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Long Non-Coding Ribonucleic Acid Microarray Profiling in β-Thalassaemia Major Patients with Prediabetes

Hong Liang[1], Yu-Wen Chen[1], Feng Liao[1] and Jian-Ming Luo[2]*

[1]*Department of Pediatrics, Hainan General Hospital, Haikou 570000, Hainan Province, China*
[2]*Department of Pediatrics, The First Affiliated Hospital of Guangxi Medical University, Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China*

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ABSTRACT

Compromised glucose metabolism in beta-thalassaemia major (β-TM) patients is linked to key long noncoding RNAs (lncRNAs) and messenger RNAs (mRNAs), revealed through gene microarray profiling. Microarray analysis compared β-TM/prediabetic patients with healthy controls. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis validated the expression of selected lncRNAs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis identified signaling pathways associated with β-TM complicated by prediabetes. Of the 26,035 lncRNAs and 17,044 mRNAs analyzed, 3,443 lncRNAs and 1,880 mRNAs showed differential expression. The qRT-PCR analysis confirmed the expression of eight dysregulated lncRNAs. The GO and KEGG analyses showed that the dysregulated mRNAs were involved in various biological processes and pathways related to glucose metabolism, insulin resistance, oxidative stress and inflammation. This study suggests a link between the pathophysiology of aberrant glucose homeostasis in β-TM and the expression of lncRNAs and mRNAs. This study provides new insights into the molecular mechanisms and potential biomarkers of β-TM and prediabetes. The differential expression of lncRNAs and mRNAs may also reflect the underlying pathophysiological changes and metabolic disturbances in β-TM and prediabetes, and thus have potential clinical applications.

INTRODUCTION

Beta-thalassaemia major (β-TM) is a complex hereditary anemia that disrupts normal glucose metabolism, predisposing patients to prediabetes a condition with significant health risks. β-TM is characterized by reduced or absent production of the β-globin chain (Needs *et al*., 2023). Blood transfusion is an important part of currently available treatments (Sharma A *et al*., 2019; Yaacoub-Kzadri *et al*., 2015). However, long-term blood transfusions can result in iron overload, which impairs insulin secretion and action, leading to abnormal glucose metabolism (Gupta *et al*., 2018; Gomber *et al*., 2018; Li *et al*., 2014; Bloomer and Brown, 2019; Ang *et al*., 2014; Teawtrakul *et al*., 2018).

Prediabetes is a condition where the blood sugar level is higher than normal, but not high enough to be diagnosed as type 2 diabetes. Prediabetes can increase the risk of developing type 2 diabetes, as well as cardiovascular disease, cancer, mental disorders, and death (Forti *et al*., 2020; Tabák *et al*., 2012; Brannick and Dagogo-Jack, 2018; Cai *et al*., 2020; Schlesinger *et al*., 2022). A meta-analysis found that the prevalence of impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and diabetes mellitus (DM) in patients with β-TM was 17.21%, 12.46% and 6.54%, respectively (He *et al*., 2019). The onset of abnormal glucose metabolism in patients with β-TM is insidious, and there is a shortage of effective biomarkers and diagnostic methods for detecting abnormal glucose metabolism in these patients (He *et al*., 2019).

* Corresponding author: luojianming1853@163.com
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Recent studies have underscored the potential role of long noncoding RNAs (lncRNAs) and messenger RNAs (mRNAs) in the pathogenesis of both β-TM and diabetes, highlighting their diagnostic and therapeutic potential. LncRNAs are transcripts of more than 200 bases that lack protein-coding potential, and are involved in various human biological processes, such as cell-cycle regulation, epigenetic modifications, chromatin remodelling, splicing and cellular differentiation (Engreitz *et al*., 2016; Dykes and Emanueli, 2017; Weidle *et al*., 2017; Leti and DiStefano, 2017). LncRNAs are also present in peripheral blood, and can serve as diagnostic molecular markers (Krause, 2018; de Gonzalo-Calvo *et al*., 2016). Diabetes and its complications have been associated with dysregulated lncRNAs and mRNAs in peripheral circulation, which can affect pancreatic β-cell function, glucose metabolism and insulin resistance (Guo *et al*., 2019; Akerman *et al*., 2017; Jin *et al*., 2017; Ruan *et al*., 2018; Sathishkumar *et al*., 2018). Moreover, lncRNAs and mRNAs have been implicated in the regulation of erythropoiesis and the pathogenesis of β-TM (Liu *et al*., 2019; Wang *et al*., 2018; Ma *et al*., 2021; Fakhr-Eldeen *et al*., 2019).

However, the role of lncRNAs and mRNAs in the molecular mechanism of β-TM associated with prediabetes has yet to be elucidated. There is a need for a comprehensive analysis of the expression profile of lncRNAs and mRNAs in the peripheral blood of β-TM patients with prediabetes, which could reveal potential targets for diagnosing and treating this condition. This study investigates the differential expression of lncRNAs and mRNAs in the peripheral blood of β-TM patients with prediabetes using microarray analysis, aiming to identify potential biomarkers for early diagnosis and targets for therapeutic intervention.

MATERIALS AND METHODS

Subjects

Between January 2021 and December 2021, five β-TM patients with prediabetes and five healthy individuals were recruited from Hainan General Hospital. Diagnosis was carried out by haemoglobin electrophoresis and gene analysis for β-TM. The American Diabetes Associations 2021 diagnostic guide (American Diabetes Association, 2021) was employed to determine whether a patient had prediabetes, which was defined as IFG and/or IGT using the oral glucose tolerance test (OGTT). Patients were excluded if they had at least one of the following conditions: (1) severe heart, liver or renal insufficiency, (2) hepatitis B or C virus infection, (3) family history of a first- or second-degree relative with type 2 diabetes, (4) previous bone marrow transplantation, (5) acute infectious disease, (6) abnormal birth history, (7) history of gestational DM in the patient's mother, (8) prior splenectomy, (9) body mass index ≥ 25 kg/m2, (10) malignant tumour, and (11) use of drugs known to cause glucose intolerance (e.g. glucocorticoids). Five healthy control participants were recruited from Hainan General Hospital in Hainan, China, for this study. The criteria for defining healthy individuals were: (1) no history of β-TM or other haematological disorders, (2) normal blood glucose levels, (3) no history of chronic diseases or infections, and (4) no use of drugs that could affect glucose metabolism.

Blood sample collection

Peripheral blood (5 ml) anticoagulated with ethylenediaminetetraacetic acid was collected from each participant after an overnight (> 8 h) fast. Ferritin, fasting glucose, fasting insulin, fasting C-peptide and 2-h plasma glucose levels were identified to evaluate OGTT. These blood parameters were chosen because they reflect the iron status, glucose metabolism and insulin secretion of the participants.

Ribonucleic acid extraction and purification

The total RNA was extracted from the blood sample using a TRIzol™ reagent (Cat. 15596026, ThermoFisher Scientific, Delaware, USA) and purified using the RNeasy Mini Kit (Cat. 74104, Qiagen, Germany). The quantity and quality of the RNA were assessed using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Delaware, USA) and analysed under A260/A280 ratios between 2.1 and 2.2, while the integrity of the RNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies Co., Ltd., California, USA) and analysed above 7.5 in terms of the RNA Integrity Number (RIN).

Microarray analysis

The participants lncRNAs and mRNAs were profiled using microarrays and the Arraystar Human LncRNA Array V5.0 (Arraystar, Maryland, USA). We chose this array because it provides a comprehensive overview of lncRNAs and mRNAs in the human genomes, and covers the most updated lncRNA annotations from authoritative databases. KangChen Bio-tech conducted the microarray study (KangChen, Shanghai, China). The human lncRNA array provides a comprehensive overview of lncRNAs and mRNAs in the human genomes. Microarray hybridisation was performed based on the manufacturer's standard protocols (Agilent Technologies Co., Ltd., California, USA), including RNA purification, transcription into fluorescent cRNA and cDNA hybridisation onto

Microarray V4.0. The hybridised arrays were washed, fixed and scanned using a Microarray Scanner (Agilent Technologies Co., Ltd., California, USA).

The scanned images were loaded into Agilent Feature Extraction software (version 11.0.1.1, Agilent Technologies Co., Ltd., California, USA) to extract the raw data. The Agilent software's quantile normalisation and robust multichip average techniques were used to normalise the expression data. Following the normalisation, probe-level files for lncRNAs and mRNAs were created. Agilent GeneSpring GX software was used to obtain the final results. The false discovery rate (FDR) and *p*-value were used to determine the significance threshold. Differentially expressed lncRNAs and mRNAs were identified using fold-change (FC) values of >2.0 or <0.5, $p < 0.05$ and FDR < 0.05.

GO and KEGG pathway analyses

Differentially expressed mRNAs were characterised using a gene ontology (GO) analysis in terms of the biological processes, cellular components and molecular functions. A biological pathway analysis that was based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was conducted to better understand the biological pathways underlying the enrichment of expressed mRNAs associated with differentially expressed lncRNAs. Significant GO and pathway analysis enrichment was defined as $p < 0.05$. Lower *p*-values indicated higher levels of enrichment.

qRT-PCR

According to the manufacturer's instructions, a reverse transcription was performed using a PrimeScript RT reagent kit with a gDNA eraser (Takara Biotechnology Co., Ltd., Dalian, China). A quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was conducted using a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) along with an SYBR™-Green PCR kit (Takara Biotechnology Co., Ltd. USA). All qRT-PCR data were quantified using the $2^{-\Delta\Delta CT}$ method. The targets for qRT-PCR validation were chosen based on their fold-change, relevance to β-TM or prediabetes, and availability of primers. The primer sequences are shown in Table I.

Statistical analysis

The results were analysed using SPSS 25.0. For the continuous variables, the Shapiro–Wilk normality test was performed. The data following a normal distribution were represented using the mean and standard deviation, with the differences assessed using an independent sample *t*-test. Quartiles were used to represent the data with a non-normal distribution, with the nonparametric Mann–Whitney U test employed to assess the differences. The chi-square or Fisher's exact test was also used to statistically evaluate the categorical data. Statistical differences were considered significant at $p < 0.05$.

Table I. Reverse transcription-quantitative polymerase chain reaction primers for randomly selected lncRNAs.

Transcript ID	Primer (5'→3')
ENST00000431759	F CCACGCAAACTCCTTCTGTA
	R GCCATTTTTTACCCTTTAGTTC
ENST00000611877	F CTTCAGAGTGGGTGGTTTCC
	R CCTTCGCTGTCCTTTGAGTT
ENST00000564152	F GTGGTTGGGTTTCTGAGTTTG
	R TGTCTGGCTTCCCTCTGTTC
ENST00000607613	F TGCTGAGAGGGGTTTAGGAA
	R GGAATCTGGAAAACTGCCCA
ENST00000581274	F ATGACATGGGGAAATGGAAGG
	R GCTGATCGCACTCAACTCTT
ENST00000449551	F TTTCAGAGGAGTGGCTGGTA
	R TGGAGTGGATCACAGGCTTA
ENST00000437035	F ACAAATCTGCCACTCAAGCC
	R TCTACTCCTGGATGTCTCTTCT
ENST00000556546	F CTGCCTCCGATCCAAATTGT
	R CCTTGGAGAAACGCCATTGA

RESULTS

The clinical and demographic characteristics of the patients with β-TM and prediabetes and the healthy volunteers are presented in Tables II and III.

Profile changes of lncRNA in patients with bTM/prediabetes

We analyzed lncRNA expression patterns in patients with β-TM complicated by prediabetes and compared these to healthy individuals. Of the 26,035 lncRNAs analyzed, 3,443 showed differential expression, with 1,511 upregulated and 1,932 downregulated (Figs. 1A, C, 2A, C). Table IV lists the 20 lncRNAs that underwent the most significant degree of change. The most substantially upregulated (FC= 8.2×10^{1}) and downregulated (FC = 1.7×10^{2}) lncRNAs were ENST00000496629 and ENST00000581274, respectively. In addition, a summary of the dysregulated lncRNA categorisation distribution was recorded. There were 1,799 intergenic, 980 antisense, 409 intronic and 255 bidirectional lncRNAs among the dysregulated lncRNAs (Fig. 3).

Table II. The clinical and demographic features of patients with β-TM complicated with prediabetes.

ID	Gender	Age (years)	Time of blood transfusion	Ferritin (ng/ml)	BMI	Fasting C-peptide (nmol/L)	Fasting insulin (pmol/L)	Fasting glucose (mmol/L)	Hour plasma glucose (mmol/L)	LIVER mri T2* (MS)	HOMA-IRI	HOMA-ISI	HOMA-βFI	HOMA-SC
Patient 1	Female	9	86	6,498	15.4	0.589	64.3	6.8	9.9	1.9	2.789	0.736	55.9	23.647
Patient 2	Female	13	89	7,704	16.4	1.04	74.94	6.6	9.8	1.5	3.15	0.613	69.35	29.075
Patient 3	Male	12	97	7,603	17.3	0.92	65.15	5.8	8.5	1.6	2.42	0.62	81.3	28.741
Patient 4	Male	9	89	6,278	14.2	0.81	61.56	5.9	8.1	1.63	2.46	0.62	78.41	28.398
Patient 5	Male	10	99	8,805	13	0.75	65.2	5.8	8.9	1.55	2.78	0.537	93.82	33.7
Control 1	Female	12	0	115.04	19	0.971	41.7	5.3	4.3	-	1.41	0.885	66.5	19.09
Control 2	Female	12	0	268	21.4	0.601	60.3	4.4	5.7	-	1.69	0.508	157.41	35.85
Control 3	Male	9	0	148.39	20.3	0.851	63.66	4.4	5.6	-	1.78	0.461	166.18	38.04
Control 4	Female	12	0	96.31	19.1	0.401	55.94	4.0	4.7	-	1.427	0.498	321.26	36.657
Control 5	Male	10	0	106	20	0.521	57.91	4.3	4.9	-	1.588	0.517	207.86	35.17

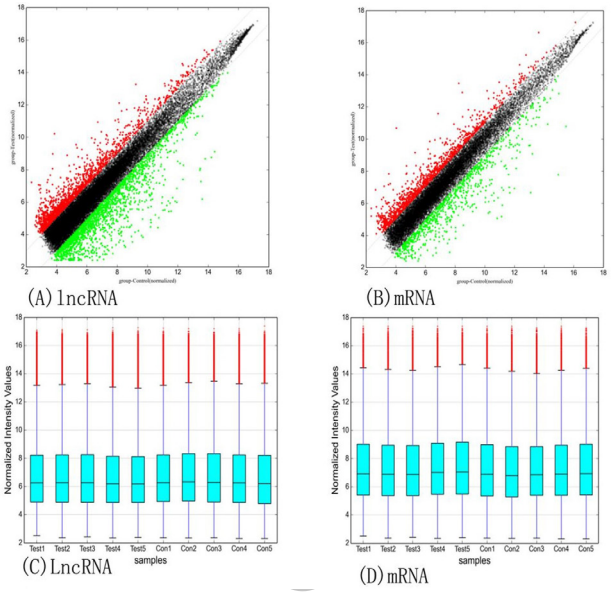

Fig. 1. The characteristics of lncRNA and mRNA differential expression. A scatter plot shows the differences between lncRNA (A) and mRNA (B) expression in patients with β-TM with prediabetes (y-axis, group-test) versus healthy controls (x-axis, group-control). Red and green represent ≥ 2-fold, $p < 0.05$. The box plots show the lncRNA (C) and mRNA (D) expression levels of each individual sample.

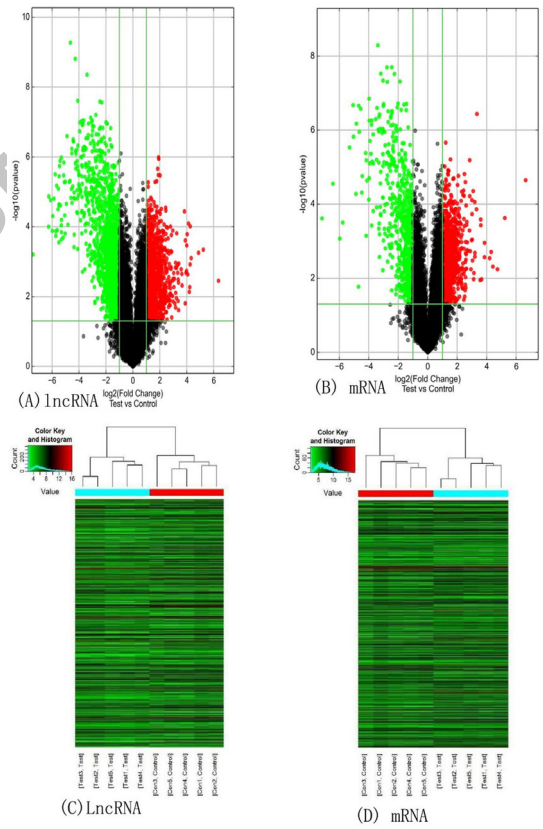

Fig. 2. Volcano plot and hierarchical clustering of differentially expressed transcripts. A, Volcano plot of differentially expressed lncRNAs; B, Volcano plot of differentially expressed mRNAs; C, Hierarchical clustering of lncRNA profiles in patients with β-TM with prediabetes compared with healthy controls (≥ 2-fold, $p < 0.05$); D, Hierarchical clustering of mRNA profiles in patients with β-TM with prediabetes compared with healthy controls (≥ 2-fold, $p < 0.05$).

Changes in the mRNA profile in patients with BTM/ prediabetes

We also examined the expression patterns of mRNAs in patients with β-TM/prediabetes compared with healthy individuals. Of the 17,044 mRNAs analyzed, 1,880 showed differential expression, with 1,128 upregulated and 752 downregulated (Figs. 1B, 1D, 2B, 2D). Table V lists the 20 most drastically changed mRNAs. The most substantially

Table III. Demographics of patients with β-TM with prediabetes and healthy controls.

Varibles	Control	Prediabetes in β-TM
ALT(u/L)	12.94 ± 4.11	72.30 ± 46.22*
Fasting glucose(mmol/L)	4.48 ± 0.49	8 ± 0.48*
hour plasma glucose(mmol/L)	5.04 ± 0.60	9.04 ± 0.79*
Fasting insulin(pmol/L)	55.90 ± 8.44	66.23 ± 5.09*
Fasting C-peptide(nmol/L)	0.67 ± 0.24	0.82 ± 0.17
HOMA-IRI	1.58 ± 0.16	2.72 ± 0.30*
HOMA-ISI	0.51(0.48–0.70)	0.62(0.58–0.68)
HOMA-βFI	183.84 ± 92.52	75.76 ± 14.14*
HOMA-SC	35.85(27.13–37.35)	28.74(26.02–31.39)

*$p < 0.05$

upregulated and downregulated mRNAs were GDF15 (FC = 1.0×10^2) and GUCA2B (FC = 1.4×10^2), respectively.

Validation of dysregulated lncRNA by qRT-PCR

To confirm the microarray data, eight dysregulated lncRNAs were randomly selected from the expression profiles for qRT-PCR verification, including four that were upregulated (ENST00000607613, ENST00000431759, ENST00000564152 and ENST00000611877) and four that were downregulated (ENST00000449551, ENST00000581274, ENST00000437035 and ENST00000556546). The criteria for selecting these lncRNAs were based on their fold-change, relevance to β-TM or prediabetes, and availability of primers.The qRT-PCR findings verified that the regulatory trends of the above lncRNAs were consistent with those of the microarray assay both in the patients with β-TM/prediabetes and the healthy controls (Fig. 4).

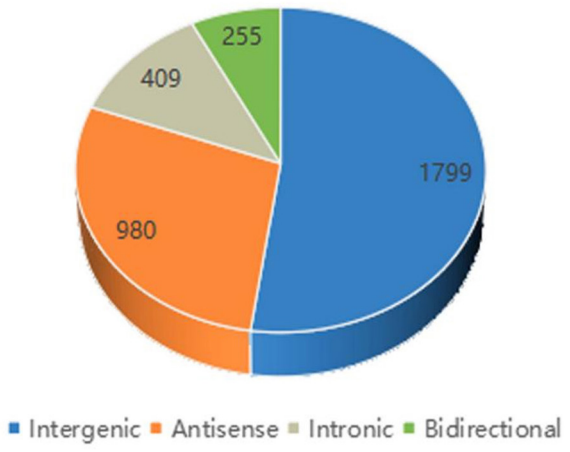

Fig. 3. Distribution of dysregulated lncRNAs.

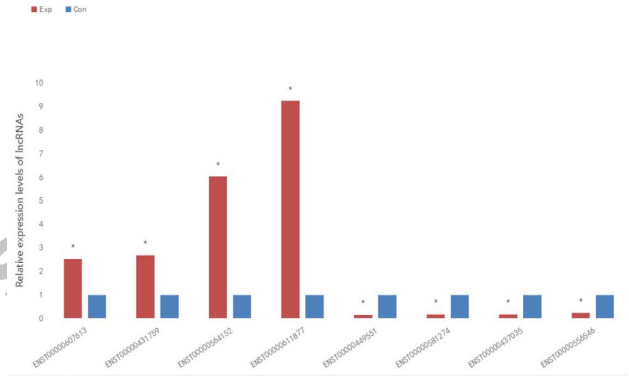

Fig. 4. The expression of selected lncRNAs. The expression level detected by qRT-PCR showed that the expression level of lncRNAs ENST00000449551, ENST00000581274, ENST00000437035 and ENST00000556546 was significantly lower and that of lncRNAs ENST00000607613, ENST00000431759, ENST00000564152 and ENST00000611877 was significantly higher in patients with β-TM with prediabetes compared with the controls (*$p < 0.05$). The qRT-PCR analysis was similar in most respects to the lncRNA microarray analysis.

GO and KEGG pathway analyses

To explore the potential functions of lncRNAs in β-TM/prediabetes, the authors conducted a GO analysis on aberrantly expressed mRNAs, which mainly covers three sections (biological process, cellular component and molecular function) and provides instructions on how to describe gene and gene product attributes in a given organism. The GO results indicated that upregulated mRNAs were involved in 126 cellular components, 767 biological processes and 92 molecular functions. In contrast, the GO analysis enriched 275 terms (214 biological processes, 28 cellular components and 33 molecular functions) in downregulated mRNAs.

Table IV. The 20 most differentially expressed lncRNAs in patients with β-TM with prediabetes relative to matched controls.

lncRNA ID	P-value	FDR	Fold change	Regulation	Chrom	Strand	Relationship	Database
ENST00000496629	0.003526083	0.032526596	82.1860551	up	chr8	-	Bidirectional	GENCODE
ENST00000431759	0.000454682	0.010781106	37.6048365	up	chr1	+	Natural antisense	GENCODE
ENST00000583516	0.000556435	0.012007394	29.3773871	up	chr3	-	Intronic antisense	GENCODE
ENST00000582591	0.000794281	0.014552508	20.1327846	up	chr18	+	Natural antisense	GENCODE
T144753	9.72507E-05	0.004628744	19.1867411	up	chr17	-	Natural antisense	RNA-SEQ: IYER *ET AL* 2015
HBMT00001229624	0.001197038	0.018044649	19.0708748	up	chr6	+	Intergenic	FANTOM5CAT
T092137	0.005372424	0.041556365	18.7283573	up	chr13	+	Natural antisense	RNA-SEQ: IYER *ET AL* 2015
ENST00000495240	0.007330467	0.049419663	18.2757294	up	chr21	-	Bidirectional	GENCODE
ENCT00000417654	8.05354E-05	0.004137151	17.5584774	up	chr7	-	Intergenic	FANTOM5CAT
ENST00000523831	0.002313282	0.025628209	16.9052281	up	chr8	+	Intergenic	GENCODE
ENST00000581274	0.000623989	0.012711699	170.1365176	down	chr18	-	Intergenic	GENCODE
T048514	9.98219E-05	0.004660449	76.2685338	down	chr10	-	Intergenic	RNA-SEQ: IYER *ET AL* 2015
ENST00000421375	1.35499E-05	0.001367337	75.6516897	down	chr3	-	Intergenic	GENCODE
ENST00000556546	1.72633E-05	0.001558422	68.9262261	down	chr14	-	Intergenic	GENCODE
ENST00000608466	0.000131548	0.005419482	63.0881264	down	chr5	-	Bidirectional	GENCODE
ENCT00000192781	4.96226E-05	0.002997505	61.6649287	down	chr18	+	Intergenic	FANTOM5CAT
ENST00000586947	1.57655E-05	0.001487151	58.2341391	down	chr18	+	Intergenic	GENCODE
ENST00000495493	3.16861E-06	0.000598221	54.343005	down	chr1	-	Exon sense-overlapping	GENCODE
AK024231	0.000182406	0.00656838	52.8044804	down	chr17	+	Intergenic	NRED
ENST00000511474	0.000135322	0.005506699	52.6697045	down	chr5	+	Intergenic	GENCODE

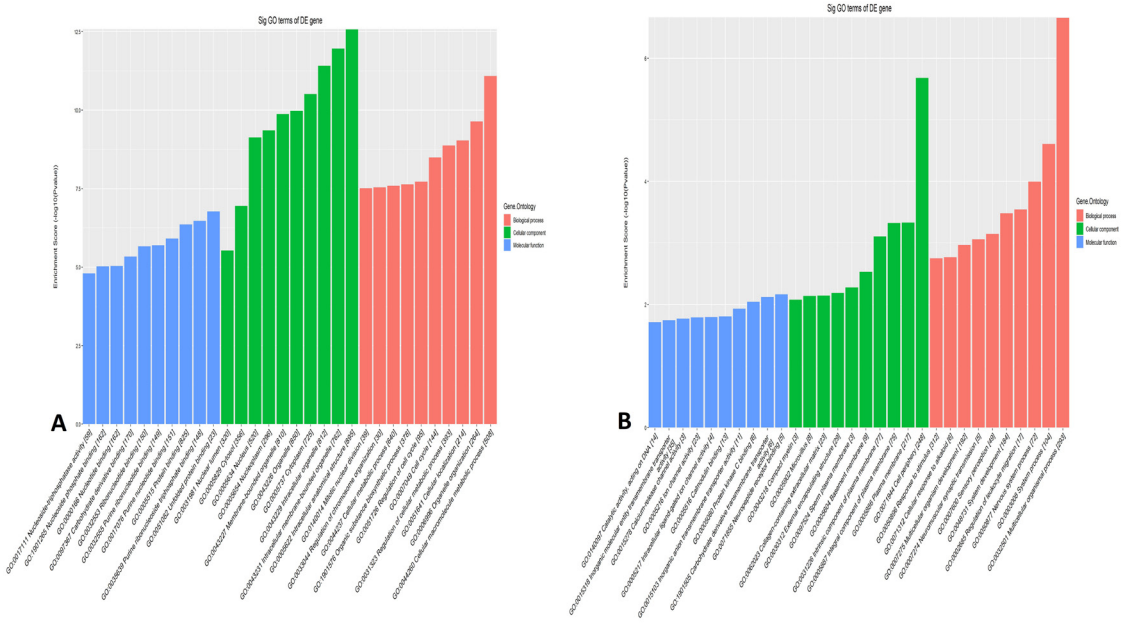

Fig. 5. Gene ontology (GO) enrichment analysis of differentially expressed mRNAs. A, Top ten GO terms enriched for genes with upregulated expression; B, Top ten GO terms enriched for genes with downregulated expression.

Unfolded protein binding (GO: 0051082), intracellular anatomical structure (GO: 0005622) and cellular macromolecule metabolic process (GO: 0044260) were the most substantially increased GO keywords in the β-TM samples with prediabetes compared with the control samples. In addition, neuropeptide receptor binding (GO: 0071855), cell periphery (GO: 0071944) and the multicellular organismal process (GO: 0032501) were the most significantly downregulated GO terms for the β-TM samples with prediabetes. Figure 5A, B show the top 10 highly enriched words and corresponding up- and downregulated transcripts.

We also performed a biological pathway analysis based on the KEGG database to better understand the biological pathways underlying the enrichment of expressed mRNAs associated with differentially expressed lncRNAs. In this study, 31 pathways were upregulated, with the most enriched network being herpes simplex virus 1 infection – *Homo sapiens* (Pathway ID: hsa05168), comprising 50 target genes. The vascular smooth muscle contraction – *Homo sapiens* (Pathway ID: hsa04270) network, which included 11 target genes, was the most substantially enriched network among the additional 17 pathways that were downregulated. Figure 6A, B display the top 10 highly enriched pathways and corresponding up- and downregulated transcripts.

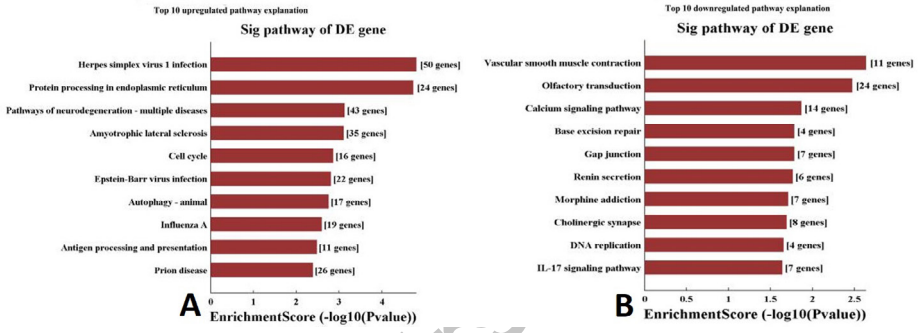

Fig. 6. Genomes pathway analysis of differentially expressed mRNAs. A, Top ten pathways enriched for genes with upregulated expression; B, Top ten pathways enriched for genes with downregulated expression.

Table V. The 20 most differentially expressed mRNAs in patients with β-TM with prediabetes relative to matched controls.

Gene symbol	P-value	FDR	Fold change	Regulation	Chrom
GDF15	2.26375E-05	0.002411457	100.2322219	up	CHR19
HBG1	0.000236675	0.008984146	37.5558847	up	CHR11
TMEM269	0.005760896	0.048273699	26.6075395	up	CHR1
ST8SIA5	0.004899657	0.045003731	21.4706818	up	CHR18
PAQR9	0.001958959	0.028087005	19.8210477	up	CHR3
TOMM5	0.002893375	0.034055763	18.4003285	up	CHR9
ACSM3	0.001112436	0.020491209	14.4928213	up	CHR16
BBS12	0.002714217	0.032809297	14.4565088	up	CHR4
SFRP2	0.010723854	0.067903233	12.6215505	up	CHR4
IFI44L	0.000374268	0.011411502	12.1742708	up	CHR1
GUCA2B	0.000243604	0.009222062	143.9714162	down	CHR1
B3GALNT2	2.82567E-05	0.002715737	86.152204	down	CHR1
TOX2	0.000854932	0.017757699	62.2958244	down	CHR20
FBXL13	0.000314764	0.010403005	54.406973	down	CHR7
OR10G4	2.96323E-06	0.000742725	39.4923179	down	CHR11
NAA80	2.12895E-07	0.000229889	33.3880965	down	CHR3
CLUH	1.51667E-06	0.000470002	32.7656707	down	CHR17
PCDHA9	5.28901E-05	0.00411625	29.1615088	down	CHR5
BCAP31	6.76081E-07	0.000338915	28.6257891	down	CHRX
CATG00000107414.1	0.01698672	0.088837575	25.8281041	down	CHR9

DISCUSSION

Our study advances the understanding of β-TM and its association with prediabetes by elucidating the differential expression of lncRNAs and mRNAs. We found that 3,443 lncRNAs and 1,880 mRNAs showed differential expression in patients with β-TM complicated by prediabetes compared to healthy individuals. Our findings build upon those of Fakhr-Eldeen *et al*. (2019) by demonstrating that lncRNAs such as MALAT1 not only contribute to β-TM pathogenesis but also to the prediabetic state. We also identified several novel lncRNAs and mRNAs that have not been previously reported in association with β-TM or prediabetes, such as ENST00000496629, ENST00000581274, GDF15 and GUCA2B. These lncRNAs and mRNAs may be involved in various biological processes and pathways related to glucose metabolism, insulin resistance, oxidative stress and inflammation, which are known to play important roles in the development and progression of β-TM and prediabetes. Our study provides new insights into the molecular mechanisms and potential biomarkers of β-TM and prediabetes.

Diabetes mellitus and its consequences have been associated with lncRNAs in peripheral circulation. Liu *et al*. (Liu *et al*., 2019) reported that in type 2 DM, the MALAT1 expression correlated with the resistin and homeostasis model assessment of insulin resistance levels. Tello-Flores *et al*. (2020) reported a significantly reduced MALAT1 expression in the serum samples or exosomes derived from patients with type 2 DM. Wang *et al*. (2018) observed substantial downregulation of the lncRNA CASC2 in the blood samples and renal tissues from diabetic patients with chronic renal failure but not in the patients with other conditions. These findings suggest that these dysregulated lncRNAs and mRNAs may significantly influence the pathophysiology of β-TM with prediabetes. Similarly, 1511 significantly upregulated lncRNAs and 1932 significantly downregulated lncRNAs were identified in this present study. The most significantly upregulated and downregulated lncRNAs were FGFR1 and AC090912.2, respectively. FGFR1 has been reported to be associated with diabetes and metabolic diseases in several literatures (Rosenstock *et al*., 2023; Kaur *et al*., 2023), but AC090912.2 has not been studied in association with diabetes and metabolic diseases. In this present study, when comparing prediabetic patients and control samples, 1128 significantly upregulated mRNAs and 752 significantly downregulated mRNAs were found. Among them, GDF15 and GUCA2B were the most prominent mRNAs up- and down-regulated, respectively. Many studies have reported that GDF15 can be used as a therapeutic target for diabetes, while GDF15 expression is increased in cardiovascular disease and is one of the indicators of poor prognosis (Gong *et al*., 2023; Bradley *et al*., 2023; Yücel and İlanbey, 2023). GUCA2B is also involved in lipid metabolism (Fernández-Sáez *et al*., 2023; Otero *et al*., 2023), Fernández-Sáez EM *et al*. (2023) reported that Increased GUCA2B patient levels contribute to improving liver injury in patients with obesity-associated NAFLD after bariatric surgery.

Iron overload in β-TM results from numerous blood transfusions and abnormally high iron absorption associated with inefficient erythropoiesis (Mangia *et al*., 2020). Excess iron in the pancreas is thought to induce oxidative stress in β-cells. Ding *et al*. (2012) revealed that lncRNA could affect the physiological functions of islets. It has been demonstrated that the downregulation of MEG3 in pancreatic tissue from the rats model of type 1 and type 2 DM, while silence of MEG3 can reduce insulin secretion and impaired glucose homeostasis. Several advances revealed that lncRNA MEG3 promotes insulin secretion and inhibits pancreatic β-cells apoptosis in DM (Li *et al*., 2022). Liu *et al*. (2019) discovered a correlation between MALAT1 expression and resistin and HOMA-IR levels in type 2 DM. Knocking down lncRNA MALAT1 in human umbilical vein endothelial cells can decrease the production of resistin, Ang II and other pro-inflammatory cytokines while increasing glucose absorption, nitric oxide concentration and p-Akt/Akt. This study's microarray results also revealed aberrant expression of the aforementioned lncRNAs, suggesting that lncRNAs may contribute to the development of β-TM with prediabetes.

This study determined the roles of differentially expressed genes using GO and KEGG pathway analyses. According to the GO analysis, dysregulated mRNAs contribute to metabolic processes, cell communication and cellular responses to numerous stimuli (inflammatory response, oxidative stress, etc.). The KEGG pathway analysis revealed several significantly enriched pathways, including protein processing in endoplasmic reticulum – *Homo sapiens* (Pathway ID: hsa04141), which were strongly associated with glycometabolism. Other examples include *Homo sapiens* cell cycle (Pathway ID: hsa04110) (Kehm *et al*., 2020), calcium signalling pathway – *Homo sapiens* (Pathway ID: hsa04020) and NOD-like receptor signalling pathway – *Homo sapiens* (Pathway ID: hsa04621). These results revealed the involvement of variously expressed mRNAs in inflammatory-mediated signalling pathways (Sidarala and Soleimanpour, 2022) and the oxidative stress response (Lundberg *et al*., 2018). A complex genetic and metabolic network governs the biochemical processes of prediabetes in patients with β-TM (Zeng *et al*., 2019; Gilon *et al*., 2014). All in all,

these lncRNAs and mRNAs may be implicated in each stage of the biological process, leading to prediabetes in patients with β-TM.

However, the study's conclusions are tempered by its small sample size, which may not capture the full diversity of lncRNA and mRNA expression patterns across the β-TM population. Moreover, the study only conducted a bioinformatics analysis to identify potential candidate lncRNAs and mRNAs without using genetic experiments to verify their regulatory role. Subsequent studies should focus on expanding the sample size and including functional assays to validate the regulatory role of the identified lncRNAs in β-cell physiology.

CONCLUSION

In conclusion, this study indicates that the altered expression of lncRNAs and mRNAs may be related to β-TM with prediabetes and function through networks or participate in various specific biological processes. This study could lay a theoretical foundation for identifying new treatment targets for patients with β-TM complicated with prediabetes, as well as diagnostic and prognostic indicators. The differential expression of lncRNAs and mRNAs may also reflect the underlying pathophysiological changes and metabolic disturbances in β-TM and prediabetes, and thus have potential clinical applications.

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IRB approval

The study was approved by the Medical Ethics Committee of Hainan Provincial People's Hospital, Haikou 570000, Hainan Province, China.

Ethics approval and consent to participate

The Helsinki Declaration was followed in the conduct of this investigation. The Hainan General Hospital's Ethics Committee gave its clearance for this research to be carried out (Approval number: NO. 2021-239). All participants gave their permission in writing after being fully informed.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Statement of conflict of interest

The authors have declared no conflict of interest.

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