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Screening of potential biomarkers in the occurrence and development of type 1 diabetes mellitus based on transcriptome analysis

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Abstract

Introduction: The aim of the study was to reveal the mechanisms for the pathogenesis and progression of type 1 diabetes mellitus (T1DM).
Material and methods: Two mRNA expression profiles and two miRNA expression profiles were downloaded from the Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs), differentially expressed miRNAs (DEMs), functional enrichment analyses, pathways, putative targets for DEMs and the miRNA-gene pairs, protein-protein pairs of DEGs, and PPI network were constructed.
Results: Based on mRNA expression profiles, 37 and 110 DEGs were identified, and named as DEGs-short and DEGs-long, respectively. Based on miRNA expression profiles, 15 and six DEMs were identified, and named as DEMs-short and DEMs-long, respectively. DEGs-short were enriched in six GO terms and four pathways, and DEGs-long enriched in 40 GO terms and 10 pathways. Seventeen miRNA-gene pairs for DEMs-short were screened out; *hisa-miR-181a* and *hisa-miR-181c* were involved in the most pairs. Twenty pairs for DEMs-long were obtained; *hsa-miR-338-3p* was involved in all the pairs. *KLRD1* was involved in more pairs in the network of DEGs-short. *ACTA2* and *USP9Y* were involved in more pairs in the network of DEGs-long.
Conclusions: *KLRD1, hisa-miR-181a*, and *hisa-miR-181c* might be pathogenic biomarkers for T1DM, *ACTA2, USP9Y*, and *hsa-miR-338-3p* progressive biomarkers of T1DM. **(Endokrynol Pol 2020; 71 (1): 58–65)**

Key words: *type 1 diabetes mellitus (T1DM); pathogenesis; progression; transcriptome analysis*

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. It is established that 415 million people had DM worldwide in 2015, and the number is predicted to reach more than 642 million by 2040 [1]. Moreover, from 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from DM [2]. DM is divided into three main types: Type 1 DM (T1DM), type 2 DM (T2DM), and gestational diabetes. Type 1 DM results from the pancreas's failure to produce enough insulin and makes up an estimated 5–10% of all diabetes cases [3]. The classical symptoms are frequent urination, increased thirst, increased hunger, and weight loss. At present, the cause of T1DM is still unknown, and genetic susceptibility, a diabetogenic trigger, and high exposure to an antigen are believed to be involved [4]. A meta-analysis involving 2238 T1DM participants showed that individuals had a higher risk for T1DM with the G allele of CTLA-4 +49A/G gene polymorphism [5]. Arroyo-Jousse et al. [6] found that T1DM patients showed a higher TNFα gene promoter methylation compared with control subjects [$p=0.00008$]. A study of genome-wide gene expression analysis revealed that CD274 up-regulation in T1DM is correlated with the pathogenesis [7]. MicroRNA (miRNAs) are involved in various biological processes and become novel biomarkers in DM. A miRNA expression profile analysis showed that eight circulating miRNAs were dysregulated in T1DM patients (miR-21-5p, miR-146a-5p, miR-148a-3p, miR-181a-5p, miR-210-5p, miR-342-3p, miR-375, and miR-1275), which might be potential circulating biomarkers of this disease [8]. Moreover, a single-nucleotide polymorphism (rs2910164) in the miRNA-146a gene is significantly associated with diabetic nephropathy in T1DM patients [9].

Transcriptomics technologies are the techniques used to study an organism's transcriptome, the sum of all of its RNA transcripts. Among them, mRNA conveys genetic information from DNA to the ribosome, and miRNA functions in RNA silencing and post-transcriptional regulation of gene expression [10]. Transcriptomic analysis can study gene expression changes in different

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organisms, which contribute to the understanding of human disease [11]. In this study, the transcriptomic analyses were performed on new-onset and long-term T1DM patients in order to reveal the mechanisms for the pathogenesis and progression of this disease.

Material and methods

Expression profiles

The expression profiles of GSE55098 [12], GSE72492, GSE55099, and GSE97123 [13] were downloaded from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/). The mRNA expression profile of GSE55098 contained 22 peripheral blood mononuclear cell (PBMC) samples from 12 newly diagnosed T1DM patients and 10 normal controls, and it was detected using the platform of [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. The mRNA expression profile of GSE72492 included 17 pancreas tissue samples from T1DM patients and seven healthy humans, and these patients had been suffering from T1DM for at least five years. Agilent-028004 SurePrint G3 Human GE 8x60K Microarray was used to conduct the detection for GSE72492. Twelve PBMC samples from newly diagnosed T1DM patients and 10 PBMC samples from normal controls were contained in the miRNA expression profile of GSE55099, and they were sequenced with the platform of miRNA-1 Affymetrix Multispecies miRNA-1 Array. Twenty-four plasma-derived exosomes from 12 T1DM patients and 12 healthy patients were concluded in the miRNA expression profile of GSE97123, and these patients had suffered from T1DM for at least 25 years. The detection platform for GSE97123 was Counter Human miRNA Expression Assay.

Data processing

For GSE55098 and GSE55099, background correction, standardisation, and expression value calculation for the raw data were conducted with the affy V1.48.0 package (http://www.bioconductor.org/packages/3.2/bioc/html/affy.html). The hgu133plus2.db package V3.2.2 (http://www.bioconductor.org/packages/3.2/data/annotation/html/hgu133plus2.db.html) was used to annotate, and the non-annotated probes were removed. For GSE72492, standardisation and logarithm calculation of expression values were performed with preprocessCore V1.32.0 (http://www.bioconductor.org/packages/3.2/bioc/html/preprocessCore.html). For GSE97123, the downloaded raw data had been normalised, and logarithm calculation was directly conducted.

Differentially expressed analysis

Based on the mRNA and miRNA profiles of GSE55098 and GSE55099, the differentially expressed genes (DEGs) and the differentially expressed miRNAs (DEMs) were separately identified in samples from newly diagnosed T1DM patients compared with those from normal controls with limma V3.32.2 (http://www.bioconductor.org/packages/3.5/bioc/html/limma.html), which were named as DEGs-short and DEMs-short, respectively. Furthermore, the DEGs and the DEMs were separately identified in samples from longstanding T1DM patients compared with those from healthy people in GSE72492 and GSE97123, and named as DEGs-long and DEMs-long, respectively. The threshold criteria was $|\log(\text{fold change})| > 1$ and $p < 0.05$.

Functional and pathway enrichment analyses of DEGs

The functional enrichment analyses of the DEGs-short and DEGs-long were performed via the Database for Annotation, Visualisation, and Integrated Discovery (DAVID) V6.8 (http://david.abcc.ncifcrf.gov/). The enriched pathway terms were screened out with the Kyoto Encyclopaedia of Genes and Genomes (KEGG) PATHWAY (http://www.genome.jp/kegg), and Reactome (http://www.reactome.org). The threshold was $P < 0.05$.

Targets prediction for DEMs

Potential targets for DEMs-short and DEMs-long were predicted by > 5 bioinformatics algorithms among the 10 algorithms in the miRWalk database: miRWalk V2.0 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/mirwalk), RNAhybrid V2.1 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/rnahybrid), DIANAmT V4.0 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/diana-microt), miRanda -rel2010 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/miranada), miRDB V4.0 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/mirdb), PICTAR4 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/pictar4), PICTAR5 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/pictar5), PITA (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/pipa), RNA22 V2 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/rna22), and Targetscan V6.2 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/targetscan). Moreover, the negative regulated miRNA-gene pairs were selected out.

The PPI network construction

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is a biological database and web resource of known and predicted protein-protein interactions (PPI). The protein-protein pairs of DEMs-short and DEMs-long were identified via STRING v10.5 (https://string-db.org/) with more than 500 scores. Afterwards, the PPI networks for DEGs-short and DEGs-long were constructed and visualised by Cytoscape V3.5.1 software (http://www.cytoscape.org/download.php).

Results

DEGs and DEMs

After differentially expressed analysis, 37 (25 up- and 12 down-regulated) and 110 (58 up- and 52 down-regulated) DEGs were identified in sets of DEGs-short and DEGs-long, respectively; and 15 (two up- and 13 down-regulated) and six (one up- and five down-regulated) DEMs were identified in sets of DEMs-short and DEMs-long, respectively. Furthermore, the top 30 most significant DEGs of DEGs-short and DEGs-long are separately shown in Table 1 and Table 2, and all the DEMs of DEMs-short and DEMs-long are shown separately in Table 3 and Table 4. Also, the overlaps of DEGs-short and DEGs-long were EIF1AY, LTF, and DDX3Y, and there was no overlap between DEMs-short and DEMs-long.

The enriched gene ontology (GO) terms and pathways

DEGs-short and DEGs-long were separately enriched in six and 40 GO terms, and all the gene ontology (GO) terms of DEGs-short and the top 10 most significant terms of DEGs-long are shown in Table 5 and Table 6, respectively. Moreover, DEGs-short were enriched in four pathway terms, namely "graft-versus-host disease", "antigen processing and presentation", "natural killer cell mediated cytotoxicity", and "signalling in immune system". DEGs-long were enriched in 10 pathways; namely,

Table 1. *The top 30 most significant differentially expressed genes (DEGs) in peripheral blood mononuclear cell samples from newly diagnosed patients with type 1 diabetes mellitus (T1DM) compared with those from normal controls (DEGs-short)*

Gene	LogFC	AveExpr	p value	Gene	LogFC	AveExpr	p value
CD160	–1.485740817	9.375468318	5.04E –06	*CMTM2*	1.43081945	8.187681273	0.006335067
KLRD1	–1.127278189	8.941906288	1.10E –05	*KCNJ2*	1.077869083	6.547479591	0.006475015
CCL4	–1.266147883	10.58044818	1.64E –05	*CHI3L1*	1.1924713	5.115614424	0.007671493
GNLY	–1.144524117	12.24886857	3.63E –05	*CYP4F3*	1.833172833	5.909274955	0.008561103
GZMH	–1.17887135	11.65018586	4.33E –05	*S100P*	1.634646917	8.568421091	0.012775649
CLIC3	–1.179784483	9.144989091	6.78E –05	*KRT23*	1.515719783	5.298236045	0.014109852
KLRF1	–1.000714933	10.69416009	0.000117664	*CRISP3*	1.10133205	4.734271773	0.02081107
FGFBP2	–1.16267335	11.11717164	0.000195317	*SLPI*	1.387085033	6.380050636	0.022275293
KLRC3	–1.267866217	8.382717909	0.000256813	*TNFAIP6*	1.364051308	5.903216886	0.022326902
HLA-DQA1	–1.78124685	5.714109242	0.001618603	*CEACAM8*	1.822226467	7.058930909	0.026668873
DUSP2	–1.289584217	8.945554227	0.001857457	*LTF*	1.407460333	9.775831136	0.029817392
CCR3	1.214328983	6.790913364	0.002497141	*RETN*	1.050559983	8.875287318	0.030854117
IFNG	–1.060417167	7.499563636	0.004650993	*MMP9*	1.185383167	7.671720636	0.030963045
FFAR2	1.626123483	5.858363545	0.004965728	*PGLYRP1*	1.002344033	6.662580909	0.03233145
MS4A3	1.222284567	8.345452523	0.006033224	*RNASE3*	1.3085789	7.113363909	0.034102172

Table 2. *The top 30 most significant differentially expressed genes (DEGs) in pancreas tissue samples from long-standing patients with type 1 diabetes mellitus (T1DM) compared with those from healthy people (DEGs-long)*

Gene	LogFC	AveExpr	p value	Gene	LogFC	AveExpr	p value
INS	–7.956409294	11.80539147	1.61E –07	*SYT7*	–1.030068536	7.626796818	0.000343623
IAPP	–4.973887634	8.748584024	6.19E –07	*ANGPT2*	1.148873747	7.086680079	0.000482281
SSC4D	–1.075597447	10.20799228	1.77E –06	*EIF1AY*	1.08379585	7.046791771	0.000545989
CHST8	–1.175932564	7.582315976	2.88E –06	*HSPB2*	1.104037749	8.503654391	0.000556139
INS-IGF2	–3.232225473	6.533042141	3.07E –06	*SLC25A34*	–1.728247111	10.11133012	0.000591585
PHYHIPL	1.18534172	7.252759135	1.51E –05	*CCDC3*	1.05894566	9.655930312	0.000628911
GCGR	–1.70586376	6.827750629	2.11E –05	*PTGIS*	1.424889976	8.260255438	0.000790294
SLC35D3	–1.679842289	6.656338606	2.39E –05	*DDX3Y*	1.992255074	6.248943021	0.000831372
G6PC2	–2.116813487	6.112831353	3.10E –05	*SLC30A8*	–1.494275889	9.141968897	0.000835193
SYT13	–1.662898167	8.209265965	3.21E –05	*RPS4Y1*	5.34177196	8.2755228	0.000839019
EPN3	–1.014739304	8.715785241	6.32E –05	*UTY*	1.428451427	5.672497188	0.000859705
VEGFC	1.186622956	8.308787518	0.00012934	*CCL21*	2.10357751	8.799136812	0.000869965
CELF4	–1.049638234	8.260741659	0.000138073	*FAM159B*	–1.685486241	7.293984476	0.000950918
ADCYAP1	–1.229689263	5.711479118	0.000153888	*UCHL1*	–1.205710231	9.847092909	0.000976663
PCSK1	–1.979802762	8.473264497	0.000227194	*RPS4Y2*	5.264040346	8.251606735	0.001024289

"smooth muscle contraction", "vascular smooth muscle contraction", "regulation of insulin-like growth factor (IGF) transport and uptake by insulin-like growth factor binding proteins (IGFBPs)", "cGMP-PKG signalling pathway", "RHO GTPases activate PAKs", "platelet degranulation", "insulin processing", "chemical carcinogenesis", "antagonism of activin by follistatin", and "insulin secretion".

The miRNA-gene pairs

A total of 17 miRNA-gene pairs were screened out for DEMs-short, and they are shown in Table 7, including nine negatively regulated pairs. Moreover, hisa-miR-181a and hisa-miR-181c were involved in the most pairs. Twenty miRNA-gene pairs in total were screened out for DEMs-long (Tab. 8), including 11

Table 3. *All the differentially expressed miRNAs (DEMs) in peripheral blood mononuclear cell samples from newly diagnosed patients with type 1 diabetes mellitus (T1DM) compared with those from normal controls (DEMs-short)*

Gene	LogFC	AveExpr	p value
hsa-miR-28-3p	–1.928257799	2.792230233	2.80E-06
hsa-miR-146b-5p	–2.666591212	4.4375168	1.12E-05
hsa-miR-181a-2	–1.887221452	2.705472828	6.15E-05
hsa-miR-28-5p	–1.383941091	3.923182481	6.17E-05
hsa-miR-1225-3p	1.023616193	1.73814695	9.27E-05
hsa-miR-181c	–1.26274394	2.593748088	0.000439044
hsa-miR-1249	1.091444886	2.36209401	0.000543799
hsa-miR-199a-5p	–1.287916775	1.850112207	0.000747813
hsa-miR-125b	–1.075210791	1.90891557	0.000922042
hsa-miR-19b	–1.14946891	5.355734538	0.001454218
hsa-let-7f	–1.664920459	5.356012127	0.001554297
hsa-miR-487b	–1.334061912	2.992923132	0.012515649
hsa-miR-342-5p	–1.333040563	5.825155044	0.013564947
hsa-miR-30c	–1.050089726	5.567514099	0.01445844
hsa-miR-494	–1.125921065	4.652256751	0.033330803

Table 4. *All the differentially expressed miRNAs (DEMs) in peripheral blood mononuclear cell samples from long-standing patients with type 1 diabetes mellitus (T1DM) compared with those from healthy people (DEMs-long)*

Gene	LogFC	AveExpr	p value
hsa-miR-378e	–1.194166667	10.26958333	2.99E-06
hsa-miR-338-3p	–1.083333333	6.218333333	1.10E-05
hsa-miR-26a-5p	–1.019166667	4.987083333	2.58E-05
hsa-miR-16-5p	–1.310833333	7.647083333	0.001228264
hsa-miR-144-3p	1.811666667	1.316666667	0.025897521
hsa-miR-451a	–1.174166667	10.32875	0.031611088

Table 5. *All the enriched gene ontology (GO) terms of DEGs-short*

Category	Term	Gene count	p value
BP	GO:0042742~defense response to bacterium	6	2.13E-06
BP	GO:0006935~chemotaxis	5	2.40E-04
CC	GO:0031226~intrinsic to plasma membrane	7	0.012653662
MF	GO:0032393~MHC class I receptor activity	2	0.03693037
BP	GO:0006026~aminoglycan catabolic process	2	0.040243639
CC	GO:0005887~integral to plasma membrane	6	0.042766631

DEGs — differentially expressed genes; BP — biological process; CC — cellular component; MF — molecular function

negatively regulated pairs. Also, hsa-miR-338-3p was involved in all the above 20 miRNA-gene pairs.

The PPI network

After STRING screening, 19 and 89 protein-protein pairs of DEGs-short and DEGs-long were separately obtained, and the PPI networks of them are shown in Figure 1 and 2, respectively. The above pairs were a clustered different functional group in the networks, and KLRD1 (dark red) was involved in more pairs in a functional group of Figure 1, and ACTA2 and USP9Y (dark red) were involved in more pairs in different functional groups of Figure 2.

Table 6. *The top 10 most significantly enriched gene ontology (GO) terms of DEGs-long*

Category	Term	Gene count	p value
CC	GO:0043292~contractile fibre	9	3.40E-07
CC	GO:0044444~cytoplasmic part	46	3.35E-05
BP	GO:0008217~regulation of blood pressure	7	3.44E-05
CC	GO:0030485~smooth muscle contractile fibre	3	1.81E-04
CC	GO:0005737~cytoplasm	57	3.52E-04
BP	GO:0031032~actomyosin structure organization	4	6.66E-04
CC	GO:0031410~cytoplasmic vesicle	12	7.96E-04
CC	GO:0030016~myofibril	5	0.003350748
BP	GO:0030334~regulation of cell migration	6	0.003896976
BP	GO:0048738~cardiac muscle tissue development	4	0.005230827

DEGs — differentially expressed genes; BP — biological process; CC — cellular component

Table 7. *The miRNA-gene pairs of DEMs-short*

MicroRNA	Gene	MicroRNA_logFC	Gene_logFC
hsa-miR-181c	*TNFAIP6*	–1.26274394	1.364051308
hsa-miR-181c	*DDX3Y*	–1.26274394	1.249616883
hsa-miR-181c	*KCNJ2*	–1.26274394	1.077869083
hsa-miR-181a	*TNFAIP6*	–1.887221452	1.364051308
hsa-miR-181a	*DDX3Y*	–1.887221452	1.249616883
hsa-miR-181a	*KCNJ2*	–1.887221452	1.077869083
hsa-miR-146b-5p	*EIF1AY*	–2.666591212	1.9180783
hsa-miR-146b-5p	*CEACAM8*	–2.666591212	1.822226467
hsa-miR-125b	*MS4A3*	–1.075210791	1.222284567
hsa-miR-181c	*KLRF1*	–1.26274394	–1.000714933
hsa-miR-181a	*KLRF1*	–1.887221452	–1.000714933
hsa-miR-181c	*IFNG*	–1.26274394	–1.060417167
hsa-miR-181a	*IFNG*	–1.887221452	–1.060417167
hsa-miR-125b	*IFNG*	–1.075210791	–1.060417167
hsa-miR-146b-5p	*FGFBP2*	–2.666591212	–1.16267335
hsa-miR-125b	*CCL4*	–1.075210791	–1.266147883
hsa-let-7f	*DUSP2*	–1.664920459	–1.289584217

DEMs — differentially expressed miRNAs

Discussion

Many genes and miRNAs have been indicated to be involved in the occurrence and development of T1DM. In this study, we parallelly identified and analysed the differential expressions for newly diagnosed and long-standing T1DM patients. Afterwards, hisa-miR-181a and hisa-miR-181c were found to be involved in the most miRNA-gene pairs of DEMs-short (Tab. 7), and the node of KLRD1 was involved in the most pairs in the PPI network of DEGs-short (Fig. 1). A meta-analysis showed that the hsa-miR-181 family are involved in the inhibition of IL-2 expression, and hisa-miR-181a and hisa-miR-181c contribute to T cell tolerance, which is very important in the pathogenesis and treatment of T1DM [14, 15]. Also, the meta-analysis also proved that hsa-miR-181c was differentially expressed in the three types of diabetes (T1DM, T2DM, and gestational diabetes). Another study found that hsa-miR-181c was down-regulated in a diabetic-like environment and up-regulated after the addition of calcitriol [16]. Endothelial dysfunction played an important role in the occurrence and development of DM, and hisa-miR-181c could attenuate nitration stress

Table 8. *The miRNA-gene pairs of DEMs-long*

MicroRNA	Gene	MicroRNA_logFC	Gene_logFC
hsa-miR-338-3p	*CCL21*	–1.083333333	2.10357751
hsa-miR-338-3p	*CXCL9*	–1.083333333	1.77456068
hsa-miR-338-3p	*ALDOB*	–1.083333333	1.580524957
hsa-miR-338-3p	*UTY*	–1.083333333	1.428451427
hsa-miR-338-3p	*PTGIS*	–1.083333333	1.424889976
hsa-miR-338-3p	*PRRX1*	–1.083333333	1.242794076
hsa-miR-338-3p	*TMOD1*	–1.083333333	1.196113314
hsa-miR-338-3p	*HOXA3*	–1.083333333	1.121206093
hsa-miR-338-3p	*TFPI*	–1.083333333	1.100948365
hsa-miR-338-3p	*EIF1AY*	–1.083333333	1.08379585
hsa-miR-338-3p	*COL12A1*	–1.083333333	1.040322198
hsa-miR-338-3p	*SYT7*	–1.083333333	–1.030068536
hsa-miR-338-3p	*RGS16*	–1.083333333	–1.031304186
hsa-miR-338-3p	*AQP2*	–1.083333333	–1.034998067
hsa-miR-338-3p	*PPP1R1A*	–1.083333333	–1.113167906
hsa-miR-338-3p	*UNC5A*	–1.083333333	–1.173571
hsa-miR-338-3p	*SLC30A8*	–1.083333333	–1.494275889
hsa-miR-338-3p	*SYT13*	–1.083333333	–1.662898167
hsa-miR-338-3p	*SLC25A34*	–1.083333333	–1.728247111
hsa-miR-338-3p	*PCSK1*	–1.083333333	–1.979802762

DEMs — differentially expressed miRNAs

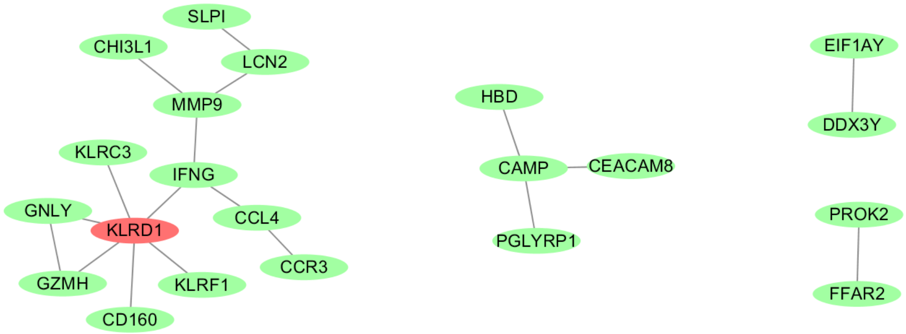

Figure 1. *The protein–protein interaction (PPI) network of DEGs-short*

through regulating FoxO1 expression and affecting endothelial cell function [17]. It might be one of the mechanisms of hisa-miR-181c in the occurrence and development of DM. Killer cell lectin-like receptor subfamily D, member 1 (KLRD1), encoded by KLRD1 gene, is an antigen preferentially expressed on NK cells, and also known as cluster of differentiation 94 (CD94). Nakata et al. [18] reported that the expression of KLRD1 was reduced in NK-enriched cells in fulminant T1DM. Goodier et al. [19] reported that there was a significant reduction in the proportion of CD94 (+) cells responding to lipopolysaccharide in T1DM compared to the non-diabetic twin (p = 0.025), which might be associated with the cause of T1DM. Therefore, we suspected that KLRD1, hisa-miR-181a, and hisa-miR-181c were novel biomarkers in the pathogenesis of T1DM. Also, this article identified some targets for hisa-miR-181a and hisa-miR-181c, such as KCNJ2, DDX3Y, KLRF1, IFNG, etc. (Tab. 7).

Furthermore, our results showed that hsa-miR--338-3p was involved in all the miRNA-gene pairs of DEMs-long (Tab. 8). The PPI network of DEGs-long was clustered different functional groups, and ACTA2 and USP9Y were involved in more pairs in different

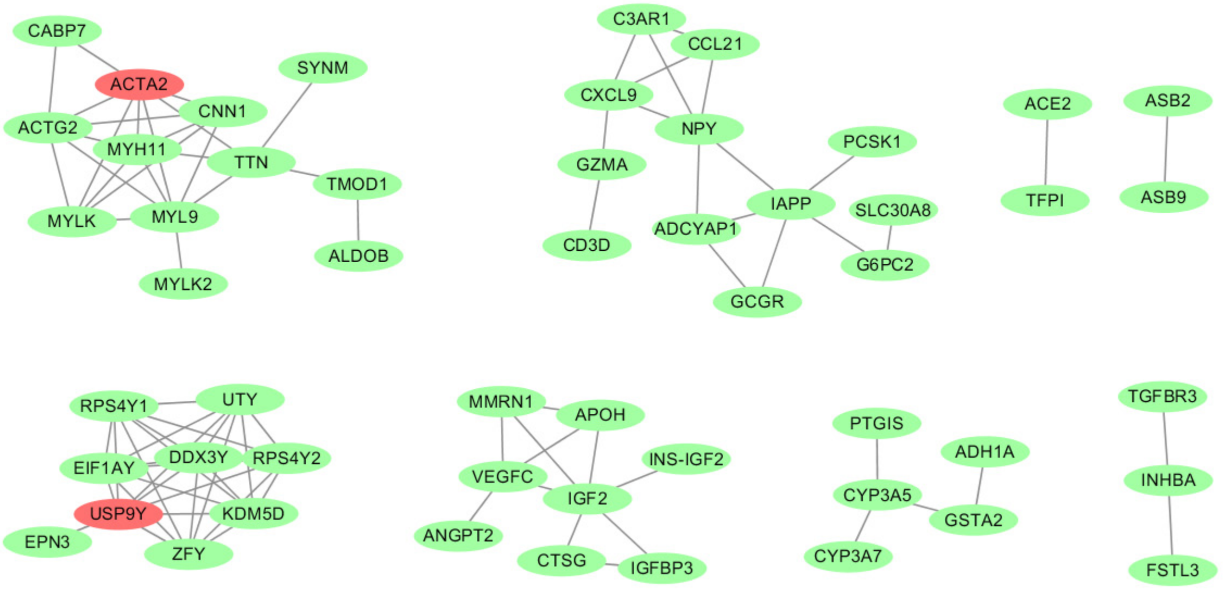

Figure 2. *The protein–protein interaction (PPI) network of DEGs-long*

functional groups (Fig. 2). Jacovetti et al. [20] found in rodents that β cell mass expansion during pregnancy and obesity is associated with the expression change of hsa-miR-338-3p; they also revealed a major role for hsa-miR-338-3p in compensatory β cell mass expansion occurring under different insulin resistance states. Subsequently, Nesca et al. [21] reported that the expression hsa-miR-338-3p displayed changes occurring before the onset of diabetes, which were positive effects on β cell activities and mass; in contrast, modification in the level of hsa-miR-338-3p primarily occurred in diabetic mice and resulted in increased β cell apoptosis. These results indicate that the expression change of hsa-miR-338-3p participates in the progression of diabetes. Alpha-actin-2 (α-SMA) is a protein encoded by the ACTA2 gene, which is commonly used as a marker of myofibroblast formation [22]. ACTA2 is the human aortic smooth muscle actin gene and is involved in cell motility, structure, and integrity [23]. Moreover, DEGs-long was enriched in GO terms of "contractile fibre", "smooth muscle contractile fibre", "actomyosin structure organisation", "myofibril", and "cardiac muscle tissue development" (Tab. 6), and ACTA2 played very important roles in the above GO terms. Although few reports revealed the relationship between ACTA2 and T1DM, our results suggest that ACTA2 is associated with the progression of this disease. USP9Y gene encodes the enzyme of ubiquitin specific peptidase 9, Y-linked (USP9Y), which locates on the Y chromosome. Mutations in this gene are associated with Sertoli cell-only syndrome (SCO) and male infertility. The gene fusion TTTY15-USP9Y score was statistically significantly higher in prostate cancer men with positive biopsy outcome than in men with negative biopsy outcome ($p < 0.001$), and thus TTTY15-USP9Y could be used to predict biopsy outcome [24]. USP9Y presents only in black people of African origin and attributes a favourable lipoprotein pattern, which is very important in the development of diabetes [25]. Previously, there was no direct evidence that USP9Y is associated with TIDM. Here, our article found that USP9Y occupied a critical position in the PPI network of DEGs-long, which suggested that USP9Y might play a role in the development of T1DM.

Conclusion

In conclusion, our study suggested that KLRD1, hisa-miR-181a, and hisa-miR-181c were involved in the onset of T1DM, and that ACTA2, USP9Y, and hsa-miR-338-3p played some important roles in its development. They are potential biomarkers in the pathogenesis or progression of T1DM, which provides further insights for T1DM.

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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