ISSN 0026-8933, Molecular Biology, 2024, Vol. 58, No. 2, pp. 246–254. © Pleiades Publishing, Ltd., 2024.

GENOMICS. TRANSCRIPTOMICS

UDC 577.29

The Expression of TP53TG1, LINC00342, MALAT1, H19, and MEG3 Long Noncoding RNAs in Type 2 *diabetes mellitus*

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Received May 12, 2023; revised October 17, 2023; accepted October 20, 2023

Abstract—Type 2 diabetes is a complex and multifactorial metabolic disorder. The frequency of type 2 diabetes has dramatically increased worldwide. Long noncoding RNAs play a regulatory role in pathological processes of type 2 diabetes. The aim of the study was to analyze TP53TG1, LINC00342, MALAT1, H19, and MEG3 lncRNAs in patients with type 2 diabetes and metabolic parameters, as well as the risk of diabetic retinopathy. Participants included 51 patients with diabetes and 70 healthy individuals. The expression of the *TP53TG1* and *LINC00342* genes was significantly decreased in the patients with diabetes compared to healthy individuals. *MALAT1* gene expression was higher in diabetes patients. *H19* gene expression was increased in the patients with diabetic retinopathy compared patients without retinopathy. TP53TG1, LINC00342, and MEG3 expression was decreased in patients with diabetic retinopathy and MALAT1 expression was increased. H19 is positively correlated with triglyceride levels; TP53TG1 and LINC00342 are positively correlated with HbA1c levels and fasting glucose levels. MALAT1 is negatively correlated with HDL levels and positively correlated with LDL levels. A decrease in the expression level of TP53TG1 and LINC00342 and an increase in the level of MALAT1 in diabetes, as well as an association with glycemic control, indicate the role of the studied noncoding RNAs in the development of type 2 *diabetes mellitus* and retinopathy and can be considered as candidates for early diagnosis of type 2 diabetes.

Keywords: type 2 *diabetes mellitus*, lncRNA, MALAT1, MEG3, H19, LINC00342

DOI: 10.1134/S0026893324020080

INTRODUCTION

Diabetes mellitus type 2 (T2DM) is a multifactorial disease caused by the combined interaction of genetic, epigenetic, and environmental factors [1].

Recent studies have revealed altered expression of long noncoding RNAs (lncRNAs), which include noncoding RNAs of more than 200 nucleotides, in many human pathological conditions [2, 3]. It is known that lncRNAs can influence transcription by interacting with chromatin remodeling proteins, as well as modulating the binding of transcription factors to target sites [4]. Some lncRNAs can influence alternative splicing of protein-coding genes or regulate protein stability in the nucleus [5]. LncRNAs are capable of regulating gene expression at the post-translational level: by interacting with complementary regions of microRNA and mRNA of target genes, they control the stability of mRNA or influence protein-protein interactions [6].

Recent studies confirm the existence of a connection between dysregulation of lncRNAs and the formation of tumor, cardiovascular, inflammatory, and metabolic diseases [7, 8]. However, the roles of lncRNAs in the development of human pathological conditions and the molecular mechanisms of their action remain poorly understood. Our work assessed the predictive significance of the differential expression of a number of lncRNAs in the formation of T2DM and diabetic retinopathy. For this purpose, the expression of the following lncRNA genes were determined: TP53TG1 (TP53 Target 1), LINC00342 (long intergenic nonprotein coding RNA 342), MALAT1 (metastasis associated lung adenocarcinoma transcript 1), H19 (H19 imprinted maternally expressed transcript), and MEG3 (maternally expressed 3).

LncRNA TP53TG1 influences alternative mRNA splicing, cell proliferation and apoptosis and is also part of the TP53 signaling pathway. Increased expression of the *TP53TG1* gene for oncological diseases leads to inhibition of cell proliferation and increases apoptosis. It has been established that gene expression of *TP53TG1* depends on glucose level; with reduced glucose levels, the expression of this gene increases significantly. According to the KEGG database, lncRNA of TP53TG1 gene takes part in inhibition of

insulin-like growth factor (IGF, Insulin-like growth factor), which is key for glucose metabolism [9]. The gene product of *TP53TG1* participates in methylation of m6A, which is important for the formation of metabolic disorders [10].

The gene product of *LINC00342* participates in glycolysis processes, while both overexpression and absence of LINC00342 lead to reprogramming of glucose metabolism in macrophages and the development of insulin resistance (IR) [11, 12]. LncRNA LINC00342 regulates the expression of the FTO demethylase gene, a key enzyme involved in the demethylation of m6A in RNA and modification of eukaryotic mRNAs. As is known, this process plays an important role in regulating the differentiation of preadipocytes into adipocytes, which promotes lipogenesis and adipose tissue deposition and determines the development of such diseases as obesity, diabetes, and metabolic syndrome [13, 14].

LncRNA MALAT1 is actively studied in T2DM. An increase in the level of lncRNA MALAT1 in the serum of patients with T2DM [3] contributes to the progression of T2DM complications such as neuropathy, retinopathy, nephropathy, and cardiovascular diseases [15, 16]. LncRNA MALAT1 participates in the regulation of the PI3K/Akt, MAPK/ERK and Wnt/β signaling pathways [15]. Gene knockdown of *MALAT1* attenuates endothelial cell apoptosis induced by high glucose [17]. The *MALAT1* gene plays an important role in the regulation of insulin sensitivity, and inhibition of the expression of the lncRNA MALAT1 gene is considered as a potential therapeutic target for T2DM. However, the data obtained in different studies are contradictory. For example, both *MALAT1* increases and decreases in gene expression have been shown in patients with T2DM with complications [3, 18].

The imprinted *H19* gene is expressed only from the maternal chromosome; it plays an important role in embryogenesis and is associated with the development of genetic disorders. Study of the expression of lncRNA H19 found that the level of *H19* gene expression is closely associated with the development of T2DM and its complications [19]. A decrease in the level of *H19* gene expression has been established in muscles with T2DM, as well as its effect on lncRNA H19 target genes, such as the insulin receptor gene and lipoprotein lipase. Reduced *H19* gene expression levels leads to the development of IR [20].

MEG3 is an imprinted gene on the maternal side. It was revealed that the *MEG3* gene is associated with the development of liver IR; in patients with T2DM, the expression level of this gene is increased [21]. Zhu X. et al. [22] showed that gene knockdown of *MEG3* enhances the expression of the microRNA 214 gene and inhibits the expression of its target gene *ATF4* (activating transcription factor 4), which in turn suppresses gene expression of *FOXO1* and a number of other genes encoding enzymes of gluconeogenesis. The level of IR decreases when gluconeogenesis is suppressed [22].

Thus, recent studies show that lncRNAs involved in the regulation of glucose homeostasis contribute to the progression of T2DM [23]. However, to date, no biomarkers have been found associated with early detection or progression of T2DM. In this regard, there is an urgent need to study biomarkers for diagnosis and detection of the disease at an early stage. The purpose of this study was to study the expression of the lncRNA genes TP53TG1, LINC00342, MALAT1, H19, MEG3 in patients with T2DM and the association of the levels of these lncRNAs with quantitative metabolic parameters characterizing T2DM, as well as with the risk of developing diabetic retinopathy.

EXPERIMENTAL

We examined 121 people, 51 patients with T2DM and 70 apparently healthy individuals (control group). The average duration of T2DM was 10.0 ± 7.11 years. The description of the groups is presented in Table 1. The following were used as criteria for inclusion in the T2DM group: verified diagnosis of T2DM as established according to WHO criteria (1999–2013) and diagnostic algorithms adopted in the Russian Federation from 2022 [24], and lack of relationship between patients. Criteria for inclusion in the control group were absence of clinical and laboratory signs of carbohydrate metabolism disorders and absence of a history of chronic endocrinological diseases. Exclusion criteria from the control group were an age of more than 35 years, hyperglycemia, and severe chronic diseases. The patients were hospitalized in City Clinical Hospital No. 21 in Ufa.

Whole blood samples were used in the analysis. Mononuclear cells (monocytes and lymphocytes) were isolated from whole blood using a Ficoll density gradient. Total RNA was isolated from mononuclear cells (lymphocytes and monocytes) of peripheral blood using the TRIzol reagent and the protocol from Invitrogen, United States or an analogue of ExtractRNA (Evrogen, Russia). The following lncRNA genes were selected for expression analysis: TP53TG1 (ID:11257), LINC00342 (ID:150759), H19 (ID:283120), MALAT1 (ID:378938), and MEG3 (ID:55384). The quality and quantity of the RNA template (in ng/μl) were assessed spectrophotometrically (NanoDrop 1000, ThermoScientific, United States) by absorbance at a wavelength of 260 nm. RNA quality was determined by the A_{260}/A_{280} ratio. RNA integrity was assessed using agarose gel electrophoresis. cDNA synthesis was carried out using the MMLV RT kit (Evrogen) and hexamer random primers. Expression of lncRNA genes was analyzed using a StepOnePlus device (Applied Biosystems, United States). PCR was carried out in 96-well plates. Reaction mixtures with a volume of 25 μL contained spe-

Table 1. The characteristics of the groups included in the study

Parameter	CONTROL $n = 70$	T2DM $n = 51$	p
Age, years, average ± Std.Dv	51.60 ± 10.31	59.88 ± 10.11	0.036
Men, *n*, %	26 (37.15)	17 (33.34)	0.213
Women, *n*, %	44 (62.85)	34 (66.66)	
Body mass index (BMI, kg/m^2), mean ± SD	25.44 ± 2.97	31.92±5.82	<0.0001
Treatment: Metformin Other drugs	–	38 (74.51) 13 (24.49)	–
Obesity, *n*, %	–	37 (72.54)	–
Duration of T2DM, median [Q1; Q3]	–	10.00 [3, 15]	–
Arterial hypertension, *n,* %	–	41 (80.40)	–
Cardiovascular diseases, *n*, %	–	4 (7.84)	–
Diabetic nephropathy, *n*, %	–	48 (94.11)	–
diabetic neuropathy, *n*, %	–	47 (92.15)	–
diabetic retinopathy, *n*, %	–	22 (43.13)	–
HbA_{1C} , %, median [Q1;Q3]	4.89 [3.8; 5.90]	9.30 [7.30; 14.10]	<0.0001
Fasting glucose (mmol/L), median [Q1; Q3]	4.82 [3.20; 5.90]	9.60 [6.95; 12.55]	<0.0001
Total cholesterol (mmol/L), median [Q1; Q3]	4.79 [3.30; 6.50]	4.94 [4.34; 5.57]	0.0007
LDL (mmol/L), median [Q1; Q3]	2.71 [0.78; 3.99]	2.98 [1.81; 3.85]	<0.006
HDL (mmol/L), median [Q1; Q3]	1.11 [0.87; 1.43]	1.13 [0.71; 1.17]	0.080
Triglycerides (mmol/L), median [Q1; Q3]	1.33 [1.10; 2.07]	2.34 [1.75; 3.49]	0.029

cific primers (shown in Table 2), fluorescent probe from DNA-Sintez (Russia) and reagents for PCR qPCRmix-HS HighROX (Evrogen). Primer design and synthesis carried out at the LLC DNA-Sintez (Russia). The housekeeping gene (B2M) was used as an endogenous control. Samples from the control group were used as a reference sample; PCR for each sample was repeated three times. A negative control containing no cDNA was included in each reaction. To remove genomic DNA, RNA was treated with DNase I (Thermo Fisher Scientific) according to the manufacturer's instructions.

Methods of statistical data analysis. Statistical processing of the data was carried out using SPSS Statistics 22 (United States) programs. GraphPad Prism version 8.0.1 (GraphPad Software Inc, United States). The relative *C*t expression level was assessed using the ΔΔ method [25]. We calculated $E^{-\Delta\Delta Ct}$, ($E = 2.0$). The results were normalized by the level of expression of a housekeeping gene and corresponding genes according to the following scheme $\Delta Ct = Ct$ (target gene) – *C*t (housekeeping gene). The ordinate axis shows the level of relative expression ($E^{-\Delta Ct}$). Differences in the level of relative expression between the group of patients and controls were calculated using the parametric *t*-Student's t-test (T-test), final gene expression results were converted to logarithmic values to use parametric statistical tests. The relationship between lncRNA expression and clinical parameters was assessed using the Pearson correlation test; $p \leq 0.05$ was considered statistically significant. To take multiple comparisons into account, the p_{FDR} correction was used (https://www.sdmproject.com/utilities/?show=FDR).

RESULTS

We did not detect significant differences in the expression levels of lncRNA TP53TG1, LINC00342, MALAT1, H19, and MEG3 genes in groups of women and men ($R = 0.81$, $p = 0.18$, $p = 0.75$, $R = 0.25$, $p = 0.27$). A comparison of the overall group of patients with the control group showed that the level of lncRNA TP53TG1 in patients was reduced by 1.7 times ($R = 0.016$, $p_{FDR} = 0.033$) and the lncRNA level of LINC00342, by 2 times ($R = 0.021$, $p_{FDR} = 0.033$). LncRNA H19 levels were similar in both groups, in patients and healthy individuals ($R = 0.20$), and the level of lncRNA MALAT1 in the patient group was increased by 3.5 times ($p = 0.0001$, $p_{FDR} = 0.0005$). The level of lncRNA MEG3 in the patient group was increased, but the differences did not reach the level of statistical significance ($R = 0.05$, $p_{FDR} = 0.062$) (Fig. 1).

The relationship between lncRNA levels and diabetic retinopathy was also assessed, since other com-

Table 2. The nucleotide sequences of the primers

dRNA	Primer
TP53TG1	F: 5'-GGCTCTTTCCTTTAATCTTCGG-3' R: 5'-GAATTGTTACCAGGGTTACTCAGAC-3' FAM-TGCCCAACTCAGGTTTAACCACCA-BHQ1
H19	F: 5'-GAATCGGCTCTGGAAGGTGA-3' R: 5'-GCTGCTGTTCCGATGGTG-3' FAM-CCAGACCTCATCAGCCCAACATC-BHQ1
LINC00342	F: 5'-TTTCATCTGAAGCAGCAGAGTG-3' R: 5'-CAGTTGTGGTGATCTTTGTTCCTG-3' FAM-CAGAGTCAGGTCACCAACCAGTGTGGA-BHQ1
MALAT1	F: 5'-GAACAAGAAGTGCTTTAAGAGGC-3' R: 5'-GCGAGGCGTATTTATAGACGG-3' FAM-AGGTGATCGAATTCCGGTGATGC-BHQ1
MEG3	F: 5'-GCCCATCTACACCTCACGAG-3' R: 5'-CCTCTTCATCCTTTGCCATCC-3' FAM-CCCACCAACATACAAAGCAGCCACT-BHQ1

plications such as arterial hypertension, diabetic nephropathy and neuropathy were present in almost all patients (groups of patients with and without complications, as well as patients with complications and the control group were compared). When comparing patients with and without retinopathy, statistically significant differences were detected only for lncRNA H19, whose level was 2.9 times higher in patients with retinopathy ($R = 0.04$, $p_{FDR} = 0.05$). In patients with diabetic retinopathy, lncRNA TP53TG1 levels was 1.4 times lower than in the control group ($R = 0.04$, $p_{FDR} = 0.05$), the level of lncRNA LINC00342 was reduced by 2 times ($R = 0.04$, $p_{FDR} = 0.05$), and the level of lncRNA MALAT1 increased by 5 times ($R = 0.0001$, $p_{FDR} = 0.0005$). In the group of patients without diabetic retinopathy, the level of gene expression of *MEG3* was 2 times higher than in the group of patients with diabetic retinopathy ($R = 0.05$, $p_{FDR} = 0.05$). However, these differences were at the level of a trend.

We further compared the expression levels of lncRNA genes in patients taking metformin or receiving other drugs and found no statistically significant differences (Fig. 1). For the lncRNA genes TP53TG1, LINC00342, MALAT1, H19, and MEG3 the significance levels (R) were 0.36, 0.98, 0.22, 0.23, and 0.75, respectively.

Analysis of the association of lncRNAs with each other and with quantitative metabolic parameters revealed a positive association between the levels of H19 and triglycerides ($r = 0.865$, $R = 0.0001$), the level of LINC00342 and fasting glucose level ($r = 0.439$, $R = 0.002$). The levels of lncRNAs of TP53TG1 and LINC00342 were positively associated with HbA1c levels ($r = 0.334$, $R = 0.02$ and $r = 0.332$, $R = 0.021$, respectively). The level of lncRNA MALAT1 is negatively associated with HDL levels ($r = -0.672$, $R = 0.012$) and positively with the LDL level ($r = 0.336$, $R = 0.017$), while the level of lncRNA LINC00342 is positively associated with the level of lncRNA TP53TG1 ($r = 0.497$, $R = 0.0001$) (Table 3).

DISCUSSION

We found a decrease in the gene expression levels of lncRNAs *TP53TG1* and *LINC00342* in T2DM patients, while the level of gene expression of *MALAT1* and *MEG3* in patients was higher than in the control group. Reduced *TP53TG1* and *LINC00342* gene expression levels were also found among patients with diabetic retinopathy compared to the control and increased gene expression of *MALAT1*; we also found a tendency to an increase in gene expression of *MEG3* in patients without retinopathy compared with patients with retinopathy.

Studying the level of gene expression of *LINC00342* and *TP53TG1* in patients with T2DM was carried out for the first time, while increasing gene expression levels of *MALAT1* and *MEG3* in the blood of patients with T2DM were confirmed by other authors [3, 26]. However, the described discrepancy between the results of analysis of the expression of lncRNAs, for example H19 and MALAT1, in patients with T2DM [27], apparently, can be explained by differences in the studied tissues, the presence or absence of complications in the subjects, as well as the treatment performed, the "experience" of T2DM, and the level of glycaemia. Inverse relationships between expression levels of lncRNAs derived from blood cells and other tissues have also been described [28]. It has been noted that lncRNA isolated from peripheral blood reflects the metabolic status of the subjects, thus

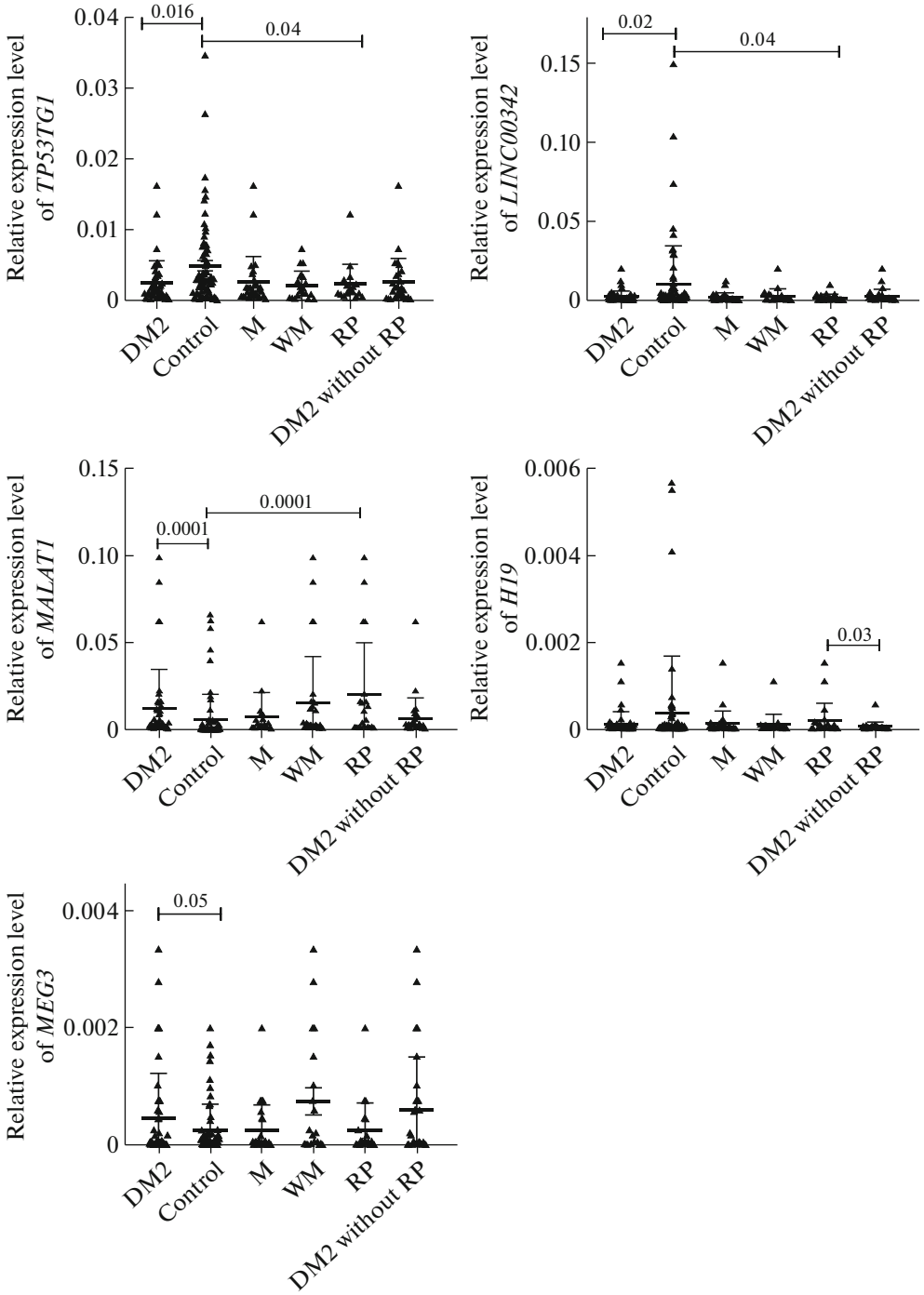

Fig. 1. Comparison of gene expression levels of *TP53TG1*, *LINC00342*, *H19*, *MALAT1* and *MEG3* in patients with T2DM, in the control group, in patients with diabetic retinopathy and in controls, and also depending on the treatment. Relative expression values ($2^{-\Delta Ct}$) are presented as mean ± SEM values. M, patients taking metformin; BM, patients taking other drugs; RP, patients with diabetic retinopathy.

blood is a valid source for early diagnosis of T2DM and its complications [29].

Genes in a co-expression network with the *LINC00342* gene relate to pathways associated with apoptosis and the inflammatory response. Previously, using the method of functional enrichment analysis, the influence of lncRNAs LINC00342 on gene expression of *PTEN* and *TP53* was shown [30]. Turning off the *LINC00342* gene led to increased gene expression of *PTEN* and *TP53* in nonsmall cell lung cancer cells [31], while increasing the level of gene expression of *LINC00342* in the blood is associated

Table 3. The correlation coefficients of lncRNA levels and metabolic parameters

Parameter	TP53TG1	LINC00342	MALAT1	H19	MEG3
Total cholesterol, mmol/L	0.096	0.193	0.219	–0.049	0.151
Triglycerides, μmol/L	–0.003	0.274	0.143	0.865**	0.348
HDL, mmol/L	–0.219	–0.332	–0.672*	0.125	–0.153
LDL, mmol/L	0.170	–0.002	0.336*	–0.314	–0.104
Fasting glucose, mmol/L	0.232	0.439**	0.088	–0.040	0.045
HbA1c, %	0.335*	0.332*	0.092	–0.080	0.048
TP53TG1	1	0.497**	0.085	0.078	0.042
LINC00342	0.497**	1	0.063	0.158	0.001
MALAT1	0.085	0.063	1	–0.045	0.141
H19	0.078	0.158	–0.045	1	0.045
MEG3	0.042	0.001	0.141	0.045	1

*Significance level less than –0.05.
**Significance level less than –0.01.

with the estimated glomerular filtration rate in chronic renal failure [30]. It has been shown that increased gene expression of *LINC00342* in malignant neoplasms leads to inhibition of apoptosis in tumor cells [31]. It was found that the level of gene expression of *LINC00342* is higher in patients with diabetic nephropathy [13]. Reduced gene expression of *LINC00342* is considered one of the key risk markers of strictly myocardial infarction [32]. LncRNA LINC00342 regulates *FTO* and *METTL3* gene expression, which are key participants in methylation processes. Abnormal methylation changes m6A, demethylases and methyltransferases affect the functioning of β-cells, causing the development of hyperglycemia and the progression of T2DM [33]. We have established a positive correlation between levels of lncRNA LINC00342 with glucose and HbAc1, suggesting the existence of a relationship between the level of gene expression of *LINC00342* and the severity of the disease. The discrepancy between expression levels may reflect differences in the degree of expression of lncRNAs in different tissues and may also indicate the influence of external factors, such as treatment or dietary habits.

The gene product of *TP53TG1* participates in the regulation of glucose metabolism by interacting with the gene products of *FOXK1* and *GCK*. Reduced gene expression levels of *TP53TG1* are noted in gastric cancer [34]. It has been shown that gene expression of *TP53TG1* is regulated by glucose: it increases when glucose levels are low and decreases when glucose levels are high [35]. Moreover, it is assumed that glucose levels determine gene expression of *TP53TG1* and the properties of this gene are both protective and carcinogenic [35]. The various effects of aberrant expression of *TP53TG1* on the pathogenesis of T2DM may be due to the actions of various mechanisms, including the influence on signaling pathways such as the WNT/β-catenin and PI3K/AKT pathways.

Participation of the lncRNA H19 in the regulation of metabolic diseases was shown in 2014 [36]. It was determined that inhibition of gene expression of *H19* small interfering RNA in mouse hepatocytes led to an increase in blood glucose levels. In HepG2 cells, *H19* gene inhibition disrupted insulin signaling mediated by increased nuclear localization of the transcriptional regulator FOXO1 [37]. It has been established that a decrease in the level of lncRNA H19 allows the P53 factor (the gene product of *TP53*) to bind to the *FOXO1* gene promoter, which leads to increased glycogen production in the liver. Increasing the level of lncRNA H19 in patients leads to increased glycogenesis [38]. A positive correlation between H19 and triglyceride levels was revealed, which coincides with the data of J. Liu et al. [39]. It has been shown that increased *H19* gene expression can be considered as a characteristic molecular change in fatty liver disease, and lncRNA H19 promotes hepatocyte steatosis and triglyceride secretion [39].

A significant increase was found in gene expression level of *MALAT1* in patients with T2DM, both with and without retinopathy. A higher level of lncRNA MALAT1 in the retina of patients with diabetic retinopathy has been established previously. It turned out that *MALAT1* is activated in the retina under high glucose conditions, while a *MALAT1* gene knockdown reduces the manifestations of retinopathy in rats [40]. A decrease in the level of lncRNA MALAT1 was revealed in the blood of patients with T2DM and metabolic syndrome, as well as its increase [3]. *MALAT1* overexpression serves as an important marker of dysfunction and proliferation of endothelial cells and microvascular complications of T2DM [3]. It is believed that increased levels of MALAT1 promotes increased cytokine levels and inflammation, making

MALAT1 one of the key molecules regulating inflammation in T2DM [41]. The existence of an inverse correlation between lncRNA MALAT1 levels and low-density lipoproteins are supported by data indicating that *MALAT1* gene silencing enhances the induction of low-density lipoproteins [42].

A tendency towards an increase in lncRNA MEG3 levels was found among patients with T2DM compared with controls and in patients without retinopathy compared with patients with this complication, but the differences did not reach the level of statistical significance. It was previously reported that MEG3 levels are increased in serum, mononuclear cells, and kidney tissue of patients with T2DM [43]. It was also found that increased *MEG3* gene expression in patients promotes the development of IR, while a decrease in the level of lncRNA MEG3 causes diabetic microvascular dysfunction in the retina and leads to diabetic retinopathy [44].

As shown previously, metformin (one of the main drugs used in the treatment of T2DM) can influence the differential expression of lncRNA genes, as shown for the lncRNAs H19 and MALAT1 [38, 45]. In our study, there was only a trend towards a decrease in the levels of lncRNAs MALAT1 and H19 in patients taking metformin. This trend is consistent with the data in [31], and the lack of statistically significant differences may be a result of the use of different doses of the drug.

LncRNAs LINC00342, TP53TG1, MALAT1, H19, and MEG3 are multifunctional, affecting the pathogenesis of T2DM through different mechanisms. Inconsistencies between the expression levels of lncRNAs in patients with T2DM in the research of different authors may be due to the choice of tissue used for analysis. It can be assumed that the features of lncRNA gene expression are determined by the cellular environment. The presence of contradictions in the results of analysis of lncRNA gene expression levels suggests the need for further in-depth studies.

The results of determining differential gene expression of *TP53TG1*, *LINC00342,* and *MALAT1*, as well as the correlation between lncRNA levels and glycemic control indicators indicate their participation in the formation of T2DM and diabetic retinopathy and can be considered as early diagnostic markers of T2DM and its complications.

FUNDING

This work was supported by the Russian Science Foundation (grant no. 22-25-00010).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in the study were in accordance with the ethical standards of the National Research Ethics Committee of the 1964 Declaration of Helsinki and its subsequent amendments. The study was approved at a meeting of the expert council on biomedical ethics of the Institute of Biochemistry and Genetics of the Ufa Federal Research Center of the Russian Academy of Sciences, protocol no. 8 dated March 14, 2012. All subjects signed informed consent after receiving an explanation of the potential risks and benefits, as well as the nature of the study.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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