association of monococyte chemo-attractant protein-1 with renal tubular damage in diabetic nephropathy. J Diabetes Complications. 2003;17:11–15.

[28] Ferre F, Colanonti A, Helmer-Citterich M. Revealing protein-IncRNA interaction. Brief Bioinform. 2016;17:106–116.

[29] Chen S, Dong C, Qian X, et al. Microarray analysis of long noncoding RNA expression patterns in diabetic nephropathy. J Diabetes Complications. 2017;31:569–576.

[30] Sattishkumar C, Prabu M, Mohan V, et al. Linking a role of IncRNAs (long non-coding RNAs) with insulin resistance, accelerated senescence, and inflammation in patients with type 2 diabetes. Hum Genomics 2018;12:41.

[31] Paraksevopoulos MD, Hatzigeorgiou AG. Analyzing MiRNA-LncRNA interactions. Methods Mol Biol (Clifton, NJ). 2016;1402:271–286.

[32] Wang X, Zhang W, Zhang J, et al. Long noncoding RNA GAS5 on apoptosis in renal ischemia/reperfusion injury. Toxicology. 2017;386:11–18.

[33] Song J, Zhang H, Sun Y, et al. Omentin-1 protects renal function in mice with type 2 diabetic nephropathy via regulating miR-27a-3p/Keap1 axis. Biomed Pharmacother. 2018;107:440–446.

[34] Burton TR, Henson ES, Baijal P, et al. The pro-cell death Bcl-2 family member, BNIP3, is localized to the nucleus of human glial cells: implications for glioblastoma multiforme tumor cell survival under hypoxia. Int J Cancer. 2006;118:1660.

[35] Huang C, Zhang Y, Kelly DJ, et al. Thioridoxin interacting protein (TXNIP) regulates tubular autophagy and mitophagy in diabetic nephropathy through the mTOR signaling pathway. Sci Rep. 2016;6:29196.

[36] Du Y, Li J, Xu T, et al. MicroRNA-145 induces apoptosis of glioma cells by targeting BNIP3 and Notch signaling. Oncotarget. 2017:8:61510–61527.

[37] Wang F, Xiong L, Huang X, et al. miR-210 suppresses BNIP3 to protect against the apoptosis of neural progenitor cells. Stem Cell Res. 2013;11:657–667.

[38] Aminzadeh A. Protective effect of tropisetron on high glucose induced apoptosis and oxidative stress in PC12 cells: roles of JNK, P38 MAPKs, and mitochondria pathway. Metab Brain Dis. 2017;32:819–826.

[39] Moriyama M, Moriyama H, Uda J, et al. BNIP3 upregulation via stimulation of ERK and JNK activity is required for the protection of keratinocytes from UVB-induced apoptosis. Cell Death Dis. 2017;8:e2576.

[40] He M, Xiang Z, Xu L, et al. Lipopolysaccharide induces human macrophage enucleating gial apoptosis by promoting mitochondrial dysfunction and activating the JNK-Bnip3-Bax pathway. Cell Stress Chaperones. 2019;24:91–104.