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Crosstalk between microRNAs, the putative target genes and the lncRNA network in metabolic diseases

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Abstract. MicroRNAs (miRNAs/miRs) are small non-coding RNAs (ncRNAs) that regulate gene expression. Emerging knowledge has suggested that miRNAs have a role in the pathogenesis of metabolic disorders, supporting the hypothesis that miRNAs may represent potential biomarkers or targets for this set of diseases. However, the current evidence is often controversial. Therefore, the aim of the present study was to determine the associations between miRNAs-target genes, miRNA-long ncRNAs (lncRNAs), and miRNAs-small molecules in human metabolic diseases, including obesity, type 2 diabetes and non-alcoholic fatty liver disease. The metabolic disease-related miRNAs were obtained from the Human MicroRNA Disease Database (HMDD) and miR2Disease database. A search on the databases Matrix Decomposition and Heterogeneous Graph Inference (MDHGI) and DisGeNET were also performed. miRNAs target genes were obtained from three independent sources: Microcosm, TargetScan and miRTarBase. The interactions between miRNAs-lncRNA and miRNA-small molecules were performed using the miRNet web tool. The network analyses were performed using Cytoscape software. As a result, a total of 20 miRNAs were revealed to be associated with metabolic disorders in the present study. Notably, 6 miRNAs (miR-17-5p, miR-29c-3p, miR-34a-5p, miR-103a-3p, miR-107 and miR-132-3p) were found in the four resources (HMDD, miR2Disease, MDHGI, and DisGeNET) used for these analyses, presenting a stronger association with the diseases. Furthermore, the target genes of these miRNAs participate in several pathways previously associated with metabolic diseases. In addition, interactions between miRNA-lncRNA and miRNA-small molecules were also found, suggesting that some molecules can modulate gene expression via such an indirect way. Thus, the results of this data mining and integration analysis provide further information on the possible molecular basis of the metabolic disease pathogenesis as well as provide a path to search for potential biomarkers and therapeutic targets concerning metabolic diseases.

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Introduction

Metabolic diseases affect millions of people in both developed and transition countries (1). In addition to conventional genetic inheritance of risk alleles, emerging evidence has shown that these diseases are also linked to lifestyle and inherited epigenetic pattern interactions, which affects gene expression and the activity of proteins involved in the onset and pathogenesis of diverse metabolic diseases (2).

The strong link between epigenetics and metabolism may offer attractive clinical applications to counteract the escalating prevalence of metabolic diseases, such as obesity, type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), among others (2,3). Regarding the epigenetic factors, MicroRNAs (miRNAs) are a class of small non-coding RNAs (ncRNAs) that regulate the expression of ~60% of protein coding genes. They control many cellular functions and metabolic pathways, and subsequently influence the development and progression of a number of diseases (4-7).

Among several activities, miRNAs are recognized as regulators of lipid and glucose metabolism and are involved in the physiopathology of metabolic diseases (6,8). The liver-enriched miR-122-5p was the first miRNA to be functionally associated with a metabolic phenotype, regulating cholesterol and lipid metabolism (9). Additionally, a miR-122 inhibitor (Miravirsen) was found as a novel therapeutic strategy against chronic hepatitis C virus (HCV) infection (10). miR-33a-5p and miR-33b-5p were also demonstrated as playing crucial roles in cholesterol and lipid turnover; while miR-34a-5p may be a key regulator of hepatic lipid homeostasis (11). miR-103a-3p and miR-107 have been reported as

regulators of hepatic insulin sensitivity (6) and as contributors of adipose growth by accelerating adipocyte differentiation (12). On the other hand, miR-375 is highly expressed in pancreatic islets and is important for insulin secretion and β cell development and maintenance (13,14). Thus, the altered expression of miRNAs and their target genes could interfere or manage the predisposition to metabolic diseases (15,16).

Furthermore, some studies have shown the interactions between miRNAs and other epigenetics mechanisms, including long ncRNAs (lncRNAs) as described elsewhere (17). Members of the lncRNA family contribute to intracellular processes by acting as host transcripts for miRNA (18,19) and lncRNAs can antagonize miRNA function by competing with miRNAs to bind to target mRNAs (20). Furthermore, lncRNAs may act as molecular decoys or sponges of miRNAs, affecting the levels and function of miRNAs (21,22). Otherwise, some lncRNAs are targeted by miRNAs, repressing lncRNAs expression (23). Besides that, lncRNA-miRNA interactions can regulate gene expression through a double-negative feedback loop (24). Moreover, accumulating evidence associates lncRNAs in the maintenance of metabolic homeostasis and the dysregulation of certain lncRNAs promotes the progression of metabolic disorders such as diabetes, obesity, chronic liver diseases, and cardiovascular diseases (25,26).

Several small molecules have been suggested as directly binding to miRNAs, then modifying miRNAs expression, thus having therapeutic potential (27). In this context, Gumireddy *et al* (28) report that the small molecule diazobenzene modifies the miR-21 expression, suggesting that miR-21 may become a druggable target.

In this study, we aimed to identify metabolic disease-related miRNAs and their target genes, and then construct miRNA-target gene and miRNA-lncRNA networks to find out putative important biological processes and determine those miRNAs that have major roles in metabolic diseases. Additionally, we aimed also to identify small molecules that interact with the miRNAs. These analyses may provide a theoretical basis for further studies and contribute to understand important complex mechanisms underlying metabolic diseases.

Materials and methods

Search for metabolic disease-related microRNAs. Metabolic disease-related miRNAs were obtained from two experiment-supported databases: Human MicroRNA Disease Database (HMDD v3.0) (29) and miR2Disease (access December 2018) (30). The miRNAs previously associated with obesity, NAFLD, or T2DM were incorporated into our analyses. After that, the results obtained from these two databases were compared to those found in the Matrix Decomposition and Heterogeneous Graph Inference (MDHGI; access December 2018) (31), a miRNA-disease predictor database. For this, we included the top 10 miRNAs predicted as associated with metabolic diseases. Thus, the inclusion of validated and predicted data increases the power of an association.

Additionally, we also investigated if the target genes of these miRNAs were previously associated with metabolic diseases (T2DM, NAFLD, and obesity) using the DisGeNET v5.0 database (32). The DisGeNET database is a discovery platform containing one of the largest publicly available collections of genes and variants associated to human diseases. For this last approach, we included only the top 10 genes associated with each disease according to the prediction score. This strategy was used to increase the association evidence power and to focus on those molecules with potential higher interest and value.

Evaluation of microRNA target genes. The list of miRNAs identified as associated with metabolic diseases was then submitted to bioinformatics analyses to search for their putative target genes. For this approach, the information from experimentally validated miRNA-target gene interactions was combined with the results from target prediction algorithms in order to retrieve a comprehensive set of target genes while controlling for false positive rates. CyTargetLinker v3.0.1 web tool (33) was used to search for validated and predicted miRNA-target gene interactions (MTI) and visualize them in a graphical way. For this study, we obtained Homo sapiens MTIs from one experimentally validated database (miRTarBase v.4.4) and from two predicted miRNA databases (MicroCosm v.5.0 and TargetScan v.7.0). Moreover, the target genes were also searched in the miRWalk v.3.0 (34) database and incorporated into the analysis. The miRNA-mRNA networks were visualized and analyzed using the Cytoscape software v3.7.0 (35).

Pathways analysis. Functional enrichment analysis of miRNA-target genes were performed to retrieve Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways annotations for the miRNAs target genes, using the plug-ins Biological Networks Gene Ontology (BiNGO; v3.0.3) (36) and ClueGO/Cluepedia (v.2.3.5) (37) on Cytoscape environment (35). The ClueGO/Cluepedia plug-in permits the visualization of the non-redundant biological terms for large clusters of gene sets in a functionally grouped network and the most representative GO term or KEGG pathways was used to name the module, considering a κ score of 0.3 and q-values >0.05.

Interactions between microRNAs and lncRNAs and associations between miRNAs and small molecules. The interactions between miRNAs and lncRNAs were analyzed using the starBase v2.0 (38) database, and the connections between miRNAs and small molecules were performed using the SM2miR (39) and PharmacomiR (40) databases. The miRNet web-tool was used to perform the search and analysis (41).

Additionally, we also investigated the subcellular location of miRNAs and lncRNAs associated with metabolic disorders using RNALocate database (42) as well as iLoc-lncRNA (43) and lncLocator (44) web tools.

Statistical analysis and visualization. Cytoscape v3.7.0 software (35) was used to illustrate the disease-related networks and analyze the network properties. The Venn diagrams were constructed using the InteractiveVenn instrument (45). The names of miRNAs, mRNAs, and lncRNAs are unified based on miRBase 22 release (46), HUGO gene nomenclature committee (HGNC), and LNCipedia v5.2 (47), respectively.

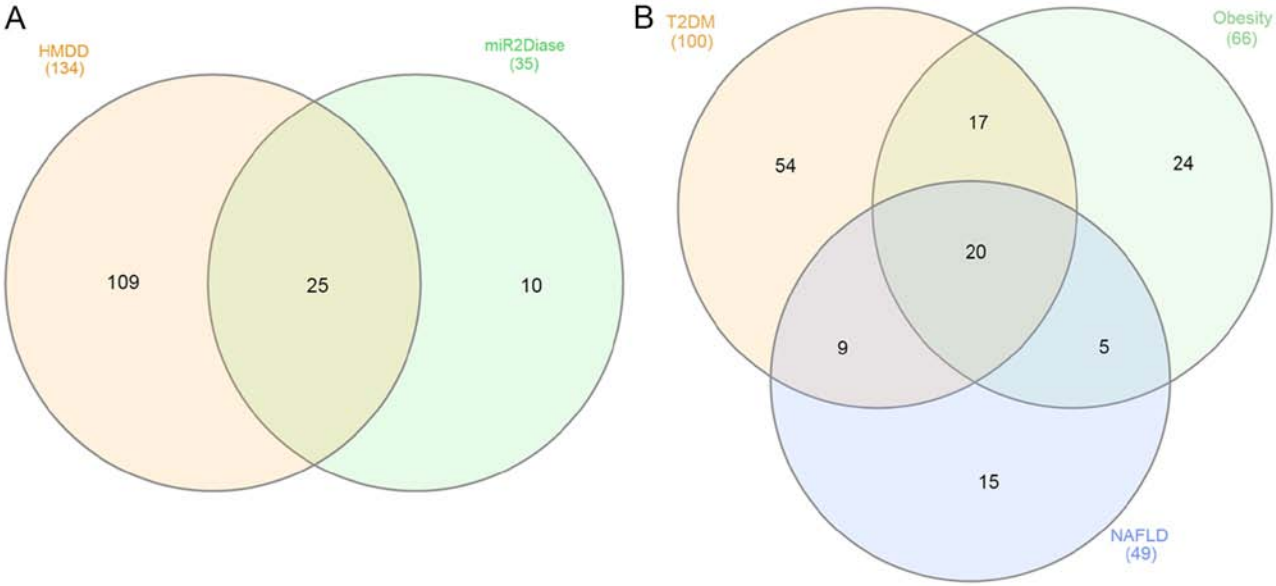

Figure 1. MicroRNAs associated with metabolic diseases. (A) MicroRNAs associated with metabolic diseases selected from the two distinct databases. (B) The number of microRNAs associated with T2DM, obesity, and NAFLD selected from the two databases combined. T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; HMDD, Human MicroRNA Disease Database.

The statistical tests used for the enrichment analysis were based on the right-sided hypergeometric test and adjusted for multiple hypotheses using the Benjamini & Hochberg False Discovery Rate (FDR) test. Interactions with a q-value <0.05 were considered strongly enriched.

Results

Identification of metabolic disease-related miRNAs. A total of 144 unique miRNAs related to metabolic disease were found mapping the two databases of human diseases (Fig. 1A and Table SI). In the HMDD database, 134 unique miRNAs were found; while in the miR2Disease, 35 unique miRNAs were found. As shown in Fig. 1A, the two databases shared 25 miRNAs. Moreover, the 10 miRNAs found only in the miR2Disease database were associated with NAFLD. The miR2Disease database had more data regarding NAFLD than HMDD; however, there is less information regarding T2DM and obesity (Table SI). Regarding the 25 miRNAs shared by the 2 databases, these miRNAs were previously associated with metabolic alterations and other human diseases, including some types of cancer (16,48,49).

Data from the two resources were systematically combined according to the metabolic disease criteria. As shown in Fig. 1B, 100 miRNAs were found to be associated with T2DM, 66 miRNAs with obesity, and 49 miRNAs with NAFLD. Moreover, 20 miRNAs were related to the three pathologies (let-7d-5p, miR-17-5p, miR-21-5p, miR-26a-5p, miR-27a-3p, miR-27b-3p, miR-29c-3p, miR-30a-5p, miR-33a-5p, miR-34a-5p, miR-103a-3p, miR-107, miR-122-5p, miR-126-3p, miR-132-3p, miR-150-5p, miR-155-5p, miR-200a-3p, miR-200b-3p, and miR-375-5p). Additionally, the results from the two databases were compared with those from the miRNA-disease predictor, MDHGI (31). Out of the 20 miRNAs, 10 miRNAs (miR-17-5p, miR-21-5p, miR-29c-3p, miR-34a-5p, miR-103a-3p, miR-107, miR-122-5p, miR-126-3p, miR-132-3p, and miR-150-5p) were also found in MDHGI database, increasing the evidence of association of these miRNAs with metabolic diseases (Table I).

Putative target genes of the selected miRNAs associated with metabolic diseases. The 20 miRNAs selected using the strategy described in the Methods Section regulate together the expression of 10.942 unique target genes (predicted or validated). miR-17-5p had the largest number of target genes (1181), followed by miR-155-5p (904) and miR-34a-5p (736). The miR-200a-3p had the lowest number of target genes (151) (Table SII). A group of 484 putative target genes was found when we analyzed only the miRNA-gene interactions reported in three online databases (Fig. S1). The largest number of interconnections was found for miR-17-5p and miR-30a-5p. Moreover, as expected, the miRNAs miR-107 and miR-103a-3p and the miRNAs miR-27a-3p and miR-27b-3p shared a great number of target genes. miR-150 did not have common target genes with other miRNAs; and we could not find target genes for miR-126-3p and miR-375 when considering only the targets that were reported as validated and predicted by at least three databases (Fig. S1).

Moreover, of these 20 miRNAs, 13 miRNAs target at least one of the top 10 candidate genes associated with each metabolic disorder: T2DM, obesity, or NAFLD, according to DisGeNET database (Tables I and II). Furthermore, some of these genes are very well described targets of the selected miRNAs. Regarding the validated and predicted target genes, *PPARG* is targeted by miR-27a-3p and miR-27b-3p, *LDLR* is targeted by miR-30a-3p, *SIRT1* by miR-132-3p, and *NEUROD1* by miR-30a-3p. However, there are genes in this list that are not regulated by the selected 20 miRNAs, suggesting that there are more miRNAs involved in the pathogenesis of metabolic diseases.

Additionally, as shown in Table I, of the 20 miRNAs, a subset of 6 miRNAs (miR-17-5p, miR-29c-3p, miR-34a-5p,

Table I. miRNAs associated with metabolic diseases from distinct databases.

miRNAs	miR2-Disease	HMDD	MDHGI	DisGe-NET
let-7d-5p	X	X		X
miR-17-5p	X	X	X	X
miR-21-5p	X	X	X	
miR-26a-5p	X	X		X
miR-27b-3p	X	X		X
miR-29c-3p	X	X	X	X
miR-30a-5p	X	X		X
miR-33a-5p	X	X		
miR-34a-5p	X	X	X	X
miR-103a-3p	X	X	X	X
miR-107	X	X	X	X
miR-122-5p	X	X	X	
miR-126-3p	X	X	X	
miR-132-3p	X	X	X	X
miR-27a-3p		X		X
miR-150		X	X	X
miR-200b-3p		X		X
miR-155-5p		X		
miR-375		X		
miR-200a-3p		X		

An 'X' indicates that the miRNA was present in this database. MiR2Disease and HMDD databases present validated interactions between the miRNAs and the selected diseases. The MDHGI database presents the predicted associations between the miRNAs and diseases according to a score of prediction. DisGeNET (v5.0) is a database of candidate genes for human diseases. HMDD, Human microRNA Disease Database (v3.0); MDHGI, Matrix Decomposition and Heterogeneous Graph Inference; miR/miRNA, microRNA.

miR-103a-3p, miR-107, and miR-132-3p) was found in the four resources (HMDD, miR2Disease, MDHGI, and DisGeNET) used for these analyses (Fig. S2). Fig. 2 summarizes the interaction of the candidate genes for metabolic diseases and the 6 selected miRNAs.

Pathway analysis of the selected miRNAs associated with metabolic diseases. To explore the biological pathways possibly affected by the target genes of the 20 analyzed miRNAs, functional enrichment analysis of their target genes using pathway maps from the GO and KEGG repositories were carried out.

GO terms were investigated for biological, cellular, and molecular processes associated with the set of predicted and validated target genes found for the 20 selected miRNAs. As a result, a total of 30 unique pathways were enriched for miRNAs. Many of these pathways are well established to be involved in metabolic diseases, such as transforming growth factor β receptor, oxidative stress, apoptosis, VEGF and angiogenesis signaling pathways (Table SIII and Fig. 3).

After that, the metabolic pathways in which participates the subgroup of the 16 genes previously associated with metabolic diseases (*HNF1A, HNF4A, AKT2, IRS1, NEUROD1, PPARG, LEP, LEPR, PCSK1, SIM1, UCP3, ADIPOQ, SIRT1, PNPLA3, PPARA* and *LDLR*) were also investigated. These genes are regulated by the 6 selected miRNAs (as shown in Table II and participate in the biological processes previously associated with metabolic diseases, such as regulation of cellular carbohydrate metabolic process, lipid homeostasis, cholesterol transport, and regulation of glucose metabolism (Fig. 4A). Additionally, these target genes were also enriched in some KEGG pathways such as transcriptional regulation of white adipocyte differentiation, AMPK signaling pathway, regulation of gene expression in β cell, and adipocytokine signaling pathway (Fig. 4B).

miRNA-lncRNAs interactions. The 20 miRNAs associated with metabolic diseases putatively interact with 423 unique lncRNAs (Table SIV). Moreover, the subgroup of 6 miRNAs putatively interplays with 210 unique lncRNAs (Fig. 5). The miRNA that connects with the largest number of lncRNAs is miR-17-5p (72 lncRNAs). The lncRNA-XIST interacts with all sub-selected 6 miRNAs. Moreover, miR-107 and miR-103a-3p share the largest number of lncRNAs (Fig. 5). Besides the miRNA-lncRNA interplay, some relations between lncRNA and genes associated with metabolic diseases were also presented in Fig. 2. Lnc-RNA-XIST interacts with *AKT2* and *IRS1*. In the same way, lncRNA-HCG18 also intercommunicates with *AKT2*, and lncRNA-MALAT1 interacts with *PPARG* (Fig. 2)

Moreover, we also searched subcellular location of the 6 miRNAs and the 6 lncRNAs associated with metabolic disorders. The RNAlocate database contains a manually curated RNA-associated subcellular localization entries with experimental evidence. In contrast, the iLoc-lncRNA and lncLocator are sequence-based predictors of subcellular locations. Based on the predicted score of iLoc-lncRNA, the majority of miRNAs associated with metabolic disorders is located on exosomes, and the lncRNAs on nucleolus, nucleus, or nucleoplasm. Similar results were found for lncLocator for lncRNA location. However, according to RNALocate database, we noted that the location of ncRNAs depends on the tissue, cell or condition they are expressed (Table SV).

Interactions between miRNAs and small molecules. The 20 miRNAs interplay with 102 unique small molecules (Table SVI). The miRNA that intercommunicates with the highest number of small molecules is miR-21-5p (70), and the miRNA that interacts with the lowest number of molecules is miR-33a-5p (4). Fig. 6 shows the connections between the 6 selected miRNAs and 42 small molecules. These miRNAs are linked with different types of molecules, including metabolites, proteins, chemicals and drugs.

Discussion

In the present study, miRNAs associated with obesity, T2DM, and NAFLD were identified through a valid text mining search strategy. For this, several bioinformatics analyses were conducted to explore the miRNA-mRNA, miRNA-lncRNA, and miRNA-small molecules interactions involved in the pathogenesis of metabolic diseases. As main result, we propose an interaction of 6 miRNAs with 13 candidate genes

Table II. Top 10 genes associated with each analyzed metabolic disease according to the DisGeNET database and the interactions with the selected miRNAs.

A, T2DM

Gene	Gene name	Score	miRNAs
GCK	Glucokinase	0.899	-
HNF1A	HNF1 homeobox A	0.812	miR-107, miR-27b-3p
HNF4A	Hepatocyte nuclear factor 4α	0.729	miR-27b-3p
HNF1B	HNF1 homeobox B	0.684	-
AKT2	AKT serine/threonine kinase 2	0.681	miR-29c-3p, miR-103a-3p
ABCC8	ATP binding cassette subfamily C member 8	0.677	-
IRS1	Insulin receptor substrate 1	0.67	miR-150-5p, let-7d-5p, miR-29c-3p
NEUROD1	Neuronal differentiation 1	0.645	miR-17-5p, miR-30a-5p
PDX1	Pancreatic and duodenal homeobox 1	0.634	-
PAX4	Paired box 4	0.618	-

B, Obesity

Gene	Gene name	Score	miRNAs
MC4R	Melanocortin 4 receptor	0.913	-
PPARG	Peroxisome proliferator activated receptor γ	0.727	miR-34a-5p, miR-27a-3p, miR-27b-3p
LEP	Leptin	0.72	miR-17-5p, miR-200b-3p, miR-132-3p, miR-150-5p
LEPR	Leptin receptor	0.688	miR-103a-3p, miR-17-5p, miR-26a-5p
POMC	Proopiomelanocortin	0.528	-
PCSK1	Proprotein convertase subtilisin/kexin type 1	0.507	miR-200b-3p
SIM1	Single-minded family bHLH transcription factor 1	0.492	miR-27b-3p, let-7d-5p
APOE	Apolipoprotein E	0.479	-
UCP3	Uncoupling protein 3	0.475	miR-17-5p, miR-200b-3p
SH2B1	SH2B adaptor protein 1	0.439	-

C, NAFLD

Gene	Gene name	Score	miRNAs
ADIPOQ	Adiponectin, C1Q and collagen domain containing	0.283	miR-103a-3p, miR-107
SIRT1	Sirtuin 1	0.282	miR-17-5p, let-7d-5p, miR-132-3p
NFE2L2	Nuclear factor, erythroid 2 like 2	0.281	-
PNPLA3	Patatin like phospholipase domain containing 3	0.231	miR-200b-3p, miR-29c-3p
TM6SF2	Transmembrane 6 superfamily member 2	0.205	-
PPARA	Peroxisome proliferator activated receptor α	0.205	miR-17-5p
SREBF1	Sterol regulatory element binding transcription factor 1	0.202	-
LEP	Leptin	0.202	miR-17-5p, miR-200b-3p, miR-132-3p, miR-150-5p
FGF21	Fibroblast growth factor 21	0.202	-
LDLR	Low density lipoprotein receptor	0.201	miR-27b-3p, miR-150-5p, miR-17-5p, miR-30a-5p

The 'Score' indicates the gene-disease association score: The score range from 0 to 1, and considers the number and type of sources, and the publications number supporting the association. miRNA/miR, microRNA; T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease.

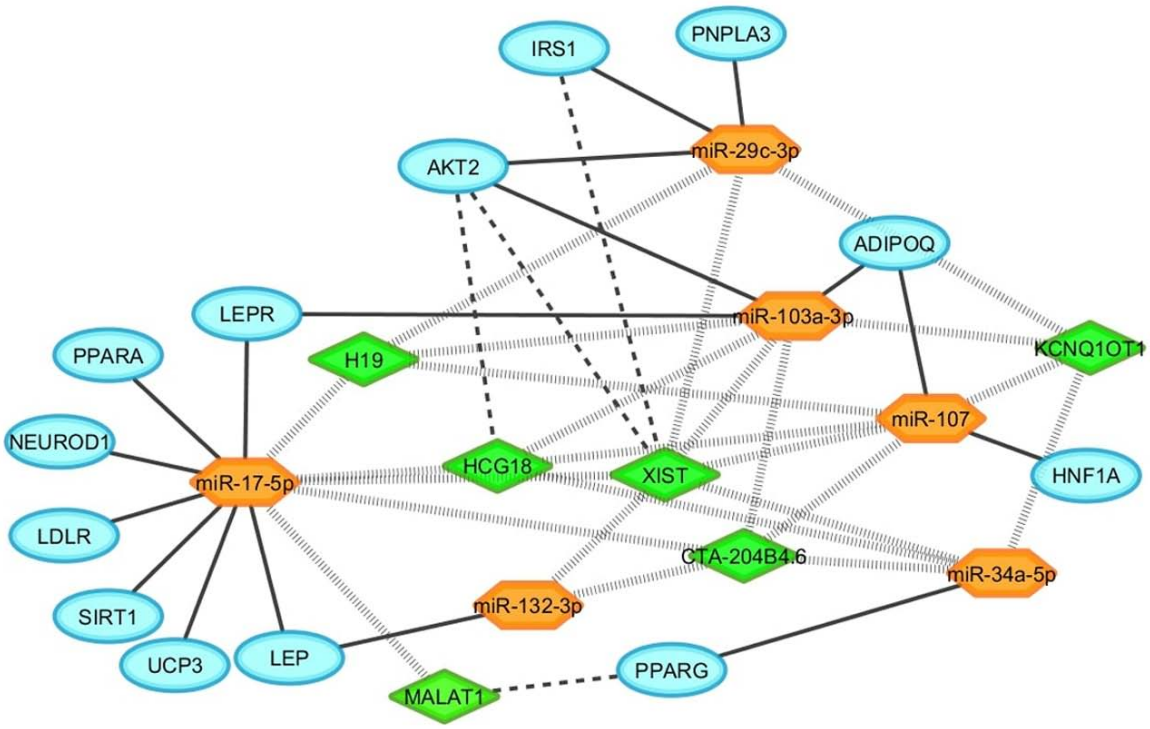

Figure 2. Interactions of 6 miRNAs with candidate genes and with lncRNAs in the context of metabolic diseases. The miRNAs are presented as hexagons, the target genes as circles, and the lncRNAs as diamonds. Solid lines represent the miRNAs-mRNAs interactions; vertical dashed-lines indicate the miRNAs-lncRNAs interactions, and dotted lines represent the lncRNAs-mRNAs interactions. miRNAs/miRs, microRNAs; lncRNAs, long non-coding RNAs.

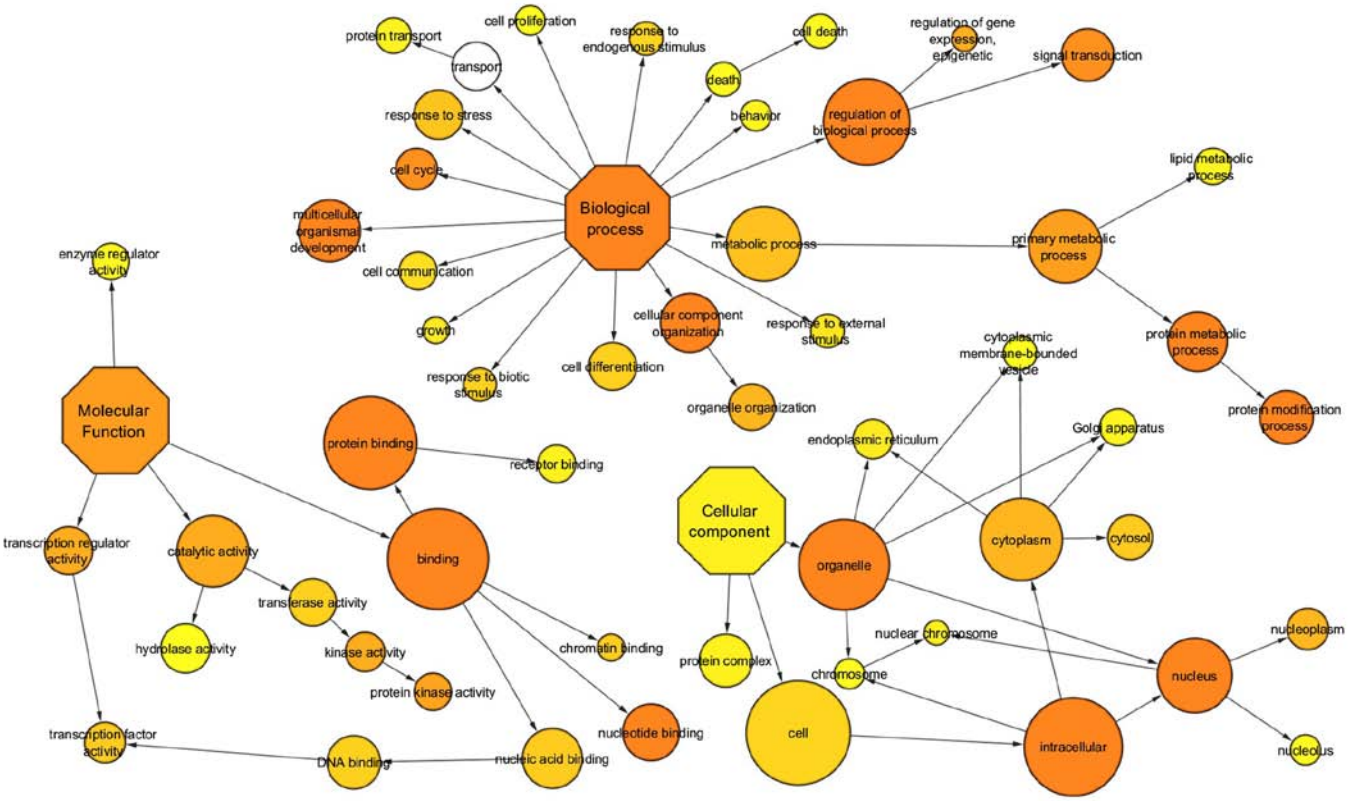

Figure 3. Enrichment pathways analysis. Gene ontology categories in biological networks of the validated and predicted target genes of the analyzed miRNAs. Data was taken from the Gene Ontology database.

to metabolic diseases, with 6 lncRNAs, and with 7 small molecules. Moreover, the functional enrichment analysis of miRNAs target genes reflected the complex biological behavior of metabolic diseases, being associated with multiple signaling

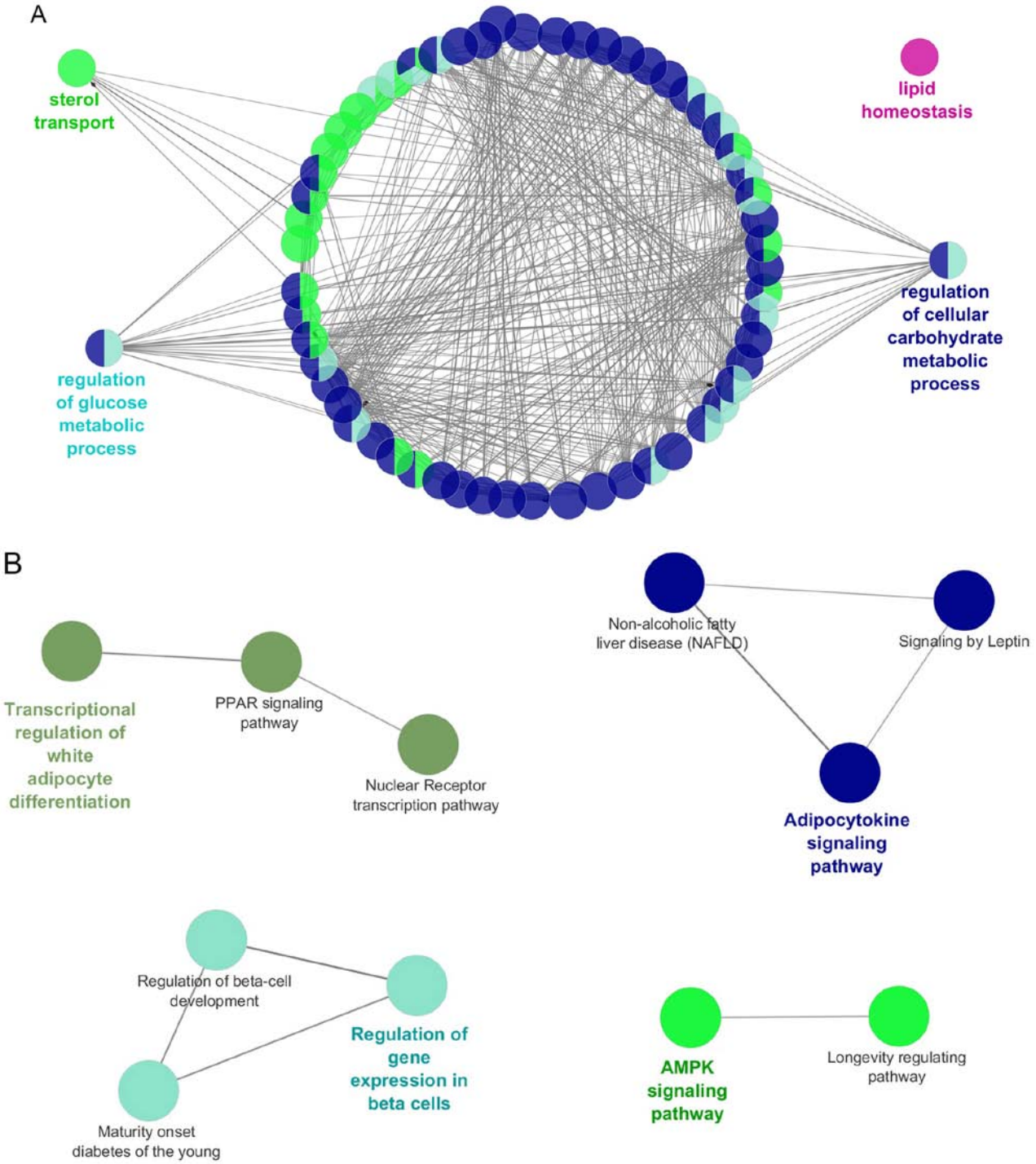

Figure 4. Enrichment pathways analysis for the candidate gene targets by the selected microRNAs. (A) GO terms according to functional cluster. (B) KEGG pathways in which the candidate genes participate. Data was taken from the GO and KEGG databases. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

pathways. In general, miRNAs are related to several human diseases (50). It is a well-known phenomenon that miRNAs show cooperativity in gene regulation, i.e. one miRNA binds with many target genes and one target gene is regulated by many miRNAs [reviewed at (50)].

Among these 6 miRNAs, miR-17-5p demonstrated the highest degree of connectivity in the present study. Several reports have linked miR-17-5p expression levels with metabolic diseases (51-53). Thus, Klöting *et al* (52) reported a significantly lower expression of miR-17-5p in the omental adipose tissue of T2DM patients compared to normal glucose tolerance (NGT) subjects and a negative correlation with visceral fat area. Also, Heneghan *et al* (54) showed a decrease in miR-17-5p expression in human omental adipose tissue and blood from obese patients. Additionally, the expression of miR-17-5p was upregulated in plasma of T2DM with NAFLD compared to those without NAFLD (51). Contrarily, miR-17-5p expression was found to be increased in skeletal muscle of T2DM rats, along with marked downregulation of GLUT4 protein level, and the miR-17 knockdown ameliorated glucose metabolism,

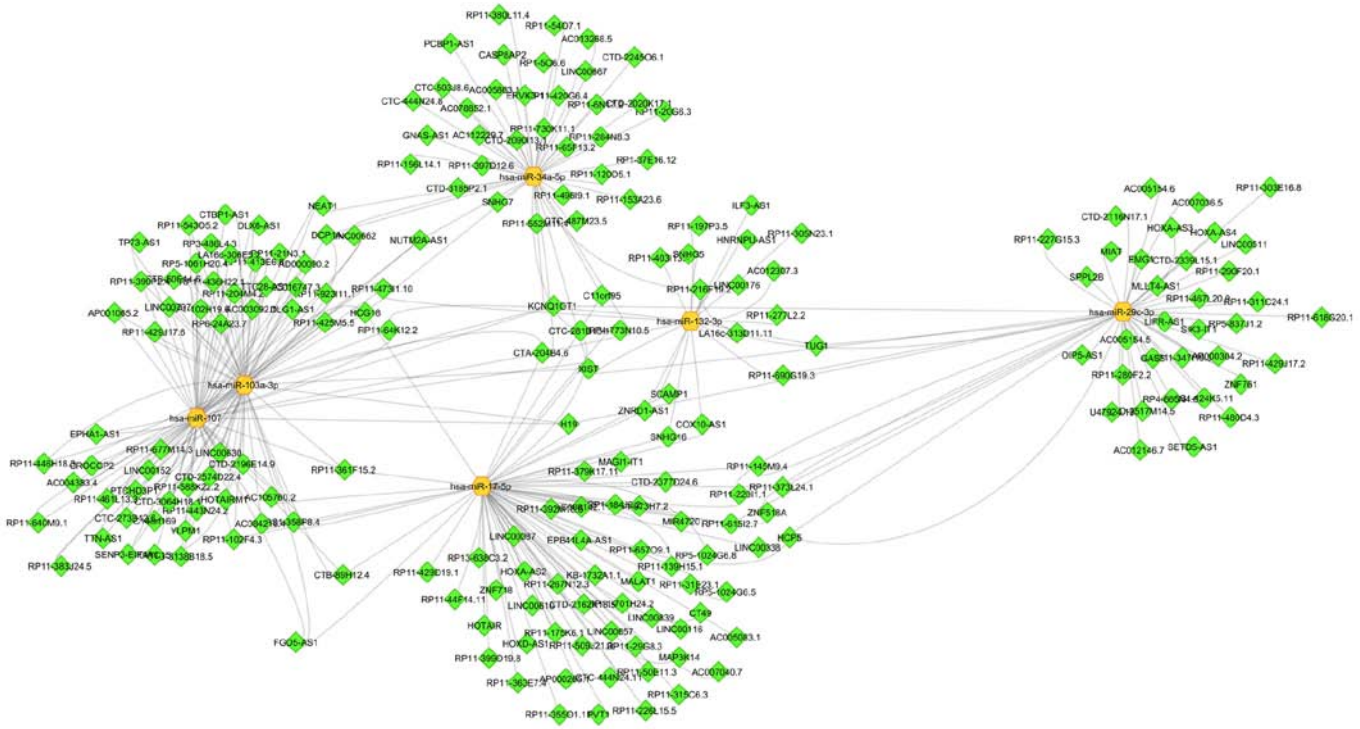

Figure 5. Interactions between miRNAs-lncRNAs. LncRNAs that interact with the 6 selected miRNAs. The miRNAs are shown as hexagons and the lncRNAs as diamonds. Data have been taken from the miRNet web tool. miRNAs, microRNAs; lncRNAs, long non-coding RNAs.

accompanied by elevation of GLUT4 protein level (53). Moreover, miR-17-5p was reported to be involved in the adipogenesis process in human adipose-derived mesenchymal stem cell (55). The miR-17-5p mimic transfection resulted in enhanced adipogenesis via repression of bone morphogenetic protein 2 (BMP2) and increased CCAAT/enhancer-binding protein α and peroxisome proliferator-activated receptor γ expression (55).

miR-103a-3p and miR-107 belong to the same cluster of miRNAs that also contains miR-15a/b, miR-16, miR-195, miR-497, miR-503, miR-424, and miR-646 (56). miR-103a-3p and miR-107 have been shown to be upregulated in the liver of T2DM patients (16), acting in the insulin signaling pathway by primarily targeting caveolin-1, which is located in lipid rafts and affects insulin receptor viability (57). Hence, the silence these two miRNAs in mice improved glucose homeostasis and insulin sensitivity (57). miR-107 is a lipid-modulated miRNA involved in modifications of the circadian system (58); interestingly, it has been reported that gut microbiota may be involved in the regulation of intestinal miR-107 levels (59). Moreover, some of the effects of miR-103a-3p and miR-107 might be mediated through other miRNAs since they strongly inhibit the miRNA-processing enzyme Dicer (60).

The miR-29 family is among the most abundantly expressed miRNA in the pancreas and liver in mice and humans (61). Moreover, the miR-29 family has been reported as a critical regulator of cholesterol turnover, fatty acid synthesis, and glucose handling (61,62). The knockdown of miR-29 family members (miR-29a, b and c) in a murine model led to a significant reduction of cholesterol and triglyceride plasma levels, reduced fatty acid content in the liver, and increased gene and protein expression levels of Ahr, Foxo3, and Sirt1 (62). Moreover, miR-29c-3p expression was increased in skeletal muscle from T2DM patients and decreased in healthy young men following exercise training. In addition to reduced IRS1 protein abundance, miR-29c-3p also decreased insulin signaling downstream of PI3K at the level of Akt and GSK3 phosphorylation in human skeletal muscle cells (63).

The main functions described for miR-34a include cell cycle arrest, apoptosis, and senescence promotion (64). Furthermore, a meta-analysis of profiling studies found that miR-34a was upregulated in T2DM patients (16). Also, the expression of this miRNA in subcutaneous fat tissue significantly correlated with BMI (kg/m^2) values (52). Similar results were found in ob/ob mice with NAFLD compared to their corresponding controls (65). Moreover, the exposure to perfluorononanoic acid (an organic pollutant with toxicological impact on the liver) induced hepatic miR-34a expression in mice (66,67).

miR-132 expression was upregulated in both blood and liver of T2DM patients (16). miR-132 targets insulin-mediated regulation of CYP2E1 (cytochrome P450, family 2, subfamily E, polypeptide 1), which is involved in the metabolism of xenobiotics in the liver (68). In omental fat, the expression levels of miR-132-3p were decreased in T2DM patients compared to NGT subjects and the number of macrophages infiltrating the fat depot was significantly associated with miR-132 expression (52).

Besides indicating a group of miRNAs associated with metabolic diseases, the present study also provides new insights into the complex molecular mechanisms involved in metabolic diseases by revealing some pathways that may be regulated by the selected miRNAs. These miRNAs potentially control genes from several important processes, including cancer, apoptosis, transcriptional regulation of white

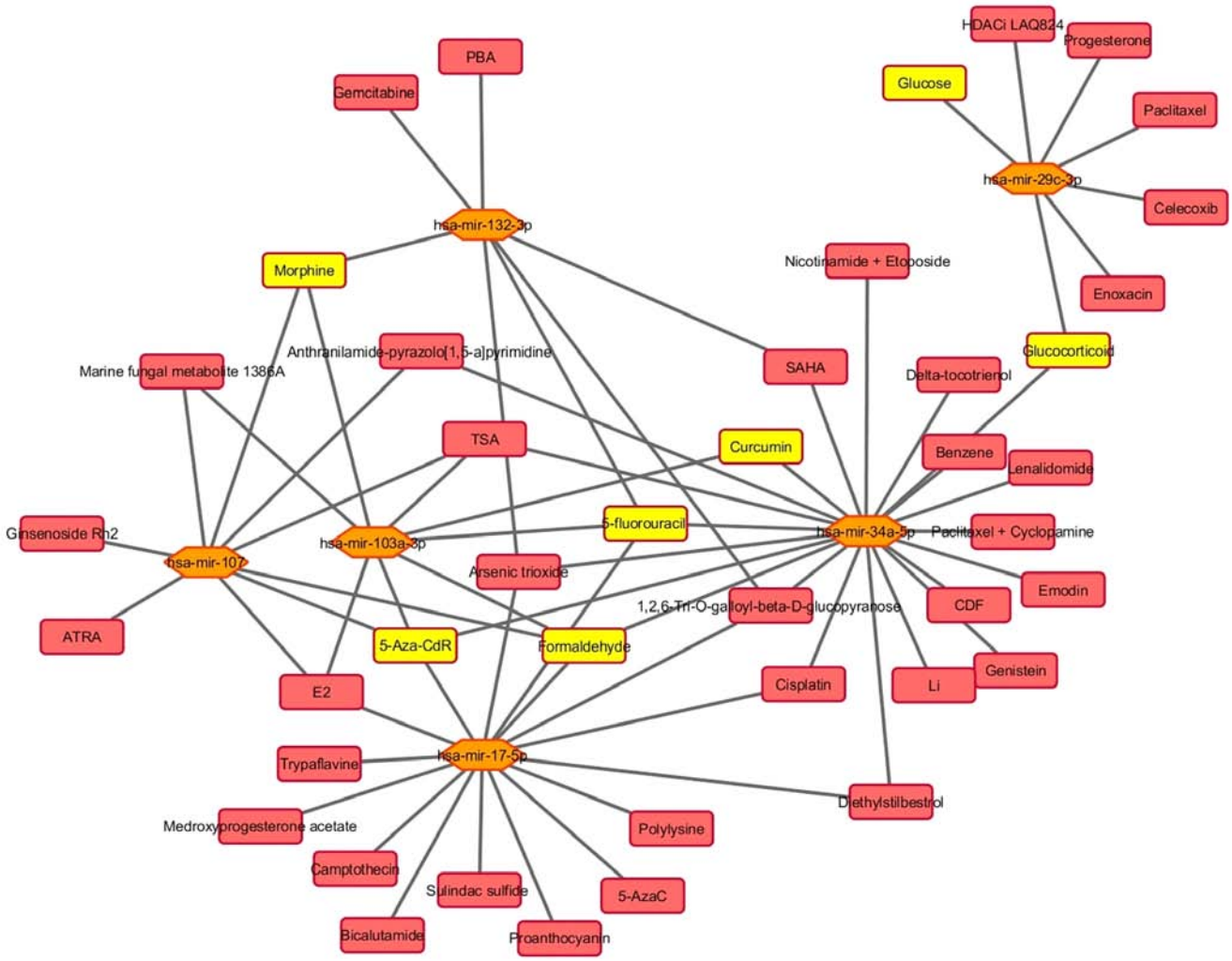

Figure 6. Interplay between miRNAs-small molecules. All small molecules that interact with the 6 selected miRNAs. The miRNAs are shown as hexagons and the small molecules as rectangles. Data were taken from the miRNet web tool. miRNAs, microRNAs.

adipocyte differentiation, regulation of gene expression in β cells, AMPK, and adipocytokine signaling pathways.

Additionally, these six miRNAs have target genes previously associated with metabolic diseases, indicating that the differential expression of this set of miRNAs could lead to metabolic diseases via dysregulation of metabolism-associated genes, including *PPARG*, *LDLR*, *SIRT1* and *NEUROD1*. In this sense, *PPARG* gene regulates fatty acid storage and glucose metabolism and has been implicated in the pathophysiology of several diseases, including obesity, T2DM, atherosclerosis, and cancer [reviewed at (69)]. The *LDLR* gene mediates the endocytosis of LDL-cholesterol, contributing to maintain the LDL plasma levels (70). *SIRT1* gene is downregulated in cells that have high insulin resistance and the overexpression of SIRT1 increases insulin sensitivity (71,72). In the same way, *NEUROD1* gene is related to increased expression of glucokinase, suggesting that this gene may play important roles in the regulation of insulin synthesis and secretion (73). Moreover, *NeuroD1* is required for normal development and maintenance of pancreatic endocrine cells and the nervous system (74).

An increasing number of publications demonstrate that miRNAs interact with lncRNAs, thereby triggering decay of lncRNA or repressing its function (18,19). Thus, it was reasonable to investigate the pathogenesis and treatment of metabolic diseases by studying the specific miRNA-lncRNA co-regulation effect. In the present study, lncRNA-XIST was found to interact with all 6 miRNAs and some other genes associated with metabolic diseases, suggesting that this lncRNA may have a physiopathological role in these diseases. In this context, lncRNA-XIST was increased in patients with T2DM compared to controls, and its expression positivity correlated with HbA1c, HOMA-IR, and fasting insulin levels (25). Based on online biology websites, we found that miR-17-5p may be targeted by lncRNA-XIST, and a study carried out on a linage of cancer cells (NSCLC) suggested that lncRNA-XIST may regulate autophagy via the miR-17/ATG7 signaling pathway (75).

It is known that the subcellular location of ncRNAs, especially lncRNAs, correlated with functionality, which could influence disease susceptibility; however, the location of ncRNAs is still controversial and little is known regarding metabolic diseases (76). LncRNA transcripts can be found in many different sites within the cell, including the chromatin, nucleus, cytoplasm, and exosomes (19,76). LncRNA subcellular location is likely dependent on several factors, such as sequence and structural motifs which can facilitate binding to proteins involved in location (77).

Small molecules can regulate multiple biological processes and have been proposed and used for therapeutic purpose in different human diseases (78). Recently, several drug-like compound libraries were screened successfully against different miRNAs in cellular assays demonstrating the possibility to target miRNAs with small molecules (79). The present article evidenced that some molecules can modulate miRNA expression, and this could be a way to indirectly regulate gene expression.

Some strengths and limitations of our study should be considered. As strengths, a comprehensive search of multiple databases was conducted. Additionally, we performed robust bioinformatic analyses to investigate the pathways in which these miRNAs are participating, explaining the association with metabolic diseases. Even though these methods are already powerful, this evaluation had some limitations. First, the results are based on the available literature about this topic. Second, we could not exclude the possibility that other miRNAs should be associated with the metabolic disorders; moreover, the results found in the online databases change over time. Third, the lack of standardization of the official nomenclature of miRNAs, without the description of which miRNA straight was analyzed (-3p or -5p). Fourth, this is an association study and because of that we could not describe the events order. These limitations should be considered when interpreting the results. Although limitations exist in the current data, the patterns uncovered here are important for understanding the association of miRNAs and metabolic diseases, and for identifying new miRNAs, pathways and target genes putatively involved in disease onset and progression.

Taken together, the present analyses demonstrate that the molecular mechanisms of metabolic diseases can be understood, and that biomarker prediction can be achieved through data mining and integration analysis. Overall, 20 candidate miRNAs were screened by bioinformatics analysis, and 6 of them (miR-17-5p, miR-29c-3p, miR-34a-5p, miR-103a-3p, miR-107 and miR-132-3p) presented the strongest association with metabolic diseases. The construction of miRNA-mRNA, miRNA-lncRNA and miRNA-small molecules networks provides a novel approach to the study of the metabolic disease pathogenesis and establishes solid knowledge for the personalized treatment of these disorders in the future. However, more studies are needed to validate these results.

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Availability of data and materials

The datasets analyzed during the current study are available in the HMDD v3.0 (www.cuilab.cn/hmdd), miR2Disease (www.miR2Disease.org), DisGeNET v5.0 (www.disgenet.org/), MDHGI (chengroup.cumt.edu.cn/tool/mdhgi/), and miRNet (www.mirnet.ca/) databases, which were accessed in December 2018.

Authors' contributions

TSA designed the study, analyzed and interpreted the data, and drafted the manuscript. FIM interpreted the data and critically reviewed the manuscript. JAM designed and supervised the study, interpreted the data and critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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