Analysis of circRNA-mRNA expression profiles and functional enrichment in diabetes mellitus based on high throughput sequencing

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Funding information
Youth Program of National Natural Science Foundation of China, Grant/Award Number: 81900431; Beijing Hospitals Authority Youth Programme, Grant/Award Number: QML20210702; Peking University International Hospital Research Grant, Grant/Award Number: YN2020ZD04

Abstract
To study the pathogenesis of diabetes mellitus (DM) and identify new biomarkers, high-throughput RNA sequencing provides a technical means to explore the regulatory network of MD gene expression. To better elucidate the genetic basis of DM, we analysed the circRNA and mRNA expression profiles in serum samples from diabetic patients. The circRNAs and mRNAs with abnormal expression in the DM group and non-diabetic group (NDM) were classified by RNA sequencing and differential expression analysis. The circRNA-miRNA-mRNA regulatory network reveals the mechanism by which competitive endogenous RNAs (ceRNAs) regulate gene expression. The biological functions and interactions of circRNA and mRNA were analysed by gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Differential expression analysis showed that 441 circRNAs (366 up-regulated, 75 down-regulated) and 683 mRNAs (354 up-regulated, 329 down-regulated) were significantly differentially expressed in the DM group compared with the NDM group. Screening of the differential genes at the nodes of the interaction network showed that a single circRNA could interact with multiple miRNAs and then jointly regulate more mRNAs. In addition, the expressions of circRNA CNOT6 and AXIN1 as well as mRNA STAT3, MYD88, and B2M were associated with the progression of diabetes. Enrichment pathway analysis indicated that differentially expressed circRNA and mRNA may participate in Nod-like receptor signalling pathway, insulin signalling pathway, sphingolipid metabolism pathway, and ribosome pathway, and play a role in the pathogenesis of diabetes. This study provides a theoretical basis for elucidating the molecular mechanism of DM occurrence and development at circRNA and mRNA levels.

KEYWORDS
ceRNA, circRNA, diabetes, mRNA
1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM), once a rare disease, has become an epidemic with the change in the social environment, lifestyle, and aging population. The significant pathophysiological features of T2DM are decreased insulin regulation of glucose metabolism (insulin resistance) and reduced insulin secretion due to impaired islet β cell function. However, due to many factors affecting the incidence of T2DM, its aetiology and pathogenesis are not completely clear at present. With the development of biological technology, in addition to some common and traditional exploration ideas, the rapid development of omics sequencing technology in recent years provides a new focus for related research.

In recent years, covalently closed circRNAs (circRNA) have become a hot topic in medical research. circRNAs were initially identified from plant viroids, yeast mitochondrial RNA, and hepatitis virus. Later, circRNAs have been found intermittently in introns and exons, and are thought to be either by-products of shearing errors or intermediates that escape from introns. However, subsequent studies have found that circRNA is ubiquitous in eukaryotes and plays an important role in eukaryotic gene expression and regulation. Subsequently, Memczak and Hansen et al. made a breakthrough in the study of the biological functions of circRNA and proposed for the first time that circRNA could serve as endogenous competitive RNA of miRNA to regulate the expression of downstream genes.

Haque et al. constructed circRNAome atlas of human islet by CircleSeq, and found that among the five most abundant circRNAs in human islet, four (circCIRBP, circZKSCAN, circRPH3AL, and circCAMSAP1) were significantly correlated with the status of diabetes. The above results suggest that circRNA has the potential as a biomarker of T2DM.

In this study, we explored the expression profiles of serum circRNA and mRNA in diabetic patients, mapped the circRNA-miRNA-mRNA regulatory network, and revealed the biological functions and interactions of circRNA and mRNA in the progression of T2DM through gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

2 | MATERIALS AND METHODS

2.1 | Patients

This study was a prospective trial and recruited from November 2018 to August 2019 type 2 diabetes and diabetes volunteers in Beijing Hospital. Inclusion criteria for type 2 diabetic participants: (a) All patients followed the diagnostic criteria of the American Diabetes Association Standards (2018); (b) Fasting blood glucose ≥7.0 mmol/L, glycosylated haemoglobin (HbA1C) ≥6.5%; (c) Patients with complete background information. Exclusion criteria: (a) Patients with severe liver, kidney, lung, or systemic diseases; (b) Patients with malignant tumour; (c) Patients with neurological diseases; (d) Patients with vascular disease, inflammation, and immune disorders. This study was approved by the Ethics Committee of our hospital (No. 2018BJYYEC-112-02). All procedures were carried out in accordance with the Declaration of Helsinki, and each participant signed a written informed consent. A total of 87 patients were screened in the diabetes mellitus (DM) group, and 9 patients were enrolled according to the inclusion criteria and patient consent (n = 9, patients D1-9). Non-diabetes group (NDM) patients were thyroid cancer patients with a total of three cases (n = 3, patients ND1-3). There was no significant difference in age and sex between the DM group and the NDM group (P > .05).

After enrollment, fasting venous blood was collected from the two groups for subsequent experiments.

2.2 | RNA extraction and sequencing

The peripheral blood of the subjects was collected and centrifuged at 3000 rpm for 15 min to extract the serum. Total RNA was isolated from collected serum samples using the Trizol reagent (Invitrogen, Carlsbad, California), and the integrity and purity of RNA were determined by Agilent 2100 bioanalyzer. RNA was purified from total RNA using the Dynabeads mRNA Purification Kit (Invitrogen, Carlsbad, California). The cDNA libraries
| Sample | Disease | Duration of diabetes (y) | Type of diabetes | Concomitant disease                                                                 | Smoking (y) | Fasting blood glucose (mmol/L) | HbA1c (%) |
|--------|---------|--------------------------|------------------|-------------------------------------------------------------------------------------|-------------|-------------------------------|-----------|
| ND1    | Thyroid carcinoma | —                        | —                | Thyroid nodule (papillary thyroid carcinoma)                                        | No          | 5.1                           | —         |
| ND2    | Thyroid carcinoma | —                        | —                | Hypertension, thyroid nodule (papillary thyroid carcinoma)                            | No          | 5.7                           | —         |
| ND3    | Thyroid carcinoma | —                        | —                | Thyroid nodule (papillary thyroid carcinoma)                                        | No          | 6.1                           | —         |
| D1     | Diabetes   | 2                        | Type 2           | Bronchial asthma, Hypertension, Dyslipidemia, obesity                                | No          | 7.7                           | 8.4       |
| D2     | Diabetes   | 10                       | Type 2           | Hypertension, hyperlipidemia, obesity                                                | No          | 11.8                          | 11.2      |
| D3     | Diabetes   | 6                        | Type 2           | Claustrophobia, Irritable bowel syndrome, Hepatitis B, Hypertension, Hyperlipidemia  | 40 y        | 7.6                           | 7.3       |
| D4     | Diabetes   | 17                       | Type 2           | Hypertension, coronary heart disease, hyperlipidemia, lower extremity atherosclerotic occlusion, old cerebral infarction | No          | 7.6                           | 8         |
| D5     | Diabetes   | 30                       | Type 2           | Hypertension, diabetes foot, benign prostatic hyperplasia, emphysema, chronic bronchitis | 50 y        | 8                             | 7.7       |
| D6     | Diabetes   | 10                       | Type 2           | Hepatitis, allergic rhinitis, hypertension, diabetes foot ulceration                 | 40 y        | 7.1                           | 6.8       |
| D7     | Diabetes   | 7                        | Type 2           | Hashimoto's hypothyroidism                                                           | No          | 6.7                           | 7.5       |
| D8     | Diabetes   | 8                        | Type 2           | Coronary heart disease, hypothyroidism, hypertension, hyperlipidemia, cholecystitis, fatty liver, obesity | 30 y        | 7.0                           | 8.3       |
| D9     | Diabetes   | 14                       | Type 2           | Hypothyroidism, secondary hypogonadism, osteoporosis, hepatitis B, liver cancer, gastric ulcer, hyperlipidemia, chronic obstructive pulmonary disease, left bundle branch block, benign prostatic hyperplasia | 40 y        | 11.5                          | 8.5       |
of all samples were sequenced using the Illumina HiSeq 2000 sequencing platform of the Beijing Huada Gene Research Institute.

## 2.3 Data quality control

In the original sequencing data, a few Reads contain artificial sequences, such as sequencing primers and connectors. In order to avoid the impact on subsequent analysis, the original data needs to be filtered to delete the low-quality areas that affect data quality and subsequent analysis. The raw data after filtering is processed to produce the required high-quality data, which is called clean data. The quality assessment report before and after data processing was generated by FastQC, and the original sequencing data were evaluated by FAST-QC.

## 2.4 Differential gene screening and analysis

Chi-square test was used for mRNA and circRNA with single repeats and more reads (both samples were greater than 20), and Fisher’s accurate test was used for those with fewer reads. For multiple repetitions, the rank-sum test is adopted without assuming the distribution. P values obtained through statistical tests are corrected with a false discovery rate (FDR) and then Q values are calculated. EdgeRSeq algorithm was used to identify genes with significant differences, and the criteria were as follows: the absolute value of \( \text{Log}_2 \) (fold change) > 1; \( P \) value < .05 (significant difference); mean expression > 1 (medium-high expression) in at least one group. Through hypothesis testing, R software was used to draw the volcano map based on the significance of differential expression of genes and transcripts between two groups, and describe the differential genes with Foldchange absolute value greater than 2 and \( P \) value < .05.

The differentially expressed genes between the DM group and the NDM group were analysed by heat map clustering, and the differentially expressed genes were analysed by an interaction network diagram. The expression levels of differentially expressed transcripts were analysed. Coexpression transcripts were screened according to correlation coefficient > 0.99 or < -0.99 and \( P < .05 \) as thresholds. The co-expression network map was drawn by Cytoscape, and the connection reflected the meaningful correlation between the two genes, and the key regulatory genes in the network were also identified.

## 2.5 KEGG pathway and GO analysis

The new gene sequences were compared with SWISS-PROT and TrEMBL databases (https://www.uniprot.org/) and functionally annotated according to genetic similarity. Genes are compared for similarity using BLAT software, which can quickly find similar sequences between two sequences and compare and score regions to determine the degree of similarity. The results of the new gene were compared with SWISS-PROT and TrEMBL, and the resulting Uniprot number was matched with GO Term. The main functions of differentially expressed mRNA were determined by GO (http://www.geneontology.org) functional significant enrichment analysis (\( P < .05 \)). GO enrichment analysis of the top 10 differentially down-regulated mRNA target genes.

| Sample | Clean reads (M) | Clean bases (G) | Clean Q20 (G) | Clean Q30 (G) | Average length (bp) |
|--------|----------------|----------------|---------------|---------------|---------------------|
| ND1    | 81.6 (97.5%)   | 12.1 (96.6%)   | 11.8 (97.5%)  | 11.3 (92.8%)  | 148.6               |
| ND2    | 69.8 (97.4%)   | 10.4 (96.4%)   | 10 (96.6%)    | 9.4 (90.9%)   | 148.4               |
| ND3    | 127.8 (97.8%)  | 19 (97.2%)     | 18.6 (97.7%)  | 17.8 (93.5%)  | 149.0               |
| D1     | 71 (93.9%)     | 10.4 (92.2%)   | 10.3 (98.2%)  | 9.9 (94.4%)   | 147.2               |
| D2     | 70.9 (94.5%)   | 10.4 (92.9%)   | 10.3 (98.3%)  | 9.9 (94.7%)   | 147.4               |
| D3     | 69.8 (94.5%)   | 10.4 (93.5%)   | 10.2 (97.7%)  | 9.8 (93.6%)   | 149.3               |
| D4     | 73.1 (93.5%)   | 10.7 (91.3%)   | 10.5 (98.2%)  | 10.1 (94.4%)  | 146.5               |
| D5     | 65.2 (94.2%)   | 9.6 (92.3%)    | 9.4 (98.2%)   | 9.1 (94.5%)   | 147.1               |
| D6     | 69.4 (94.7%)   | 10.4 (93.5%)   | 10.1 (97.8%)  | 9.7 (93.7%)   | 149.2               |
| D7     | 67.9 (97.0%)   | 10.1 (96.4%)   | 9.9 (98.0%)   | 9.5 (94.0%)   | 149.0               |
| D8     | 68.4 (94.9%)   | 10.2 (93.8%)   | 10 (97.8%)    | 9.6 (94.0%)   | 149.3               |
| D9     | 105.6 (96.2%)  | 15.1 (91.8%)   | 14.7 (97.7%)  | 14 (92.9%)    | 143.1               |
KEGG pathway analysis (http://www.genome.jp/kegg/) using hypergeometric inspection (phyper), find out the significant enrichment of the target genes of KEGG term, determine the target genes to exercise the main biological metabolic pathways. The first 10 differentially down-regulated mRNA target genes were analysed by KEGG enrichment.

### 2.6 Statistical analysis

SPSS (Version 20.0, IBM SPSS Statistics) was used for statistical analysis. All results are expressed as ($\bar{x} \pm s$). The independent sample T test was used for pairwise comparison and one-way ANOVA was used for multiple comparisons. $P < .05$ indicated statistically significant differences.

### 3 RESULTS

#### 3.1 Analysis of clinical data of two groups of subjects

All subjects in the NDM group had thyroid cancer, and all three patients had no smoking history. Except for the history of hypertension in NDM2, the other patients had no other comorbidities. Fasting blood glucose in three patients was in the normal range. The mean duration of disease in the DM group was (11.55 ± 7.82) years (2–30 years). The common complications in the DM group were hypertension, dyslipidemia, obesity, coronary heart disease, diabetic foot, bronchitis, and prostatic hyperplasia. Five patients in the DM group had a smoking history of more than 30 years. The mean

![Figure 1](image)

**Figure 1** Differentially expressed circRNA and mRNA in diabetes mellitus (DM) and NDM. (A,B) Each dot in the figure represents an mRNA or circRNA. The horizontal axis represents Log2 (fold change) of the multiple difference in RNA expression between the two groups. The ordinate is the negative log of the change in RNA expression. (B,C) The differentially expressed circRNAs or mRNAs were bidirectional clusterings with groups. The colour depth represented the expression level of genes, red represented high expression level, and blue represented low expression level. (E) Red nodes, green nodes, and blue nodes represent circRNA, miRNA, and mRNA, respectively.
### Table 3. The top 40 upregulated and downregulated mRNAs in diabetic mellitus vs non-diabetes mellitus

| Gene_name | Regulation | Log2 (fold change) | P value |
|-----------|------------|--------------------|---------|
| MAP3K11   | Down       | −14.5961           | 1.04E−40|
| PTPRJ     | Down       | −14.3032           | 6.47E−30|
| THEMIS2   | Down       | −13.1795           | 1.30E−18|
| KDM7A     | Down       | −13.0246           | 7.29E−19|
| APSZ1     | Down       | −12.9379           | 8.61E−20|
| NCLN      | Down       | −12.9353           | 8.90E−19|
| RP11-463O12.5 | Down | −12.6569           | 1.20E−15|
| NRBFI2    | Down       | −12.6043           | 3.68E−20|
| LILRA2    | Down       | −12.5016           | 8.52E−18|
| PPP3CB    | Down       | −12.2835           | 4.26E−20|
| UBE4A     | Down       | −12.1589           | 5.21E−17|
| CCNDBP1   | Down       | −11.9528           | 6.53E−17|
| ZEB2      | Down       | −11.9496           | 3.31E−19|
| UBN1      | Down       | −11.9128           | 3.48E−17|
| PPP1R3B   | Down       | −11.9024           | 1.12E−15|
| HSPD1     | Down       | −11.5267           | 2.29E−12|
| SPEC1I    | Down       | −11.4986           | 1.95E−18|
| FKBPO     | Down       | −11.4339           | 4.31E−17|
| STAT3     | Down       | −11.3275           | 1.16E−15|
| PDZD4     | Down       | −11.2461           | 3.01E−15|
| CYB561D1  | Down       | −11.1792           | 3.32E−15|
| PPP4C     | Down       | −11.1567           | 2.83E−13|
| STX7      | Down       | −11.0493           | 2.79E−13|
| B2M       | Down       | −10.987            | 1.11E−08|
| MIR6749   | Down       | −10.9479           | 8.87E−18|
| NUP98     | Down       | −10.9222           | 1.14E−13|
| SAMSN1    | Down       | −10.776            | 1.74E−08|
| RBM39     | Down       | −10.6893           | 1.73E−08|
| TK2       | Down       | −10.6864           | 8.68E−12|
| DNAH1     | Down       | −10.67             | 5.28E−11|
| KLHL8     | Down       | −10.644            | 1.39E−14|
| VPS37B    | Down       | −10.6342           | 1.99E−08|
| RP11−473I1.9 | Down | −10.5847           | 3.20E−08|
| MYD88     | Down       | −10.5633           | 2.31E−12|
| SUN1      | Down       | −10.5589           | 2.01E−14|
| ADAM19    | Down       | −10.5396           | 2.68E−08|
| STX10     | Up         | 11.01652           | 5.54E−15|
| FYB       | Up         | 11.1549            | 7.74E−16|
| STX10     | Up         | 11.2958            | 6.46E−16|
| PPP1R12A  | Up         | 11.30557           | 4.82E−16|

### Table 4. The top 40 upregulated and downregulated circular RNAs in diabetic mellitus vs non-diabetes mellitus

| Gene_name | Regulation | Log2 (fold change) | P value |
|-----------|------------|--------------------|---------|
| PHC3      | Down       | −11.934            | 2.38E−16|
| USP3      | Down       | −10.8112           | 1.39E−08|
| DENND4C   | Down       | −10.0627           | 8.61E−08|
| MTMR1     | Down       | −8.8613            | 1.91E−06|
| CDYL      | Down       | −7.87129           | 2.28E−05|
| PARP9     | Down       | −7.43445           | 5.23E−05|
| GFI1B     | Down       | −7.34648           | 8.43E−05|
| NUP88     | Down       | −7.34594           | 3.65E−05|
| SPG7      | Down       | −7.26616           | 4.13E−08|
| KIAA1841  | Down       | −6.79335           | 0.00269|
| SIK3      | Down       | −6.457             | 0.00706|
| RAB18     | Down       | −6.41203           | 7.25E−06|
| R3HDM2    | Down       | −6.08236           | 1.44E−09|
| CHEK2     | Down       | −5.51574           | 0.04004|
| PHF20     | Down       | −5.50292           | 2.36E−05|
| CDK7      | Down       | −5.14883           | 8.58E−05|
| DLEU2     | Down       | −4.75964           | 0.03293|
| NBR1      | Down       | −4.52277           | 8.95E−05|
| CNOT6     | Up         | 4.577776           | 2.95E−06|
| KIAA1715  | Up         | 4.637315           | 0.0367|
| MBNL3     | Up         | 4.730478           | 6.53E−05|
| OGDH      | Up         | 5.185212           | 0.02865|
| CFAP44    | Up         | 5.249135           | 0.00449|
| LNX2      | Up         | 5.304185           | 0.00348|
| UBP1      | Up         | 5.409766           | 0.01784|
| TMEM116   | Up         | 5.626273           | 0.01181|
| RP11−233J.4 | Up | 5.759975           | 0.01042|
| RP11−274B21.4 | Up | 5.837293          | 0.00122|
| AXIN1     | Up         | 5.941428           | 0.00139|
| C2orf76   | Up         | 6.04159            | 0.00269|
| RP11−706O13.1 | Up | 6.266376          | 0.00232|
| ATF7IP2   | Up         | 6.355302           | 0.00202|
| CCDC22    | Up         | 7.048212           | 9.70E−07|
| DLGAP5    | Up         | 7.219386           | 7.64E−12|
| SOX6      | Up         | 7.547414           | 1.58E−06|
| MAPRE2    | Up         | 7.672044           | 1.52E−06|
| CNOT4     | Up         | 7.746042           | 1.17E−06|
| DROSHA    | Up         | 8.371408           | 6.60E−08|
| TRIP11    | Up         | 9.550322           | 7.07E−11|
| CBFA2T2   | Up         | 11.93007           | 7.57E−21|
fasting blood glucose and HbA1c in the DM group were (8.33 ± 1.81) mmol/L and (8.18 ± 1.19) %, respectively (Table 1).

3.2 | Data quality

According to the experimental design, 12 human serum samples were divided into the DM group and NDM group for quality detection. After mass pretreatment, the proportion of reads and bases in each sample was greater than 90%. The proportion of quality scores ≥20 points (Q20) was higher than 97%, the proportion of quality scores ≥30 points (Q30) was higher than 92%, and the single base error rate was 0.001 (Table 2).

3.3 | Differentially expressed circRNA and mRNA in DM and NDM groups

To explore the molecular mechanism of DF pathogenesis, serum samples from 12 patients were examined and whole-transcriptome analysis using total RNA sequencing was performed using total RNA. The data are cleaned and mapped to the reference genome and spliced into a putative transcript. After bioinformatics analysis, differentially expressed mRNA and circRNA in the DM group and DF group were screened out. A volcanic map was used to show the differential expression of mRNA and circRNA, as shown in Figure 1A, B. The absolute value of Fold Change 2 and P < .05 were used as the criteria to judge the down-regulation and up-regulation of RNAs (FDR...
and For example, Zhou et al.\textsuperscript{15-18} CircRNA is a kind of non-coding RNA. Today, more and more circRNAs have been found to regulate gene expression at the transcriptional, post-transcriptional, and translational levels. They participate in the pathological process of many diseases, such as Alzheimer’s disease, diabetic atherosclerosis, glioma, gastric cancer, and osteosarcoma, by regulating gene splicing, regulating miRNA, and binding functional proteins.

3.4 GO enrichment analysis and KEGG pathway analysis

According to the relative expression levels between the two groups, the differential genes can be divided into up-regulated genes and down-regulated genes, and the GO enrichment analysis results are shown in Figure 2A-C. GO enrichment analysis was performed on the mRNA predicted by the differentially expressed circRNA, and the results were basically consistent with the differentially expressed circRNA. Differentially expressed mRNAs are enriched in signalling pathways such as biological regulation, cellular programming, cellular components, metabolic process, and catalytic activity.

KEGG pathway analysis was performed on the differential genes, and the KO number corresponded to the KEGG pathway. KEGG enrichment analysis was performed according to mRNA predicted by differential circRNA and differential mRNA, and pathways obtained by KEGG enrichment analysis were shown in a bubble diagram (Figure 2D,E). Analysis of the enrichment pathways of the predicted mRNA and the differentially expressed mRNA predicted by circRNA showed that nod-like receptor signalling pathway, insulin signalling pathway, sphingolipid metabolism pathway, and ribosomal pathway play an important role in the occurrence and development of DM.

4 DISCUSSION

Type 2 diabetes is characterised by a long duration and no cure. T2DM patients with elevated blood glucose will also bring many complications, seriously affecting the quality of life of patients. Studies have shown that post-transcriptional modified proteins or lipids oversynthesize advanced glycation end (AGE) products under high sugar levels or excessive diet, which can destroy islet β cells by disrupting defense mechanisms. In addition, AGEs and their polyligands bind to form receptors for advanced glycation end products, and further promote the synthesis of more AGEs, thereby triggering the cellular destruction mechanism that leads to diabetic retinopathy, diabetic nephropathy, diabetic foot, and other diabetic complications in diabetic patients.\textsuperscript{11,12}

Non-coding RNAs (ncRNAas) are called ‘dark matter’ in life, but they are not non-functional RNAs. NcRNA has not been eliminated in the process of biological evolution, suggesting that it is closely related to the level of biological evolution. NcRNA not only has important biological functions, but also is closely related to the occurrence of many major diseases, and has become a research hotspot in life science.\textsuperscript{13} CircRNA is a kind of non-coding RNA widely existing in animal cells. At present, it is generally believed that most circRNA are the products of the reverse shearing of the exon of the precursor mRNA (pre-mRNA), that is, the 3’-5’ phosphate diester bond is formed between the shearing site downstream of the 5’ end and the shearing site upstream of the 3’ end, thus forming circRNA.\textsuperscript{14} Today, more and more circRNAs have been found to regulate gene expression at the transcriptional, post-transcriptional, and translational levels. They participate in the pathological process of many diseases, such as Alzheimer’s disease, diabetic atherosclerosis, glioma, gastric cancer, and osteosarcoma, by regulating gene splicing, regulating miRNA, and binding functional proteins.\textsuperscript{15-18} For example, Zhou et al.\textsuperscript{19} obtained differential circRNA-0008717 from clinical osteosarcoma tissue samples and normal human serum.
samples through high-throughput gene sequencing analysis, and its expression in osteosarcoma was significantly increased. After further examination, circRNA-0008717 with overexpression was proved to enhance the expression of BMI-1 through the circRNA-0008717-miR-203-BMI-1 network, thus promoting the proliferation and invasion of osteosarcoma cells. Studies have shown that circRNA Cdr1as acts as a powerful inhibitor of miR-7 in the brain of developing zebrafish, and miR-7 is highly expressed in islet cells. Overexpression of miR-7 in mouse experiments can lead to diabetes. Therefore, strongly interacting circRNA Cdr1as/ miR-7 may be a new target for improving β cell function in diabetic patients. In addition, the expression level of circANKRD36 is positively correlated with glucose and glycosylated haemoglobin, and may also be involved in inflammatory pathways related to T2DM through its interaction with miRNA. In this study, we investigated the differences in circRNA and mRNA expression between non-diabetic and diabetic subjects. We found that 441 circRNAs (366 up-regulated, 75 down-regulated) and 683 mRNAs (354 up-regulated, 329 down-regulated) were significantly differentially expressed in T2DM compared with NDM groups. The expressions of circRNA CNOT6 and AXIN1 and mRNA STAT3, MYD88, and B2M were correlated with the progression of diabetes. At present, the roles of CircRNA CNOT6 and AXIN1 in various diseases have not been studied, so we need to further explore their roles in the development of diabetes in later experiments.

Myeloid differentiation factor 88 (MyD88) is an important link molecule in the toll-like receptor (TLR) signalling pathway and plays an important role in the pathogenesis and development of diseases. The MYD88-dependent signalling pathway activates the downstream NF-κB through a series of signal transductions (MyD88, IRAK, and TRAF-6). MyD88 is a downstream ligand of the TLR4 receptor complex, which promotes the expression and release of inflammatory cytokines (IL-6, TNF-α). Activation of the TLR4 pathway and downstream MyD88 is also activated in patients with diabetes, which affects the progression of diabetes by regulating inflammatory response. Serum β-2 microglobulin (B2M) is also closely related to the progression of diabetes. Some studies have found that B2M can predict the mortality of patients with diabetes and can also be used as a predictor of diabetes complications. As an important signal pathway of life activities, STAT3 is closely related to the occurrence and development of diabetes. Stat3-related signalling pathways regulate the progression of diabetes by regulating inflammatory response, cell proliferation, apoptosis, or autophagy.

Studies have shown that circRNA can enhance the function of proteins, acting as a mediator to mediate the binding of enzymes to substrates and recruit proteins to specific sites. Immunoprecipitation data show that circRNA can interact with different RNA-binding proteins to act as a ‘molecular sponge’ for proteins. When the binding sites on miRNA bind to circRNA, the number of binding sites on miRNA for mRNA will be reduced, and there are a large number of competitive sites for circRNA to compete with endogenous mRNA for the function of binding miRNA. This is also the core of the ceRNA regulatory network, that is, circRNA acts on targeted miRNAs and regulates the level of miRNA target genes by competitively binding miRNAs. The results of this study showed that a single circRNA could interact with multiple miRNAs, and then jointly regulate the target gene mRNAs of more miRNAs, thus regulating the progression of diabetes.

The results showed that the enrichment of target genes in GO enrichment analysis was mostly concentrated in biological processes, including biological regulation, cellular program, metabolic process, and catalytic activity, etc. KEGG pathway mainly focuses on the insulin signalling pathway, sphingolipid metabolism pathway, and ribosome pathway. Most of these pathways are related to glycolipid and energy metabolism and may play an important role in diabetes metabolism. In later studies, we need to further explore the regulatory mechanism of differentially expressed circRNA-miRNA-mRNA in the progression of diabetes, so as to provide more potential targets for the treatment of diabetes.

**FUNDING INFORMATION**

This work was supported by the Youth Program of National Natural Science Foundation of China to Dr Wanni Zhao (No. 81900431); Beijing Hospitals Authority Youth Programme to Dr Wanni Zhao (No. QML20210702), and Peking University International Hospital Research Grant to Dr Jianfeng Liang (No. YN2020ZD04).

**DATA AVAILABILITY STATEMENT**

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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How to cite this article: Zhao W, Meng X, Liang J. Analysis of circRNA-mRNA expression profiles and functional enrichment in diabetes mellitus based on high throughput sequencing. Int Wound J. 2022;19(5):1253-1262. doi:10.1111/iwj.13838