Original Paper

Aberrant Expression of Long Non-Coding RNAs in Newly Diagnosed Type 2 Diabetes Indicates Potential Roles in Chronic Inflammation and Insulin Resistance

Xiaoli Wang^a Xiangyun Chang^a Peipei Zhang^a Ling Fan^a Ting Zhou^b Kan Sun^a

^aDepartments of Endocrinology and Metabolism, ^bMedical Experimental Laboratory, First Affiliated Hospital, School of Medicine, Shihezi University, Shihezi, Xinjiang, China

Key Words

Type 2 diabetes • lncRNA • Inflammation • Insulin resistance

Abstract

Background/Aims: Long non-coding RNAs (lncRNAs) have emerged as key players in several biological processes and complex diseases. The risk of type 2 diabetes (T2D) is determined by a combination of environmental factors and genetic susceptibility. The purpose of this study was to identify aberrant lncRNAs involved in T2D pathogenesis. **Methods:** Microarray analysis was performed using whole blood samples from patients newly diagnosed with T2D and healthy controls. Pathway and Gene Ontology (GO) analyses were utilized to annotate the target genes. Coding non-coding co-expression (CNC) analysis was performed to construct a co-expression network. **Results:** We found 55 lncRNAs and 202 mRNAs were differentially expressed in the T2D group compared to the healthy control group. Pathway and GO analyses demonstrated that dysregulated mRNAs were mainly associated with immune regulation, inflammation, and insulin resistance, whereas CNC analysis identified 10 pairs of co-expressed lncRNA-mRNAs in our patient cohort ($R > 0.99$). Furthermore, expression of the top three upregulated lncRNAs in the T2D group was correlated with measures of glycometabolism ($P < 0.05$). **Conclusion:** This study identified aberrantly expressed lncRNAs and mRNAs in Han Chinese patients with T2D, and demonstrated that dysregulated lncRNAs may have roles in T2D pathogenesis through regulation of inflammation and insulin resistance.

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Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases in the world characterized by elevated plasma glucose levels, and type 2 diabetes (T2D) accounts for most patients with DM. According to the World Health Organization (WHO), in 2015 there

X. Wang and X. Chang contributed equally to this work.

Kan Sun

Department of Endocrinology and Metabolism, First Affiliated Hospital, School of Medicine
Shihezi University, No.107, Bei'er Road, Shihezi, Xinjiang, (China)
Tel. +86-0993-2858109, E-Mail sunkan2015@sina.com, wxldoctor@126.com

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were 415 million patients with T2D globally, which is predicted to rise to 642 million by 2040 [1]. The risk of acquiring T2D is determined by a combination of environmental factors and genetic susceptibility.

Long non-coding RNAs (lncRNAs) are defined as non-encoding RNA transcripts longer than 200 bp [2]. lncRNAs were previously considered as “transcriptional noise” because of their poor conservation among species and low expression. To date, over 7000 lncRNAs have been identified in the human genome, of which only a small number have been ascribed a function [3]. It has been realized that lncRNAs regulate multiple biological processes [4], with an increasing number of studies demonstrating that lncRNAs play important roles in cell differentiation, tissue development, chromosome inactivation, and epigenetic modification [5–7]. Furthermore, the link between genetically controlled lncRNA expression and human disease has been established [8], with growing evidence of their involvement in glucose homeostasis and DM [9–12]. Over 1000 lncRNAs that are relevant to β cell programming and diabetes pathophysiology have been identified in pancreatic beta cells [13].

A core mechanism of T2D is insulin resistance (IR), which is a condition in that targeted cells fail to respond to insulin. IR is characterized by a decrease in insulin signaling [14]. Although it is widely accepted that low-grade chronic inflammation is an important factor in the pathogenesis of IR [15, 16], the molecules and mechanisms that regulate inflammation and the IR process in T2D are not fully understood. However, non-coding RNAs were implicated in the regulation of these processes [17–20].

The purpose of this study is to identify lncRNAs associated with T2D by examining lncRNA and mRNA expression profiles from whole blood samples of patients newly diagnosed with T2D compared to healthy individuals. We also investigated whether there were correlations between the expression level of aberrantly expressed lncRNAs and different measures of metabolic processes. We demonstrate that dysregulated lncRNAs may have roles in the pathogenesis of T2D through regulation of inflammation and IR.

Materials and Methods

Ethics

This study was conducted in accordance with the Declaration of Helsinki and performed with the approval from the Ethics Committee of the First Affiliated Hospital, School of Medicine Shihezi University (China). Informed consent was provided by all participants before entering the study.

Subjects

We enrolled individuals ≥ 35 years old at the time of their visit at the First Affiliated Hospital in Shihezi (China) from October 2015 to December 2016. The diagnosis of T2D was based on the 1999 WHO diagnostic criteria [21]. Our control group consisted of healthy volunteers without DM who were recruited from communities in Shihezi in China. Demographic and clinicopathological information, such as age, ethnicity, previous diseases, medication, smoking, and drinking habits, was obtained via a questionnaire. All participants were Han Chinese and had not been treated with any hypoglycemic agents. Individuals with a history of cancer, pregnancy, lactation, immunodeficiency, chronic organ disease, or infectious disease or patients who were receiving immunosuppressive or hormone-containing drugs were excluded.

The initial ‘microarray cohort’ (discovery cohort) was composed of six patients newly diagnosed with T2D and six healthy volunteers, whereas the validation cohorts were composed of 60 newly diagnosed patients with T2D and 60 healthy control individuals.

Anthropometric and biochemical measurements

For all participants, anthropometric and body composition measurements were performed before breakfast. Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m). A standard 75 g oral glucose tolerance test was conducted for all subjects after overnight fasting (longer than 10 h). In brief, 75 g of glucose was ingested within 5 min and peripheral blood samples were collected in the fasting state and 2 h after glucose ingestion. Blood samples were withdrawn from the cubital vein

and collected in evacuated sample tubes containing preservatives (heparin sodium). The tubes were stored at 4 °C until centrifugation. In addition, 1 mL fresh whole blood samples collected in evacuated sample tubes containing ethylene diamine tetraacetic acid were collected for measurement of hemoglobin A1c (HbA1c) level using an automated D10 analyzer (Bio-Rad, Hercules, CA, USA). Fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were analyzed enzymatically using an autoanalyzer (Hitachi, Tokyo, Japan). Fasting and 2-hour plasma insulin (2hINS) levels were detected using electrochemiluminescence (E170, Roche, Basel, Switzerland).

Total RNA extraction and purification

Total RNA was extracted from peripheral blood using the PAXgene Blood RNA kit (Qiagen, Dusseldorf, Germany) for isolation and purification according to the manufacturer's instructions and as described previously [22]. Briefly, approximately 2.5 mL of blood from each subject was incubated for 2 h at room temperature in PAXgene® Blood RNA tubes (Becton Dickinson, Franklin Lakes, NJ, USA) followed by centrifugation for 10 min at 3000 × g. Cell pellets were then incubated with a mixture of 300 µL binding buffer and 40 µL of proteinase K for 10 min at 55 °C with shaking. The resultant lysate was centrifuged for 3 min in a PAXgene® Shredder spin column at 16 300 × g, and the supernatant was then mixed with 350 µL of 100% ethanol in the PAXgene® RNA spin column followed by centrifugation for 1 min at 16 300 × g. The purified RNA was eluted by elution buffer, denatured by incubating at 65 °C for 5 min, chilled on ice, and stored at -20 °C until use in microarray and gene expression studies.

RNA labeling and hybridization

Double-stranded complementary DNA (cDNA) was synthesized from total RNA according to the manufacturer's instructions and labeled with a fluorescent dye. Labeled cDNA was denatured at 95 °C for 3 min in hybridization solution and hybridized for 16 h at 45 °C on GeneChip Human Transcriptome Array 2.0 microarrays (Affymetrix, Santa Clara, CA, USA), which covers > 245 000 protein-coding transcripts and > 40 000 non-coding transcripts. Following hybridization, slides were washed and dried prior to data collection using Microarray Scanner 3000 (Affymetrix, Santa Clara, CA, USA).

Microarray analysis

Transcriptome Analysis Console software (Affymetrix) was used to analyze the expression profiles of lncRNAs and mRNAs. Differentially expressed genes were identified based on threshold changes of ≥ 2-fold or ≤ -2-fold and *P* values < 0.05. The data were normalized and hierarchically clustered with CLUSTER 3.0. Data were performed to be Tree Visualization with Java Treeview software (Stanford University School of Medicine, Stanford, CA, USA).

Pathway and Gene Ontology (GO) analysis

Pathway analysis was a functional analysis for mapping genes to pathways based on Kyoto Encyclopedia of Genes and Genomes (KEGG). The enriched genes in the KEGG pathway were statistically calculated by hypergeometric distribution. Gene Ontology (GO) was also used to explore the potential molecular function of the differentially expressed mRNAs (<http://geneontology.org/>).

Construction of the coding non-coding gene co-expression network

The coding non-coding co-expression (CNC) network was constructed based on correlation analysis between the differentially expressed lncRNAs and mRNAs. The network was drawn using the open source bioinformatics software Cytoscape (<http://www.cytoscape.org>).

Quantitative real-time PCR and statistical analysis

Primers for quantitative real-time PCR (qRT-PCR) were designed based on the lncRNA sequences from NONCODE (<http://www.noncode.org>), and were synthesized and purified at Sheng Gong (Shanghai, China). The reverse transcription reactions on extracted total RNA were performed using a cDNA synthesis kit (Qiagen, Dusseldorf, Germany) and a 7500 Fast RT-PCR System (Applied Biosystems). qRT-PCR was performed using the ABI 7500 Fast. The qRT-PCR cycle was 95 °C for 2 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s, and a final melting curve analysis from 60–95 °C. qRT-PCR results were quantified

using the $2^{-\Delta\Delta ct}$ method against β -actin (ACTB) for normalization. The data represent the means of three experiments. The primers used in qRT-PCR were listed in Table 1. Because data of qRT-PCR were abnormally distributed, the expression level of lncRNAs between healthy control and T2D group were analyzed using Mann – Whitney *U* test. Spearman’s correlation analyses were employed to verify the relationship between expression level of lncRNAs and metabolic features. *P* < 0.05 was considered statistically significant.

Results

lncRNA expression profiles in patients with T2D

The microarray cohort was composed of six patients newly diagnosed with T2D and six healthy volunteers. Their clinical and demographic characteristics were shown (for all online suppl. material, see www.karger.com/doi/10.1159/000484388) in Table S1. From our microarray analysis, we detected 13 921 lncRNAs, of which 55 lncRNAs were differently expressed in the T2D group compared to the healthy control group (Fig. 1a). Of these 55 lncRNAs identified in our patient cohort, we found that 39 lncRNAs were upregulated and 16 lncRNAs were downregulated (see online suppl. material, Tables S2 and S3).

Table 1. Primers for qRT-PCR detection of lncRNAs

Target	Primer
n342533	Forward, 5'-TGGCTGTTCTGAAGAGTGTACTG-3' Reverse, 5'-GCCAGAGGGTCTGTAGTCTGAT-3'
n341578	Forward, 5'-GCTAAATCAGAGAGGCAGAAAG-3' Reverse, 5'-ATGCATCAATATCCTCCCAATTA-3'
n341270	Forward, 5'-AGGCTGCTGGACTGGAATC-3' Reverse, 5'-ATACACTGGGTGGGCTCCTT-3'
n336302	Forward, 5'-CAATAATCACGGCTGCCAAG-3' Reverse, 5'-GGCCTTTCCAGGTTGGAAC-3'
n405950	Forward, 5'-GCGGTACAGTCTGGGCATT-3' Reverse, 5'-AGATCAGGAGGGCTGCATT-3'
n341520	Forward, 5'-GGAGGCAAACGCTGTGTTATC-3' Reverse, 5'-AGGAGAAGCAGCAGCATCAGA-3'
n338909	Forward, 5'-TCCCACCCACTCAGCTCACT-3' Reverse, 5'-ACCAGGAAGCTCACGGACAC-3'
n325833	Forward, 5'-TGTGGAGTGGGTAGCCTCCA-3' Reverse, 5'-CCGAGCTTTAGGTAATTCTCACAG-3'
n410159	Forward, 5'-ATTGCTGTGGCGTCTGC-3' Reverse, 5'-TCGATTACCCCCATCAGGC-3'
n342476	Forward, 5'-CTCTGCCTCGCAGGTTCAAG-3' Reverse, 5'-GGTGGATCACCTGAGGTCAAG-3'
ACTB	Forward, 5'-ACTGGGACGACATGGAGAAA-3' Reverse, 5'-TAGCACAGCCTGGATAGCAA-3'

Fig. 1. Differential expression of long non-coding RNAs (lncRNAs) and mRNAs in patients with type 2 diabetes (T2D) and controls. Hierarchical clustering analysis of (a) 55 lncRNAs and (b) 202 mRNAs that were differentially expressed in the two groups of participants. Expression values are represented as red and green, which indicate upregulation and downregulation respectively, in patients (D1, D2, D3, D4, D5, and D6) compared to that found in control individuals (C1, C2, C3, C4, C5, and C6).

Fig. 2. The pathways (a) or Gene Ontology (GO) (b) terms identified with the aberrantly expressed mRNAs found in the patient cohort compared to the control cohort.

Table 2. Correlation between lncRNAs and mRNAs (only correlation > 0.99 genes are shown). ^a Probe set identity of lncRNAs in microarray; ^b Noncode identity of lncRNA in NONCODE database ([HTTP://NONCODE.org](http://NONCODE.org)); ^c Probe set identity of mRNAs in microarray

lncRNA (Probe set ID) ^a	Noncode ID ^b	Correlation	P value	mRNA (Probe set ID) ^c	Gene ID	Gene symbol
TC19002237.hg.1	n336109	0.995690	0.001282	TC06002121.hg.1	8875	VNN2
TC04002272.hg.1	n379706	0.995335	0.032954	TC06000051.hg.1	9450	LY86
TC21000719.hg.1	n384014	0.995106	0.002139	TC21000167.hg.1	3772	KCNJ15
TC13000982.hg.1	n382000	0.994872	0.018456	TC01003260.hg.1	6283	S100A12
TC06003855.hg.1	n409772	0.994590	0.001282	TC06002121.hg.1	8875	VNN2
TC03002627.hg.1	n382000	0.994056	0.004603	TC07000509.hg.1	948	CD36
TC19002237.hg.1	n336109	0.993600	0.001156	TC0X000052.hg.1	51311	TLR8
TC0X001624.hg.1	n408024	0.991440	0.012212	TC01003261.hg.1	6279	S100A8
TC08002477.hg.1	n341587	0.991300	0.004603	TC07000509.hg.1	948	CD36
TC19002237.hg.1	n336109	0.990320	0.000051	TC21000167.hg.1	3772	KCNJ15

mRNA expression profiles in T2D

We also identified 44 699 coding RNAs from microarray analysis. Of the 202 mRNAs that have significantly different expression between the T2D group and the healthy control group (Fig. 1b), 106 mRNAs were upregulated (see online suppl. material, Table S4) and 96 mRNAs were downregulated (see online suppl. material, Table S5).

Pathway and GO analyses

We found using pathway analysis that the differentially expressed mRNAs identified in our Han Chinese cohort with T2D were involved in 13 pathways, including natural killer cell-mediated cytotoxicity, fat digestion and absorption, the nuclear peroxisome proliferator-activated receptor (PPAR) signaling pathway, the adipocytokine signaling pathway, the NF-kappa B signaling pathway, et al. (Fig. 2a). Using GO biological process analysis, we also found that the dysregulated mRNAs were involved in 29 items, including innate immune response, positive regulation of the inflammation response, neutrophil chemotaxis, cytokine production, negative regulation of insulin secretion, et al. (Fig. 2b).

Fig. 3. The coding non-coding co-expression network of differentially expressed long non-coding RNAs and mRNAs found in the patient cohort compared to the control cohort. Red box represents mRNA, blue triangle represents lncRNA.

Fig. 4. Validation of differentially expressed long non-coding RNAs (lncRNAs) in microarrays by quantitative PCR (qPCR). (a) Five upregulated and (b) five downregulated lncRNAs from microarray data were assayed by qPCR using β -actin (ACTB) as a housekeeping gene for normalization.

CNC network of lncRNAs and mRNAs

There were 55 lncRNAs and 202 mRNAs for CNC network analysis, which derived 256 network nodes and 2070 co-expression pairs of lncRNAs and mRNAs (Fig. 3). Our analysis of the CNC network indicated that a single lncRNA is correlated with one to tens of mRNAs and *vice versa*. Ten lncRNA-mRNA pairs have a co-expression relationship ($R > 0.99$). The target mRNAs were lymphocyte antigen 86 (LY86), S100 calcium binding protein A12 (S100A12), S100 calcium binding protein A8 (S100A8), CD36, toll-like receptor 8 (TLR8), Vanin-2 (VNN2), and potassium inwardly-rectifying channel, subfamily J, member 15 (KCNJ15).

Table 2 lists the differentially expressed mRNAs and their co-expressed lncRNAs in the CNC network with a correlation coefficient > 0.99 and $P < 0.05$.

qRT-PCR validation

To independently validate the identified dysregulated lncRNAs, five upregulated and five downregulated lncRNAs were randomly chosen from differentially expressed lncRNAs (fold changes ≥ 2.0 or ≤ -2.0). qRT-PCR was performed to compare the expression levels of these differentially expressed lncRNAs in the T2D group ($n = 60$) versus those found in the healthy control group ($n = 60$). As shown in Fig. 4, our qRT-PCR results are consistent with the findings from our microarray data.

Correlation of lncRNA expression with metabolic features

To investigate whether there is a correlation between differentially regulated lncRNAs in our cohort of T2D and metabolic features, expression of the top three upregulated lncRNAs (n342533, n335556, and n336109) were examined by qPCR in 60 pairs of whole blood RNA samples. The clinical features of the T2D group and the healthy control group are shown in Table 3. In addition to confirming that expression of n342533, n335556, and n336109 was significantly increased in the T2D group compared to the healthy control group (all $P < 0.05$) (Fig. 5), we found using correlation analysis that n342533 was positively correlated with BMI, FPG, 2hPG, HbA1c, homeostatic model assessment (HOMA)-IR and ALT (all $P < 0.05$) and negatively correlated with HDL-C and HOMA- β (both $P < 0.05$). We also found that n335556 was positively correlated with FPG, HbA1c, and HOMA-IR (all $P < 0.05$) and negatively correlated with 2hINS ($P < 0.05$), whereas n336109 was positively correlated with FPG, 2hPG, HbA1c, and HOMA-IR (all $P < 0.05$) and negatively correlated with HDL-C, 2hINS, and

Table 3. Demographic and metabolic characteristics of the T2D group and healthy control group. Data are reported as means $\pm$ standard deviation, number (%) or median (interquartile range). BMI, body mass index; FPG, fasting plasma glucose; 2hPG, 2-hour plasma glucose; HbA1c, haemoglobin A1c; FINS, fasting insulin; 2hINS, 2-hour insulin; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA- β , homeostatic model assessment of β cell function; TG, Triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate aminotransferase

Table 3 Demographic and metabolic characteristics of the T2D group and healthy control group			
Variable	T2D (n = 60)	Healthy control (n = 60)	P value
Male (%)	61.7	58.3	0.426
Age (year)	50.4 $\pm$ 13.4	51.0 $\pm$ 9.0	0.792
Current smokers (%)	21.7	20.0	0.798
Current drinkers (%)	20.0	21.7	0.500
Systolic blood pressure (mmHg)	129 $\pm$ 13	127 $\pm$ 14	0.366
Diastolic blood pressure (mmHg)	77 $\pm$ 11	77 $\pm$ 10	0.871
BMI (kg/m ²)	26.3 $\pm$ 3.0	25.4 $\pm$ 2.7	0.086
FPG (mmol/L)	9.56 $\pm$ 3.13	4.93 $\pm$ 0.47	<0.001
2hPG (mmol/L)	17.61 $\pm$ 4.31	6.40 $\pm$ 0.80	<0.001
HbA1c (%)	9.1 $\pm$ 2.1	5.5 $\pm$ 0.4	<0.001
FINS (μ U/mL)	58.42 $\pm$ 40.53	57.82 $\pm$ 16.50	0.916
2hINS (μ U/mL)	193.15 $\pm$ 117.76	288.44 $\pm$ 113.66	<0.001
HOMA - IR	3.17 (1.72 - 4.72)	1.58 (1.31 - 2.19)	<0.001
HOMA - β	31.84 $\pm$ 19.93	124.77 $\pm$ 61.64	< 0.001
TG (mmol/L)	2.21 $\pm$ 1.28	1.43 $\pm$ 0.75	< 0.001
TC (mmol/L)	4.67 $\pm$ 0.88	4.40 $\pm$ 0.89	0.105
HDL - C (mmol/L)	0.96 $\pm$ 0.25	1.27 $\pm$ 0.35	<0.001
LDL - C (mmol/L)	2.83 $\pm$ 0.88	2.71 $\pm$ 0.78	0.434
ALT (U/L)	25.8 $\pm$ 11.1	21.2 $\pm$ 14.6	0.047
AST (U/L)	22.8 $\pm$ 10.9	21.9 $\pm$ 8.9	0.608
Urea (mmol/L)	5.54 $\pm$ 1.71	5.11 $\pm$ 1.43	0.144
Creatinine (μ mol/L)	65.44 $\pm$ 17.06	64.21 $\pm$ 20.59	0.732

Table 4. Correlations between expressions of top 3 upregulated lncRNAs and metabolic characteristics. BMI, body mass index; HDL-C, high density lipoprotein cholesterol; FPG, fasting plasma glucose; 2hPG, 2-hour plasma glucose; HbA1c, haemoglobin A1c; FINS, fasting insulin; 2hINS, 2-hour insulin; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA- β , homeostatic model assessment of β cell function; ALT, alanine transaminase

Demographic and metabolic characteristics	n342533		n335556		n336109	
	r	P value	r	P value	r	P value
Model 1						
BMI (kg/m ²)	0.288	0.023	0.187	0.146	0.198	0.123
HDL-C (mmol/L)	-0.313	0.014	-0.156	0.229	-0.309	0.016
FPG (mmol/L)	0.611	< 0.001	0.360	0.004	0.674	< 0.001
2hPG (mmol/L)	0.546	< 0.001	0.217	0.090	0.604	< 0.001
HbA1c (%)	0.578	< 0.001	0.332	0.009	0.602	< 0.001
FINS (μ U/mL)	0.177	0.173	0.167	0.198	-0.081	0.536
2hINS (μ U/mL)	-0.179	0.164	-0.300	0.018	-0.307	0.015
HOMA - IR	0.323	0.010	0.441	< 0.001	0.374	0.003
HOMA - β	-0.510	< 0.001	-0.227	0.075	-0.649	< 0.001
ALT (mmol/L)	0.335	0.008	0.036	0.784	0.223	0.084
Model 2 adjusted age, gender, BMI and HDL - C						
FPG (mmol/L)	0.391	0.003	0.305	0.022	0.614	< 0.001
2hPG (mmol/L)	0.272	0.042	0.418	0.001	0.620	< 0.001
HbA1c (%)	0.420	0.001	0.357	0.007	0.584	< 0.001
FINS (μ U/mL)	0.385	0.003	0.255	0.058	0.062	0.650
2hINS (μ U/mL)	-0.007	0.958	-0.247	0.066	-0.257	0.056
HOMA - IR	0.587	< 0.001	0.437	0.001	0.528	< 0.001
HOMA - β	-0.188	0.165	-0.253	0.060	0.381	0.004

Fig. 5. Expression levels of the long non-coding RNAs (a) n342533, (b) n335556, and (c) n336109 in 60 patients with type 2 diabetes (T2D) compared to those found in 60 healthy controls (HC) by quantitative PCR (qPCR).

HOMA- β (all $P < 0.05$; Table 4). Following adjustments for age, gender, BMI, and HDL-C, we found that these top three lncRNAs were positively correlated with FPG, 2hPG, HbA1c, and HOMA-IR (all $P < 0.05$), and n336109 remained negatively correlated with HOMA- β (both $P < 0.05$; Table 4).

Discussion

The present study compared lncRNA and mRNA expression profiles in whole blood of six patients newly diagnosed with T2D and six healthy individuals. We identified differentially expressed lncRNAs ($n = 55$) and mRNAs ($n = 202$) in our T2D group compared to our control group, subsets of both were validated by qPCR. We also performed pathway and GO analyses and found that the dysregulated mRNAs associated with T2D were mainly involved in inflammation, immune response, insulin resistance, and the regulation of insulin secretion, whereas using CNC analysis, we found 10 pairs of lncRNAs and mRNAs were co-expressed.

Furthermore, the expression level of the top three upregulated lncRNAs were correlated with variables of glycometabolism and HOMA-IR in our cohort of Han Chinese patients with T2D. To our knowledge, this is the first study to investigate lncRNA expression profile in T2D of Han Chinese population.

T2D is characterized by impaired insulin secretion and IR. The pathogenesis of T2D has been studied for several decades, and although the main cause of the disease is not properly understood, it is widely accepted that interactions between genetic and environmental factors contribute to this widespread condition [23]. The development of IR is contributed by chronic low-grade systemic inflammation [24] that is found in adipose tissue, liver, endocrine pancreas, skeletal muscle, and immune cells [25]. A previous study described lncRNA expression profiles of human pancreatic islets in deceased donors [10]. Although these findings were important towards understanding the involvement of islets and β -cell dysfunction during the development of T2D, they could not clarify the pathogenesis of IR. Using whole blood samples to examine the expression profiles of lncRNAs and mRNAs in T2D and healthy control groups, the findings from our study contribute towards an improved understand of the pathogenesis of T2D and related conditions, such as immunometabolic disorder, systematic inflammation, and IR.

In the present study, pathway analysis indicated that dysregulated mRNAs were mainly involved in natural killer cell mediated cytotoxicity, antigen processing and presentation, the PPAR signaling pathway, and the adipocytokine signaling pathway in our cohort of Han Chinese patients with T2D. These pathways concerned biological processes of immune regulation, inflammation, or metabolism. Several studies reported a role of the immune system in glucose intolerance and T2D. Defects in invariant natural killer T (INKT) cell populations, which are a subset of natural killer T cells, have been reported in patients with T2D or obesity [26]. Furthermore, INKT cells present in adipose tissue may exert a regulatory role against obesity and associated metabolic disorders [27]. PPARs are fundamentally involved in regulating energy homeostasis. For example, PPAR γ is a master regulator of gene expression for metabolism, inflammation, and other pathways in many cell types [28]. Adipose tissue plays a key role in the development of T2D through the secretion of adipokines, such as leptin, adiponectin, and monocyte chemotactic protein-1 [29]. In this study, we found using GO analysis that differentially expressed mRNAs were also most frequently involved in inflammation and immune processes, which coincide with our findings from pathway analysis.

The major function of lncRNAs is to regulate the expression of protein-coding genes [30]. In this study, we identified the biological processes associated with those differentially expressed mRNAs identified in our cohort of T2D. While, all the dysregulated lncRNAs were never reported in T2D. To uncover the potential function of these lncRNAs, we further investigated the relationship between differentially expressed lncRNAs and mRNAs constructing a gene co-expression network to identify correlated targets of dysregulated lncRNAs in our Han Chinese patients with T2D. Target mRNAs included LY86, S100A12, S100A8, CD36, TLR8, VNN2, and KCNJ15. We identified 10 pairs of co-expressed lncRNA-mRNAs in our cohort. Previous studies reported that LY86 was involved in obesity, inflammation, and insulin resistance [31]. S100A8 and S100A12, belonging to the calgranulin gene family, play key immune response roles in inflammatory disorders, like diabetes and cardiovascular disease [32]. In addition, S100A8 can recruit macrophages to adipose tissue during inflammation and in the setting of hyperglycemia and obesity [33, 34]. It was reported that the elevated expression of TLR8 found in the adipose tissue of patients with obesity or T2D had consensus with inflammatory signatures and may represent an immune marker of metabolic inflammation [35]. Upregulation of CD36 is significantly associated with IR and hyperinsulinemia [36]. The vanin gene family member VNN2 encodes secreted and membrane-bound ectoenzymes that convert pantetheine into pantothenic acid and cysteamine [37]. Human VNN2 was characterized as a protein involved in the regulation of adherence and migration of neutrophils [38]. Whereas *KCNJ15* is a type 2 diabetes-associated risk gene, overexpression of which suppresses insulin secretion in rat insulinoma (INS1) cells

[39]. Although the roles of these target mRNAs in inflammation, IR, and insulin secretion are known, the functions of the co-expressed lncRNAs are not; however, we speculate they may have important roles in the pathophysiological processes of T2D by regulating expression of their co-expressed mRNA. Future studies are warranted to determine the precise regulatory mechanisms, which will provide a better understanding of the molecular processes involved in the pathogenesis of T2D.

Correlations between the top three upregulated lncRNAs and clinical features were identified in our cohort of patients. We found that these lncRNAs were correlated with metabolic indices, including FPG, 2hPG, HbA1c, and HOMA-IR. Fadista and colleagues [10] demonstrated an association between lncRNAs and HbA1c level, while those lncRNAs were specifically expressed in islet tissue. In the present study, we found that lncRNAs in peripheral whole blood were also associated with metabolic indices. Our findings suggest that these identified lncRNAs may play a role in the regulation of glycometabolism. One of the tested lncRNAs, n336109, was also negatively correlated with HOMA- β , a finding which indicates it may affect insulin secretion. Previous study reported that lncRNAs could affect apoptosis and insulin secretion in pancreatic β cells [40]. Based on our CNC analysis, n336109 was positively co-expressed with KCNJ15, and additionally, microarray data showed that both n336109 and KCNJ15 were upregulated in our T2D group. Okamoto et al. [39]. reported that KCNJ15 overexpression decreases the insulin response to glucose in rat INS1 cells. The authors then demonstrated that *KCNJ15* was a new susceptibility gene in Asian patients with T2D [41]. Although little is known about the function of n336109, its potential role in the pathogenesis of T2D is suggested not only by its expression change in our cohort of Han Chinese with T2D, but also by our correlation analysis using clinical data of our cohort.

Several limitations exist in this study. First, although the different expression patterns of lncRNAs suggest potential roles in T2D pathogenesis, the precise underlying regulatory mechanism(s) was not directly identified. Second, because these lncRNA expression profiles were not detected in other types of diabetes such as type 1 diabetes we cannot determine the specificity of these differentially expressed lncRNAs in T2D. Third, because IR is involved in adipose tissue, liver, and muscle, testing using samples of these tissues may be more suitable than peripheral blood. Nevertheless, to further elucidate the roles of lncRNAs in T2D, more laboratory and clinical research is needed.

Conclusion

This study characterizes the profiles of lncRNAs in a cohort of Han Chinese patients with T2D. Furthermore, although dysregulated lncRNAs may play potential roles in the pathogenesis of T2D through regulation of inflammation, IR, and insulin secretion, more research is needed to illuminate the functions of lncRNAs in the pathogenesis of T2D.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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