The relevance of the non-invasive biomarkers lncRNA GAS5/miR-21 ceRNA regulatory network in the early identification of diabetes and diabetic nephropathy

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Research Article

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Abstract

Background

To investigate the diagnostic value of serum lncRNA growth arrest-specific transcript 5 (lncRNA GAS5) and microRNA-21 (miR-21) in patients with type 2 diabetes mellitus (T2DM) and diabetic nephropathy (DN), and elucidate their roles in the pathogenesis.

Methods

A microarray technology was used to assess lncRNA GAS5 and miR-21 expression profiles in non-anticoagulant blood from 44 patients including T2DM without DN group (DM), T2DM with DN group (DN), and healthy controls group (N), followed by real-time PCR validation. Logistic regression and receiver operating characteristic (ROC) curves were applied to evaluate the clinical indicators among normal, T2DM, and DN patients.

Results

The serum lncRNA GAS5 expression in T2DM and DN patients was significantly down-regulated compared with the N group, while the expression of miR-21 was significantly up-regulated (all \( P < 0.05 \)). Fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) were negatively correlated with serum lncRNA GAS5, and FBG was independently correlated with serum lncRNA GAS5. Urinary microalbumin, total cholesterol (TC), creatinine (Cr), urea, and systolic blood pressure (SBP) were significantly positively correlated with serum miR-21. Glomerular filtration rate (GFR) and albuminuria (ALB) were negatively correlated with serum miR-21, and ALB was independently correlated with serum miR-21. Serum lncRNA GAS5, miR-21 and lncRNA GAS5/miR-21 showed good diagnostic efficiency as the "diagnostic signature" of T2DM and DN.

Conclusion

The lncRNA GAS5/miR-21 diagnostic signature may be a more effective non-invasive biomarker for detecting T2DM. In addition, miR-21 alone may be a more accurate serum biomarker for the early screening of DN patients.

Background

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia and caused by defects in insulin secretion, insulin action, or both. The incidence of DM has been gradually increasing, causing serious health issues globally. The global prevalence of diabetes, which was about 9.3% (463 million people) in 2019, is expected to rise by 10.2% (578 million people) by 2030 and 10.9% (700 million people)
Type 2 diabetes mellitus (T2DM) is the most common type of diabetes, accounting for more than 90% of diabetes patients. If hyperglycemia not effectively controlled, kidney, heart, retina, and peripheral neuropathy complications may occur as the disease progresses (2). Diabetic nephropathy (DN), which refers to chronic kidney disease, is one of the most common and severe chronic microvascular complications of diabetes, and it is an important cause of death and disability in patients with DM (2). In 2019, there were 2.62 million incident cases, 134.58 million patients, 405.99 thousand deaths, and 13.09 million disability-adjusted life-years of DN worldwide (3). Patients with DN are usually prescribed medications that control and delay the disease progression; severe cases require renal replacement therapy (4). The diagnosis of DN and its severity is currently based on histological changes observed in the kidney biopsy samples and clinical features such as proteinuria, glomerular filtration rate (GFR), and albuminuria (ALB). However, kidney biopsy is an invasive procedure not always well accepted by patients. On the other hand, there are limitations in using ALB as a marker of DN, as many patients experience GFR loss without deterioration in albuminuria (5). Therefore, searching for a rapid, safe, non-invasive screening method for early diagnosis of DM and DN is crucial.

Non-coding RNAs (ncRNAs), small RNAs that account for 98% of the human genome (6), are classified into small non-coding RNAs and long non-coding RNAs (lncRNAs) based on the transcript size. Small non-coding RNAs are short RNA transcripts of 18–24 nucleotides that regulate the expression of target genes by base-pairing with the 3' UTR (non-coding region) and directly cleaving mRNA or by inhibiting the synthesis of protein, which in turn leads to degradation or translational inhibition of mRNA (7). In recent years, studies have found that miRNAs negatively regulate the expression of target proteins at the post-transcriptional level and participate in the development of DN (8). For example, studies have found that miR-21 is a pivotal pathogenic factor in the development of DN; miR-21 can affect cell growth, proliferation and apoptosis by regulating PTEN (phosphatase and tensin homolog)/AKT signaling pathway, TGF-β/Smad pathway, and MMPS/TIMPS signaling pathway (9). Thus, miR-21 may serve as a useful biomarker for diagnosis and prognosis in DN. Our previous study also found that high glucose conditions induce up-regulation of miR-21 expression in mesangial cells and podocytes, resulting in the derepression of PTEN, as in the endogenous target of miR-21. With the increase of expression of PTEN, the hypertrophy and proliferation of mesangial cells are promoted, while the autophagy of mesangial cells and podocyte are inhibited, causing the accumulation of extracellular matrix protein and the injury of podocytes (10).

lncRNAs, a class of noncoding transcripts with lengths > 200 nucleotides, are pivotal regulators of genome structure and gene expression. They regulate miRNA expression and guide chromatin-modifying complexes. Recent studies have found that lncRNAs mediate disease pathogenesis. For example, GAS5, a lncRNA located at 1q25, has been reported to be involved in lung, breast, and gastric cancer (11). Mechanistically, GAS5 can inhibit apoptosis and cell cycle arrest at the G0/G1 phase. Moreover, recent study found studies have found a complementary region between GAS5 and miR-21 using RNA22 program software (http://cbcsrv.watson.ibm.com/ma22.html) (Fig. 1)(12). Furthermore, GAS5, as a negative regulator of miR-21, mediates the survival of chondrocytes and participates in the occurrence of osteoarthritis (13). In addition, few studies interpreted the relationship between lncRNA GAS5 and miR-21...
in DM and DN. Ge et al. (14) suggested that lncRNA GAS5 can inhibit cell proliferation and fibrosis in DN by sponging miR-221 and modulating SIRT1 expression.

This study analyzed the expression changes of serum lncRNA GAS5 and miR-21 in patients with DM and DN and their correlation with clinical and pathological parameters. At the same time, we verified the role of lncRNA GAS5 and miR-21 in the pathogenesis of DM and DN and sought new therapeutic targets and biomarkers.

**Materials and methods**

**Participants**

A total of 44 patients were recruited at the Department of Nephrology at the First Affiliated Hospital of China Medical University between March 2020 and September 2022. These patients were divided into three groups: T2DM without DN group (DM group), T2DM with DN group (DN group), and healthy controls group (N group). DM group included 10 patients diagnosed with diabetes according to the WHO Diabetes Marks and Urine albumin creatinine ratio < 30 mg/g, including 4 males and 6 females. Their average age was 52.40 ± 11.03 years old. DN group included 25 patients with DN confirmed by renal biopsy and accurately diagnosed according to the DN pathological classification issued by the 2010 Renal Pathology Association Research Committee (15). Among DN patients (14 males and 11 females), there were 2 cases with type IIb, 19 cases with type III, and 4 cases with type IV. The average age of patients was 44.08 ± 10.49 years old. N group included 9 healthy people, 4 males and 5 females, with a normal range of fasting blood glucose (FBG), random blood glucose (2hPG), glycosylated hemoglobin (HbA1c), serum creatinine (Cr), urinary microalbumin (MA), urine Cr, and urine protein. Their average age is 42.78 ± 13.43 years old. Participants with cancer, cardiovascular disease, liver damage, rheumatic immune system diseases, and other kidney diseases were excluded from this study. Besides, patients with malnutrition and patients who took angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) drugs were excluded. The case and control groups were matched for gender, smoking history, and blood pressure.

This study was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University (approval number: KT2020024). All subjects signed the informed consent.

**Sample collection**

A total of 5 ml of non-anticoagulant blood was obtained from a patient with an empty stomach in the morning. The peripheral non-anticoagulant blood was left to solidify at room temperature for 1 hour, after which it was centrifuged at 4°C (1700 g for 10 min). Next, the serum was collected, centrifuged (2000 g for 10 min), and stored in a refrigerator at -80°C.

**Real-time PCR**
We detected the expression of the target gene through real-time PCR. The DNA standard curve was diluted according to the gradient, and the machine directly generated the concentrations of the target gene and housekeeping genes of each sample. The target gene concentration of each sample was divided by the concentration of the housekeeping gene, which is the corrected relative content of this gene for this sample. Total RNA extraction was performed using the TRIzol method. Reverse transcription synthesizers were used to detect IncRNAs and microRNAs of cDNAs. All cDNA samples were configured with a real-time PCR reaction system, operating PCR reaction, and relative quantification. The sequence of PCR primers, including GAS5 (Invitrogen, Shanghai, China), hsa-miR-21, and hsa-miR-191-5p (Guangzhou, China) are shown in Table 1. The tested genes were corrected with internal parameters (β-actin and hsa-miR-191-5p), and the data for analysis were analyzed by the $2^{-\Delta\Delta CT}$ method.

Table 1

<table>
<thead>
<tr>
<th>LP and TG</th>
<th>BPS</th>
<th>AT (℃)</th>
<th>LP (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin(H)</td>
<td>F:5' GTGGCCGAGGACTTTGATTG3' R:5' CCTGTAACAACGCATCTCATATT3'</td>
<td>60</td>
<td>73</td>
</tr>
<tr>
<td>IncRNA GAS5</td>
<td>F:5'GCAAGCCTAACTCAAGCCATT3' R:5'CTCCACCATTTCACAATTCCAG3'</td>
<td>60</td>
<td>66</td>
</tr>
<tr>
<td>hsa-miR-191-5p</td>
<td>GSP:5'GGCAACGGAATCCCAAAG3' R:5'GTGCGTGTCGTGGAGTGC3'</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>hsa-miR-21</td>
<td>GSP:5'GGGGGCTAGTTATGCAGACTG3' R:5'CAGTGCCTGTGCGGAGT3'</td>
<td>60</td>
<td>66</td>
</tr>
</tbody>
</table>

Abbreviations: LP and TG: internal parameters and tested genes; BPS: bidirectional primer sequence; AT: annealing temperature; LP: length of products; GSP is a specific primer for the corresponding. miRNA and R are the primers that match the RT primer.

Statistical analysis

Experimental data are expressed in ± s. Data from multiple groups were compared using one-way ANOVA, and differences between groups were subjected to Fisher’s least significant difference test for multiple comparisons. Pearson, Spearman test and multiple linear regression analysis were used to analyze the relativity of clinical indicators among normal, diabetic, and DN patients. The correlation was analyzed by logistic regression and area under the ROC curve for the diagnostic efficacy of the IncRNA GAS5, miR-21, and IncRNA GAS5/miR-21 “diagnostic signature” of diabetes and DN. All data were statistically analyzed by SPSS 20.0, GraphPad software, and a two-tailed test. P < 0.05 indicated statistically significant differences.
Results

The changes in expression of serum lncRNA GAS5 and miR-21 in DM, DN, and N groups

In order to detect the different expressions of lncRNA GAS5 and miR-21 in serum, we performed PCR experiments by using 5 ml of non-anticoagulant blood from a fasted patient in the morning. The result showed that expression of serum lncRNA GAS5 in the DN group and DM group was lower than that in the N group, and it was obviously down-regulated in the DM group; the differences among the three groups were statistically significant (P < 0.05) (Fig. 2A). On the other hand, the serum miR-21 expression in the DN and DM groups was higher than that in the N groups and greatly up-regulated in the DN group; the differences among the three groups were statistically significant (P < 0.05), as shown in Fig. 2B.

Stratified analysis of serum lncRNA GAS5 and miR-21 in relation to clinical and Pathological parameters in patients with DN

To assess the connection between the serum lncRNA GAS5 and miR-21 and other pathological factors, patients were stratified based on sex, the 24-hour urine protein test, HbA1c, and chronic kidney disease (CKD), pathological grading of renal biopsy, and age, using SPSS. We found that in patients with DN, the expression of serum lncRNA GAS5 was gradually increased as the 24-hour urinary protein quantification progressed (P = 0.028) (Fig. 3A). With the progression of pathological grades in renal biopsy (type IIb-IV), we found that serum miR-21 expression was highest at stage 3 and did not increase gradually (P = 0.038) (Fig. 3B). Table 2 shows the expression of serum lncRNA GAS5 and miR-21 in different renal biopsy pathological grades. The expression of serum lncRNAs GAS5 and miR-21 was not correlated with patients age, HbA1c, and CKD stages.
Table 2
The correlation between serum lncRNA GAS5 and miR-21 and clinical and pathological parameters in patients with DN

<table>
<thead>
<tr>
<th>Clinical and pathological parameters</th>
<th>Cases (%)</th>
<th>IncRNA GAS5</th>
<th>miR-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratifications</td>
<td></td>
<td>±s</td>
<td>P</td>
</tr>
<tr>
<td>24h UTP (g)</td>
<td>25(100)</td>
<td>0.00 ± 0.00</td>
<td>0.028</td>
</tr>
<tr>
<td>24h UTP &lt; 3.5</td>
<td>5(20)</td>
<td>0.00 ± 0.00</td>
<td>0.028</td>
</tr>
<tr>
<td>3.5 ≤ 24h UTP &lt; 8.0</td>
<td>15(60)</td>
<td>0.01 ± 0.00</td>
<td>1.68 ± 1.08</td>
</tr>
<tr>
<td>24h UTP ≥ 8.0</td>
<td>5(20)</td>
<td>0.01 ± 0.01</td>
<td>2.06 ± 1.07</td>
</tr>
<tr>
<td>CKD stages</td>
<td></td>
<td>±s</td>
<td>P</td>
</tr>
<tr>
<td>CKD1</td>
<td>8(32)</td>
<td>0.00 ± 0.00</td>
<td>0.238</td>
</tr>
<tr>
<td>CKD2</td>
<td>8(32)</td>
<td>0.01 ± 0.00</td>
<td>1.77 ± 1.14</td>
</tr>
<tr>
<td>CKD3-4</td>
<td>9(36)</td>
<td>0.01 ± 0.01</td>
<td>1.70 ± 0.85</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td></td>
<td>±s</td>
<td>P</td>
</tr>
<tr>
<td>HbA1c ≤ 6.5</td>
<td>8(32)</td>
<td>0.01 ± 0.01</td>
<td>0.185</td>
</tr>
<tr>
<td>HbA1c &gt; 6.5</td>
<td>17(68)</td>
<td>0.01 ± 0.00</td>
<td>1.81 ± 1.05</td>
</tr>
<tr>
<td>Pathological grading of renal biopsy</td>
<td></td>
<td>±s</td>
<td>P</td>
</tr>
<tr>
<td>Type IIb</td>
<td>2(8)</td>
<td>0.01 ± 0.00</td>
<td>0.820</td>
</tr>
<tr>
<td>Type III</td>
<td>19(76)</td>
<td>0.01 ± 0.01</td>
<td>1.97 ± 1.00</td>
</tr>
<tr>
<td>Type IV</td>
<td>4(16)</td>
<td>0.01 ± 0.00</td>
<td>0.94 ± 0.12</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>±s</td>
<td>P</td>
</tr>
<tr>
<td>18 ~ &lt; 40</td>
<td>10(40)</td>
<td>0.01 ± 0.01</td>
<td>0.56</td>
</tr>
<tr>
<td>40 ~ &lt; 50</td>
<td>4(16)</td>
<td>0.01 ± 0.00</td>
<td>1.75 ± 0.83</td>
</tr>
<tr>
<td>50 ~ &lt; 60</td>
<td>11(44)</td>
<td>0.01 ± 0.00</td>
<td>1.79 ± 1.16</td>
</tr>
</tbody>
</table>

Correlation analysis of serum lncRNA GAS5 and/or miR-21 with clinical and/or pathological parameters

We examined a correlation between serum lncRNA GAS5 and/or miR-21 and clinical and/or pathological parameters. FBG ($r=-0.381$, $P = 0.011$) (Fig. 4A) and HbA1c ($r=-0.366$, $P = 0.001$) (Fig. 4B) were significantly negatively correlated with serum lncRNA GAS5. Stepwise regression analysis revealed that FBG ($\beta=-0.001$, $P = 0.022$) was independently correlated with serum lncRNA GAS5. Urinary MA ($r = 0.692$, $P = 0.001$) was significantly positively correlated with serum lncRNA GAS5. Further, we found that urinary MA was significantly positively correlated with miR-21 ($r=0.755$, $P = 0.001$).
P < 0.001) (Fig. 4C), SBP (r = 0.431, P = 0.003) (Fig. 4E), Cr (r = 0.506, P < 0.001) (Fig. 4F), Urea (r = 0.516, P < 0.001) (Fig. 4G), and TC (r = 0.400, P = 0.007) (Fig. 4I) were significantly positively correlated with serum miR-21; ALB (r=-0.510, P < 0.001) (Fig. 4D) and eGFR (r=-0.536, P < 0.001) (Fig. 4H) were significantly negatively correlated with serum miR-21. Stepwise regression analysis revealed that ALB (β=0.054, P < 0.001) was independently correlated with serum miR-21.

The diagnostic efficiency of serum IncRNA GAS5 and miR-21 for DM and DN

To examine whether the serum IncRNA GAS5 and miR-21 obtained the diagnostic efficiency for DM and DN, the area under the curve (AUC) analysis was performed. When analyzing patients with DM, the AUC of IncRNA GAS5 was 0.7302 (95% CI, 0.54 to 0.92, P = 0.03), the cut-off point was 0.0056, the sensitivity was 62.86%, and the specificity was 77.78% (Fig. 5A). The AUC of miR-21 was 0.8397 (95% CI, 0.71 to 0.97, P = 0.002), the cut-off point was 0.66, the sensitivity was 77.14%, and the specificity was 77.78%, as shown in Fig. 5B.

When analyzing patients with DN, the diagnostic efficiency of serum IncRNA GAS5 for DN was poor; the area under the ROC curve was not statistically significant, while the diagnostic efficiency of serum miR-21 for DN was better. The AUC of miR-21 was 0.9179 (95% CI, 0.84 to 1.00, P < 0.0001), the cut-off point was 0.9900, the sensitivity was 76.00%, and the specificity was 94.74% (Fig. 6).

The diagnostic efficiency of IncRNA GAS5/miR 21 "diagnosis signature" for DM and DN

Given the limited diagnostic efficiency of a single serum marker, we used logistic regression to analyze serum IncRNAs GAS5 and miR-21 of the enrolled groups. The regression coefficient was used to establish a "diagnosis signature" model, which was effectively combined with serum IncRNA GAS5 and miR-21. DM diagnostic signature was: 8.188×lg(miR-21)-3.779×lg(lncRNA GAS5)-6.008, while the DN diagnostic signature was: 10.571×lg(miR-21)-0.33×lg(lncRNA GAS5)-0.287. Our results showed the following results:

1) As shown in Fig. 7A, the AUC was 0.8984 (95% CI, 0.77(1.03, P = 0.0003); the cut-off point of 1.101 showed the best diagnostic efficiency (sensitivity 85.71%, specificity of 88.89%). Among them, the control group accounted for 20% above the cut-off point, and the patients with diabetes accounted for 15% below the cut-off point. A significant difference in the diagnostic signature between the non-diabetic subjects (N group) (median: -0.10, interquartile range (IQR): -2.44(0.79) and diabetes subjects (DM + DN group) (median: 2.91, IQR: 1.64–4.96, P < 0.001) (Fig. 7B); Therefore, the IncRNA GAS5/miR 21 diagnostic signature could well distinguish patients with normal blood glucose from those with diabetes, with or without nephropathy.

2) As shown in Fig. 7C, The AUC was 0.9158 (95% CI: 0.72(0.95, P < 0.0001); the cut-off point of -0.4523 showed the best diagnostic efficiency (sensitivity 88.00%, specificity 84.21%). Among them, the non-nephrotic group accounted for 16% above the cut-off point, and the nephropathy group accounted for 12% below the cut-off point. A significant difference in the diagnostic signature between the nephrotic subjects (DN group) (median: 1.33, IQR: 0.15(4.56) and non-nephrotic subjects (N + DM group) (median: -2.07, IQR: -3.02(-0.57, P < 0.001). (Fig. 7D) Therefore, the IncRNA GAS5/miR 21 diagnostic signature could provide a good distinction between diabetic nephropathy patients and non-nephropathy patients with or without diabetes.
Discussion

DN is a major terminal complication of diabetes, which occurs in approximately 40% of patients with DM. Clinical features such as proteinuria, GFR, and ALB are the most common biomarkers for assessing patients with DN, yet, they all have certain limitations (5). Therefore, searching for rapid and effective serum biomarkers are essential for early prediction of the risk of DM and DN. Especially for some patients who are not suitable for kidney biopsy, it is of great clinical significance to find non-invasive clinical indicators that can identify the early stage of DN.

MiRNA-21 is among the most abundant and highly conserved miRNAs expressed in most cells. It performs vital regulatory roles in health, including the heart and kidneys (16). Alteration in miRNA-21 can lead to endothelial dysfunction (16). In this study, we found that serum levels of miR-21 in DM and DN groups increased. With the progression of pathological grades in renal biopsy (type IIb-IV), serum miR-21 expression was highest at stage 3. Furthermore, we found that serum miR-21 expression levels were positively correlated with urinary MA levels, decreased renal function, and decreased serum ALB, which further suggests that miR-21 is a good early biomarker for the diagnosis and identifying DN in patients with T2DM. Similarly, Found et al. (17) examined 340 participants (including 100 healthy participants, 120 patients with T1DM with < 5 years duration, and 120 patients with T1DM with > 5 years duration) and indicated that plasma miRNA-21 could serve as an early marker for diagnosis and identifying DN in patients with type 1 diabetes. A year later, Liu et al. (9) performed a systematic search; 29 relevant studies suggested that miR-21 is an attractive potential prognostic, diagnostic, and predictive biomarker for DN in clinical practice.

Moreover, the ROC analysis showed that the sensitivity and specificity of diagnosing DM were higher when serum miR-21 reached 0.66 (cut-off point), and the specificity of diagnosing DN was even higher when miR-21 reached 0.99, suggesting that serum miR-21 expression level can be used as a noninvasive diagnostic biomarker to predict the occurrence of DM and DN. Yet, miR-21 levels in DN group were up-regulated 2.60 times compared to the DM group, which suggests that miR-21 might differentiate patients with DN compared to those with DM.

In addition to microRNAs, lncRNAs have emerged as critical players in DM progression. GAS5 is a key player associated with regulating cell development but is also involved in different pathogenesis, including cancer (11), bone disease (18), etc. In 2015, Carter and colleagues first reported that serum lncRNA GAS5 decreases in patients with type 2 diabetes; the results indicated that individuals with absolute GAS5 < 10 ng/µl have almost twelve times higher odds of having diabetes (19). In our study, serum lncRNA GAS5 was significantly down-regulated in the DM and DN groups compared with the control groups. Moreover, the serum lncRNA GAS5 was gradually up-regulated along with 24-hour urine protein quantification progression. Also, serum lncRNA GAS5 resulted as an independent protective factor of fasting blood glucose, indicating that down-regulation of lncRNA GAS5 may induce hyperglycemia. Importantly, the area under the ROC curve indicated that serum lncRNA GAS5 had better diagnostic
efficacy in detecting DM patients than DN, which suggest that IncRNA GAS5 may be a good biomarker for predicting the occurrence of diabetes.

Next, we assessed the sensitivity of combined IncRNA GAS5/miR-21 for detecting DM and DN. This study further verified the correlation between the two. The expression of IncRNA GAS5 and miR-21 showed moderate diagnostic efficacy, respectively. The AUC of IncRNA GAS5 and miR-21 combined diagnostic features was increased to 0.8984 (DM), the sensitivity was increased to 85.71%, and the specificity was increased to 88.89%. This suggested that a combination of GAS5 and miR-21 has higher diagnostic efficacy for diabetes than IncRNA GAS5 and miR-21 alone, while miR-21 alone has the highest diagnostic efficacy for DN.

Moreover, through RNA22 program software (12), we first learned that the derived sequence of exon 4 of GAS5 contains the binding site of miR-21, which can constitute the complementary region. Previous data suggested that IncRNA GAS5 can theoretically form an RNA-induced silencing complex (RISC) with miR-21, thus forming a mutually inhibitory regulatory ring (7). Previous studies have also shown that the expression of miR-21 and GAS5 in samples of breast cancer patients is negatively correlated. In the occurrence of osteoarthritis, GAS5 acts as a negative regulator of miR-21 to regulate the survival of chondrocytes (13). Yet, the regulatory network of IncRNA/miRNA has not been reported in the pathogenesis of diabetic kidney injury.

This study has some limitations. First, the study has a small sample size. Two, the regulatory network involving IncRNA GAS5 and miR-21 should be further explored using molecular biology.

Conclusions

A combination of GAS5 and miR-21 may be an accurate diagnostic tool for screening patients with DM, while miR-21 alone may be more accurate for the screening of DN patients. This provides insight into the future diagnosis and treatment of diabetes and diabetic nephropathy.

Declarations

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The authors would like to thank the participants who completed the study.

Authors’ contributions

Conceptualization and formal analysis, HS, TC, and QF; methodology, HS, TC; data curation, HS, TC, XL, YZ, SZ, PH, YP; funding acquisition, QF; HS and TC were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Ethics approval and consent to participate

All patients provided written informed consent before the beginning of the study. The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of China Medical University (approval number: KT20200024).

Consent for publication

Written informed consent has been obtained from the patients to publish this paper.

Competing interests

The authors declare that they have no competing interests.

References


**Figures**
Figure 1

GAS5 (top) consists of 12 exons with a putative binding site in exon 4.

Figure 2

The Changes in Expression of Serum IncRNA GAS5 and miR-21 in DM, DN, and N groups. (A, B) The average expression. *P<0.05 vs. the N group, *P<0.01 vs. the DM group. DN: diabetic nephropathy group; DM: diabetic group; N: normal control group.
Figure 3

Stratified Analysis of Serum IncRNA GAS5 and miR-21 in Relation to Clinical and Pathological Parameters in Patients with DN. (A) The expression of serum IncRNA GAS5. $a P<0.05$ vs. the 3.5g≤24h UTP<8.0g group; B was the expression of serum miR-21 in various renal biopsy pathological grades. $a P<0.05$ vs. the IIb group.
Linear correlation analysis of serum lncRNA GAS5 and miR-21 in relation to clinical and pathological parameters. FBG (r=-0.381, P=0.011) and HbA1c (r=-0.366, P=0.001) were significantly negatively correlated with serum lncRNA GAS5. Stepwise regression analysis revealed that FBG (β=-0.001, P=0.022) was independently correlated with serum lncRNA GAS5; TC (r=0.400, P=0.007), while MA (r=0.692, P<0.001), Cr (r=0.506, P<0.001), Urea (r=0.516, P<0.001), SBP (r=0.431, P=0.003) were significantly positively correlated with serum miR-21. Also, ALB (r=-0.510, P<0.001) and eGFR (r=-0.536, P<0.001) were significantly negatively correlated with serum miR-21. Stepwise regression analysis revealed that ALB (β=-0.054, P<0.001) was independently correlated with serum miR-21.
Figure 5

The ROC curve of serum IncRNA GAS5 and miR-21 for DM. (A) ROC curve of serum IncRNA GAS5. (B) The ROC curve of serum miR-21. The abscissa is 1-specificity, and the ordinate is the sensitivity.
Figure 6

The ROC curve of serum miR-21 for diagnosis of DN. The ROC curve of serum miR-21 above the abscissa is 1-specificity, and the ordinate is the sensitivity.
Figure 7

(A, B) Diagnostic signatures using logistic regression models of the diabetic group (DM+DN) and normal controls (N). (C, D) Diagnostic signatures using logistic regression models of DN and non-nephrotic group (DM+N). DN+DM: the diabetic group includes patients with diabetes mellitus and diabetic nephropathy; N+DM: non-nephrotic group includes the normal control group and patients with solely diabetes mellitus.