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Comparison of Transcriptome Between Type 2 Diabetes Mellitus and Impaired Fasting Glucose

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The aim of this study was to compare the transcriptome between impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM), and further research their molecular mechanisms.

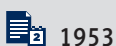
Material/Methods: The original microarray GSE21321, including miRNA and mRNA expression profiles, was downloaded from the GEO database. Data preprocessing was processed by limma package, and differentially expressed genes (DGs) and miRNA (DMs) were screened. Then, the regulatory relationships among miRNA, TF, and genes were screened and the regulatory network was constructed. Finally, DAVID was used for KEGG enrichment analysis.

Results: There were 11 upregulated IFG-related DMs and five upregulated T2DM-related DMs. Three of the DMs overlapped. In addition, there were eight downregulated IFG-related DMs and two downregulated T2DM-related DMs. Only one downregulated DM overlapped. Similarly, there were 264 upregulated IFG-related DGs and 331 upregulated T2DM-related DGs; and 196 overlapping genes were obtained. In addition, there were 400 downregulated IFG-related DMs and 568 downregulated T2DM-related DMs. A total of 326 downregulated DMs were overlapped. The overlapped DGs were enriched in various pathways, including hematopoietic cell lineage, Fc gamma R-mediated phagocytosis, and MAPK signaling pathway. *TAF1* (upregulated gene) and *MAFK* (downregulated gene) were hub nodes both in IFG- and T2DM-related miRNA-TF-gene regulatory network. In addition, miRNAs, including hsa-miR-29a, hsa-miR-192, and hsa-miR-144, were upregulated hub nodes in the two regulatory networks.

Conclusions: Genes including *TAF1* and *MAFK*, and miRNAs including hsa-miR-29a, hsa-miR-192, and hsa-miR-144 might be potential target genes and important miRNAs for IFG and T2DM.

MeSH Keywords: **Blood Glucose • Diabetes Mellitus, Type 2 • Transcriptome**

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Background

Type 2 diabetes mellitus (T2DM) is a metabolism disorder that is characterized by hyperglycemia in the context of insulin resistance and relative deficiency of insulin secretion, and is becoming a major public health challenge worldwide [1]. The main symptoms of patients with T2DM are excess thirst, frequent urination, and constant hunger [2]. Impaired fasting glucose (IFG) is commonly regarded as pre-diabetes, where fasting blood glucose level is consistently higher than normal but not high enough to be diagnosed as diabetes mellitus [3]. IFG is one of the insulin-resistant states that exists between normal glucose tolerance and overt T2DM, and is closely related to increased risk of cardiovascular disease [4].

Previous studies have shown that various physiological parameters, including liver enzyme, cardiopulmonary function, and endogenous hydrogen sulphide, were closely related to IFG and T2DM [5]. In addition, IFG and T2DM to some extent share some common pathophysiological mechanisms. Wu et al. suggested that many genetic variants, such as *CDKAL1* rs9465871, were found in IFG patients and associated with the increased risk of T2DM [6]. Menni et al. provided evidence that multiple metabolites from carbohydrates, proteins, and lipids are risk factors for both IFG and T2DM [7]. In addition, miR-126 was confirmed to be a biomarker for pre-diabetes and T2DM [8]. The different pathomechanisms between them have been widely researched. The expression level of growth differentiation factor-15, which could be a novel biomarker for IFG, was found lowest in patients with normal glucose tolerance, highest in the T2DM patients, and intermediate in IFG patients [9]. In 2013, Nesca et al. [10] found that there was significant change in the level of miR-146a in the early stage of T2DM based on miRNA expression profile. Another study found some diabetes-related miRNAs, including miR-192, miR-29a, and miR-30d, could be used to distinguish IFG and T2DM [11]. However, more research on the molecular mechanism of T2DM and IFG is needed. Therefore, we explored the molecular mechanism of the two diseases by comparing the transcriptome between IFG and T2DM.

Gene expression profile GSE21321 [11] is composed of mRNA and miRNA expression profiles from IFG and T2DM patients, as well as healthy controls. It is rarely analyzed and it is therefore appropriate to explore these genes and miRNAs involved in the molecular pathomechanism of IFG and T2DM. In this study, the original dataset was downloaded to compare the transcriptome of IFG and T2DM. Differentially expressed genes (DGs) and miRNA (DMs) were screened and the relationship among miRNAs, transcription factors (TFs), and genes were analyzed. The overlapping DGs between IFG and T2DM were processed by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. This study may improve the

understanding of the relationship between IFG and T2DM, and may help to identify the important pathomechanism involved in the progression of impaired glucose tolerance (IGT) to T2DM.

Material and Methods

Data acquirement

The gene expression profile of GSE21321 [11] was downloaded from Gene Expression Omnibus (GEO) database. This dataset is composed by mRNA expression profiling and miRNA expression profiling generated by Karolina et al. [11] from male adult patients (age range, 21 to 70 years). The mRNA expression profiles were obtained from 24 participants: eight healthy controls with fasting glucose <6.1 mmol/L, seven IFG patients (fasting glucose >6.1 mmol/L and <7.0 mmol/L), and nine T2DM patients (fasting glucose ≥7.0 mmol/L). The miRNA expression profiles were generated from 10 healthy controls, seven IFG patients and nine T2DM patients. In addition to two healthy control samples, the others samples of miRNA expression profile were the same as those of the mRNA expression profile. The clinical characteristics of participants are shown in Karolina et al. [11]. The microarray platforms for analysis of miRNA and mRNA expression were miRCURY LNA microRNA Array v.11.0 and Illumina Human Ref-8 v3.0 expression Beadchip, respectively.

Data preprocessing of microarray miRNA profiling

Probes where the signal value was negative in more than 20% of samples were eliminated. After screening, the negative values in the expression matrix were replaced using the 10 nearest neighbor averaging. Then, RMA background correction, quantile normalization, and log₂ transformation were processed by limma package [12]. Median value was taken from repetitions.

Data preprocessing of microarray mRNA expression profiling

The raw data was preprocessed, including background correction, quantile normalization, and log₂ transformation using the limma package. Median value was taken as the expression value of a gene when multiple probes were mapped to the same GeneID.

Differential expression analysis

Limma package was used for differential expression analysis of gene and miRNA expression matrix. The thresholds |log₂FC| ≥1 and *p*-value <0.05 were used to screen DGs and DMs.

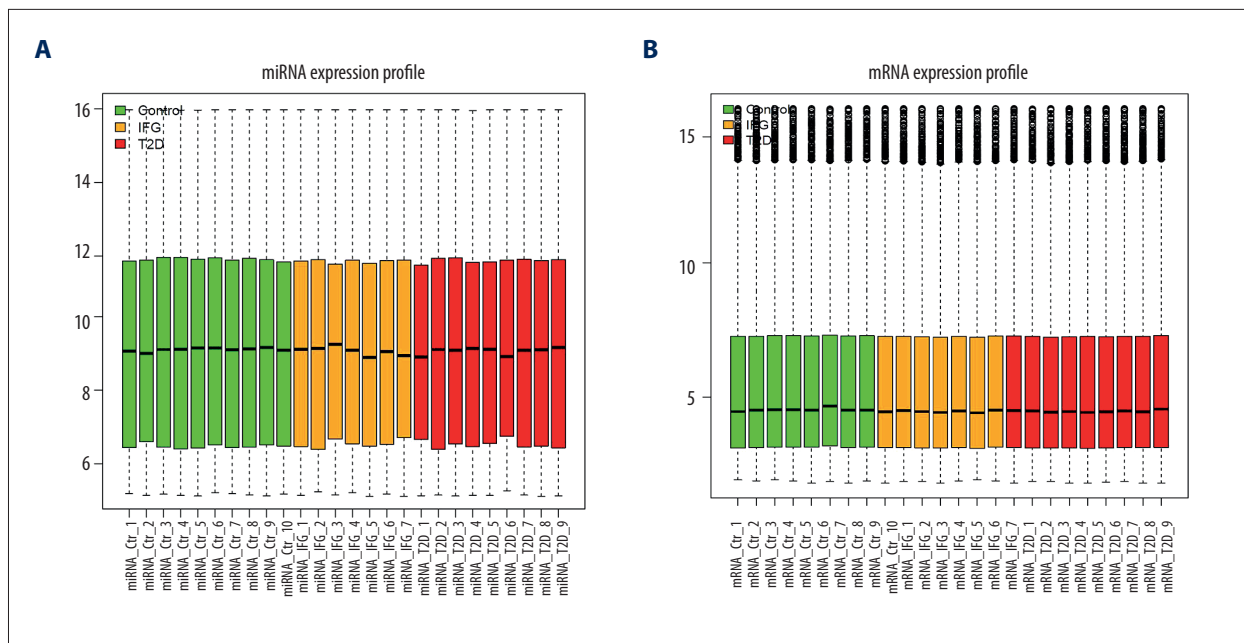


Figure 1. Box plot of miRNA and mRNA expression profile. **(A)** miRNA expression profiles; **(B)** mRNA expression profiles.

Functional enrichment analysis

Database for Annotation, Visualization and Integrated Discover (DAVID) (<http://david.abcc.ncifcrf.gov/>) was the online tool used for the functional enrichment analysis. In this study, multiple overlapping DGs between IFG and T2DM were performed for KEGG pathway enrichment analysis. The cut-off criterion was p -value <0.05 .

MiRNA-TF-gene regulatory network construction

The information on TF binding sites was provided by Encyclopedia of DNA Elements (ENCODE) database (<http://genome.ucsc.edu/ENCODE/>). Statistical analysis was performed on repeatability of TF binding sites, then the TF binding site that existed in no less than 2 samples was included for further analysis. Combined with annotation of gene transcription area, TFs in the promoter region were screened. Then the regulatory relationships between TFs and genes were established, and redundancy regulated pairs were excluded.

A total of six prediction algorithms, including targetScan (http://www.targetscan.org/mamm_31/), PicTar, RNA22, miRanda, and PITA [13], were used for obtaining the miRNA-gene regulatory relationship. The miRNA-gene regulatory relationships were predicted by at least three prediction algorithms.

The TF-miRNA regulatory network was extracted from TranMir (v1.2). TranMir has curated 735 entries, which include 201 TFs, 209 miRNAs, and 16 organisms from 268 publications.

Based on the above regulatory networks, the integrated miRNA-TF-gene regulatory network was constructed.

Results

Normalized microarray data

The normalized miRNA expression matrix is shown in Figure 1A, and the normalized mRNA expression matrix is displayed in Figure 1B. Raw expression values were well normalized to the median array intensity.

Screened DGs and DMs

The Venn plot shows DGs and DMs in IFG and T2DM patients (Figure 2). Compared with normal samples, there were 11 up-regulated IFG-related DMs and five up-regulated T2DM-related DMs, among which three were overlapping genes. In addition, there were eight downregulated IFG-related DMs and two downregulated T2DM-related DMs. Only one downregulated DM overlapped. Similarly, there were 264 upregulated IFG-related DGs and 331 upregulated T2DM-related DGs. A total of 196 overlapping genes were found among them. In addition, there were 400 downregulated IFG-related DMs and 568 downregulated T2DM-related DMs. A total of 326 downregulated DMs were overlapping. A total of three miRNAs were differentially expressed in both IFG and T2DM samples, including hsa-miR-144 (IFG, p value=5.38E-07; T2DM, p value=4.62E-09), hsa-miR-192 (IFG, p value=0.001716; T2DM, p value=1.88E-05) and hsa-miR-144 (IFG, p value=0.0094; T2DM, p value=0.00013).

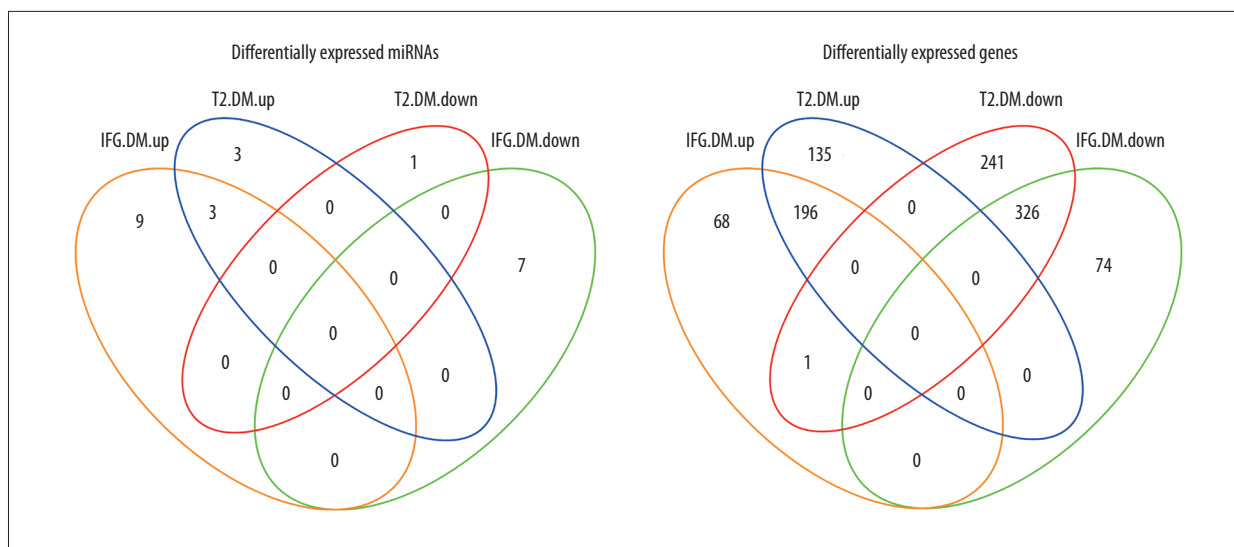


Figure 2. Venn plot of differentially expressed genes (DGs) and miRNAs (DMs).

Table 1. KEGG pathway enrichment result.

Term	Count	%	P Value
hsa04640: Hematopoietic cell lineage	9	0.188916877	0.006632212
hsa04666: Fc gamma R-mediated phagocytosis	9	0.188916877	0.011869958
hsa04010: MAPK signaling pathway	17	0.356842989	0.012989433
hsa04912: GnRH signaling pathway	9	0.188916877	0.014156486
hsa04062: Chemokine signaling pathway	13	0.272879933	0.018527986
hsa04810: Regulation of actin cytoskeleton	14	0.293870697	0.022659152
hsa04620: Toll-like receptor signaling pathway	8	0.167926113	0.046087399
hsa00601: Glycosphingolipid biosynthesis	4	0.083963056	0.046424127

Functional enrichment analysis of overlapped DGs

As shown in Table 1, the overlapped DGs were significantly enriched in various pathways, including hematopoietic cell lineage (p value=0.0066), Fc gamma R-mediated phagocytosis (p value=0.0118699) and MAPK signaling pathway (p value=0.012989).

Regulatory network construction

Figure 3 shows the IFG miRNA-TF-gene regulatory network. In this network, miRNAs including hsa-miR-519d, hsa-miR-505, and hsa-miR-490-3p, and TFs including TATA box binding protein (TBP)-associated factor 1 (*TAF1*), nuclear transcription factor Y, alpha (*NFYA*) and MAX interactor 1, dimerization protein (*MXI1*) were the hub nodes with high degree. The T2DM-related miRNA-TF-gene regulatory network is shown in Figure 4. The hub nodes of this network include hsa-miR-29a, hsa-miR-192, zinc

finger and BTB domain containing 7A (*ZBTB7A*) and TAF1 RNA polymerase II, and TATA box binding protein (TBP)-associated factor (*TAF1*). Comparing the miRNA-TF-gene regulatory network of IFG and T2DM, *TAF1* and v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog K (*MAFK*) were with the common hub nodes.

Discussion

We downloaded the microarray data of IFG and T2DM to compare the transcriptome between them. The miRNA-TF-gene regulatory network of IFG and T2DM were separately constructed. In addition, *TAF1* (upregulated gene) and *MAFK* (downregulated gene) were the hub nodes in both regulatory networks. In addition, miRNAs including hsa-miR-29a, hsa-miR-192, and hsa-miR-144, were upregulated hub nodes in the two regulatory networks.

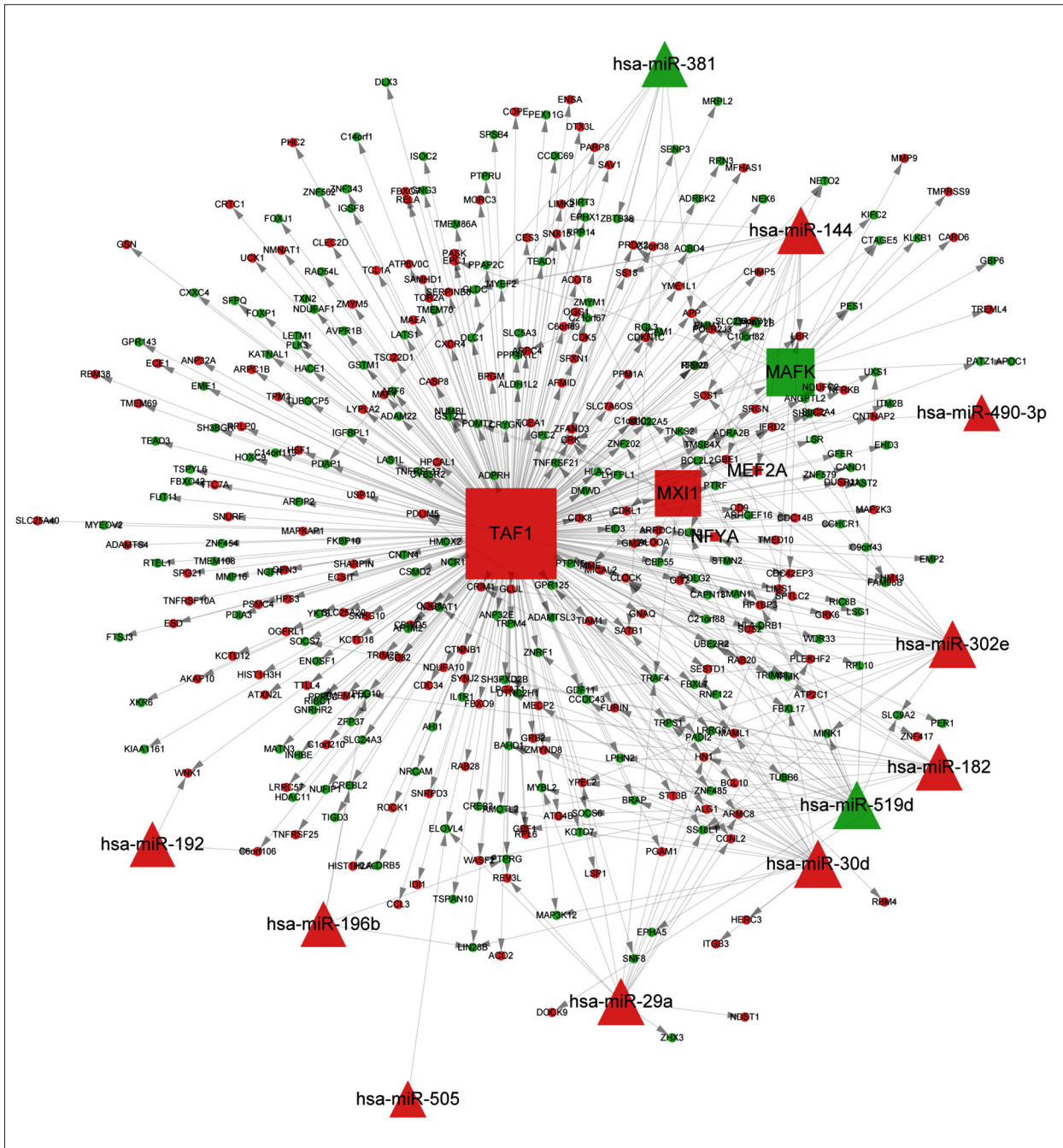


Figure 3. The miRNA-TF-gene regulatory network of impaired fasting glucose (IFG). The red node represents upregulated genes and miRNA. The green node represents upregulated genes. Rectangle, triangle and circle represent TFs, miRNA and gene, respectively. The size of nodes correlate with the degree of genes.

TAF1 is the largest component of the TFs *TFIIF* which is composed of TBP and a variety of TBP-associated factors. The level of *TAF1* in obese diabetic patients has been found to be higher than in healthy subjects [14]. In addition, a previous study showed that *TAF1* was independently related to markers of insulin resistance and glycated hemoglobin [15]. In addition,

TAF1 could promote Mdm2-regulated p53 turnover and further affect impaired wound healing in diabetes [16]. In our study, *TAF1* was confirmed to be upregulated in IFG and T2DM samples. Moreover, it was targeted to various genes such as adrenoceptor alpha 2B (*ADRA2B*), v-akt murine thymoma viral oncogene homolog 2 (*AKT2*), and acyl-CoA thioesterase 8

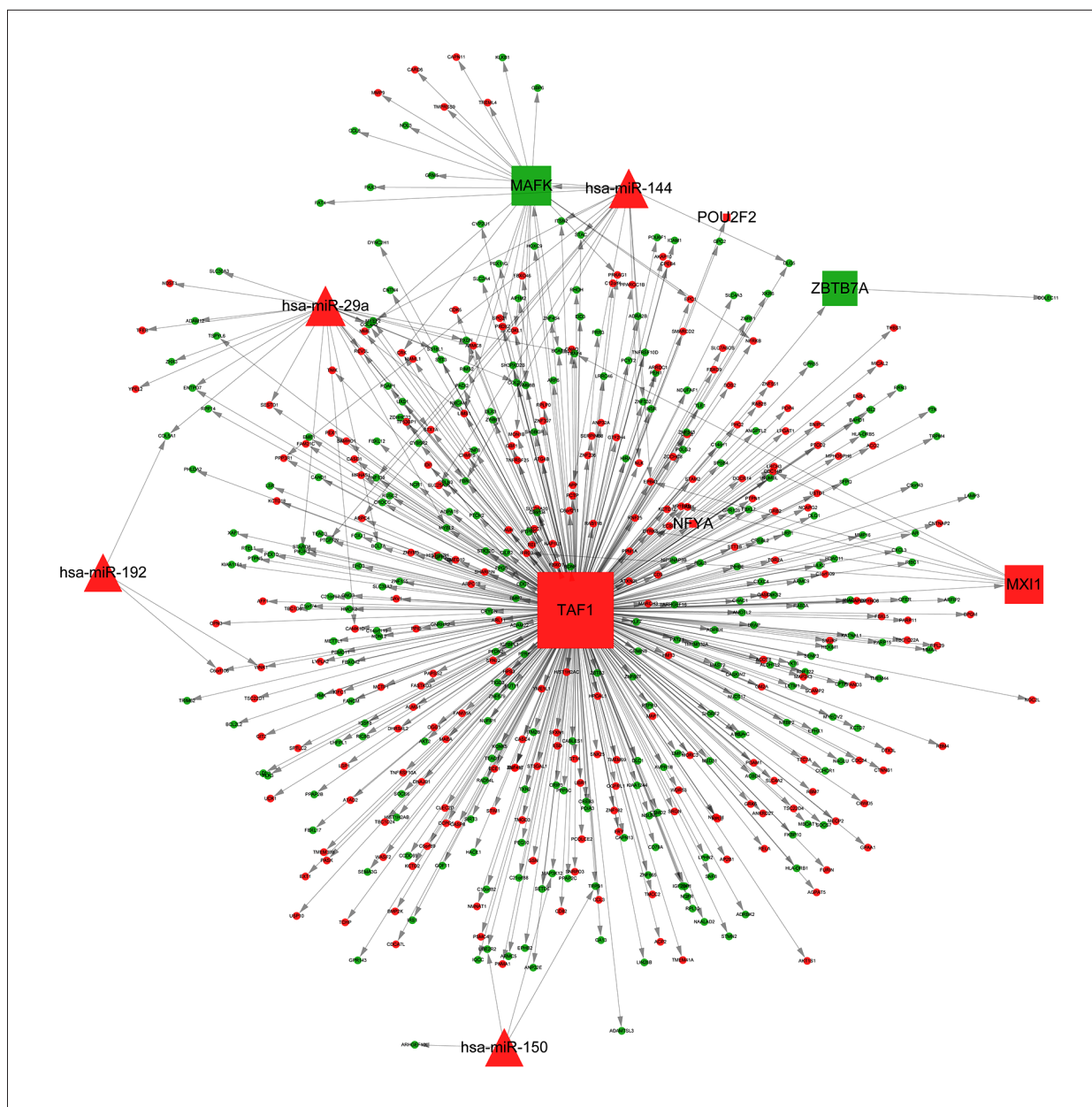


Figure 4. The miRNA-TF-gene regulatory network of type 2 diabetes mellitus (T2DM). The red node represents upregulated genes and miRNA. The green node represents upregulated genes. Rectangle, triangle, and circle represent TFs, miRNA and gene, respectively. The size of node size represents the degree of genes.

(*ACOT8*). In a previous study, the polymorphism of *ADRA2B* was closely associated with insulin secretion and impaired glucose tolerance [17]. In addition, the expression levels of *AKT2* and *ACOT8* have also been found to significantly change in T2DM patients [18,19]. Therefore, *TAF1* might be a critical gene in the molecular mechanism of IFG and T2DM by targeting various genes including *ADRA2B*, *AKT2*, and *ACOT8*.

In this study *MAFK* was also screened as an important gene for IFG and T2DM. This gene targeted various genes such as

branched chain amino-acid transaminase 1, cytosolic (*BCAT1*) and matrix metalloproteinase 9 (*MMP9*). Research regarding *MAFK* associated with molecular mechanism of IFG and T2DM has not been described. However, SNPs such as rs2242400 in *BCAT1* have been confirmed to be related to T2DM [20]. In addition, it has been shown that decreased *MMP-9* level affects the attenuation of vascular dysfunction in T2DM samples [21]; and there was a higher circulating plasma level of *MMP-9* in T2DM samples than controls [22]. Therefore, *MAFK* might be a potential target gene for IFG and T2DM.

Moreover, the over-expression of hsa-miR-29a could repress the progresses of glucose uptake and further induce insulin resistance [23]. In our study, the miRNA targeted phosphoinositide-3-kinase, regulatory subunit 3 (*PIK3R3*), cell division cycle 7 (*CDC7*), and *BCL71*, and also further participated in the molecular mechanism of IFG and T2DM. The research of de Bruind et al. showed that *PIK3R3* could affect insulin resistance via the IGF-1 signaling pathway [24]. In addition, hsa-miR-144 was also confirmed to downregulate the expression of insulin receptors, hinder the uptake of glucose, and delay the progression of insulin signaling [25]. In our study, this miRNA targeted several genes including *GRM5*, *ARPC4*, *APP*, and *FAT4*. A previous study showed that *APP* was a critical gene in regulating insulin secretion from pancreatic islets [26]. This suggests that hsa-miR-29a and hsa-miR-144 might be potential target miRNAs for IFG and T2DM.

In our study, hsa-miR-192 was also upregulated in T2DM and IFG samples. Whereas in a previous study, altered expression of hsa-miR-192 was observed in T2DM subgroup [27]. Ortega et al. [28] showed that in T2DM patients, miR-192 in plasma was decreased, but increased after metformin

treatment. Thus miR-192 might be the target of metformin for T2DM. In another study, it was shown to be a marker of pre-diabetes and was also regulated by an exercise intervention [29]. In our present study, hsa-miR-192 targeted chromosome 6 open reading frame 106 (*C6orf106*) and collagen, type V, alpha 1 (*COL5A1*). Genome-wide association studies showed that SNP of *COL5A1* was associated with T2DM [30].

Conclusions

There are many similarities of transcriptome between IFG and T2DM. Genes including *TAF1* and *MAFK*, and miRNAs including hsa-miR-29a, hsa-miR-192, and hsa-miR-144 might be the potential target genes and important miRNAs for IFG and T2DM. However, the results need to be further confirmed by additional experiments.

Conflict of interests

All authors declare that they have no conflict of interests to state.

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