Endocrine Journal 2019, **66** (5), 431-441

ORIGINAL

Expression profile of circular RNAs in placentas of women with gestational diabetes mellitus

Huiyan Wang *, Guangtong She *, Wenbai Zhou, Kezhuo Liu, Jun Miao and Bin Yu

Changzhou Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University, Changzhou, Jiangsu 213003, China

Abstract. Forty-five pregnant women who underwent cesarean section, including 30 cases of gestational diabetes mellitus (GDM) and 15 normal pregnant women, were enrolled in this study to examine the differential expression of circular RNAs (circRNAs) in the placentas of women with GDM by RNA sequencing (RNA-seq) analysis. The differentially expressed circRNAs were analyzed bioinformatically using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment and circRNA-microRNA (miRNA) interaction prediction. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to verify the results. A total of 8,321 circRNAs were identified in the human placenta, among which 46 were differentially expressed (fold change ≥2 and $p < 0.05$), including three that were upregulated and 43 that were downregulated. According to the GO and KEGG enrichment results, these circRNAs may be associated with vital biological processes, cellular components, molecular functions, and signaling pathways. In particular, KEGG analysis shown they may be involved in advanced glycation end products-receptor for advanced glycation end products (AGE-RAGE) signaling pathway in diabetic complications, indicating that these circRNAs might participate in the occurrence and pathogenesis of GDM. qRT-PCR verified that the expression of circ_5824, circ_3636, and circ_0395 was consistent with RNA-seq analysis; their expression levels were significantly lower in the GDM group than in the control group. The circRNA-miRNA interaction was analyzed according to the molecular sponge mechanism, and its potential function is discussed. These results shed light on future functional studies of circRNAs related to GDM.

Key words: Gestational diabetes mellitus, CircRNA, RNA sequencing, MicroRNA, Placenta

THE PRESENT STUDY suggests that circRNAs are associated with the occurrence and development of GDM. Our results may open a new chapter in the study of GDM.

Introduction

Gestational diabetes mellitus (GDM), also known as glucose intolerance with onset or first recognition during pregnancy [1], can cause severe maternal and neonatal complications, such as increased risk of preeclampsia, macrosomia, depression, and stillbirth. It is particularly noteworthy that uncontrolled GDM has long-term adverse effects on mothers and children, such as susceptibility to obesity and metabolic syndrome [2]. Because of its high incidence (affecting 3–9% of pregnancies [3]), GDM has attracted the attention of prenatal medical experts.

GDM is a heterogeneous disorder in which pregnancy can make recessive diabetes dominant (make pregnant women with no previous diabetes have GDM) or aggravate the condition of a woman with preexisting diabetes. The hallmark of GDM is increased insulin resistance [4]. Pancreatic beta cells are no longer able to compensate for the increased insulin resistance during pregnancy. However, the precise mechanisms underlying GDM remain unknown. On the other hand, Ilekis *et al.* [5] suggested that the occurrence of adverse pregnancy outcomes associated with GDM could be related to placental issues. As the main channel of energy transfer between mother and fetus, it should play an important role in the abnormal metabolism of GDM. Placental lactogen, prolactin, and estradiol also seem to contribute to the development of insulin resistance during pregnancy, among which cortisol and progesterone are the main culprits. Other studies have focused on the placental microenvironment, including inflammatory factors, genes, and

Submitted Jul. 24, 2018; Accepted Jan. 28, 2019 as EJ18-0291
Released online in J-STAGE as advance publication Feb. 27, 2019
Correspondence to: Bin Yu, Changzhou Maternity and Child Health Care Hospital affiliated to Nanjing Medical University, No. 16 Bo Ai Road, Changzhou, Jiangsu Province, 213003, China.
E-mail: ybcz0519@163.com
*These two authors contributed equally to this work.

proteins [6-8]. The recent discovery of small molecules with potential regulatory effects, such as microRNAs (miRNAs) [9, 10] and long non-coding RNAs (lncRNAs) [11, 12], may reveal the essence of GDM more accurately.

Circular RNAs (circRNAs) are a special type of non-coding RNA with characteristics of evolutionary conservation, structural stability, and tissue specificity [13, 14]. Because of these biological features, circRNAs have attracted wide attention in recent years. They play important roles in tumor development and nervous system diseases [15-17]. circRNAs act as miRNA sponges and affect the expression of downstream genes [18, 19]. Some reports have shown that circRNAs are also present in the human placenta and may be related to the occurrence of pregnancy complications [20]. circRNAs may also play a role in the pathogenesis of diabetes and thus serve as novel molecular targets for clinical therapy [21, 22]. For example, Zhao *et al.* reported that hsa_circ_0054633 presented a certain diagnostic capability for pre-diabetes and type 2 diabetes mellitus, and high glucose exposure profoundly altered circRNA expression in endothelial cells [23]. However, studies on the relationship between circRNAs and GDM are lacking.

In the present study, we examined the differential expression of circRNAs in placentas from women with GDM by RNA sequencing (RNA-seq) and preliminarily investigated their biological functions *via* bioinformatics analysis. We hope to clarify the relationship between circRNAs and the occurrence and pathogenesis of GDM.

Materials and Methods

Patients

A total of 45 pregnant women who underwent cesarean section from August 2016 to June 2017, including 30 cases of GDM and 15 normal pregnant women, were enrolled in this study. Their baseline characteristics are shown in Table 1. The diagnosis of GDM was made by an oral glucose tolerance test (75 g) during the second trimester (24–28 weeks of gestation). All pregnant women with multiple gestations, infection, other pregnancy complications, congenital or chromosomal abnormalities of the fetus, or a family history of diabetes were excluded. The study was approved and reviewed by the ethics committee of Changzhou Women and Children Health Hospital (Changzhou, China, Approval No: CZFY20160103). Informed consent was obtained prior to cesarean section.

Sample collection

Placentas were obtained within 15 min after cesarean section. Placental fragments were collected in the middle of the initial placental depth. The decidual layer, chorionic surface, and membranes were removed. All placental samples were washed with saline and stored at −80°C following the addition of 1 mL Trizol (Invitrogen, Carlsbad, CA, USA). Three cases and three paired controls were chosen for RNA-seq analysis.

RNA extraction and sequencing

Total RNA was extracted from the placenta tissues using the mirVana miRNA Isolation Kit (Ambion, Inc., Foster City, CA, USA) following the manufacturer's protocol. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples with an RNA integrity number ≥7 were subjected to subsequent analyses. Libraries were constructed using TruSeq Stranded Total RNA with Ribo-Zero Gold according to the manufacturer's instructions. Libraries were then sequenced on the Illumina sequencing platform (HiSeq 2500) and 150 bp/125 bp paired-end reads were generated.

Table 1 The information of patients with GDM and normal control groups

Characteristics	GDM ($N = 30$)	Control ($N = 15$)	p value
Age (year)	32.57	31.47	0.39
Fasting glucose (mmol/L)	5.09	4.35	0.03
2 h postgrandial glucose (mmol/L)	6.86	5.28	0.001
OGTT 1 h (mmol/L)	10.27	7.32	<0.01
OGTT 2 h (mmol/L)	8.98	6.06	<0.01
HbA1C (%)	5.18	4.85	0.13
BMI at delivery (kg/m^2)	28.97	27.27	0.21
Pre-pregnancy BMI (kg/m^2)	22.12	21.34	0.22
Birth weight (g)	3,445.67	3,362.85	0.61
Neonatal sex (male/female)	0.87	0.88	0.55

Identification and quantification of human circRNAs

circRNAs were predicted by CIRCexplore2 [24] and compared with those in circBase (http://www.circbase.org/). DESeq [25] software was used to standardize the number of junction reads of each sample. A fold change (FC) >2 and $p < 0.05$ were considered to indicate significant differences.

Functional enrichment analysis and circRNA-miRNA associations

The differentially expressed circRNA genes were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. GO enrichment analysis was based on three aspects: biological process (BP), cellular component (CC), and molecular function (MF). circRNA-targeted miRNAs were identified and predicted by miRanda [26, 27]. The circRNA-miRNA interaction network was constructed based on the functional annotation of the miRNA target genes.

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

We randomly selected 10 circRNAs from the RNA-seq results to verify by qRT-PCR. RNase R-treated RNAs were diluted with water and used as a PCR template. cDNAs were obtained using a Reverse Transcription Kit (M-MLV; Promega, Madison, WI, USA). SYBR Master Mix (TaKaRa Bio Inc., Kusatsu, Japan) was used to examine the expression of circRNAs according to the manual. All primers were designed and synthesized by Ribo Bio (Guangzhou, China). Amplification was performed on an ABI 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) under the following conditions: denaturation at 95°C for 10 min, followed by 38 cycles of amplification at 95°C for 10 s and 60°C for 1 min. The relative expression levels of the circRNA genes were calculated using the $2^{-\Delta\Delta Ct}$ method. GAPDH was employed as the internal reference to normalize the expression levels of the target genes.

Statistical analysis

Statistical analyses were performed using SPSS 19 software. The *t*-test was used to analyze data between two groups. $P < 0.05$ was considered statistically significant.

Results

Identification and quantification of human circRNAs

A total of 8,321 circRNAs were indentified in human placentas from GDM and normal control pregnant women. A total of 7,804 circRNAs had already been reported in circBase, whereas 517 were newly discovered, among which 46 were differentially expressed in the placenta tissues of GDM women (FC > 2 and $p < 0.05$), three that were upregulated and 43 that were downregulated. Their related information is shown in Table 2. A heatmap (Fig. 1A) and volcano plot (Fig. 1B) reveal the differential expression profiles of circRNAs between GDM women and the control group.

Functional analysis of differentially expressed circRNAs

The differentially expressed circRNA genes were analyzed by GO (Fig. 2A, B, C) and KEGG (Fig. 2D) enrichment. Based on the results, these differentially expressed circRNAs may be associated with GO functional annotation of biological processes (*e.g.*, smoothened signaling pathway), cellular components (*e.g.*, nuclear speck, transcriptional repressor complex), and molecular function (*e.g.*, small ubiquitin-like modifier [SUMO] binding, ubiquitin−like protein binding). According to KEGG analysis, the host genes of these differentially expressed circRNAs are associated with focal adhesion, shigellosis, bacterial invasion of epithelial cells, endocrine resistance, and the advanced glycation end products-receptor for advanced glycation end products (AGE−RAGE) signaling pathway in diabetic complications. In particular, the AGE/RAGE pathway plays an important role in a variety of diabetic complications [28] and is associated with adverse outcomes in GDM [29].

qRT-PCR analysis

To validate the RNA-seq results, 10 circRNAs were randomly subjected to qRT-PCR analysis, among which the results of three circRNAs (circ_5824, circ_3636, and circ_0395) were consistent with RNA-seq data. Compared with the control group, their expression in the GDM group was significantly reduced (Fig. 3A). Their primer sequences are shown in Table 3. Images of the PCR products of these three circRNAs on 1.5% agarose gels did not show obvious primer dimers or non-specific PCR products (Fig. 3B). All bands of the GDM group were less bright than those in the control group, indicating their differential expression.

circRNA-miRNA interaction analysis

As target molecules of miRNAs, the interaction analysis of circRNA-miRNA can help explore the function and mechanism of circRNAs. Three circRNA (circ_5824, circ_3636, and circ_0395)-targeted miRNAs were identified and their potential functions were eluci-

Table 2 Statistic of differently expressed transcrips

ID	CircBase name	region	gene symbol	chr	log2FC	*p*	regulation
circ_0395	NA	exonic	*PAPPA2*	1	–4.69	0.02	down
circ_1698	hsa_circ_0058092	exonic	*FN1*	2	–6.46	0.05	down
circ_1840	hsa_circ_0081006	exonic	*KRIT1*	7	–6.68	0.04	down
circ_1926	hsa_circ_0000155	exonic	*DCAF6*	1	–6.74	0.04	down
circ_2308	hsa_circ_0120939	exonic	*EXOC6B*	2	–6.61	0.05	down
circ_2372	hsa_circ_0006260	exonic	*SLC41A2*	12	–7.90	0.01	down
circ_2415	hsa_circ_0007430	exonic	*NRDC*	1	7.17	0.03	up
circ_2523	hsa_circ_0000857	exonic	*ZNF236*	18	–7.19	0.03	down
circ_3003	hsa_circ_0005362	exonic	*PHC3*	3	–6.65	0.04	down
circ_3223	hsa_circ_0002466	exonic	*TTBK2*	15	–7.69	0.02	down
circ_3636	NA	exonic	*ADAM12*	10	–5.90	0.03	down
circ_3798	hsa_circ_0005243	exonic	*TMEM184B*	22	–8.17	0.04	down
circ_3869	hsa_circ_0001578	exonic	*RANBP9*	6	–7.85	0.01	down
circ_3993	hsa_circ_0008192	exonic	*PTBP3*	9	–6.44	0.05	down
circ_4046	hsa_circ_0088249	exonic	*PAPPA*	9	–6.55	0.05	down
circ_4390	hsa_circ_0006670	exonic	*SIPA1L3*	19	–6.87	0.03	down
circ_4524	hsa_circ_0006380	exonic	*TCF12*	15	–6.46	0.05	down
circ_4718	hsa_circ_0005029	exonic	*EPT1*	2	–6.65	0.04	down
circ_4792	hsa_circ_0000417	exonic	*CPSF6*	12	–6.87	0.03	down
circ_4802	hsa_circ_0002702	exonic	*RUSC2*	9	–7.40	0.04	down
circ_5036	hsa_circ_0009049	exonic	*PLPP3*	1	6.90	0.03	up
circ_5124	hsa_circ_0028319	exonic	*TMEM116*	12	–6.49	0.05	down
circ_520	hsa_circ_0004919	exonic	*CARF*	2	–6.69	0.04	down
circ_5754	NA	exonic	*LOC100507487*	4	–6.45	0.05	down
circ_5824	hsa_circ_0005243	exonic	*TMEM184B*	22	–2.37	0.04	down
circ_6525	hsa_circ_0134318	exonic	*GLI3*	7	–6.63	0.04	down
circ_6998	hsa_circ_0013218	exonic	*DNTTIP2*	1	–7.01	0.03	down
circ_7167	hsa_circ_0002226	exonic	*ETFA*	15	–6.77	0.04	down
circ_7224	hsa_circ_0002795	exonic	*SPAST*	2	–6.99	0.03	down
circ_730	hsa_circ_0042170	exonic	*NCOR1*	17	–6.52	0.04	down
circ_7360	hsa_circ_0002634	exonic	*ATXN7*	3	–6.44	0.05	down
circ_7367	hsa_circ_0003218	exonic	*BMPR2*	2	–7.88	0.01	down
circ_7402	hsa_circ_0126389	exonic	*SLC30A9*	4	–6.69	0.04	down
circ_7466	hsa_circ_0125310	exonic	*LARP1B*	4	6.76	0.04	up
circ_7540	hsa_circ_0091581	exonic	*GPC3*	X	–6.37	0.05	down
circ_7687	hsa_circ_0008667	exonic	*ADAMTS6*	5	–7.19	0.03	down
circ_780	hsa_circ_0002814	exonic	*HERC2*	15	–6.54	0.04	down
circ_7965	hsa_circ_0025641	exonic	*RASSF8*	12	–6.95	0.03	down
circ_8068	hsa_circ_0017310	exonic	*CNST*	1	–6.73	0.04	down
circ_8086	hsa_circ_0008234	exonic	*FOXP1*	3	–7.11	0.03	down
circ_8122	NA	splicing	*LIMS2*	2	–6.64	0.04	down
circ_8133	hsa_circ_0000139	exonic	*GON4L*	1	–8.05	0.01	down
circ_8210	hsa_circ_0002968	exonic	*MAPK8*	10	–7.34	0.02	down
circ_8271	hsa_circ_0091206	exonic	*PCDH11X*	X	–7.10	0.03	down
circ_967	hsa_circ_0035472	exonic	*RNF111*	15	–7.25	0.05	down
circ_986	NA	exonic	*PAPPA2*	1	–6.70	0.04	down

Note: NA, not available; chr, chromosome.

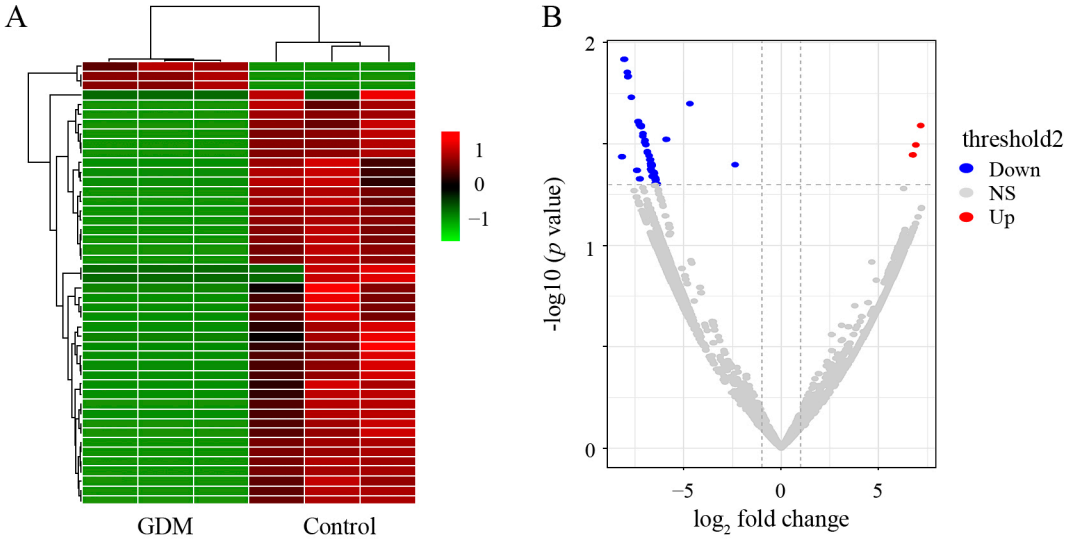

Fig. 1 Prediction and identification of circRNAs expressed in the placentas of women with GDM

A. Expression profiles of the circRNAs are displayed in a heatmap. Each column represents a sample and each row represents a circRNA. High expression is indicated in red and low expression is indicated in green. B. Differentially expressed circRNAs are displayed in volcano plots. Gray dots indicate circRNAs with no significant difference. Red dots indicate significantly upregulated circRNAs, whereas blue dots indicate significantly downregulated circRNAs. NS, not significant.

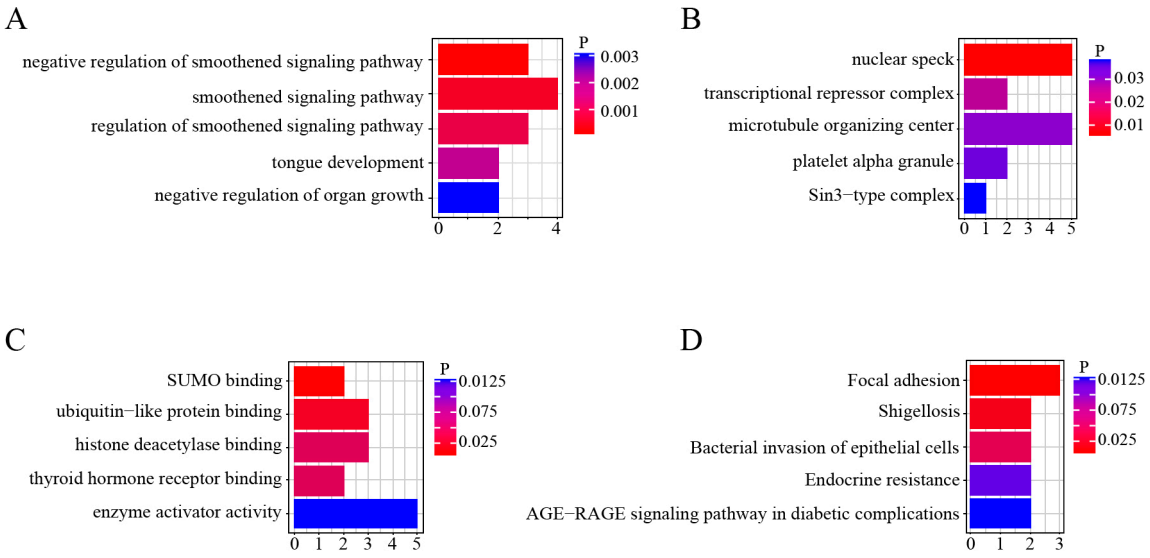

Fig. 2 GO and KEGG enrichment terms of differentially expressed circRNA transcript genes

A. Top five classes of biological process (BP) enrichment terms. B. Top five classes of molecular function (MF) enrichment terms. C. Top five classes of cellular component (CC) enrichment terms. D. Top five classes of KEGG pathway terms.

dated according to the miRNA target genes. The miRNAs that interact with these three circRNAs, as predicted using miRanda software, are shown in Table 4. circRNA_0395 was targeted by 88 miRNAs. The top three total scores of these miRNAs were hsa-miR-8485, hsa-miR-3135b and hsa-miR-1273g-3p, whereas circRNA_3636 and circRNA_5824 had three and eight miRNA binding sites, respectively. Furthermore, a network of circRNA-miRNA-mRNA interactions was established based on these three circRNAs and their target miRNAs (Fig. 4).

Discussion

We successfully discovered 46 differentially expressed circRNAs in the placentas of women with GDM *via* RNA-seq analysis and confirmed the results using qRT-PCR. Their biological functions were predicted by bioin-

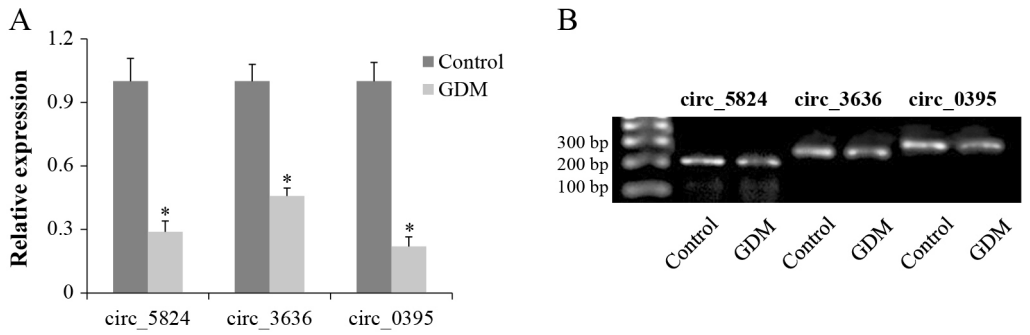

Fig. 3 qRT-PCR verification of differentially expressed circRNAs

A. The expression of circ_5824, circ_3636, and circ_0395 was confirmed by qRT-PCR, which was consistent with the sequencing results. * $p < 0.05$. B. Images of PCR products of the three circRNAs on a 1.5% agarose gel.

Table 3 Primer sequences of the circRNAs

Gene	5'-3'	3'-5'
circ_5824	CACCGGACAGGCATCTAGTGA	CAGTGTTGCAGGCTCTTTGA
circ_0395	AGACAGGAATTTGGGTACATC	GAGTGCCATCCACATACAGG
circ_3636	GTGCTATGGTGCTCTGTCTA	TGAGTGAGCCGAGTTGTTCT
GAPDH	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA

formatics analysis. These results suggest that the differentially expressed circRNAs are associated with the occurrence and development of GDM.

The roles of several small molecules in the occurrence of GDM have recently attracted our attention, namely, miRNAs and lncRNAs. Thus far, more than 600 miRNAs expressed in the human placenta have been reported [30]. Human placenta tissue exhibits specific miRNA expression in a time-dependent manner during pregnancy and is reflected in the maternal plasma. Some placental miRNAs are dysregulated in plasma and are involved in GDM [9]. As a newly discovered non-coding RNA, circRNAs have gained increasing attention from researchers. Several functional circRNAs that act as competitive endogenous RNAs by effectively adsorbing miRNAs and regulating their target genes were recently identified [31, 32]. To date, there have been few reports on the relationship between circRNAs and pregnancy. Maass *et al.* detected 63 circRNAs in the human placenta. By functional prediction, they reported that some circRNAs may be related to pregnancy complications, such as early onset preeclampsia, fetal growth restriction, and infection during pregnancy [20]. In this study, we identified 46 differentially expressed circRNAs in the placentas of women with GDM and preliminarily discussed the possible mechanisms of their participation in GDM. Yan *et al.* [33] recently reported differentially expressed circRNAs in placenta tissues from patients with GDM. Their circRNA expression profile differed from that described herein, which could be due to the different prediction tools used (different algorithms have different sensitivities and accuracy rates; dramatic differences between the algorithms were observed specifically regarding the highly expressed circRNAs and the circRNAs derived from proximal splice sites) [34]. Different regions and populations, as well as database sequencing systems, will also cause differences.

Among the differentially expressed circRNAs, the qRT-PCR results of three circRNAs (circ_5824, circ_3636 and circ_0395) were consistent with RNA-seq analysis. circRNA_0395 attracted our attention; it was significantly decreased in the placentas of women with GDM and overlaps with the PAPPA2 (pregnancy-associated plasma protein A 2) gene, which encodes a pregnancy-related protein. PAPPA2 can be used to predict macrosomia at birth in GDM pregnancies [35] and is related to metabolic diseases beyond total adiposity [36]. Thus, it was important to examine the interactions between circRNAs and miRNAs that could play a key role in the occurrence and development of GDM. Accumulating evidence has indicated that circRNAs have a series of important biological functions, acting as miRNA sponges [16, 37]. Bioinformatics analysis revealed that circRNA_0395 is targeted by 88 types of miRNAs. We then performed a systematic review of the literature to examine these miRNAs more closely. miRNA-1273g-3p piqued our interest. miR-1273g-3p, a member of the miR-1273 family, was first identified as

Table 4 Differentially expressed circRNAs and their targeted-miRNAs

Transcript	miRNA	Total score	Total energy	miRNA length	Position
circRNA_0395	hsa-miR-197-3p	163	–30.67	22	16149
	hsa-miR-143-5p	171	–30.17	22	6098
	hsa-miR-363-5p	173	–32.57	22	21029
	hsa-miR-328-5p	156	–30.21	23	29421
	hsa-miR-328-3p	171	–33.9	22	15185
	hsa-miR-504-3p	490	–91.63	21	17820 6102 37955
	hsa-miR-619-5p	192	–42.14	22	5885
	hsa-miR-33b-3p	163	–31.13	22	723
	hsa-miR-762	152	–31.86	22	4010
	hsa-miR-877-3p	173	–33.32	21	37405
	hsa-miR-937-5p	164	–32.56	20	1045
	hsa-miR-939-3p	161	–32.92	21	32279
	hsa-miR-1226-3p	173	–39.96	22	2418
	hsa-miR-1207-5p	325	–61.96	21	45023 52033
	hsa-miR-1285-3p	183	–33.2	22	45215
	hsa-miR-1303	184	–34.28	22	31482
	hsa-miR-1304-3p	167	–30.42	22	17797
	hsa-miR-1254	167	–30.39	24	29504
	hsa-miR-1273a	195	–40.51	25	20806
	hsa-miR-1911-5p	174	–31.86	23	27861
	hsa-miR-1912	167	–30.6	22	3402
	hsa-miR-1913	154	–32.71	22	57642
	hsa-miR-1972	176	–34.62	22	21085
	hsa-miR-1976	162	–30.12	20	30368
	hsa-miR-2276-5p	164	–34.09	22	44308
	hsa-miR-3127-3p	175	–34.76	22	3440
	hsa-miR-3137	154	–30.13	24	29438
	hsa-miR-3151-5p	154	–30.84	21	4010
	hsa-miR-3184-3p	175	–31.45	23	35510
	hsa-miR-3192-5p	168	–31.82	23	12012
	hsa-miR-3200-5p	180	–31.15	22	58686
	hsa-miR-4254	172	–33.9	23	6254
	hsa-miR-4269	169	–30.86	21	22629
	hsa-miR-3619-5p	161	–30.17	22	15775
	hsa-miR-3135b	633	–134.88	22	6104 35487 37957 31448
	hsa-miR-4518	159	–30.96	26	41381
	hsa-miR-4640-3p	161	–30.85	22	22760
	hsa-miR-4644	183	–31.99	23	29307
	hsa-miR-4651	157	–30.53	20	45812
	hsa-miR-4656	164	–30.76	23	29419
	hsa-miR-4685-3p	174	–36.05	22	18357
	hsa-miR-4687-5p	165	–33.59	22	44117
	hsa-miR-4722-5p	163	–30.19	23	58192
	hsa-miR-4728-5p	166	–30.49	23	45800
	hsa-miR-4741	167	–32.11	23	254
	hsa-miR-4758-5p	155	–34.04	23	870
	hsa-miR-4763-5p	153	–30.28	21	22772
	hsa-miR-4763-3p	174	–36.51	24	52031
	hsa-miR-4436b-5p	162	–32.29	22	8141
	hsa-miR-5090	162	–30.65	23	9284

Table 4 Cont.

Transcript	miRNA	Total score	Total energy	miRNA length	Position
	hsa-miR-5095	187	–41.97	21	5879
	hsa-miR-1273g-3p	548	–112.25	21	29358 20828 8006
	hsa-miR-5096	179	–32.37	21	5957
	hsa-miR-5187-5p	176	–30.51	22	31084
	hsa-miR-5189-5p	168	–31.45	24	27993
	hsa-miR-5196-3p	157	–36.54	21	302
	hsa-miR-6089	340	–84.82	24	32436 21040
	hsa-miR-6727-5p	157	–30.15	23	9285
	hsa-miR-6734-5p	167	–32.06	23	15550
	hsa-miR-6734-3p	173	–30.38	23	38422
	hsa-miR-6751-5p	177	–32.76	23	34482
	hsa-miR-6756-5p	533	–118.76	23	4014 45811 56027
	hsa-miR-6764-5p	160	–31.16	22	32
	hsa-miR-6771-5p	320	–65.79	22	12050 20896
	hsa-miR-6776-3p	168	–31.44	23	22795
	hsa-miR-6777-3p	166	–32.42	20	30373
	hsa-miR-6782-5p	161	–30.39	25	4011
	hsa-miR-6787-3p	166	–31.64	22	17799
	hsa-miR-6793-5p	175	–34.09	22	41435
	hsa-miR-6797-5p	165	–30.74	25	29034
	hsa-miR-6799-3p	159	–31.14	23	905
	hsa-miR-6803-5p	160	–33.98	22	6836
	hsa-miR-6810-5p	165	–30.76	23	9210
	hsa-miR-6810-3p	171	–39.89	23	57643
	hsa-miR-6812-5p	328	–66.88	25	45808 56018
	hsa-miR-6780b-5p	172	–31.94	23	3663
	hsa-miR-6846-5p	162	–32.59	22	549
	hsa-miR-6849-5p	171	–30.56	23	23141
	hsa-miR-6856-5p	173	–31.35	24	31975
	hsa-miR-6884-3p	179	–38.62	23	37374
	hsa-miR-7108-3p	164	–31.3	20	8900
	hsa-miR-7111-3p	347	–66.25	22	38374 38427
	hsa-miR-7160-3p	157	–33.98	21	44310
	hsa-miR-1273h-5p	334	–65.67	21	12016 29392
	hsa-miR-7851-3p	176	–32	22	12018
	hsa-miR-8085	171	–30.52	21	12693
	hsa-miR-8089	160	–32.08	24	45819
	hsa-miR-8485	928	–184.2	21	37704 44413 37720 44429 44383
circRNA_3636	hsa-miR-6734-5p	158	–32.46	23	111
	hsa-miR-6852-5p	162	–31.45	21	73
	hsa-miR-7155-5p	167	–30.87	19	78
circRNA_5824	hsa-miR-331-3p	154	–33.14	21	287
	hsa-miR-486-3p	162	–30.4	21	340
	hsa-miR-4486	170	–34.58	17	111
	hsa-miR-4649-5p	153	–30.89	24	100
	hsa-miR-4786-3p	168	–31.26	22	194
	hsa-miR-6165	156	–34.53	19	26
	hsa-miR-6729-5p	160	–32.59	22	105
	hsa-miR-8073	176	–33.56	22	34

A total of 99 miRNAs could be combined with these 3 circRNAs. Total Score: the cumulative prediction score. The higher the value is, the more accurate. Total Energy: the accumulative complementary pair matches the free energy. The smaller the energy is, the more reliable.

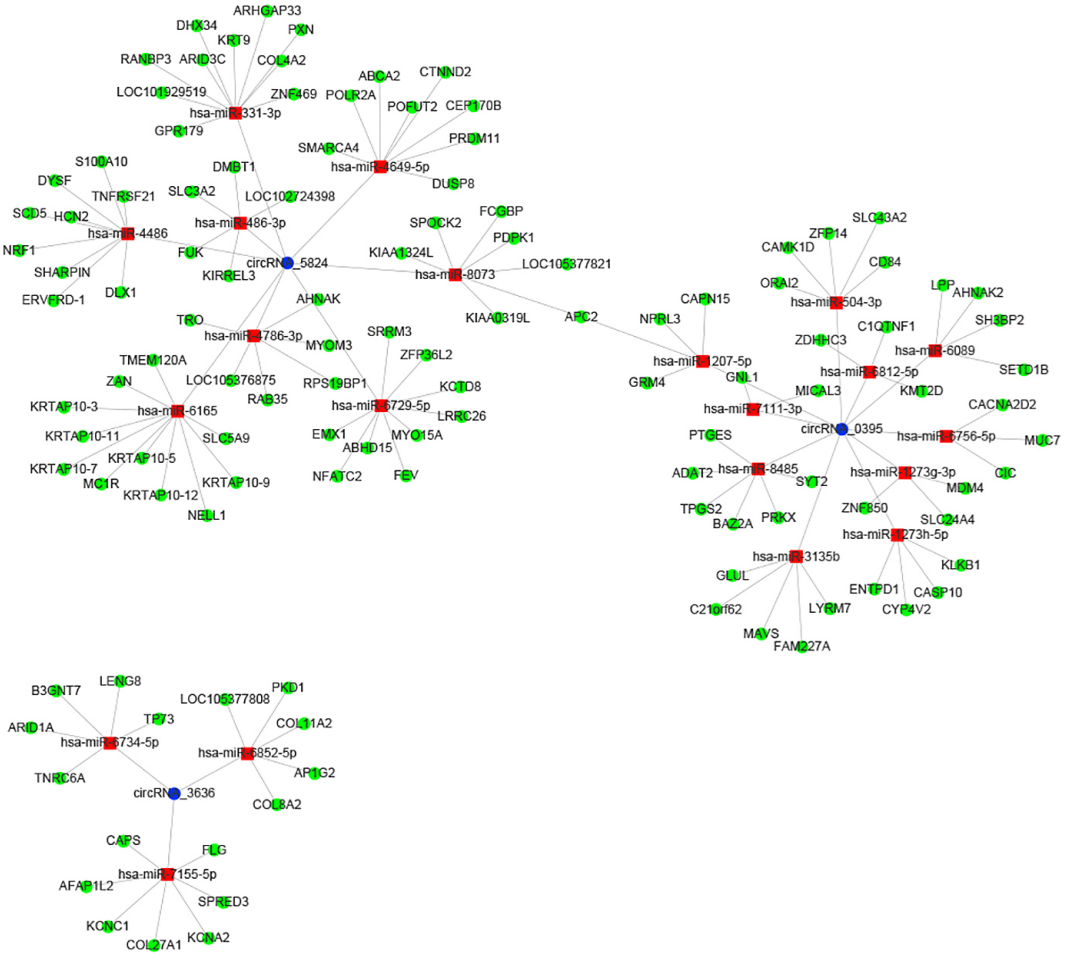

Fig. 4 circRNA-miRNA-mRNA interaction network
The circRNA-miRNA-mRNA network consists of three circRNAs (blue), 21 miRNAs (red) and 120 disease-related genes (green).

an miRNA in 2011 and can bind to 1,074 genes [30]. Guo *et al.* [38] reported that miR-1273g-3p participates in acute glucose fluctuation and is an important factor that leads to endothelial dysfunction and autophagy. It is also involved in the progression of several complications caused by diabetes by modulating the autophagy-lysosome pathway and could serve as a new target for disease therapy [39]. However, there has been no report on the role of miRNA-1273g-3p in pregnancy. Thus, without direct evidence that circRNA_0395 is targeted by miRNA-1273g-3p, further research was needed to confirm their relationship.

In conclusion, our study provides a preliminary landscape of the differential expression of circRNAs that may be involved in the occurrence and pathogenesis of GDM. The current study also provides new insight into the molecular mechanism of GDM.

Authors Contribution Statement

Huiyan Wang, Guangtong She, Wenbai Zhou and Bin Yu carried out the assays and participated in designing the study. Huiyan Wang, Guangtong She, Kezhuo Liu, Jun Miao carried out clinical consultation. Guangtong She, Wenbai Zhou and Bin Yu carried out sample collection, laboratory tests and performed the statistical analysis. Guangtong She and Bin Yu conceived the study, participated in its design and coordination and draft the manuscript.

Acknowledgements

We thank all of the project participants for their contributions.

Disclosure of Interests

The authors declare that they have no competing interests.

Details of Ethics Approval

The study design and protocol were reviewed and approved by the ethics committee of Changzhou Maternity and Child Health Care Hospital affiliated to Nanjing Medical University (Approval No: CZFY20160103).

Funding

This work was supported by grants from Jiangsu province health and family planning commission (F201439, QNRC305), Changzhou health and family planning commission (ZD201412), Changzhou municipal bureau of science and technology (CJ20159055).

References

1. Jovanovic L, Pettitt DJ (2001) Gestational diabetes mellitus. *JAMA* 286: 2516–2518.
2. Reece EA, Leguizamón G, Wiznitzer A (2009) Gestational diabetes: the need for a common ground. *Lancet* 373: 1789–1797.
3. Shaat N, Groop L (2007) Genetics of gestational diabetes mellitus. *Curr Med Chem* 14: 569–583.
4. Mastrogiannis DS, Spiliopoulos M, Mulla W, Homko CJ (2009) Insulin resistance: the possible link between gestational diabetes mellitus and hypertensive disorders of pregnancy. *Curr Diab Rep* 9: 296–302.
5. Ilekis JV, Tsilou E, Fisher S, Abrahams VM, Soares MJ, *et al.* (2016) Placental origins of adverse pregnancy outcomes: potential molecular targets: an Executive Workshop Summary of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. *Am J Obstet Gynecol* 215: S1–S46.
6. Jin B, Liu L, Zhang S, Cao X, Xu Y, *et al.* (2017) Nuclear magnetic resonance-assisted metabolic analysis of plasma for mild gestational diabetes mellitus patients. *Metab Syndr Relat Disord* 15: 439–449.
7. Pantham P, Aye IL, Powell TL (2015) Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta* 36: 709–715.
8. Peiris HN, Lappas M, Georgiou HM, Vaswani K, Salomón C, *et al.* (2015) Myostatin in the placentae of pregnancies complicated with gestational diabetes mellitus. *Placenta* 36: 1–6.
9. Poirier C, Desgagné V, Guérin R, Bouchard L (2017) MicroRNAs in pregnancy and gestational diabetes mellitus: emerging role in maternal metabolic regulation. *Curr Diab Rep* 17: 35.
10. Li J, Song L, Zhou L, Wu J, Sheng C, *et al.* (2015) A microRNA signature in gestational diabetes mellitus associated with risk of macrosomia. *Cell Physiol Biochem* 37: 243–252.
11. Zhang Y, Wu H, Wang F, Ye M, Zhu H, *et al.* (2017) Long non-coding RNA MALAT1 expression in patients with gestational diabetes mellitus. *Int J Gynecol Obstet* 140: 164–169.
12. Shi Z, Zhao C, Long W, Ding H, Shen R (2015) Microarray expression profile analysis of long non-coding rnas in umbilical cord plasma reveals their potential role in gestational diabetes-induced macrosomia. *Cell Physiol Biochem* 36: 542–554.
13. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, *et al.* (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495: 333–338.
14. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, *et al.* (2013) Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 19: 141–157.
15. Lukiw WJ (2013) Circular RNA (circRNA) in Alzheimer's disease (AD). *Front Genet* 4: 307.
16. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, *et al.* (2013) Natural RNA circles function as efficient microRNA sponges. *Nature* 495: 384–388.
17. Xu H, Guo S, Li W, Yu P (2015) The circular RNA Cdr1as, *via* miR-7 and its targets, regulates insulin transcription and secretion in islet cells. *Sci Rep* 5: 12453.
18. Geng HH, Li R, Su YM, Xiao J, Pan M, *et al.* (2016) The circular RNA Cdr1as promotes myocardial infarction by mediating the regulation of miR-7a on its target genes expression. *PLos One* 11: e0151753.
19. Liu Q, Zhang X, Hu X, Dai L, Fu X, *et al.* (2016) Circular RNA related to the chondrocyte ECM regulates MMP13 expression by functioning as a MiR-136 'Sponge' in human cartilage degradation. *Sci Rep* 6: 22572.
20. Maass PG, Glažar P, Memczak S, Dittmar G, Hollfinger I, *et al.* (2017) A map of human circular RNAs in clinically relevant tissues. *J Mol Med (Berl)* 95: 1179–1189.
21. Poirier C, Desgagné V, Guérin R, Bouchard L (2017) MicroRNAs in pregnancy and gestational diabetes mellitus: emerging role in maternal metabolic regulation. *Curr Diabetes Rep* 17: 35.
22. Gu Y, Ke G, Wang L, Zhou E, Zhu K, *et al.* (2017) Altered expression profile of circular RNAs in the serum of patients with diabetic retinopathy revealed by microarray. *Ophthalmic Res* 58: 176–184.
23. Zhao Z, Li X, Jian D, Hao P, Rao L, *et al.* (2017) Hsa_circ_0054633 in peripheral blood can be used as a diagnostic biomarker of pre-diabetes and type 2 diabetes mellitus. *Acta Diabetol* 54: 237–245.
24. Zhang XO, Dong R, Zhang Y, Zhang JL, Luo Z, *et al.* (2016) Diverse alternative back-splicing and alternative splicing landscape of circular RNAs. *Genome Res* 26: 1277–1287.
25. Anders S, Huber W (2013) Differential expression of RNA-Seq data at the gene level—the DESeq package. http://www.bioconductor.org/packages/devel/bioc/vignettes/DESeq/inst/doc/DESeq.pdf
26. John B, Enright AJ, Aravin A, Tuschl T, Sander C, *et al.* (2004) Human microRNA targets. *PLOS Biol* 2: e363.

27. Enright AJ, John B, Gaul U, Tuschl T, Sander C, *et al.* (2003) MicroRNA targets in *Drosophila*. *Genome Biol* 5: R1.
28. Goh SY, Cooper ME (2008) Clinical review: the role of advanced glycation end products in progression and complications of diabetes. *J Clin Endocrinol Metab* 93: 1143–1152.
29. Luo SJ, Yang HX (2012) Roles of advanced glycation end products and its receptor on the fetal brain injury in pregnant rats with gestational diabetes mellitus. *Zhonghua Fu Chan Ke Za Zhi* 47: 364–367 (In Chinese).
30. Morales-Prieto DM, Ospina-Prieto S, Schmidt A, Chaiwangyen W, Markert UR (2014) Elsevier trophoblast research award lecture: origin, evolution and future of placenta miRNAs. *Placenta* 35 Suppl: S39–S45.
31. Li C, Li X, Ma Q, Zhang X, Cao Y, *et al.* (2017) Genome-wide analysis of circular RNAs in prenatal and postnatal pituitary glands of sheep. *Sci Rep* 7: 16143.
32. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO (2012) Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 7: e30733.
33. Yan L, Feng J, Cheng F, Cui X, Gao L, *et al.* (2018) Circular RNA expression profiles in placental villi from women with gestational diabetes mellitus. *Biochem Biophys Res Commun* 498: 743–750.
34. Hansen TB, Venø MT, Damgaard CK, Kjems J (2016) Comparison of circular RNA prediction tools. *Nucleic Acids Res* 44: e58.
35. Kuc S, Wortelboer EJ, Koster MP, de Valk HW, Schielen PC, *et al.* (2011) Prediction of macrosomia at birth in type-1 and 2 diabetic pregnancies with biomarkers of early placentation. *BJOG* 118: 748–754.
36. Alvarez ML, Khosroheidari M, Eddy E, Kiefer J (2013) Role of microRNA 1207-5P and its host gene, the long non-coding RNA Pvt1, as mediators of extracellular matrix accumulation in the kidney: implications for diabetic nephropathy. *PLoS One* 8: e77468.
37. Thomas LF, Sætrom P (2014) Circular RNAs are depleted of polymorphisms at microRNA binding sites. *Bioinformatics* 30: 2243–2246.
38. Guo J, Sang Y, Yin T, Wang B, Yang W, *et al.* (2016) miR-1273g-3p participates in acute glucose fluctuation-induced autophagy, dysfunction, and proliferation attenuation in human umbilical vein endothelial cells. *Am J Physiol Endocrinol Metab* 310: E734–E743.
39. Zhao Z, Li X, Jian D, Hao P, Rao L, *et al.* (2017) Hsa_circ_0054633 in peripheral blood can be used as a diagnostic biomarker of pre-diabetes and type 2 diabetes mellitus. *Acta Diabetol* 54: 237–245.