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Bioinformatic identification of signature miRNAs associated with fetoplacental vascular dysfunction in gestational diabetes mellitus

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ABSTRACT

Background: Intrauterine exposure to gestational diabetes mellitus (GDM) poses significant risks to fetal development and future metabolic health. Despite its clinical importance, the role of microRNAs (miRNAs) in fetoplacental vascular endothelial cell (VEC) programming in the context of GDM remains elusive. This study aims to identify signature miRNA genes involved in this process using bioinformatics analysis via multiple algorithms.
Methods: The dataset used in this study was acquired from Gene Expression Omnibus (GEO). Firstly, differentially expressed miRNA genes (DEMGs) were evaluated using limma package. Thereafter, an enrichment analysis of DEMGs was performed. Then, the least absolute shrinkage and selection operator (LASSO) and support vector machine (SVM) were used as the other algorithms for screening candidate signature miRNA genes. Genes from the intersection of limma, LASSO, and SVM genes were used as the final signature miRNA genes. The receiver operator characteristic curve (ROC), the nomogram diagram, gene set enrichment analysis (GSEA), and signature miRNAs-target genes interaction network were implemented further to explore the features and functions of signature genes.
Results: A total of 32 DEMGs, with 21 upregulated and 11 downregulated miRNA genes, were obtained from limma analysis. LASSO and SVM analyses identified 15 and 12 candidate signature miRNA genes, respectively. After the intersection of genes from limma, LASSO, and SVM analyses, MIR34A and MIR186 were found as the final signature genes related to fetoplacental VEC programming. MIR34A and MIR186 were highly expressed and were associated with an increased risk of fetoplacental VEC programming in GDM mothers. The area under the curve (AUC) of ROC for MIR34A and MIR186 were 0.960 and 0.935, respectively. GSEA analysis revealed that these signature genes positively participate in cellular processes related to VEC migration, cell differentiation, angiogenesis, programmed cell death, and inflammatory response. Finally, miRNAs-target genes interaction network analysis provides the interaction of signature miRNAs and their critical target genes, which may help further studies for miR-34a and miR-186 in GDM.
Conclusions: MIR34A and MIR186 are novel signature miRNA genes related to fetoplacental VEC programming that may represent critical genes associated with placental function and fetal programming under GDM conditions.

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1. Introduction

GDM is a metabolic disorder characterized by hyperglycemia during pregnancy, affecting approximately 6–9% of pregnancies worldwide [1]. Intrauterine exposure to GDM has been associated with adverse outcomes for both the mother and the fetus, including increased risk of macrosomia, neonatal hypoglycemia, and long-term metabolic complications for the offspring [2,3]. Understanding the molecular mechanisms underlying these adverse effects is crucial for the development of targeted interventions to improve maternal and fetal outcomes.

Fetoplacental VECs serve as a good target for studying the interaction between maternal diseases and fetal programming since they are exposed directly to the maternal environment and are responsible for nutrient transport from the mother to the fetus [4]. Fetoplacental VEC dysfunction secondary to maternal diseases, therefore, may influence fetal development and health later in life [5,6]. Previous studies regarding maternal GDM on fetoplacental VEC programming mostly focus on changes in protein expression genes; however, few studies focus on the role of maternal GDM on fetoplacental VEC programming from the perspective of miRNAs.

miRNAs are a group of small, non-coding RNAs that play key roles in the post-transcriptional regulation of gene expression [7]. Recently, the role of miRNAs in the pathogenesis and gestational outcomes in GDM has aroused wide concern. Evidence showed that miRNAs may participate in glucose metabolism in GDM [8,9]. Moreover, it was suggested that miRNAs may also play crucial roles in regulating insulin resistance and inflammation processes in GDM [10]. Additionally, increasing evidence showed that specific changes in circulating miRNAs may serve as good biomarkers for the diagnosis and predicting adverse maternal and offspring outcomes in GDM [11,12]. Nowadays, the specific miRNAs involved in fetoplacental VEC programming in GDM mothers remain poorly understood. Recent advancements in bioinformatics have provided powerful tools for the analysis of complex biological datasets, allowing researchers to uncover novel molecular pathways and regulatory networks involved in disease pathogenesis [13,14]. In the context of GDM, bioinformatics analysis offers a promising approach to elucidate the role of miRNAs in fetoplacental VEC programming, a process critical for placental development and function.

The identification of signature miRNA genes and their function analysis from fetoplacental VECs have the potential to provide insights into the molecular mechanisms underlying GDM-associated placental dysfunction and fetal programming. Furthermore, these miRNAs may serve as potential biomarkers for the early detection and risk stratification of GDM, as well as targets for the development of novel therapeutic interventions aimed at improving maternal and fetal outcomes in affected pregnancies. Therefore, this study aims to identify signature miRNA genes related to fetoplacental VECs programming under GDM conditions based on powerful bioinformatics algorithms.

2. Materials and methods

2.1. Data sources

The dataset GSE103552 (Platform: GPL6244; Affymetrix Human Gene 1.0 ST Array) was downloaded from GEO (https://www.ncbi.nlm.nih.gov/geo/). In this dataset, a total of 37 primary human fetoplacental endothelial cells from arteries and veins were isolated after a healthy pregnancy (8 arteries, 8 veins) and after a pregnancy complicated by

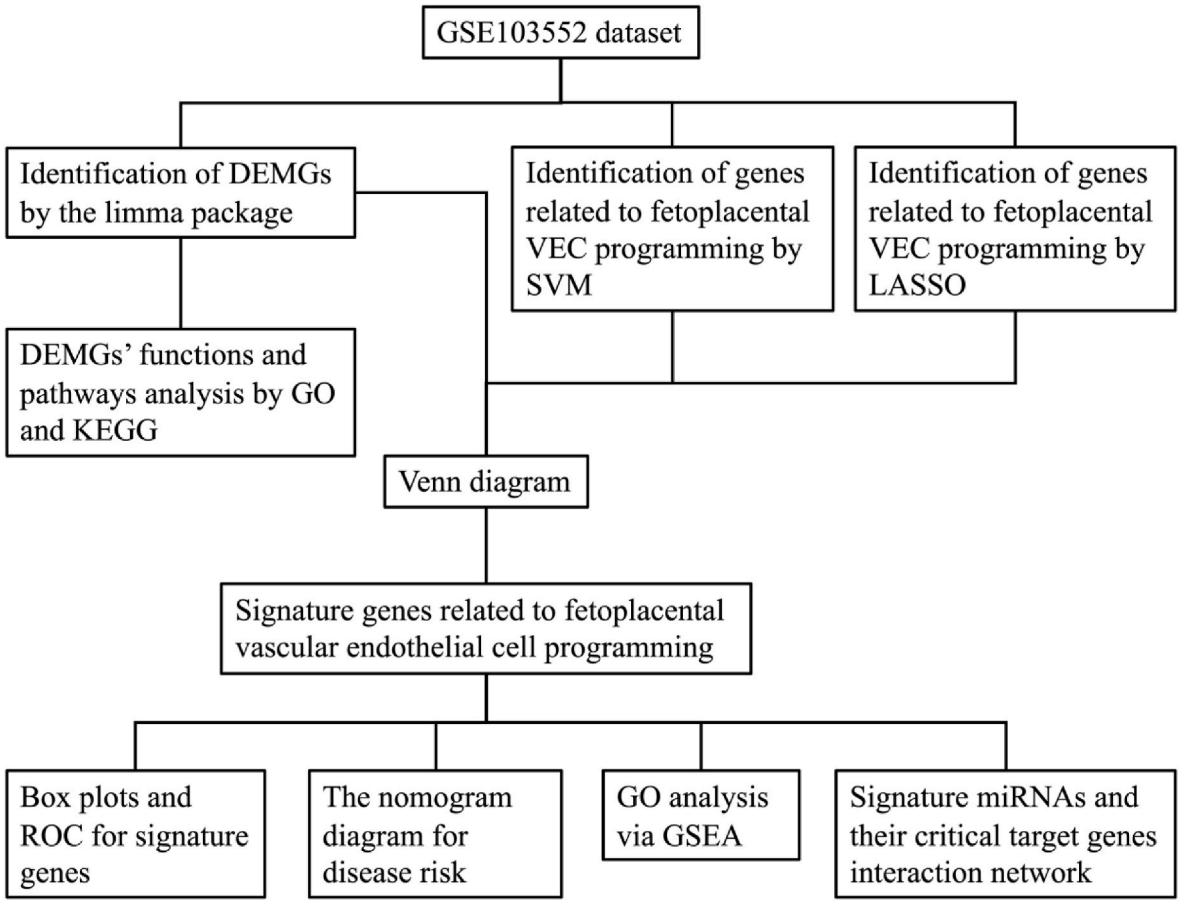

Fig. 1. The study protocol. DEMGs: Differentially expressed miRNA genes; SVM: Support vector machine; LASSO: Least absolute shrinkage and selection operator; GO: gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; ROC: receiver operating characteristic; GSEA: Gene set enrichment analysis.

GDM (11 arteries, 10 veins). The flow chart of the study is shown in Fig. 1.

2.2. Identification of differentially expressed miRNA genes by the limma package

The differentially expressed miRNA genes (DEMGs) were screened by using the limma package [15], with criteria as listed below: Adjusted p-value less than 0.05 and |log fold change (FC)| larger than 0.3. The results of DEMGs were generated and displayed using a volcano plot. Additionally, the DEMGs were also shown by heatmap in terms of up-regulated and down-regulated.

2.3. Enrichment analysis of the DEMGs via GO and KEGG

The enrichment analysis of DEMGs was evaluated by the org.Hs.eg.db, enrichplot, ggplot2, and clusterProfiler packages on account of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) [16]. Three categories, biological process (BP), cellular component (CC), and molecular function (MF), were used to explore the biological functions of the identified DEMGs. KEGG was used to study the signaling pathways in which DEMGs may participate.

2.4. Identification of signature miRNA genes by intersecting genes from multiple bioinformatic algorithms

The LASSO and SVM analyses were used to further screening of miRNA genes related to fetoplacental VEC programming in GDM. The LASSO analysis was performed by the glmnet R package [17]. The SVM analysis was achieved by the e1071 package. Genes from the intersection of genes of limma, LASSO, and SVM analyses were used as the final signature miRNA genes related to fetoplacental VEC programming. The AUC from the ROC was used to evaluate the representativeness of identified signature miRNAs with fetoplacental VEC programming. An AUC larger than 0.80 was used to class diagnostic performance.

2.5. Multi-factor regression analysis for predicting adverse fetoplacental VEC programming based on signature genes

Multi-factor regression analysis was used to generate a nomogram diagram to evaluate the contribution of signature genes to adverse fetoplacental VEC progrmaming. Briefly, the rms and rmda packages were used to perform multi-factor regression analysis based on signature genes and their expression data in the normal pregnancy and GDM groups.

2.6. GSEA analysis of the signature miRNA genes

GO analysis was carried out to evaluate the role of signature genes on account of biological processes (BP), cellular components (CC), and molecular functions (MF). The packages used during the analyses included limma, org.Hs.eg.db, clusterProfiler, and enrichplot. BP, CC, and MF, which have a p-value of less than 0.05, were included. The top five BP, CC, and MF enriched in the low and high expression groups in the GO analysis for the signature genes were shown in the figure based on their score ranking.

2.7. Construction of miRNAs-target genes interaction network

We further predicted the target genes of these signature miRNAs and constructed their interaction network, aiming to provide more comprehensive functional information for the signature miRNAs. We used the miRWalk (http://mirwalk.umm.uni-heidelberg.de/) to predict their target genes by searching the official names of miRNAs (hsa-miR-34a-3p, hsa-miR-34a-5p, hsa-miR-186–3p, and hsa-miR-186–5p) on the website and exported the CSV workbooks. The final critical target genes for signature miRNAs were from the intersection of the differentially expressed genes (DEGs) from the GSE103552 dataset (Screening criterion: $|\log FC|>0.7$ and adjust $p < 0.05$) and their correlated predicted target genes. The interaction network was constructed using Cytoscape software (Version: 3.10.1).

2.8. Statistical analysis

Statistical analyses in this study were carried out using R software (Version 4.3.1). A p-value less than 0.05 was used as statistically significant.

3. Results

3.1. Identification of DEMGs in fetoplacental VECs from normal pregnant and GDM mothers

32 DEMGs were identified, of which, 21 of them were up-regulated while 11 were down-regulated (Fig. 2A). The heatmap demonstrated the up-regulated and down-regulated DEMGs in the VECs of normal pregnant and GDM mothers (Fig. 2B). All DEMGs are listed in Table 1.

3.2. Results for GO and KEGG analyses of the DEMGs

The GO analysis consists of three categories: BP, CC, and MF. As is shown in Fig. 3A, the top three enriched BP are negative regulation of amide metabolic process, negative regulation of cellular macromolecule biosynthetic process, and miRNA-mediated gene silencing by inhibition of translation, respectively. In the CC analysis, RISC complex, RNAi effector complex, and blood microparticle appeared to be the top three locations. The KEGG analysis revealed that these DEMGs are involved in cancer pathologies (Fig. 3B). The details of the results for the GO and KEGG analyses are shown in Supplementary Table 1.

3.3. Signature miRNA genes related to fetoplacental VEC programming in mothers with GDM

A total of 15 candidate signature miRNA genes were obtained from LASSO analysis (Fig. 4A and B, and Table 2). For the SVM analysis, 12 candidate signature miRNA genes were found (Fig. 4C and D). Finally, after intersecting genes from limma, LASSO, and SVM, two miRNA genes, MIR34A and MIR186, were found as final signature genes (Fig. 4E).

3.4. Relative expression and diagnostic performance of the signature miRNA genes in the fetoplacental VECs of mothers with normal pregnancy and GDM

The expression of MIR34A and MIR186 genes was significantly increased in the fetoplacental VECs of mothers with GDM compared to those with normal pregnancy (Fig. 5A and B). Moreover, the AUC of MIR34A and MIR186 in ROC were 0.960 and 0.935, respectively (Fig. 5C and D). Additionally, the AUC by combining MIR34A and MIR186 in ROC was 0.964. These results indicate that MIR34A and MIR186 have a relatively good value in representing fetoplacental VEC programming under GDM conditions.

3.5. MIR34A and MIR186 are associated with an increased risk of adverse fetoplacental VEC programming in mothers with GDM

The calibration curve of the signature genes in multi-factor regression analysis showed that the predicted probability was consistent with the actual probability (Fig. 6A). This result suggested that the expression change of the signature miRNA genes is effective in predicting the outcomes of GDM. The nomogram plot shows that the high expression of MIR34A and MIR186 is associated with an increased risk of

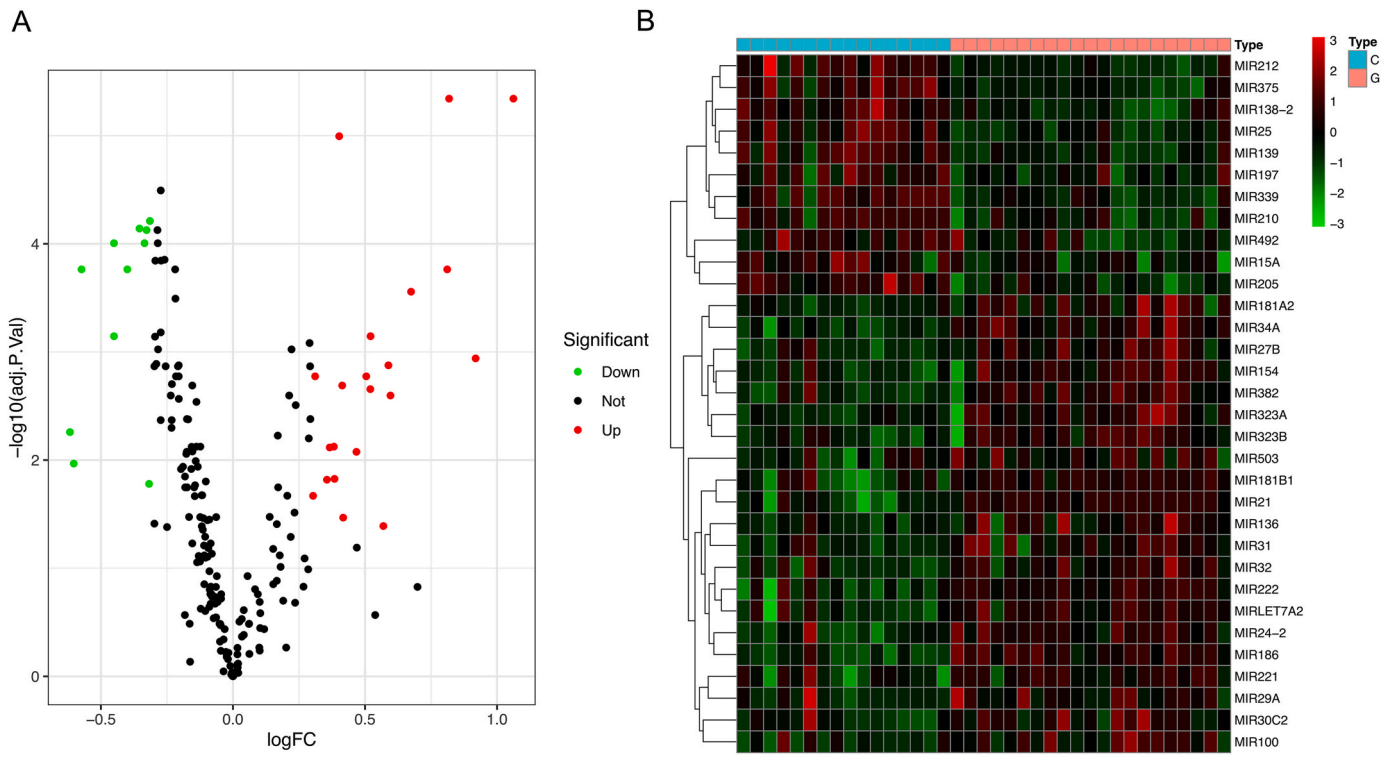

Fig. 2. Identification of the DEMGs between normal pregnant and GDM mothers by the limma package. (A) Volcano showed the expression of DEMGs. (B) The heatmap of the DEMGs in normal and gestational diabetes mothers. In Fig. 2B, C means normal control while G means gestational diabetes. DEMGs: Differentially expressed miRNA genes; GDM: Gestational diabetes mellitus.

Table 1
Differentially expressed miRNA genes identified from the GSE103552 dataset.

Genes	logFC	t	p-value	Adj p-value
MIR186	1.062	6.950	3.390E-08	4.550E-06
MIR21	0.818	6.860	4.480E-08	4.550E-06
MIR34A	0.402	6.468	1.500E-07	1.010E-05
MIR205	−0.314	−5.723	1.520E-06	6.150E-05
MIR212	−0.354	−5.613	2.130E-06	7.220E-05
MIR138-2	−0.327	−5.551	2.590E-06	7.470E-05
MIR375	−0.335	−5.367	4.580E-06	9.880E-05
MIR25	−0.451	−5.317	5.350E-06	9.880E-05
MIR222	0.811	4.991	1.470E-05	1.724E-04
MIR339	−0.573	−4.983	1.510E-05	1.724E-04
MIR139	−0.400	−4.978	1.530E-05	1.724E-04
MIR181B1	0.674	4.806	2.600E-05	2.773E-04
MIR210	−0.451	−4.446	7.760E-05	7.156E-04
MIR24-2	0.521	4.431	8.110E-05	7.156E-04
MIRLET7A2	0.919	4.208	1.583E-04	1.148E-03
MIR382	0.588	4.125	2.031E-04	1.330E-03
MIR27B	0.505	3.992	3.001E-04	1.681E-03
MIR323A	0.311	3.969	3.215E-04	1.681E-03
MIR323B	0.414	3.876	4.211E-04	2.044E-03
MIR154	0.520	3.840	4.679E-04	2.209E-03
MIR32	0.596	3.784	5.510E-04	2.529E-03
MIR492	−0.617	−3.426	1.521E-03	5.515E-03
MIR181A2	0.382	3.286	2.240E-03	7.514E-03
MIR136	0.366	3.264	2.374E-03	7.649E-03
MIR31	0.467	3.213	2.729E-03	8.393E-03
MIR15A	−0.603	−3.104	3.663E-03	1.078E-02
MIR100	0.384	2.949	5.514E-03	1.492E-02
MIR221	0.356	2.937	5.692E-03	1.520E-02
MIR197	−0.318	−2.893	6.379E-03	1.660E-02
MIR30C2	0.303	2.750	9.177E-03	2.141E-02
MIR29A	0.417	2.531	1.578E-02	3.407E-02
MIR503	0.569	2.430	2.008E-02	4.076E-02

Note: Adj: Adjusted; logFC: log fold change.

fetoplacental VEC programming in GDM mothers (Fig. 6B).

3.6. Results for the GO analysis of MIR34A and MIR186

In the GO analysis for MIR34A and MIR186, the top five BP, CC, and MF enriched in the low and high expression groups were listed based on their score ranking. All results of GO analysis for MIR34A and MIR186 are listed in Tables 3 and 4. The results showed that MIR34A is associated with cell cycle process, negative regulation of blood vessel endothelial cell migration, negative regulation of cell migration involved in sprouting angiogenesis, negative regulation of cellular response to growth factor stimulus, and negative regulation of epithelial cell migration in the low expression group (Fig. 7A). In the high expression group, MIR34A is associated with epithelial cell differentiation, interleukin 6 production, negative regulation of interleukin 6 production, regulation of epithelial cell differentiation, and tumor necrosis factor superfamily cytokine production (Fig. 7B). MIR186 is associated with amyloid precursor protein metabolic process, positive regulation of intracellular signal transduction, positive regulation of AMPK cascade, regulation of intracellular signal transduction, and regulation of sprouting angiogenesis (Fig. 7C).

3.7. The interaction network of hsa-miR-34a-3p, hsa-miR-34a-5p and hsa-miR-186–3p, and hsa-miR-186–5p with their critical target genes

The human MIR34A and MIR186 genes can be transcribed into hsa-miR-34a-3p, hsa-miR-34a-5p, hsa-miR-186–3p, and hsa-miR-186–5p, respectively. As predicted from miRWalk, 6431 target genes with 16408 binding regions were identified for hsa-miR-34a-3p. 16208 target genes with 64567 binding regions were identified for hsa-miR-34a-5p. 7804 target genes with 20395 binding regions were identified for hsa-miR-186–3p. 9316 target genes with 26936 binding regions were identified for hsa-miR-186–5p. A total of 105 DEGs were identified in the filter criteria of $|\log FC| > 0.7$ and adjusted $p < 0.05$ from the same dataset.

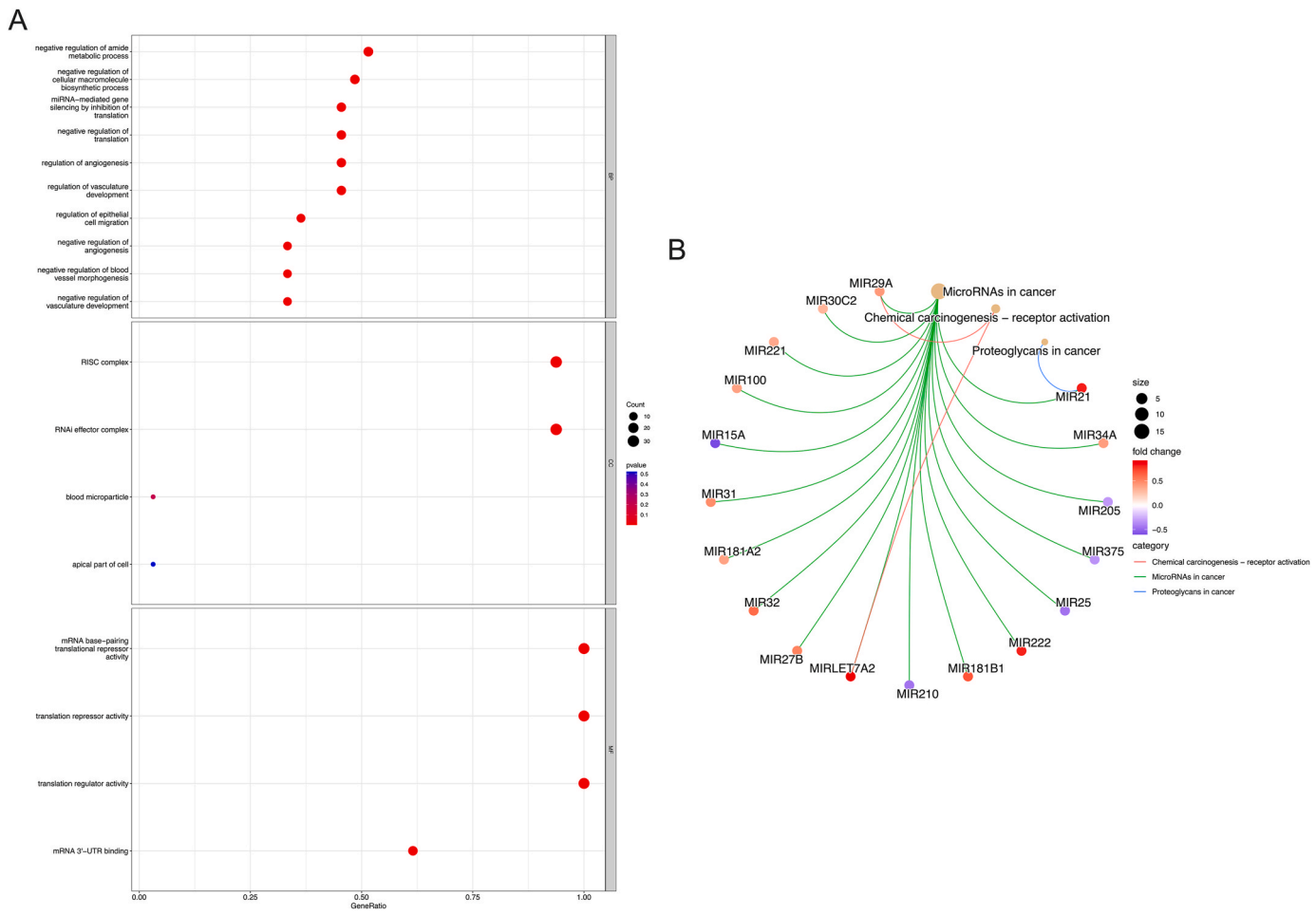

Fig. 3. Enrichment analysis of DEMGs via GO and KEGG. (A) GO analysis of DEMGs on account of BP, CC, and MF, (B) The KEGG analysis of DEMGs. DEMGs: Differentially expressed miRNA genes; GO: gene ontology; BP: Biological process; CC: Cellular component; MF: Molecular function; KEGG: Kyoto encyclopedia of genes and genomes.

Finally, 35, 76, 36, and 47 genes were found as critical target genes for hsa-miR-34a-3p, hsa-miR-34a-5p, hsa-miR-186–3p, and hsa-miR-186–5p, respectively after intersecting predicted target genes from miRWalk and DEGs from the same dataset as signature miRNA genes. The details of the predicted target genes (Supplementary Table 2-5) DEGs (Supplementary Table 6), and the intersection genes are shown in Table 5 and in the supplementary files of this article. The interaction network is shown in Fig. 8.

4. Discussion

The major findings of this study are summarized as follows: 1) We identified 32 DEMGs related to fetoplacental VEC programming, of which 21 were up-regulated while 11 were down-regulated. 2) GO and KEGG analyses of the 32 DEMGs were performed which may help in studying the association of these DEMGs and fetoplacental VEC programming under GDM background. 3) MIR34A and MIR186 genes were identified as signature genes related to adverse fetoplacental VEC programming in GDM when highly expressed. 4) GO analyses for the function of MIR34A and MIR186 were performed which may help future research regarding these signature genes and fetoplacental VEC programming in GDM. 5) We constructed the interaction network of hsa-miR-34a-3p, hsa-miR-34a-5p and hsa-miR-186–3p, and hsa-miR-186–5p with their critical target genes. This may help to uncover mechanisms in signature miRNAs-target proteins expression in GDM.

As a group of small, non-coding RNA, miRNAs play crucial roles in post-transcriptional regulation of gene expression [18]. miRNAs have also been reported to actively participate in the pathogenesis of GDM [19–22]. The dysregulation of miRNAs was previously used as diagnosis markers or candidates for therapeutic targets of GDM [23,24]. Abnormal expression of miRNA genes in the fetoplacental VECs in the context of GDM may impair placental development and function by regulating their target genes. Here, by employing multiple bioinformatic algorithms, we report for the first time on the effect of maternal GDM on fetoplacental VEC programming from the perspective of miRNAs via analyzed data from GEO. By implementing strict screening criteria, we identified 32 DEMGs, followed by an enrichment analysis of their functions and pathways. We found that their functions are enriched in the negative regulation of metabolic processes, cellular macromolecule biosynthetic, and gene expression. The placenta is a master regulator of nutrient supplementation and the intrauterine environment which are of vital importance in fetal development [25]. Therefore, dysregulation of the expression of these miRNA genes may affect placental function, resulting in fetal programming. KEGG analysis revealed that they may also participate in cancer pathogenesis. This is consistent with the previous reports that maternal GDM increases the risk of childhood cancers [26]. The results from our study, therefore, offer potential targets for miRNAs and childhood cancers in the context of GDM.

We then further screened the miRNA genes by intersecting genes from limma, LASSO, and SVM analysis and got the final signature miRNA genes, namely MIR34A and MIR186. MIR34A and MIR186 can be transcribed into miR-34a and miR-186, respectively. Here, we found that the expression of MIR34A and MIR186 were increased in the fetoplacental VECs of mothers with GDM compared to those from normal

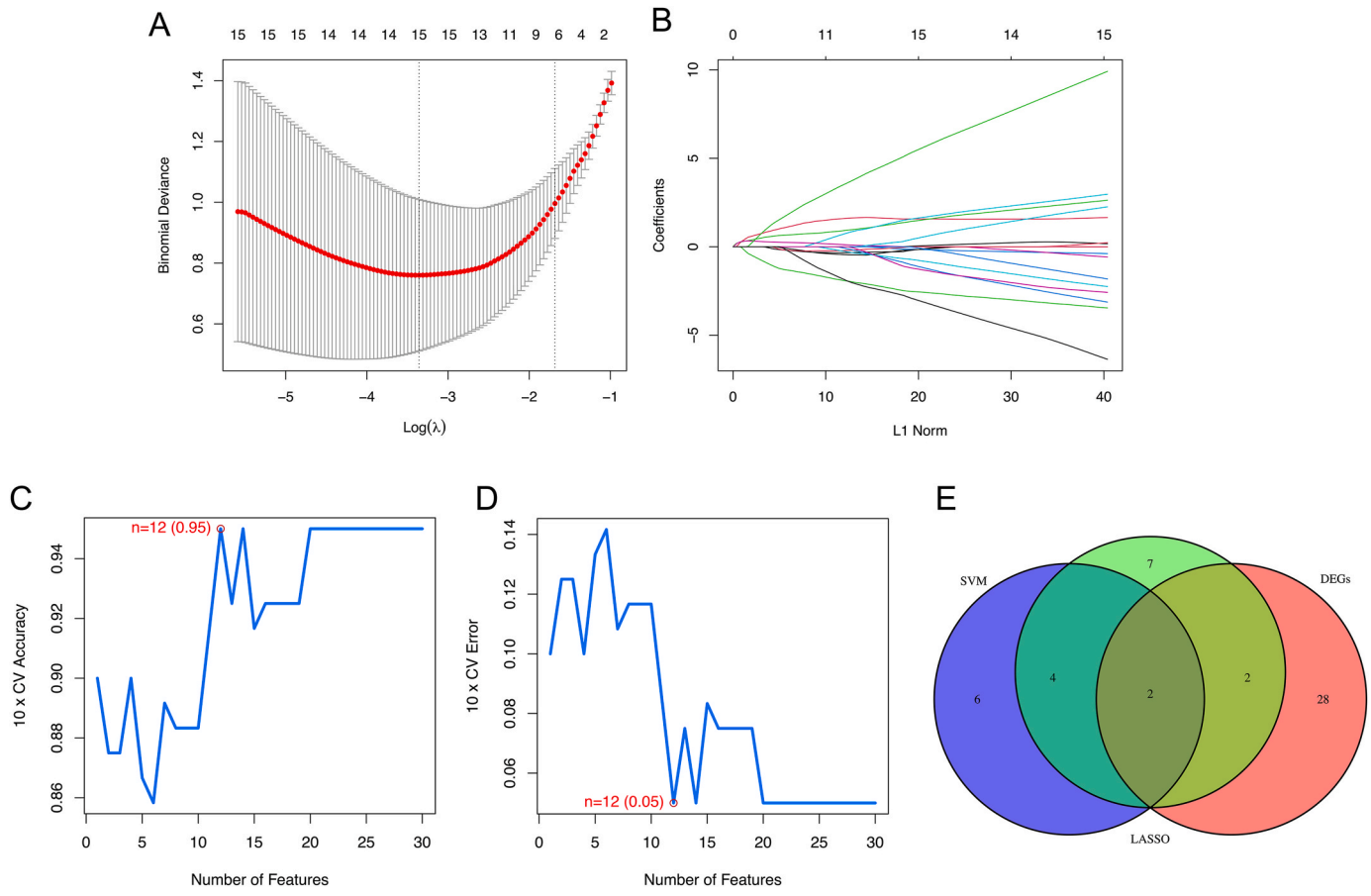

Fig. 4. Identification of the signature miRNA genes through multiple bioinformatic algorithms. (A) Penalty plot of the LASSO model with error bars denoting standard errors. (B) The LASSO coefficients for candidate signature miRNA genes associated with fetoplacental VEC programming in GDM, (C) The optimal accuracy value obtained by the SVM algorithm, (D) The lowest error rate determined by the SVM algorithm, (E) The interaction of the genes from limma, LASSO, and SVM analyses by Venn diagram. LASSO: Least absolute shrinkage and selection operator; VEC: vascular endothelial cell; GDM: Gestational diabetes mellitus; SVM: Support vector machine.

Table 2
Candidate signature genes identified from LASSO and SVM analyses.

Methods	Candidate signature miRNA genes
LASSO	MIR205, MIR34A, MIR186, MIR492, MIR423, MIR196A1, MIR133A1, MIRLET7E, MIR27A, MIR217, MIR99A, MIR93, MIR320A, MIR98, and MIR19B2
SVM	MIR186, MIR99A, MIR15B, MIR133A1, MIR150, MIR208A, MIR320C1, MIR34A, MIR423, MIR455, MIR33A, and MIR98

Note: LASSO: Least absolute shrinkage and selection operator; SVM: Support vector machine.

pregnancies, with an AUC higher than 90 representing good diagnostic values. It is, therefore, imperative to further uncover the mechanisms that miR-34a and miR-186 may participate in the fetoplacental VEC programming in GDM.

MiR-34a has been shown to play a crucial role in regulating VEC migration, inflammatory response, oxidative stress, and programmed cell death in previous studies. MicroRNA-34a-5p regulates the migration and invasion of VEC by targeting specific genes such as Smad4 [27]. In addition, the significance of miR-34a in inflammation is emphasized due to its ability to regulate immune responses, while its involvement in oxidative stress is evident through its impact on cellular redox balance [28]. Selective knockout of miR-34a in adipocytes can resist glucose intolerance, insulin resistance, and systemic inflammation caused by obesity, which is related to the polarization transition of adipose tissue resident macrophages from pro-inflammatory M1 type to anti-inflammatory M2 type. miR-34a inhibits M2 macrophage polarization and promotes inflammatory response, which can be effectively reversed by forcibly expressing Klf4 in bone marrow-derived macrophages and adipose tissue of obese mice [29]. In terms of placental function and fetal development, studies have shown that miR-34a affects the proliferation, migration, and invasion of trophoblast cells by targeting key genes, which is crucial for the normal development of the placenta [30]. Additionally, the expression of miR-34a is associated with the regulation of specific genes in the placenta, which are involved in angiogenesis and amino acid transport, both of which are crucial for fetal growth and placental function [31]. The role of miR-34a in pregnancy complications, especially in the context of GDM, is receiving increasing attention. Previous studies have shown that miR-34a is associated with the regulation of genes related to glucose metabolism and insulin signaling, suggesting that it may contribute to the development of GDM [32,33]. Furthermore, miR-34a, as a key epigenetic regulator, is likely to exert a significant influence on the long-term health outcomes for offspring of mothers with GDM by modulating DNA methylation and histone modification [34]. Consistent with previous studies, functional prediction in our study showed that the function of the MIR34A gene involves blood vessel endothelial cell migration, sprouting angiogenesis, mitogen-activated protein kinases (MAPK) cascade, and inflammatory cytokine production (Interleukin-6 and tumor necrosis factor superfamily cytokines). In summary, previous studies and our own research indicate that miR-34a may play a pivotal role in fetal-placental VEC programming and placental function and, subsequently, fetal programming and future risk of adverse

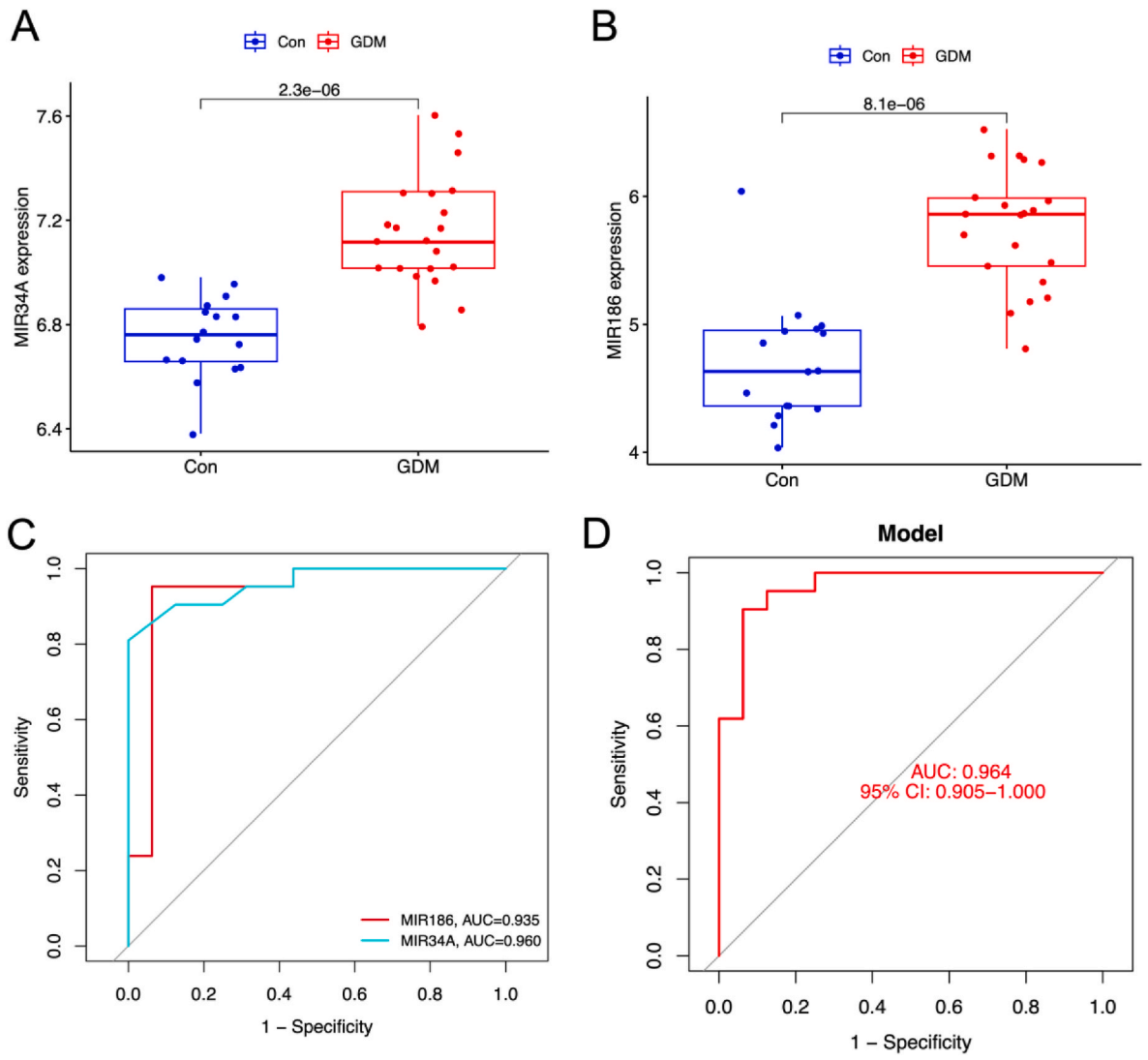

Fig. 5. The performance of the signature genes. (A–B) The expression of signature genes in the VECs between normal pregnancy and GDM mothers. (C–D) ROC curves for the diagnostic performance of the signature miRNAs. VEC: vascular endothelial cell; GDM: Gestational diabetes mellitus; ROC: receiver operating characteristic.

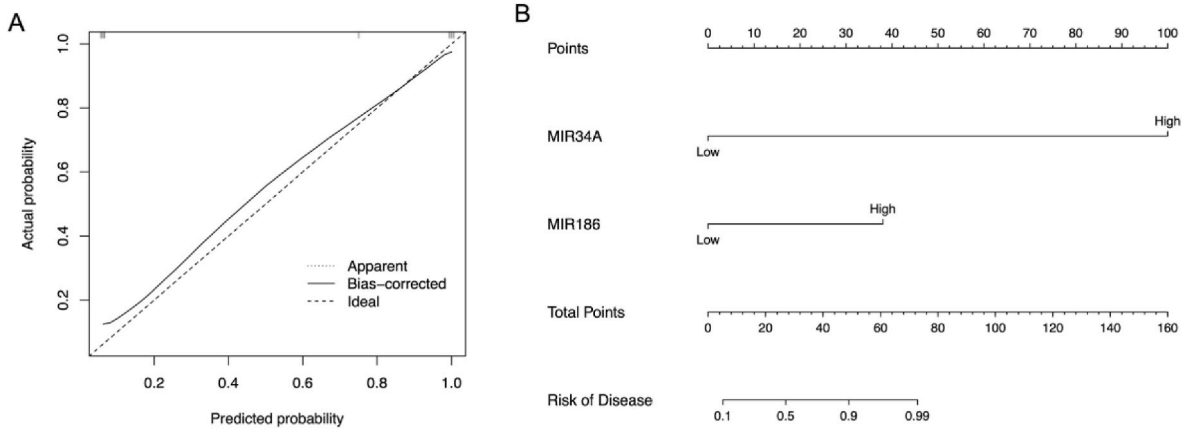

Fig. 6. Contribution of the signature miRNA genes to the risk of fetoplacental programming in mothers with GDM predicted by the nomogram diagram. (A) Calibration curve of the signature genes, (B) The nomogram of the signature genes.

Table 3
GO analysis for MIR34A.

ID	Enrichment Score	p-value	rank
GOBP_NEGATIVE_REGULATION_OF_BLOOD_VESSEL_ENDOTHELIAL_CELL_MIGRATION	−0.695	2.904E-4	40
GOBP_NEGATIVE_REGULATION_OF_EPITHELIAL_CELL_MIGRATION	−0.679	1.777E-4	40
GOBP_NEGATIVE_REGULATION_OF_CELL_MIGRATION_INVOLVED_IN_SPROUTING_ANGIOGENESIS	−0.760	3.412E-4	37
GOBP_NEGATIVE_REGULATION_OF_CELLULAR_RESPONSE_TO_GROWTH_FACTOR_STIMULUS	−0.717	0.005	45
GOBP_INTERLEUKIN_6_PRODUCTION	0.716	0.011	2
GOBP_TUMOR_NECROSIS_FACTOR_SUPERFAMILY_CYTOKINE_PRODUCTION	0.710	0.012	4
GOBP_NEGATIVE_REGULATION_OF_INTERLEUKIN_6_PRODUCTION	0.723	0.009	2
GOBP_CELL_CYCLE_PROCESS	−0.598	0.021	49
GOBP_POSITIVE_REGULATION_OF_EPITHELIAL_CELL_DIFFERENTIATION	0.719	0.030	8
GOBP_FAT_CELL_DIFFERENTIATION	0.716	0.032	4
GOBP_REGULATION_OF_FAT_CELL_DIFFERENTIATION	0.716	0.032	4
GOBP_ENDOTHELIAL_CELL_MIGRATION	−0.498	0.019	40
GOBP_REGULATION_OF_ENDOTHELIAL_CELL_MIGRATION	−0.498	0.019	40
GOBP_BLOOD_VESSEL_ENDOTHELIAL_CELL_MIGRATION	−0.490	0.021	40
GOBP_REGULATION_OF_BLOOD_VESSEL_ENDOTHELIAL_CELL_MIGRATION	−0.490	0.021	40
GOBP_REGULATION_OF_EPITHELIAL_CELL_MIGRATION	−0.476	0.028	40
GOBP_TISSUE_MIGRATION	−0.476	0.028	40
GOBP_AMEBOIDAL_TYPE_CELL_MIGRATION	−0.467	0.021	40
GOBP_NEGATIVE_REGULATION_OF_GENE_EXPRESSION	0.485	0.033	60
GOBP_POST_TRANSCRIPTIONAL_REGULATION_OF_GENE_EXPRESSION	0.485	0.033	60
GOBP_REGULATORY_NCRNA_MEDIATED_GENE_SILENCING	0.485	0.033	60
GOBP_POSITIVE_REGULATION_OF_MAPK_CASCADE	0.711	0.036	21
GOBP_NEGATIVE_REGULATION_OF_CELL_CYCLE_PROCESS	−0.614	0.045	49
GOCC_RIBONUCLEOPROTEIN_COMPLEX	0.408	0.046	35
GOCC_RNAI_EFFECTOR_COMPLEX	0.408	0.046	35

Table 4
GO analysis for MIR186.

ID	Enrichment Score	p-value	rank
GOBP_POSITIVE_REGULATION_OF_INTRACELLULAR_SIGNAL_TRANSDUCTION	0.656	0.008	35
GOBP_POSITIVE_REGULATION_OF_MAPK_CASCADE	0.772	0.010	41
GOBP_REGULATION_OF_SPROUTING_ANGIOGENESIS	0.678	0.008	30
GOBP_AMYLOID_PRECURSOR_PROTEIN_METABOLIC_PROCESS	0.646	0.017	9
GOBP_REGULATION_OF_INTRACELLULAR_SIGNAL_TRANSDUCTION	0.526	0.006	30
GOBP_PHOSPHATIDYLINOSITOL_3_KINASE_PROTEIN_KINASE_B_SIGNAL_TRANSDUCTION	0.588	0.042	13
GOBP_AMYLOID_BETA_METABOLIC_PROCESS	0.707	0.039	9
GOBP_POSITIVE_REGULATION_OF_CELL_MIGRATION_INVOLVED_IN_SPROUTING_ANGIOGENESIS	0.661	0.044	47
GOBP_POSITIVE_REGULATION_OF_EPITHELIAL_CELL_MIGRATION	0.578	0.045	38
GOBP_MAPK_CASCADE	0.579	0.047	41
GOBP_SPROUTING_ANGIOGENESIS	0.504	0.014	42
GOBP_POSITIVE_REGULATION_OF_LOCOMOTION	0.544	0.031	52
GOBP_REGULATION_OF_PROGRAMMED_CELL_DEATH	0.482	0.011	35
GOBP_POSITIVE_REGULATION_OF_SIGNALING	0.517	0.042	52
GOBP_APOPTOTIC_PROCESS	0.479	0.027	30
GOBP_CELL_MOTILITY	0.444	0.028	30
GOBP_LOCOMOTION	0.444	0.028	30
GOBP_REGULATION_OF_LOCOMOTION	0.444	0.028	30

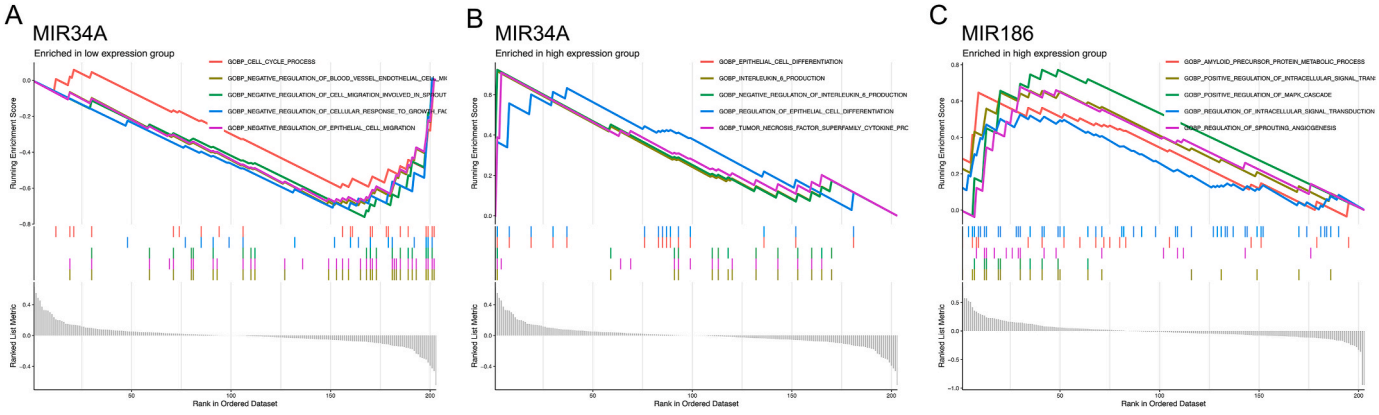

Fig. 7. GSEA analysis for MIR34A and MIR186. (A) Top five BP, CC, and MF enriched in the low expression group in the GO analysis for the MIR34A gene, (B) Top five BP, CC, and MF enriched in the high expression group in the GO analysis for the MIR34A gene, (C) Top five BP, CC, and MF enriched in the high expression group in the GO analysis for the MIR186 gene. MIR34A: microRNA34a; MIR186: microRNA186; GO: gene ontology.

Table 5
Critical target genes for hsa-miR-34a-3p, hsa-miR-34a-5p, hsa-miR-186–3p, and hsa-miR-186–5p.

Signature miRNAs	Critical target genes for signature miRNAs
hsa-miR-34a-3p	CDK1, MGST1, POLE2, GINS2, SHCBP1, NDC80, WDR76, CDC6, TSPAN8, KIF14, PRC1, CLSPN, NEK2, KIF11, DHFR, KRT19, DHCR24, SCD, ASPM, BORA, RFC3, TIPIN, STIL, TMEM97, ORC1, ARG2, CENPV, EXO1, TMC7, TUFT1, FHOD3, ADCY4, FBN1, SLC6A15, and CCDC144A
hsa-miR-34a-5p	CDK1, CDKN3, CCNB2, SKA1, SKA3, NUF2, MGST1, BUB1B, GINS2, SHCBP1, NDC80, RAD51, DLGAP5, DTL, SPAG5, DDIAS, DEPDC1, WDR76, ASF1B, FOXM1, NUSAP1, RAD51AP1, CDCA8, CDC6, EXOSC8, TSPAN8, FANCI, KIF14, PRC1, CLSPN, FAM111B, NEK2, AURKB, CDC20, ZWINT, DHFR, KIF2C, TOP2A, SPC24, KRT19, KIF18A, PLK1, NXN, MCM10, ASPM, BORA, RFC3, TIPIN, STIL, TMEM106C, NGDN, TMEM97, ORC1, CKS1B, ARG2, ETV4, RBBP8, EXO1, TICRR, BLM, LTBP2, RSRP1, TMC7, TAS2R50, TUFT1, RIMKLB, BST2, ADCY4, FBN1, ANGPTL4, SLC6A15, TAS2R31, CCDC144A, TAS2R20, BLID, and POSTN
hsa-miR-186–3p	CDK1, SKA1, BUB1B, NDC80, DLGAP5, DEPDC1, OIP5, PRIM1, CDC6, EXOSC8, FANCI, KIF14, PRC1, CLSPN, FAM111B, NEK2, KIF11, ZWINT, DHFR, KIF2C, TOP2A, MCM10, SCD, ASPM, BORA, STIL, TMEM97, ORC1, ETV4, RBBP8, TICRR, TMC7, RIMKLB, FHOD3, SLC6A15, and TAS2R20
hsa-miR-186–5p	CDK1, SKA1, NUF2, POLE2, BUB1B, GINS2, SHCBP1, NDC80, RAD51, DLGAP5, DTL, ASF1B, FOXM1, CEP55, NUSAP1, PRIM1, FANCI, KIF14, FAM111B, NEK2, AURKB, ZWINT, DHFR, DHCR24, PLK1, NXN, MCM10, SCD, ASPM, BORA, TIPIN, STIL, ETV4, RBBP8, CENPV, TICRR, BLM, LTBP2, TMC7, TUFT1, RIMKLB, TAGLN, FHOD3, ADCY4, SLC6A15, CCDC144A, and POSTN

Note: The target genes for hsa-miR-34a-3p, hsa-miR-34a-5p, hsa-miR-186–3p, and hsa-miR-186–5p were predicted from the official website of miRWalk. The final critical target genes for these miRNAs were obtained from the intersection of the differentially expressed genes from the GSE103552 dataset ($|logFC|>0.7$ and adjusted $p < 0.05$) and their correlated predicted target genes.

consequences.

In the context of GDM, placental inflammation is closely related to placental function, which is of critical importance for the development of a growing fetus. It was reported that miR-186 can induce reactive changes in VEC through its regulation of inflammation-related genes [35]. For example, miR-186 may affect the expression of inflammatory genes and regulate the inflammatory response of VEC by targeting key molecules in the NF-κB signaling pathway [36]. MiR-186 may also participate in the proliferation, migration, and tubular structure formation of endothelial cells by modulating the expression and function of vascular endothelial growth factor, which is crucial for the development and function of placental blood vessels [37]. In addition, precise control of cell cycle and apoptosis are necessary for the development of blood vessels [37]. MiR-186 has been shown to be able to target and regulate genes related to cell cycle and apoptosis, subsequently impacting the delicate balance between cell survival and death [38]. In terms of placental function, the placenta serves to transport nutrients, secrete hormones, and provide defense. MiR-186 can influence the nutrient transport function of the placenta by regulating the expression of glucose transporter 1 (GLUT1). The expression of GLUT1 in the placental vascular endothelium and cytotrophoblasts is crucial for maternal-fetal glucose circulation [39]. miRNAs can also influence the secretion levels of placental hormones, including human chorionic gonadotropin (hCG) and human placental lactogen (HPL), which are pivotal in maintaining pregnancy and facilitating fetal growth and development [40]. Furthermore, miR-186, as a member of the oxidative stress regulatory network, may play a role in regulating the placental oxidative stress response by modulating the expression of antioxidant enzymes [41]. Meanwhile, oxidative stress may further exacerbate placental dysfunction by influencing biological processes such as the proliferation, apoptosis, and differentiation of placental cells [42]. Therefore, the increased expression of miR-186 observed in our study may contribute to the occurrence of placental VEC programming in GDM patients. Additionally, we found that both miR-186 and miR-34a have relatively good value in representing fetoplacental VEC programming under GDM conditions with an AUC higher than 90. In summary, miR-186 may contribute to GDM-related placental dysfunction and fetoplacental VEC programming through various mechanisms, including regulating inflammatory response, influencing cell proliferation and apoptosis, as well as modulating oxidative stress. These discoveries offer crucial insights into the mechanism of miR-186 in GDM and may serve as potential targets for developing novel therapeutic strategies.

Considering the important role miR-34a and miR-186 may play in fetoplacental VEC programming and placental function, we constructed the interaction network of hsa-miR-34a-3p, hsa-miR-34a-5p, hsa-miR-186–3p, and hsa-miR-186–5p with their critical target genes. These target genes are involved in various cellular processes and pathways related to endothelial function, inflammation, and metabolism. By modulating the expression of these target genes, miR-34a and miR-186 may influence the pathophysiology of GDM and its associated complications. The interaction network constructed by this study may offer a reference for future studies related to miR-34a and miR-186 and fetal programming in the context of GDM.

In summary, our findings provide essential insights into the potential roles of MIR34A and MIR186 genes in fetoplacental VEC programming in GDM and their possible implications for fetal health. The alterations of these signature genes expression in GDM may lead to long-term fetal programming, which could affect cardiovascular, metabolic, and other physiological functions in the offspring. Therefore, the identification of these biomarkers can provide valuable information for developing new diagnostic and therapeutic strategies to manage GDM and prevent its associated complications. Future studies are needed to investigate the functional roles of MIR34A and MIR186 in GDM and their potential roles as therapeutic targets.

Certainly, our research still has some limitations. Firstly, owing to the lack of a similar dataset, this study was performed based on a single dataset with a relatively small sample size, which may result in bias in data interpretation. In addition, although miR-34a and miR-186 were identified as promising biomarkers in GDM-related fetoplacental VEC programming that may be associated with placental dysfunction and fetal programming, there is a lack of experimental validation of the functions and mechanisms of these miRNAs. Therefore, the function and mechanisms of these signature miRNAs still need to be further validated by experimental and molecular studies.

5. Conclusions

This study identified 32 DEMGs, of which MIR34A and MIR186 were the final signature genes involved in fetoplacental VEC programming in GDM. The upregulation of MIR34A and MIR186 expression may contribute to the pathophysiology of GDM, probably through mediating endothelial cell migration, inflammation, oxidative stress, and programmed cell death, leading to the programming of fetal health outcomes.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The dataset used in this study is available on the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The other data presented in the manuscript are included in this published

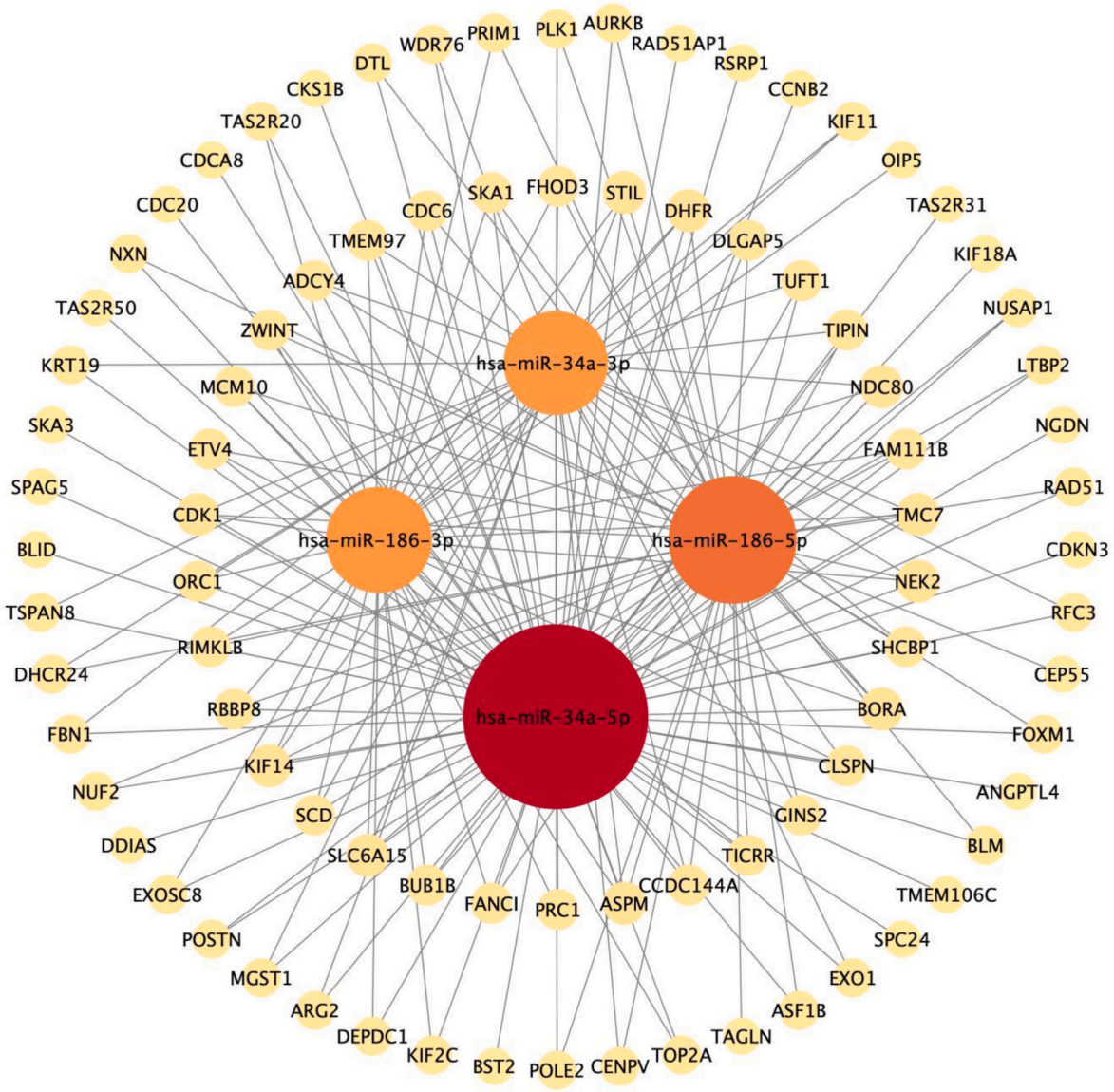

Fig. 8. Prediction and construction of the miRNA-mRNA interaction network of hsa-miR-34a-3p, hsa-miR-34a-5p and hsa-miR-186–3p, and hsa-miR-186–5p and their critical target genes in fetoplacental VECs in the context of GDM. VEC: vascular endothelial cell; GDM: Gestational diabetes mellitus.

article and its supplementary files.

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Declaration of competing interest

The authors declare that there are no conflicts of interest related to this study. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101888.

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Huatuo Huang was initially listed as the first author due to his pivotal role in project initiation and preliminary research design. However, as the research progressed, the contributions of other authors became more prominent. To accurately reflect the actual contributions of each author, we have decided to rearrange the author order.

Chunhong Liu provided continuous experimental support and data analysis throughout the entire research process, and his contribution was crucial throughout the project. Therefore, he remains in the position of second author to reflect his ongoing and significant contributions to the research.

Xiaoxia Pang was responsible for key experimental operations and data collection, and her contributions were crucial to the success of the research. Her position remains unchanged to ensure recognition of her contributions.

Chunfang Wang has made significant contributions in literature review and preliminary data analysis. Her contribution is crucial for the framework construction of the research, highlighting her leadership role throughout the entire research project. We have decided to rearrange her order in the author list.

Yulan Lu undertook the main data analysis and paper writing work in the later stage of the research. She played a decisive role in interpreting the research results and ultimately forming the paper. Therefore, in order to more accurately reflect her significant contribution to the success of the research, she was promoted to first author.

Xinghong Chen joined the team in the later stages of the research and made significant contributions in experimental design and data analysis. His contribution is crucial for the in-depth research and the improvement of the paper, therefore he has been added as the fourth author.