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Research article

Upregulation of MMPs in placentas of patients with gestational diabetes mellitus: Involvement of the PI3K/Akt pathway

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ABSTRACT

In recent years, there has been a notable rise in the incidence of pregnancies complicated by gestational diabetes mellitus (GDM), characterized by glucose intolerance first identified during pregnancy. Analysis of placental tissue has revealed that placentas from women with GDM tend to be larger and heavier compared to control placentas, indicating potential changes in trophoblast proliferation, differentiation, and apoptosis. In this study, transcriptome sequencing was conducted on placentas obtained from both normal pregnancies and pregnancies with GDM to investigate the molecular mechanisms underlying this condition. The original sequencing data were subjected to sequencing analysis, resulting in the identification of 935 upregulated genes and 256 downregulated genes. The KEGG and GO analysis techniques on differential genes uncovered evidence suggesting that the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway may contribute to the pathogenesis of GDM. Subsequent analysis indicated that the expression levels of matrix metalloproteinases (MMP) 11, MMP12, MMP14, and MMP15, which are regulated by the PI3K/Akt pathway, were upregulated in the placentas of patients with GDM when compared to those of individuals with normal placental function. Additionally, our investigation into alternative splicing patterns revealed an increase in exon skipping alternative splicing of CSF3R in the placenta of patients with GDM compared to that in the control group. The CSF3R–PI3K-MMP pathway is speculated to regulate the pathogenesis of GDM.

1. Introduction

The prevalence of gestational diabetes mellitus (GDM) is increasing owing to the worldwide obesity epidemic and advancing maternal age. While GDM may improve after childbirth, a growing body of research suggests that it is linked to unfavorable outcomes for both the mother and fetus, resulting in complications during pregnancy and delivery, as well as an increased risk of developing

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postpartum type 2 diabetes mellitus (T2DM). Despite its transient nature, GDM can have lasting effects beyond the perinatal period, influencing the health of both the mother and fetus in the short and long term [1,2]. Common pathological changes observed in GDM include increases in placental volume and weight [3,4]. GDM occurs when insulin resistance during pregnancy is not regulated. As the placenta grows, the likelihood of insulin resistance also increases [5]. Previous research has suggested that the PI3K/Akt signaling pathway plays a pivotal role in cellular physiology by facilitating growth factor signaling during organismal growth and crucial cellular processes, such as glucose homeostasis, lipid metabolism, cell proliferation, and cell function [6–8]. The activation of PI3k/Akt signaling in beta cells can potentially enhance beta cell proliferation by influencing the cell cycle regulators p21, p27, CDK4, and CyclinD1 [9]. Furthermore, the activation of the PI3K/Akt pathway has been observed in placentas affected by preeclampsia (PE), possibly exhibiting a crucial role in the regulation of cell proliferation [10]. Accumulating evidence indicates that the PI3K/Akt signaling pathway is indispensable for maintaining normal metabolic processes owing to its unique attributes. However, dysregulation of the PI3K/Akt pathway, characterized by its overexpression or mutation, has been found to play a significant role in the development of various human diseases. These diseases include obesity, T2DM, and cancer [11]. Furthermore, the PI3K/Akt signaling pathway is involved in insulin metabolism and signaling, and is closely related to placental growth and development. Abnormalities in the PI3K/Akt signaling pathway may lead to poor placental development and restricted fetal growth. These factors may also be associated with the occurrence of GDM [12]. we found that the expression levels of MMP11, MMP12, MMP14, and MMP15, which are regulated by the PI3K/Akt pathway, were upregulated in the placentas of patients with GDM when compared to those of individuals with normal placental function.The deregulation of membrane type-matrix metalloproteinases (MT-MMP) expression is associated with GDM [13]. The regulation of MMP expression through the PI3K/Akt pathway may play a role in the onset and progression of GDM by influencing placental function, islet function, and the inflammatory response [14].

Over 90 % of transcribed human genes undergo alternative RNA splicing, thereby enhancing proteomic diversity through the generation of isoforms with variable functions from a single gene [15]. Abnormal pre-mRNA splicing is now acknowledged as the fundamental etiology of numerous human diseases, such as cancer, Alzheimer's, frontotemporal dementia, spinal muscular atrophy (SMA), and retinitis pigmentosa [16]. Moreover, three colony-stimulating factor 3 receptor (CSF3R) transcripts that arise from alternative RNA splicing have been identified in humans [17]. In the context of CSF3R, three documented splice variants exhibited distinct functional characteristics. Therefore, these three CSF3R splice variants, which possess inherently diverse signaling properties, have been hypothesized to interact with one another within the same cellular environment. Furthermore, alterations in their expression levels relative to other isoforms may determine cellular responses to proliferation or differentiation [18]. Our investigation revealed upregulation of CSF3R splice variants in the placenta of individuals with GDM. Previous studies have provided evidence that differential expression of various splice variants of CSF3R can influence cellular signaling pathways. Given the available literature on the expression of colony-stimulating factor 3 (CSF3) and its receptor CSF3R in placental tissues [19,20]and trophoblast [21], this cytokine has been hypothesized to play a role in placental development.Bioinformatics methodologies offer a fresh perspective for understanding GDM and its related complications, further investigating the potential influence of CSF3 and CSF3R.

Table 1
Comparison of the study group characteristics.

	Normal group (n = 60)	GDM group (n = 60)	p-value
Age(Years)	29.40 ± 5.34	31.20 ± 5.12	0.055
Gravidity	2.69 ± 1.21	2.97 ± 1.18	0.187
Parity	1.07 ± 0.80	1.23 ± 0.81	0.278
Gestational age at delivery (weeks)	38.97 ± 0.81	38.77 ± 0.72	0.126
Body mass gain during pregnancy (kg)	14.43 ± 6.18	13.56 ± 5.93	0.419
BMI (Kg/)	28.15 ± 3.17	29.94 ± 3.39	0.005
SBP in late pregnancy (mmHg)	118.61 ± 9.21	120.38 ± 9.68	0.293
DBP in late pregnancy (mmHg)	73.87 ± 8.25	74.82 ± 8.63	0.524
HB (g/L)	119.07 ± 13.08	120.41 ± 12.36	0.555
PLT (s)	227.33 52.85	219.38 61.82	0.434
PT (s)	10.84 ± 1.35	11.19 ± 0.98	0.100
APTT (s)	27.67 ± 1.89	27.51 ± 2.71	0.717
FIB (s)	4.40 ± 0.78	4.62 ± 1.63	0.347
HDL (mmol/L)	1.90 ± 0.29	1.77 ± 0.27	0.356
LDL (mmol/L)	3.22 ± 0.69	3.31 ± 1.05	0.795
VLDL (mmol/L)	0.51 ± 0.27	0.93 ± 0.34	0.004
TG (mmol/L)	2.94 ± 1.61	3.37 ± 1.03	0.545
TC (mmol/L)	5.62 ± 0.76	6.02 ± 1.29	0.500
Urea (mmol/L)	2.82 ± 0.67	3.22 ± 0.71	0.03
CR (μmol/L)	44.87 ± 8.04	47.20 ± 7.43	0.118
UR (μmol/L)	272.73 ± 70.26	278.45 ± 105.25	0.735
OGTT fasting glucose	4.16 ± 0.32	5.07 ± 0.52	0.000
OGTT1-h glucose	7.13 ± 1.51	9.61 ± 1.65	0.000
OGTT2-h glucose	6.21 ± 1.13	7.65 ± 1.54	0.000
HbA1C	4.94 ± 0.28	5.32 ± 0.55	0.000
Birth Weight (g)	3303.51 ± 330.15	3618.69 ± 462.76	0.000
Placenta weight (g)	552.07 ± 88.38	631.67 ± 126.05	0.024

2. Experimental materials and methods

2.1. Placental samples collection and analysis

Fresh consecutively delivered placentae were collected from women with normal pregnancies (n = 60) and women with pregnancies complicated by GDM (n = 60), all of whom had singleton term pregnancies (37–42weeks) and underwent caesarean delivery before the onset of labor at the Affiliated Hospital of Jining Medical University (Jining, China) between March 2020 and December 2022. The exclusion criteria were as follows: age <18 years old; a history of diabetes and other disorders affecting glucose metabolism; mental illness; heart, liver or kidney failure; intrahepatic cholestasis during pregnancy; central type placenta previa; hypertensive disorder complicating pregnancy; placental abruption; none of the participants were subjected to any dietary restrictions. Finally, 120 pregnant women were registered to our study. Initially, the mean placental weights were calculated. Subsequently, four fragments of 1 cm^3 were precisely excised from the central region of the placenta on the maternal side. Three of these fragments were promptly frozen at a temperature of −80 °C and intended for use within 30 min after collection, while the remaining fragment was designated for examination under light microscopy. This study was approved by the Ethics Committee of the Affiliated Hospital of Jining Medical University (Jining, China; ethical approval no. 2021B139). Each participant who was over 18 years of age provided written informed consent and signed an informed consent form. GDM was diagnosed according to International Association of Diabetes and Pregnancy Study Group criteria(IADPSG criteria) [22]. In the approach, a 75-g 2-h OGTT is administered to the fasting woman. Those whose FPG was ≥5.1 mmol/L (92 mg/dL) and/or 1-h PG was ≥10.0 mmol/L (180 mg/dL) and/or 2-h PG was ≥8.5 mmol/L (153 mg/dL) were defined as GDM by IADPSG criteria.The characteristics of the patients are listed in Table 1.

2.2. HE staining

Placental tissue samples were immersed in ice-cold PBS to remove blood contamination. A piece of placenta tissue 1 cm × 1 cm was fixed in 4 % paraformaldehyde, and tissue blocks were embedded in paraffin. Sections with a thickness of 4 μm were cut from the block and stained with HE. The resulting slides were examined and photographed under a light microscope (Olympus).

2.3. Experimental method of sequencing

For the experimental sequencing method, three placentas from the normal control group and three placentas from the GDM group were selected for transcriptome analysis. Total RNA was extracted from these samples to compare the expression levels of differentially expressed genes (DEGs). Total RNA was extracted, and RNA quality was assessed. After the RNA samples were qualified, common transcriptome libraries were constructed, and Illumina NovaSeq 6000 was used for sequencing.

2.4. Bioinformation analysis

After obtaining the sequencing data, basic data quality control was carried out; these high-quality sequences were then compared to the reference genome, and gene expression level and gene structure analyses were performed. The Illumina NovaSeq high-throughput sequencing platform was used to sequence cDNA libraries and produce numerous base sequences (reads). The reads or bases produced by the sequencing platform are referred to as raw data. High-quality clean reads were obtained from the original data by strict quality filtering for subsequent data analysis. The raw reads sequenced were not all effective, as they contained a small number of repeated low-quality reads with joints. These reads may affect subsequent comparison and analysis; therefore carefully filtering the sequenced raw reads is necessary for obtaining an effective and high-quality number of clean reads. Clean reads were compared to the silva database using bowtie 2 software to remove rRNA, and the remaining reads were used for subsequent analysis. FastQC was used to count the content of different bases in read pairs to determine whether the content of G and C bases and A and T bases was separated. Reads with rRNA removed were compared with the reference genome using the Hisat2 software. The ratio of comparison between the sequencing data and the reference genome, and the regional distribution of comparison between the sequencing data and the reference genome were calculated according to the gene location information specified in the genome annotation file (GTF format). According to the comparison results, the distribution of protein-coding region (CDS), Intron, Intergenic region, 3′ untranslated region (3′ UTR) and 5 ’untranslated region (5′ UTR) of the genome was statistically analyzed, and the source of sequencing reads on the genome was detected.

2.5. Transcription factor prediction

Differentially expressed transcription factors (TFs) are likely involved in the regulation of biological processes related to sample handling or stress. Predictive analysis of the TFs of DEGs is effective in exploring the differentially expressed TFs of different groups and their differential regulatory mechanisms. Predictive analysis of TFs can help identify key regulators that are driving the observed gene expression patterns and understand how these TFs are differentially regulated in different conditions or disease states.This approach can be particularly useful in identifying potential therapeutic targets or biomarkers for specific diseases or conditions. By understanding the regulatory networks involving TFs and DEGs, researchers can uncover novel pathways and mechanisms that may be dysregulated in disease states, providing new opportunities for targeted interventions. Overall, predictive analysis of TFs of DEGs can provide valuable insights into the molecular mechanisms underlying gene expression changes and help uncover potential therapeutic

targets for various diseases and conditions.

2.6. Statistical analysis

SPSS (version 25.0; IBM Corp.) was used for statistical analyses. The relative expression of the investigated factors was expressed as mean ± standard deviation. Differences between the two groups were analyzed using an independent sample *t*-test. Spearman's correlation analysis was also conducted. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics

The clinical characteristics compared between the GDM group and the control group are described in Table 1. The group diagnosed with GDM exhibited significantly higher body mass index (BMI), urea levels, fasting plasma glucose, 1- and 2-h plasma glucose levels during the oral glucose tolerance test (OGTT), HbA1C levels, very low-density lipoprotein (VLDL) levels, birth weight, and placental weight ($P < 0.05$) compared with the control group. Placental weight was measured in both groups, as depicted in Fig. 1a. The placental weight in the GDM group was heavier than that of the control group ($P < 0.05$), as illustrated in Fig. 1b.

3.2. Histological examination

The morphological features of term placental tissues from both the GDM and control groups were thoroughly examined using light microscopy. Fig. 2a presented the typical morphological characteristics of term placentas from the control group, which included the presence of syncytiotrophoblasts lining the villous trees with multigrade branching and clearly visible erythrocytes within multiple placental villi. No indications of calcification, fibrin deposition, or villous edema were observed. The term placentas from the GDM group displayed the expected morphological features, as illustrated in Fig. 2b–f. Qualitative observations of the terminal villi in certain areas of GDM placenta slides revealed the presence of chorioangiopathy (Fig. 2b), intervillous stenosis (Fig. 2c), an apparent increase in the number of syncytial knots (Fig. 2d), a reduction in free villi with uneven villi thickness and degeneration of villi interstitium, villous edema, edematous stroma (Fig. 2e), and perivillous fibrin deposition (Fig. 2f).

3.3. Identification of DEGs

The volcano plot in Fig. 3a illustrates that a total of 1191 DEGs were identified, with 935 upregulated and 256 downregulated genes in the GDM group compared to the control group. To visually represent the expression patterns of these DEGs under various experimental conditions and identify novel functional genes, differential gene heat maps were generated (Fig. 3b).

In our study, we found that the upregulation of MMP11, MMP12, MMP14, and MMP15 in the placenta of individuals with GDM compared to the control group, as depicted in the heat map Fig. 3c.

3.4 Gene Ontology (GO) annotation analysis of DEGsGO enrichment analysis was conducted on the entire set of DEGs. The results, depicted in Fig. 4a, demonstrate the top 20 upregulated biological processes (BPs). The DEGs primarily encompassed BPs associated with multicellular organismal processes, multicellular organism development, and developmental processes. This suggests a potential association between GDM and the formation of multicellular organisms. The initiation of GDM is intricately associated with the physiological mechanisms of multicellular organisms [23], specifically encompassing hormone regulation, placental function, cellular differentiation, and proliferation. Metabolic processes in multicellular organisms are associated with GDM. GDM patients experience heightened blood glucose levels during pregnancy because of inadequate insulin secretion or heightened insulin resistance, thereby affecting the glucose metabolism process in pregnant women. This correlation may be attributed to the regulatory mechanisms governing metabolism in multicellular organisms, including insulin synthesis and secretion and glucose uptake and utilization [24].

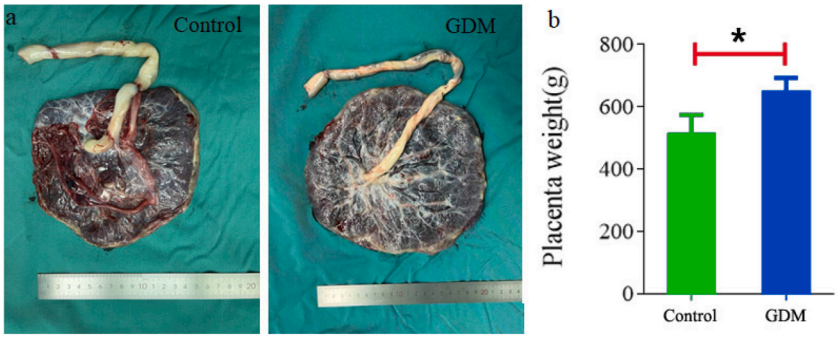


Fig. 1. Photos of the placenta were compared between the control group and the GDM group (a).The placental weight of the GDM group was heavier than that of the control group (b).*p < 0.05.

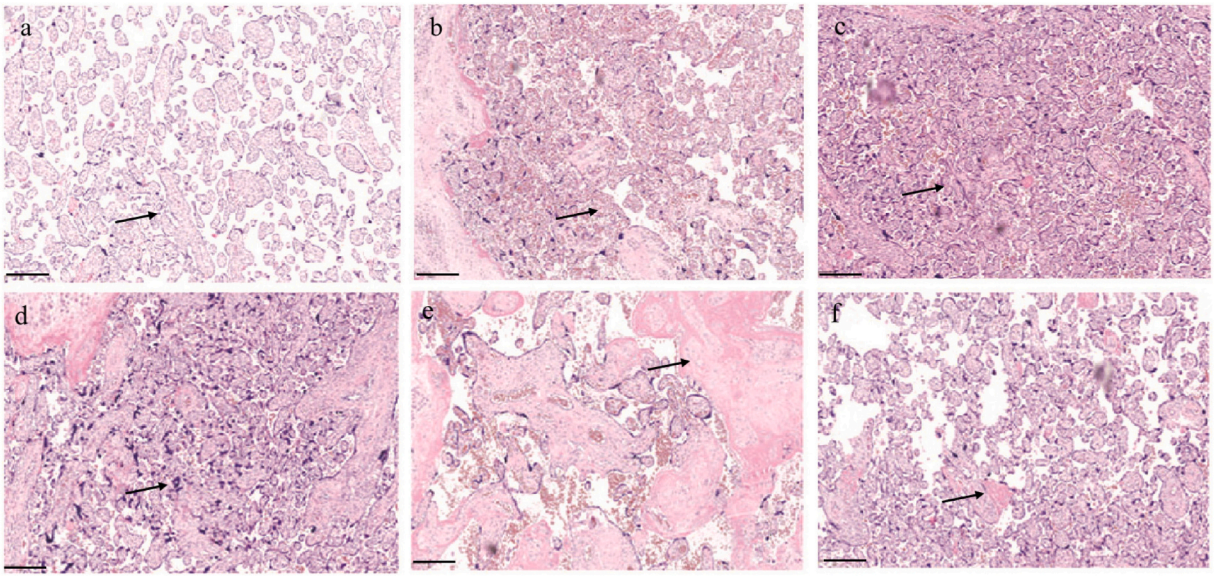


Fig. 2. Pathological morphology of placentas from control group (a) and GDM group (b,c, d,e,f). Placental sections were HE-stained. (a)The normal branching of villous trees, (b) Chorioangiopathy,(c)Intervillous stenosis, (d)The increased syncytial knotting(arrow), (e) Free villi reduce with uneven villi thickness and degeneration of villi interstitium, villous edema (arrows), (f) Perivillous fibrin depositions in GDM group. Scar Bar = 100 μm.

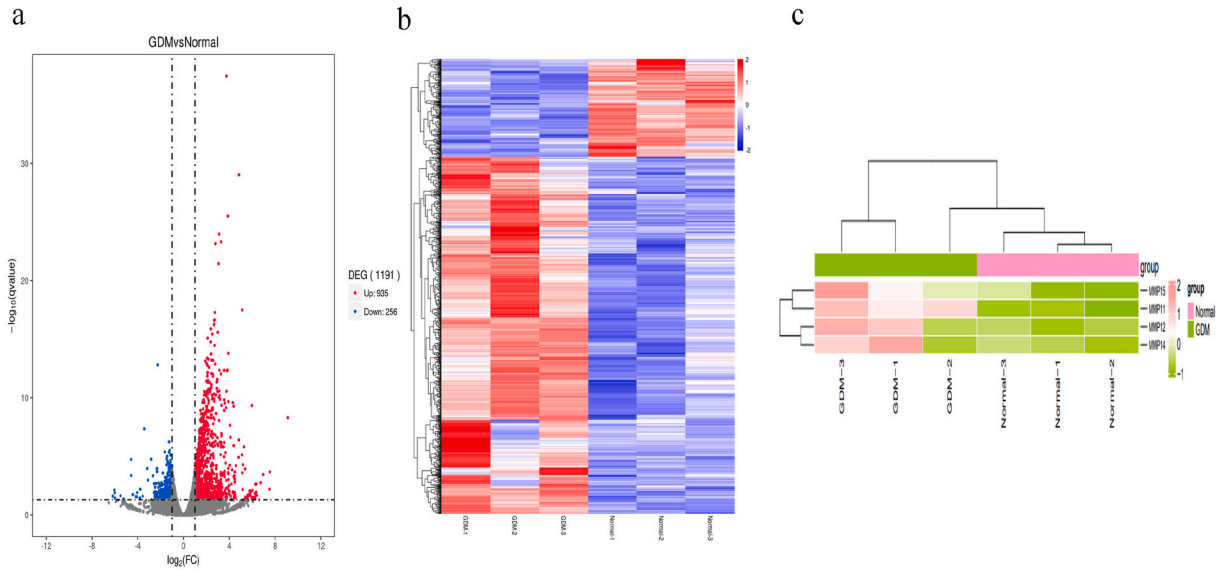


Fig. 3. Transcriptome sequencing of placental tissues in the control group and the GDM group. (a) Volcano plot analysis of 935 upregulated DEGs and 256 downregulated DEGs. (b) Differential gene heat maps were performed for the DEGs. (c) The upregulation of MMP11, MMP12, MMP14, and MMP15 in the placenta of individuals with GDM compared to the control group.

The top 20 upregulated cellular components (CCs) are depicted in Fig. 4b, revealing that the majority of DEGs are associated with membrane-related components. These include the membrane, its intrinsic components, and its integral components. The top 20 upregulated molecular functions (MFs) are illustrated in Fig. 4c, indicating that the predominant MFs of the DEGs are related to various functions involving signaling receptors and transmembrane transporters. These include signaling receptor binding, transmembrane transporter activity, and inorganic molecular entity transmembrane transporter activity.

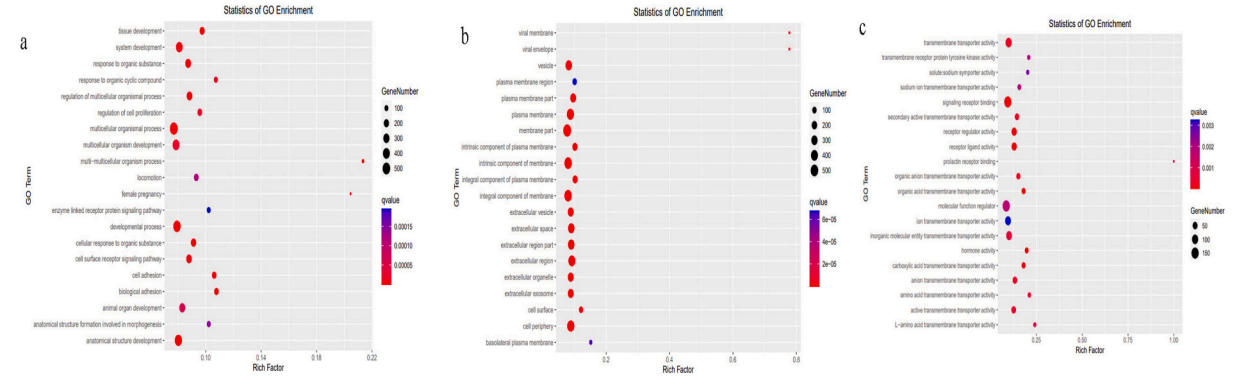


Fig. 4. Gene Ontology (GO) analyses of DEGs in the control group and the GDM group.(a)Top 20 GO-BP terms for the total DEGs.(b)Top 20 GO-CC terms for the total DEGs.(c) Top 20 GO-MF terms for the total DEGs.

3.4. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the DEGs

KEGG pathway analysis indicated that the DEGs were associated with various signaling pathways, including the PI3K/Akt, MAPK, and cAMP signaling pathways, proteoglycans in cancer, cytokine-cytokine receptor interactions, and cell adhesion molecules (CAMs) (Fig. 5a and. b).

3.5. TFs prediction

In our study, we observed differential expression of MMP11, MMP12, MMP14, and MMP15 between the GDM and control groups. Additionally, our analysis of the KEGG pathway suggests that transcription factors within the signaling pathway may play a role in promoting the transcription of these MMPs. Specifically, the activation of the PI3K signaling pathway has been shown to promote the activation of transcription factors, such as CREB3L2, CREB5 [25], Stat5B [26], FOX04 [27], and SREBF1, which are involved in the regulation of gene expression. To further investigate the potential regulatory mechanisms, we used the JASPAR database to analyze these five transcription factors and predict the transcription of MMP genes. These five transcription factors had binding sites in the MMP11, MMP12, MMP14, and MMP15 promoter regions, the results are shown in Table 2.

3.6. Variable splicing analysis

Variable splicing is a significant factor in gene regulation, protein functionality, and disease onset. In humans, three CSF3R transcripts, resulting from alternative RNA splicing, have been identified, each of which exhibits distinct functional characteristics

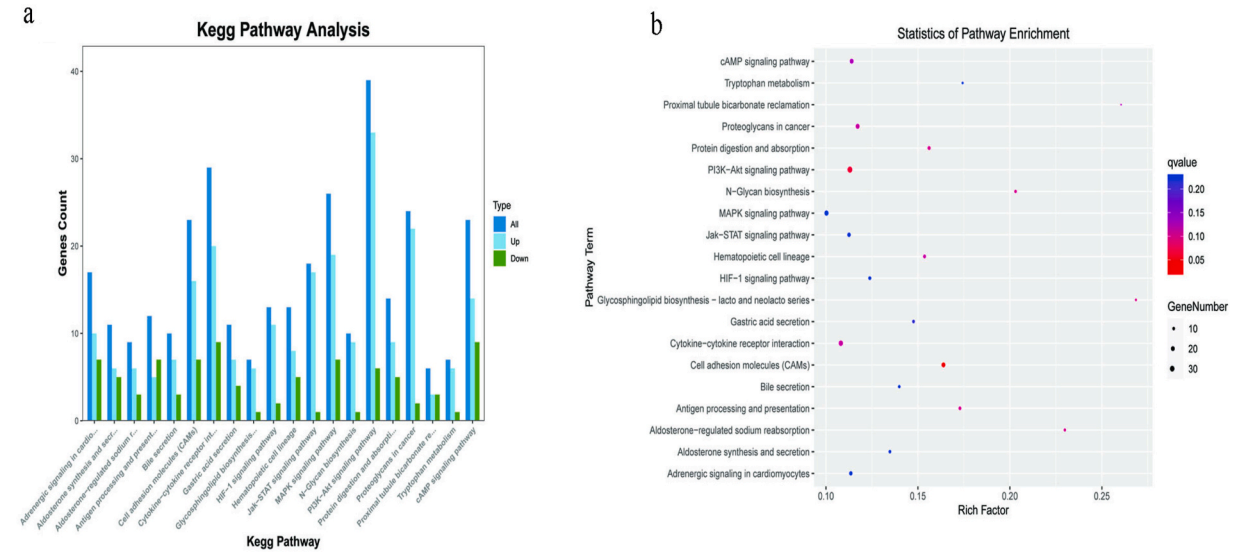
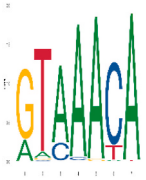
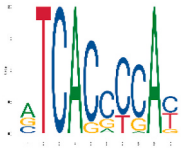
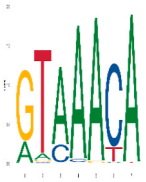
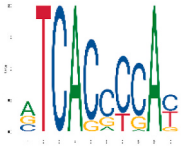
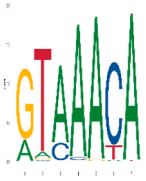
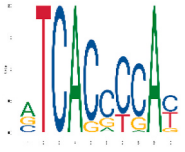
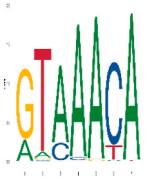
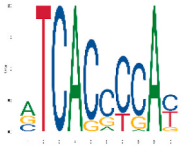


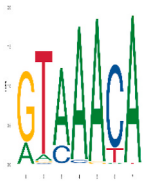
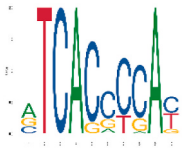
Fig. 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of DEGs.(a) The top 20 pathways of significant enrichment were selected and displayed by bar chart,(b) The top 20 pathways of significant enrichment were selected and displayed by scatter plots.

Table 2
Binding situation between transcription factors and the promoter regions.

Transcription factor	Target gene	NO	score	start	end	Predicted sequences	Sequence logo
FOXO4	MMP1	1	6.59	231	237	GTAAAAA	
		2	6.21	1113	1119	GTAAACT	
		3	5.61	547	553	GTCCACA	
		4	4.91	29	35	ATAAAAA	
		5	4.91	1500	1506	ATAAAAA	
		6	4.71	1296	1302	GCCAACA	
		7	4.68	23	29	AAAAATA	
SREBF1	MMP1	1	12.74	1438	1447	ATCACACCAC	
		2	10.35	1625	1634	ATCACACCAC	
		3	7.61	572	581	CTCACCTCCT	
		4	7.15	138	147	ATCGCGTCAC	
		5	6.26	1263	1272	ATCACTTGAG	
FOXO4	MMP12	1	8.57	1788	1794	ATAAATA	
		2	7.47	322	328	GGAAACA	
		3	7.47	971	977	GGAAACA	
		4	7.15	1888	1894	AAAAACA	
SREBF1	MMP12	1	12.69	1085	1094	ATCACACCAT	
		2	11.9	1265	1274	ATCACCTCAG	
		3	8.52	627	636	ATCAGACCAG	
		4	6.42	27	36	ATCAAATCAC	
FOXO4	MMP15	1	5.20	1853	1859	GTAAACG	
		2	4.60	656	662	TTAAACA	
		3	3.94	1594	1600	ATATACA	
		4	3.54	1596	1602	ATACATA	
SREBF1	MMP15	1	10.29	1341	1350	CTCACCCCAA	
		2	8.63	202	211	ATCACCAGAC	
		3	7.72	1777	1786	GTCACCCCCG	
FOXO4	MMP3	1	11.04	740	746	ATAAACA	
		2	8.57	1303	1309	ATAAATA	
		3	6.59	1670	1676	GTAAAAA	
		4	6.36	354	360	GAAAATA	
SREBF1	MMP3	1	10.44	942	951	ATCACCACAT	
		2	8.33	453	462	GTCACGGCAC	

(*continued on next page*)

Table 2 (*continued*)

Transcription factor	Target gene	NO	score	start	end	Predicted sequences	Sequence logo
FOXO4	MMP14	1	10.64	73	79	GTCAACA	
		2	6.78	2059	2065	GCAAACA	
		3	6.21	1559	1565	GTAAACT	
		4	5.11	107	113	ACAAACA	
SREBF1	MMP14	1	11.95	216	225	GTCACACCAT	
		2	8.86	1708	1717	ATCAAGCCAC	
		3	7.52	1300	1309	TTCACCCAAC	
		4	6.80	740	749	CACACACCAT	

[17]. Previous studies have documented the activation of the PI3K signaling pathway through CSF3R alternative splicing [28]. In our study, we analyzed alternative splicing in DEGs and observed a higher occurrence of alternative splicing in the CSF3R gene in the GDM group than in the control group, as depicted in Fig. 6.

4. Discussion

Placental development plays a crucial role in fetal health. Pregnancies complicated by GDM are characterized by maternal insulin resistance, low-grade inflammation, and endothelial cell dysfunction, which are considered to be key factors. However, the molecular mechanisms underlying the pathogenesis of GDM remain largely unknown. Many signaling pathways are involved in the pathophysiology of GDM, including the peroxisome proliferator-activated receptors (PPARs) signaling pathway [29], HIF1α signaling pathway, mTOR and JAK/STAT signaling pathway, nuclear factor-κB (NF-κB) signaling pathway, nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, and PI3K/mTOR and MAPK signaling pathway [30]. In this study, a total of six placental tissue samples were selected for transcriptome sequencing. These findings revealed significant differences in the molecular expression profiles of placental tissues between the GDM and control groups. Furthermore, analysis of the DEGs using the KEGG pathway demonstrated that the DEGs were primarily concentrated in the PI3K/Akt signaling pathway. Notably, the PI3K/Akt pathway has been extensively validated as a crucial factor that facilitates placental development and fetal growth in both humans and rodents [31]. The PI3K/Akt signaling pathway is involved in the regulation of trophoblast proliferation [10], migration, and invasion [32–34]. The diabetic placenta is characterized by increased size and weight [35,36], which is consistent with our results. This may be caused by the increased active proliferation and decreased placental apoptosis of trophoblast cells among patients with diabetes [37–39]. Because activation of the PI3K/Akt signaling pathway further regulates cellular physiological functions, such as promoting cell proliferation and survival, hyperglycemia may possibly stimulate proliferation and reduce apoptosis of trophoblast cells through the PI3K/Akt signaling pathway, thereby increasing placental hypertrophy and weight.

The PI3K/Akt pathway is activated in preeclamptic placentas, and the inhibition of the PI3K/Akt pathway may be a useful therapeutic treatment for preeclampsia [10]. The PI3K/Akt signaling pathway could increase insulin sensitization and act as an anti-hyperglycemic [40]; therefore, dysfunctional PI3K/Akt-mediated glucose transport and glycogen synthesis play important roles in the development of obesity and T2DM [41]. The treatment of obesity and T2DM is possible by altering the PI3K/AKT pathway [42–45]. Additionally, certain studies have found that gene mutations or abnormal PI3K expression may be related to the development of diabetes. For example, studies have found that certain genetic mutations in PI3K may lead to abnormalities in insulin signaling, which can lead to diabetes [46–48]. Other studies have found that overexpression of PI3K may lead to the overactivation of insulin signaling, which can lead to insulin resistance and diabetes [49,50]. Therefore, the PI3K/Akt signaling pathway is closely related to the pathogenesis of diabetes and may be one of the important molecular mechanisms of diabetes. However, the role of the PI3K/Akt signaling pathway in GDM has not yet been studied.

In the present study, the PI3K/Akt pathway was significantly upregulated in the GDM group. Additionally, upregulation of MMP11, MMP12, MMP14, and MMP15, along with increased expression of CSF3R splice variants, was found in the placenta of individuals with GDM compared to the control group. Placental MT-MMPs are required for cytotrophoblast migration, invasion of the uterine wall, and remodeling of spiral arteries, which participate in the fusion of cytotrophoblasts to generate syncytiotrophoblasts, as well as in the formation of new blood vessels (angiogenesis). Temporal and spatial regulation of MT-MMP activity is crucial. Aberrant MT-MMP expression has been associated with pregnancy complications, including PE [51], fetal growth restriction (FGR), and GDM [13]. Previous investigations have demonstrated the upregulation of MT1-MMP (MMP14) protein levels in the placenta during the third trimester in patients with GDM, which aligns with our findings.

CSF3R is a cell membrane receptor associated with the PI3K signaling pathway [52]. Variable splicing of CSF3R refers to the generation of multiple mRNA transcripts via a splicing mechanism during gene transcription. Three annotated variants of CSF3R have

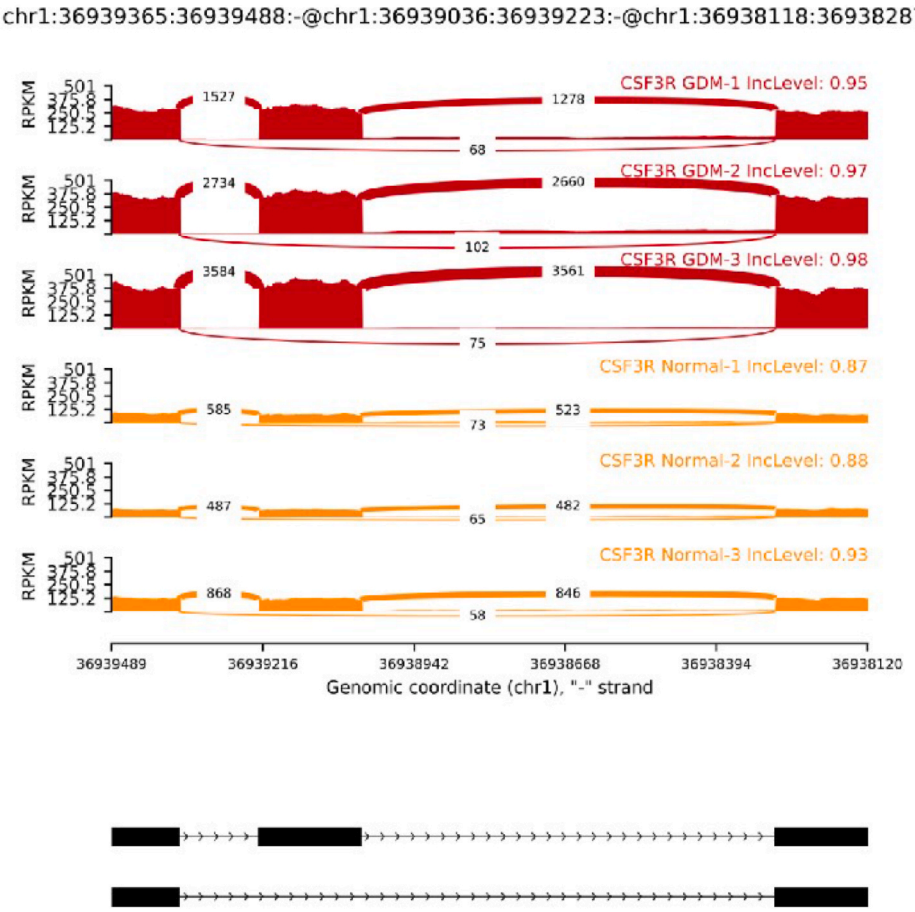


Fig. 6. An analysis of alternative splicing in DEGs and a higher occurrence of alternative splicing in the CSF3R gene within the GDM group compared to the control group.

been described, which are identical in their extracellular domains but differ in their downstream sequences [53,54]. These alternatively spliced forms of CSF3R may exhibit different functions and regulatory mechanisms. Accumulating evidence has shown that CSF3 plays an essential role in placental metabolism, trophoblast development, decidualization of endometrial stromal cells, and ovulation by binding to CSF3R to regulate multiple signaling pathways [52–55]. CSF3 could promote trophoblast invasion and migration through activating the PI3K/Akt signaling pathway, thereby involving the normal pregnancy program [56]. CSF3 upregulates metalloproteinase-2 and VEGF through activating PI3K/Akt signaling pathway in human trophoblast Swan 71 cells, stimulating placental blood vessel formation, which is essential for placental formation [52]. CSF3 upregulates β1 integrin and increases migration of human trophoblast Swan 71 cells via PI3K and MAPK signaling pathway activation,

The above evidence indicates that CSF3 should be considered an additional regulatory factor that contributes to successful embryo implantation and placental development via the PI3K/Akt signaling pathway [57]. Specifically, when the CSF3R receptor is activated by its ligand (CSF3), CSF3R interacts with relevant proteins in the PI3K signaling pathway through its intracellular structure, thereby activating the PI3K signaling pathway [58]. We speculate that the alternative splicing of CSF3 and CSF3R results in the formation of three CSF3R splicing variants. These variants activate the PI3K/Akt signaling pathway in cells, leading to the activation of specific transcription factors. These transcription factors then bind to the promoter regions of MMPs, regulating their expression. This dysregulation of MMP expression ultimately leads to glucose metabolism disorders and insulin resistance in gestational diabetes mellitus (GDM). However, additional investigations are necessary to understand the importance of the interaction between CSF3 and CSF3R in trophoblasts. Further studies are required to fully understand the specific mechanisms by which MMPs contribute to GDM.

5. Conclusion

The CSF3R–PI3K-MMP pathway is speculated to regulate the pathogenesis of GDM. Our study found for the first time that the PI3K pathway is upregulated in GDM, which may be related to the regulation of the signal axis of CSF3R–PI3K-MMP. This study provides a foundation for an in-depth understanding of the mechanisms of GDM and for the development of drugs related to the treatment of GDM.

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Ethics statement

This study was approved by the human research Ethics Committee of the Affiliated Hospital of Jining Medical University (Jining, China; ethical approval no. 2021B139). All patients provided informed consent to participate in the study.

Data availability statement

The data presented in the study (sequencing raw data) were uploaded to GEO repository accession number GEO24488221 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GEO24488221).

CRediT authorship contribution statement

Yanan Zhang: Funding acquisition, Formal analysis, Data curation. **Yufen Liu:** Data curation. **Yanyan Shi:** Formal analysis. **Chunyu Bai:** Data curation. **Ting Wang:** Data curation. **Fang Ruan:** Data curation. **Chuanbing Hu:** Funding acquisition, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] B.E. Metzger, et al., Hyperglycemia and adverse pregnancy outcomes, N. Engl. J. Med. 358 (19) (2008) 1991–2002.
[2] S.R. Murray, R.M. Reynolds, Short- and long-term outcomes of gestational diabetes and its treatment on fetal development, Prenat. Diagn. 40 (9) (2020) 1085–1091.
[3] A. Edu, et al., Placenta changes in pregnancy with gestational diabetes, Rom. J. Morphol. Embryol. 57 (2) (2016) 507–512.
[4] J. Huynh, et al., A systematic review of placental pathology in maternal diabetes mellitus, Placenta 36 (2) (2015) 101–114.
[5] R. Arshad, N. Karim, J. Ara Hasan, Effects of insulin on placental, fetal and maternal outcomes in gestational diabetes mellitus, Pakistan J. Med. Sci. 30 (2) (2014) 240–244.
[6] P. Abeyrathna, Y. Su, The critical role of Akt in cardiovascular function, Vasc. Pharmacol. 74 (2015) 38–48.
[7] Q. Lu, et al., TIPE2 overexpression suppresses the proliferation, migration, and invasion in prostate cancer cells by inhibiting PI3K/Akt signaling pathway, Oncol. Res. 24 (5) (2016) 305–313.
[8] X. Peng, et al., Oxyfadichalcone C inhibits melanoma A375 cell proliferation and metastasis via suppressing PI3K/Akt and MAPK/ERK pathways, Life Sci. 206 (2018) 35–44.
[9] J. Li, et al., Pre-eclampsia-associated reduction in placental growth factor impaired beta cell proliferation through PI3k signalling, Cell. Physiol. Biochem. 36 (1) (2015) 34–43.
[10] J.K. Park, et al., Inhibition of the PI3K-Akt pathway suppresses sFlt1 expression in human placental hypoxia models in vitro, Placenta 31 (7) (2010) 621–629.
[11] G.L. Dornan, J.E. Burke, Molecular mechanisms of human disease mediated by oncogenic and primary immunodeficiency mutations in class IA phosphoinositide 3-kinases, Front. Immunol. 9 (2018) 575.
[12] C. Feng, et al., SHBG expression is correlated with PI3K/AKT pathway activity in a cellular model of human insulin resistance, Gynecol. Endocrinol. 34 (7) (2018) 567–573.
[13] A. Majali-Martinez, et al., Placental membrane-type metalloproteinases (MT-MMPs): key players in pregnancy, Cell Adhes. Migrat. 10 (1–2) (2016) 136–146.
[14] N. Houttu, et al., The impacts of fish oil and/or probiotic intervention on low-grade inflammation, IGFBP-1 and MMP-8 in pregnancy: a randomized, placebo-controlled, double-blind clinical trial, Biomolecules 11 (1) (2020).
[15] A. Nellore, et al., Human splicing diversity and the extent of unannotated splice junctions across human RNA-seq samples on the Sequence Read Archive, Genome Biol. 17 (1) (2016) 266.
[16] G.S. Wang, T.A. Cooper, Splicing in disease: disruption of the splicing code and the decoding machinery, Nat. Rev. Genet. 8 (10) (2007) 749–761.
[17] J.E. Maxson, et al., Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML, N. Engl. J. Med. 368 (19) (2013) 1781–1790.
[18] A. Lance, et al., Altered expression of CSF3R splice variants impacts signal response and is associated with SRSF2 mutations, Leukemia 34 (2) (2020) 369–379.
[19] S.C. Shorter, G.S. Vince, P.M. Starkey, Production of granulocyte colony-stimulating factor at the materno-foetal interface in human pregnancy, Immunology 75 (3) (1992) 468–474.
[20] S.A. McCracken, et al., Gestational regulation of granulocyte-colony stimulating factor receptor expression in the human placenta, Biol. Reprod. 60 (4) (1999) 790–796.
[21] J.Y. Zhang, et al., Vitamin D promotes trophoblast cell induced separation of vascular smooth muscle cells in vascular remodeling via induction of G-CSF, Front. Cell Dev. Biol. 8 (2020) 601043.
[22] National Institutes of Health consensus development conference statement: diagnosing gestational diabetes mellitus, March 4-6, 2013, Obstet. Gynecol. 122 (2 Pt 1) (2013) 358–369.
[23] Y. Jin, H. Wang, Identification of hub genes affecting gestational diabetes mellitus based on GEO database, Biotechnol. Genet. Eng. Rev. (2023) 1–11.
[24] P. Schwartsburd, Insulin resistance is a two-sided mechanism acting under opposite catabolic and anabolic conditions, Med. Hypotheses 89 (2016) 8–10.
[25] X. Xiao, et al., Methylation-mediated silencing of ATF3 promotes thyroid cancer progression by regulating prognostic genes in the MAPK and PI3K/AKT pathways, Thyroid 33 (12) (2023) 1441–1454.
[26] G. Fouquet, et al., Iron-loaded transferrin potentiates erythropoietin effects on erythroblast proliferation and survival: a novel role through transferrin receptors, Exp. Hematol. 99 (2021) 12–20.e3.
[27] L. Ren, et al., [Synergistic regulation of the erythroid differentiation of K562 cells by KLF1 and KLF9], Yi Chuan 40 (11) (2018) 998–1006.

[28] J.M. Liu, et al., [PRDM1 expression and its relationship with PI3K/AKT pathway activation in extranodal NK/T cell lymphoma-nasal type], Zhonghua Xue Ye Xue Za Zhi 39 (12) (2018) 1010–1016.
[29] Á. Lendvai, et al., The peroxisome proliferator-activated receptors under epigenetic control in placental metabolism and fetal development, Am. J. Physiol. Endocrinol. Metab. 310 (10) (2016) E797–E810.
[30] C. Nguyen-Ngo, et al., Molecular pathways disrupted by gestational diabetes mellitus, J. Mol. Endocrinol. 63 (3) (2019) R51–r72.
[31] J. Li, et al., A MicroRNA signature in gestational diabetes mellitus associated with risk of macrosomia, Cell. Physiol. Biochem. 37 (1) (2015) 243–252.
[32] J. Prast, et al., Human chorionic gonadotropin stimulates trophoblast invasion through extracellularly regulated kinase and AKT signaling, Endocrinology 149 (3) (2008) 979–987.
[33] Q. Qiu, et al., EGF-induced trophoblast secretion of MMP-9 and TIMP-1 involves activation of both PI3K and MAPK signalling pathways, Reproduction 128 (3) (2004) 355–363.
[34] X. Zhu, et al., Knockdown of TACC3 inhibits trophoblast cell migration and invasion through the PI3K/Akt signaling pathway, Mol. Med. Rep. 14 (4) (2016) 3437–3442.
[35] A. Ramos, et al., In women with gestational diabetes mellitus factors influencing growth have a larger effect on placental weight than on birth weight, Eur. J. Obstet. Gynecol. Reprod. Biol. 202 (2016) 60–65.
[36] M. Kucuk, F. Doymaz, Placental weight and placental weight-to-birth weight ratio are increased in diet- and exercise-treated gestational diabetes mellitus subjects but not in subjects with one abnormal value on 100-g oral glucose tolerance test, J. Diabet. Complicat. 23 (1) (2009) 25–31.
[37] H.T. Chen, et al., [Adiponectin exerts antiproliferative effect on high glucose-induced BeWo cell proliferation in vitro], Zhonghua Fu Chan Ke Za Zhi 51 (3) (2016) 204–208.
[38] G. Unek, et al., The proliferation mechanism of normal and pathological human placentas, Histol. Histopathol. 32 (4) (2017) 339–349.
[39] T.H. Hung, et al., Decreased placental apoptosis and autophagy in pregnancies complicated by gestational diabetes with large-for-gestational age fetuses, Placenta 90 (2020) 27–36.
[40] Y. Gao, et al., Effects of D-pinitol on insulin resistance through the PI3K/Akt signaling pathway in type 2 diabetes mellitus rats, J. Agric. Food Chem. 63 (26) (2015) 6019–6026.
[41] X. Huang, et al., The PI3K/AKT pathway in obesity and type 2 diabetes, Int. J. Biol. Sci. 14 (11) (2018) 1483–1496.
[42] C. Wang, et al., FAM3A activates PI3K p110α/Akt signaling to ameliorate hepatic gluconeogenesis and lipogenesis, Hepatology 59 (5) (2014) 1779–1790.
[43] J.R. Kuang, et al., Dapper1 attenuates hepatic gluconeogenesis and lipogenesis by activating PI3K/Akt signaling, Mol. Cell. Endocrinol. 447 (2017) 106–115.
[44] T. Chattopadhyay, et al., Bone morphogenetic protein-7 (BMP-7) augments insulin sensitivity in mice with type II diabetes mellitus by potentiating PI3K/AKT pathway, Biofactors 43 (2) (2017) 195–209.
[45] M. Kuai, et al., A novel formula Sang-Tong-Jian improves glycometabolism and ameliorates insulin resistance by activating PI3K/AKT pathway in type 2 diabetic KKAy mice, Biomed. Pharmacother. 84 (2016) 1585–1594.
[46] Y. Terauchi, et al., Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase, Nat. Genet. 21 (2) (1999) 230–235.
[47] D. Chen, et al., p50alpha/p55alpha phosphoinositide 3-kinase knockout mice exhibit enhanced insulin sensitivity, Mol. Cell Biol. 24 (1) (2004) 320–329.
[48] F. Mauvais-Jarvis, et al., Reduced expression of the murine p85alpha subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes, J. Clin. Invest. 109 (1) (2002) 141–149.
[49] C. Wang, et al., Hepatic overexpression of ATP synthase β subunit activates PI3K/Akt pathway to ameliorate hyperglycemia of diabetic mice, Diabetes 63 (3) (2014) 947–959.
[50] T.Y. Liu, et al., Irisin inhibits hepatic gluconeogenesis and increases glycogen synthesis via the PI3K/Akt pathway in type 2 diabetic mice and hepatocytes, Clin. Sci. (Lond.) 129 (10) (2015) 839–850.
[51] J. Chen, R.A. Khalil, Matrix metalloproteinases in normal pregnancy and preeclampsia, Prog Mol Biol Transl Sci 148 (2017) 87–165.
[52] V.A. Furmento, et al., The granulocyte colony-stimulating factor (G-CSF) upregulates metalloproteinase-2 and VEGF through PI3K/Akt and Erk1/2 activation in human trophoblast Swan 71 cells, Placenta 35 (11) (2014) 937–946.
[53] B.R. Avalos, Molecular analysis of the granulocyte colony-stimulating factor receptor, Blood 88 (3) (1996) 761–777.
[54] P. Dwivedi, K.D. Greis, Granulocyte colony-stimulating factor receptor signaling in severe congenital neutropenia, chronic neutrophilic leukemia, and related malignancies, Exp. Hematol. 46 (2017) 9–20.
[55] S. Makinoda, et al., Granulocyte colony-stimulating factor (G-CSF) in the mechanism of human ovulation and its clinical usefulness, Curr. Med. Chem. 15 (6) (2008) 604–613.
[56] J. Ding, et al., M2 macrophage-derived G-CSF promotes trophoblasts EMT, invasion and migration via activating PI3K/Akt/Erk1/2 pathway to mediate normal pregnancy, J. Cell Mol. Med. 25 (4) (2021) 2136–2147.
[57] V.A. Furmento, et al., Granulocyte colony-stimulating factor (G-CSF) upregulates β1 integrin and increases migration of human trophoblast Swan 71 cells via PI3K and MAPK activation, Exp. Cell Res. 342 (2) (2016) 125–134.
[58] Q. Li, et al., Targeted regulation of osteoblasts and osteoclasts in osteosarcoma patients by CSF3R receptor inhibition of osteolysis caused by tumor inflammation based on transcriptional spectrum analysis and drug library screening, Recent Pat. Anti-Cancer Drug Discov. 19 (5) (2023) 635–651.